Toxinology

P. Gopalakrishnakone *Editor-in-Chief*

Abul Faiz · Ravindra Fernando
Christeine Ariaranee Gnanathasan
Abdulrazaq Garba Habib · Chen-Chang Yang *Editors*

Clinical Toxinology in Asia Pacific and Africa

Springer Reference

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In recent years, the field of toxinology has expanded substantially. On the one hand it studies venomous animals, plants and micro organisms in detail to understand their mode of action on targets. While on the other, it explores the biochemical composition, genomics and proteomics of toxins and venoms to understand their three interaction with life forms (especially humans), development of antidotes and exploring their pharmacological potential. Therefore, toxinology has deep linkages with biochemistry, molecular biology, anatomy and pharmacology. In addition, there is a fast-developing applied subfield, clinical toxinology, which deals with understanding and managing medical effects of toxins on human body. Given the huge impact of toxin-based deaths globally, and the potential of venom in generation of drugs for so-far incurable diseases (for example, diabetes, chronic pain), the continued research and growth of the field is imminent. This has led to the growth of research in the area and the consequent scholarly output by way of publications in journals and books. Despite this ever-growing body of literature within biomedical sciences, there is still no all-inclusive reference work available that collects all of the important biochemical, biomedical and clinical insights relating to toxinology.

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Clinical Toxinology in Asia Pacific and Africa

With 170 Figures and 43 Tables

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Series Preface

The term TOXIN is derived from the Greek word *Toeikov* and is defined as a substance derived from tissues of a plant, animal, or microorganism that has a deleterious effect on other living organisms. Studying their detailed structure, function, and mechanism of action as well as finding an antidote to these toxins is the field of TOXINOLOGY, and the scientists are called TOXINOLOGISTS.

In recent years, the field of toxinology has expanded substantially. On the one hand, it studies venomous animals, plants, and microorganisms in detail to understand their habitat, distribution, identification, as well as mode of action on targets, while on the other, it explores the biochemical composition, genomics, and proteomics of toxins and venoms to understand their interaction with life forms (especially humans), the development of antidotes, and their pharmacological potential for drug discovery. Therefore, toxinology has deep linkages with biochemistry, molecular biology, anatomy, pharmacology, etc. In addition, there is a fast-developing applied subfield, clinical toxinology, which deals with understanding and managing medical effects of venoms and toxins on the human body following envenomations. Given the huge impact of envenomation-based deaths globally and the potential of venom in the generation of drugs for debilitating diseases (e.g., diabetes, chronic pain, and cancer), the continued research and growth of the field is imminent.

Springer has taken the bold initiative of producing this series, which is not an easy target of producing about 11 volumes, namely, biological toxins and bioterrorism, clinical toxinology, scorpion venoms, spider venoms, snake venoms, marine and freshwater toxins, toxins and drug discovery, venom genomics and proteomics, evolution of venomous animals and their toxins, plant toxins, and microbial toxins.

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Singapore

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Volume Preface

Clinical toxinologic conditions are becoming increasingly frequent, more so than is generally recognized. The conditions comprise of clinical aspects such as the diagnosis, management, and prevention of snakebite envenoming, scorpion sting, mushroom toxins, plant toxins, and other natural toxins. Clinical toxinology also deals with the ecology, epidemiology, regional differences, and varieties of fauna accounting for different envenoming manifestations.

This handbook includes 30 chapters addressing various topics on clinical toxinology such as the epidemiology and management of snakebites in different Asian and African countries, disability following snakebite, effect of snake venoms on hemostasis, socioeconomic aspects of snakebites, therapeutic application of snake venom, scorpion sting in the Middle East, jellyfish sting, etc. These titles are written by experts currently working in the subspecialty, many of whom have first-hand experience in relevant research field. In virtually all the topics, appropriate illustrations are provided to simplify comprehension including tables, figures, pictures of snakes, etc.

A variety of clinical patterns and toxidromes commonly observed in practice are described and depicted. Thus, this handbook will be very useful to students and specialists that work or study expedition and wilderness medicine, traveller's health, tropical and geographic medicine, and health economics, among others.

Snakebite is a major clinical toxinologic issue worldwide, especially in the rural areas of developing countries in tropical and subtropical regions, with an estimate of at least 400,000 envenomings and 20,000–50,000 deaths annually worldwide. The actual figures may be much higher due to under-reporting of the bitten incidents. Therefore, clinical toxinologic issues related to venomous snakebites are heavily covered in this handbook, and various important aspects of snakebites are discussed. For example, in the management of snakebites, the only specific treatment is antivenom; however, antivenom is often unavailable in the rural areas and remote health centers in developing countries. The vast majority of the estimated burden of venomous snakebites is thus derived from South and Southeast Asia, sub-Saharan Africa, and Central and South America. Several chapters in this handbook have addressed the epidemiology and management of venomous snakebites in different Asian and African countries.

Moreover, the use of antivenoms produced by the purification of IgG immunoglobulins from large animals immunized against specific snake venoms is life-saving. Nevertheless, very little has been changed on the way these antivenoms are produced in the last few decades. The advances on the transcriptomic analysis of venom glands from different snake species with a focus on the efforts to develop antivenom sera by DNA immunization and its efficacy in neutralizing the toxic effects elicited by the envenomation from snakebite are also discussed in this handbook.

In this modern era of science and technology, this volume, *Clinical Toxinology in Asia Pacific and Africa*, in the series, *Toxinology*, is designed to keep abreast with new knowledge and experience in toxinology regionally and globally. Toxinologists, researchers, scientists, and experts in this field from various working areas considered it necessary to collect all the aspects of clinical toxinology in a single, handy handbook. This can be used by medical students, postgraduate students, general practitioners, specialists in internal medicine, critical care physicians, emergency physicians, and anesthetists worldwide.

We are certain that this publication in the field of toxinology will advance knowledge and understanding of clinical toxinological issues at different levels and that it will entice actions through cognitive, curative, and preventive measures aimed at making improvements in this discipline worldwide.

March 2015

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His research studies include structure function studies, toxin detection, biosensors, antitoxins and neutralization factors, toxinogenomics and expression studies, antimicrobial peptides from venoms and toxins, and PLA2 inhibitors as potential drug candidates for inflammatory diseases. The techniques he employs include quantum dots to toxinology, computational biology, microarrays, and protein chips.

Prof. Gopalakrishnakone has more than 160 international publications, 4 books, about 350 conference presentations, and 10 patent applications.

He has been an active member of the International Society on Toxinology (IST) for 30 years and was president from 2008 to 2012. He is also the founder president of its Asia Pacific Section, a council member, as well as an editorial board member of *Toxicon*, the society's official journal.

His research awards include the Outstanding University Researcher Award from the National University of Singapore (1998); Ministerial Citation, NSTB Year 2000 Award in Singapore; and the Research Excellence Award from the Faculty of Medicine at NUS (2003).

His awards in teaching include Faculty Teaching Excellence Award 2003/2004 and NUS Teaching Excellence Award 2003/2004. Prof. Gopalakrishnakone also received the Annual Teaching Excellence Award in 2010 at both university and faculty levels.

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Abul Faiz, MBBS, FCPS, FRCP, PhD, is a professor of medicine. He was formerly an administrative chief of the Dhaka Medical College, a dean of the Faculty of Medicine of the University of Dhaka, and a director general of Health Services of the Government of Bangladesh. Professor Faiz is a member of the World Health Organization Malaria Treatment Guideline Committee, the National Steering Committee for the elimination of kala-azar, Bangladesh, and Regional Technical Advisor for malaria, SEARO WHO. Currently, he is the president of both Bangladesh Association for Advancement of Tropical Medicine (BAATM) and Toxicology Society of Bangladesh (TSB). He is member of the Board of Drugs for Neglected Diseases Initiative (DNDi); International Advisory Board, *Davidson's Principles and Practice of Medicine*; and editorial board, *Asian Neurology* and *Journal of Bangladesh Society of Medicine*. He has been involved as principal investigator in key clinical studies on malaria. He has the credit of publication of several hundred articles in peer-reviewed journals.

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Professor Fernando was the founder secretary general of the Indo-Pacific Association of Law, Medicine and Science and a past president of the Ceylon College of Physicians, Sri Lanka Medical Association, and the College of Forensic Pathologists of Sri Lanka and Asia-Pacific Association of Medical Toxicology.

He was a member of the World Health Organization's Expert Advisory Panel for Vector Biology and Control, and a member of the Scientific Committee on Pesticides of the International Commission on Occupational Health.

Professor Fernando was the founder head of the National Poisons Information Centre, in Sri Lanka. He has also served as the chairman of the National Dangerous Drugs Control Board.

He is an international editor, *Medicine*, *Science and the Law*, the official journal of the British Academy of Forensic Sciences, and *Journal of Forensic Medicine and Toxicology*, and the editor-in-chief of the *International Journal of Prevention and Treatment of Substance Use Disorders*, published by the Colombo Plan.

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In her capacity as a medical teacher and a medical specialist, she has contributed to undergraduate and postgraduate teaching, development of the medical curriculum, and for continuing medical education of doctors and nurses. She has been

instrumental in developing the Herpetarium and Snake Venom Research Laboratory in the Department of Clinical Medicine, Faculty of Medicine, at the University of Colombo. Professor Gnanathasan has contributed to venom research and clinical trials on antivenom and is working on an indigenous anti–snake-venom serum. Awarded the Master of Philosophy degree, her thesis, "A National, Hospital Based Survey of Snakes (Venomous and Non Venomous) Responsible for Human Bites in Sri Lanka – A Clinico-Epidemiological Study," examined clinico-epidemiological surveys of identified snakebites in Sri Lanka. She has published about 50 scientific papers in peer-reviewed international medical journals, authored 2 books for her credit, and is recipient of several research awards and prizes. Snakebites, toxicology, and internal medicine are her main research interests.

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Professor Habib trained and worked at university hospitals in Nigeria, Saudi Arabia, England, and Singapore. His areas of interest include community-acquired infections, emerging infections, human immunodeficiency virus (HIV) infections, immunology, tropical diseases, and snakebites. He participated in the initial characterization of a new emerging infection CoV SARS in Singapore (2003). He served as director medical services (2005–2007) and subsequently as a consultant to the Institute of Human Virology, Nigeria (an affiliate of Institute of Human Virology University of Maryland, USA), where they provided care to over one-third of HIV-infected patients in the country.

He serves on several advisory boards and committees in Nigeria: National Drug Safety Advisory Committee (2008–date), the National Taskforce on Multidrug-Resistant Tuberculosis (2007–2008), the writing team of the *National Antiretroviral Therapy Guidelines* (2007), EchiTab Study Group for the National Snakebite Control Programme (1994 to date), and the National Expert Committee on Adverse

Events Following Immunizations (AEFI) (2012–date). Professor Habib facilitated the development of the *National TB HIV Strategic and Clinical Management Guidelines* (2006–2007), the Nigerian Field Epidemiology and Laboratory Training Program (FELTP) on TB HIV of the Centers for Disease Control, Nigeria/USA, and the Federal Ministry of Health, Nigeria (2008), and is medical advisor, National Rapid Response Team on Avian Influenza (2006–2007).

He is a fellow of West African College of Physicians, Academy of Medical Sciences Infectious Diseases (Singapore), Royal College of Physicians (London), Royal Society of Tropical Medicine and Hygiene (UK); a member of Royal College of Physicians (UK), International Society of Infectious Diseases, International AIDS Society, International Society of Toxinology, International Union Against Tuberculosis and Lung Diseases, and International Travel Society; and the current president, Nigerian Infectious Diseases Society.

He has over 100 publications on global health and is a recipient of several awards and prizes including the third prize of "World Oxoid infection control team of the year award in 2007" (Basingstoke, UK, corecipient), Singapore Prime Minister's medal and certificate of appreciation and courage fund medal for valor and selfless dedication during the SARS epidemic in 2003, and Ayo-Iyun Prize for best result in WACP examinations (winner).

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APAMT between 2010 and 2012 and was the secretary general and treasurer of Asian Society of Toxicology (ASIATOX) between 2006 and 2009 and between 2009 and 2012, respectively. Dr. Yang is also a consultant to the Food and Drug Administration and Council of Agriculture in Taiwan.

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Part I

Snake Envenomation and Snake Venoms

Epidemiology of Snake Envenomation in Taiwan

1

Yan-Chiao Mao and Dong-Zong Hung

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Abstract

There are six major venomous snakes in Taiwan including 3 crotalids, *Trimeresurus (Viridovipera) stejnegeri*, *Protobothrops mucrosquamatus*, and *Deinagkistrodon acutus*; 1 viperid, *Daboia russelli siamensis*; and 2 elapids, *Naja atra* and *Bungarus multicinctus*. The annual incidence of these venomous snakebites has increased from 361.3 to 965.5 cases in the past 70 years, while the incidence rate declines from 8.8 to 4.3 cases per 100,000 person-years. Overall, the highest frequency of bites is observed for, in descending order, *T. stejnegeri*, *P. mucrosquamatus*, *B. multicinctus*, *N. atra*, *D. acutus*, and *D. r. siamensis*. However, the distribution of snakebites varies throughout the country and between hospitals and has changed with time. In northern and southern Taiwan, *T. stejnegeri* and *P. mucrosquamatus* snakebites account for the majority of cases; in central Taiwan, cases of *N. atra* bites predominate, whereas cases of *D. r. siamensis* bites only scattered in the southern and eastern areas. In Sawai's studies during 1960s–1970s, the case fatality rate for *T. stejnegeri*, *P. mucrosquamatus*, *N. atra*, and *B. multicinctus* bites was 0 %, 1.4 %, 1.6 %, and 7.1 %, respectively. In a recent study, three deaths were reported among 3,862 snakebite cases during 2002–2005. Snakebite is an occupational and environmental disease in Taiwan, generally involving middle-aged males, with a ratio of male to female victims of 2–3:1. Snakebites usually occur in the warm season (April–October) on farmlands, in homes, or on roads. Knowledge of the habitats and habits of venomous snakes could be helpful in the identification of offending snakes and the prevention of snakebites.

Introduction

Taiwan is an island in southeastern Asia with an area of 35,883 km^2. This island is characterized by the contrast between the eastern two-thirds, consisting mostly of rugged mountains (highest peak, 3,952 m) and the flat to gently rolling Chianan Plains in the west. The Tropic of Cancer runs through the middle of Taiwan and is associated with tropical and subtropical weather. The average annual temperature is 22 °C (71.6 °F) in the plain area. There are no extremes of temperature in winter and summer; thus the island is a suitable environment for many animals including snakes. A total of 61 (52) snake species, belonging to seven families, inhabit Taiwan and its territorial sea: Typhlopidae, Pythonidae, Colubridae, Homalopsidae, Pareatidae, Elapidae, and Viperidae (Hsiang et al. 2009). Among these species, 29 (22) are venomous, including 16 (15) land snakes and 13 (7) sea snakes (Hsiang et al. 2009; Tu 2008). Reported numbers of snake species are variable (shown in brackets) because some species are extremely rare or may even be exotic. Among

the 16 venomous land snakes, only six are medically important, namely, *Trimeresurus (Viridovipera) stejnegeri*, *Protobothrops mucrosquamatus*, *Deinagkistrodon acutus*, *Daboia russelli siamensis*, *Naja atra*, and *Bungarus multicinctus* (Table 1.1). The other venomous snakes have rarely or never been reported to cause significant envenomation.

Study of snakebites in Taiwan began during the Japanese colonial period (1895–1945). Statistical records of this injury were previously maintained by the government. However, after World War II (1945), the reporting system was discontinued and unfortunately the original data were lost (Sawai and Tseng 1969). In the following 70 years, rapid expansion of human population, environmental change (Lin 2001), and altered healthcare-seeking behaviors may have additively changed the epidemiology of snakebites. Understanding the local epidemiologic data will be helpful in the better management of snakebites in that country.

History of Snake Venom and Antivenom Study

Systemic study of reptiles in Taiwan began in the 1890s and only 18 reptile species were recorded till 1897 (Liu 2005). Since 1909, Masamitsu Ōshima, a herpetologist, had investigated Taiwan's snakes and published several scientific articles. In 1918, Yonetaro Kikuchi described a small venomous snake found in Mt. Noko, Nanto (Nenggao Mountain in Nantou county). This snake was named as "*Trimeresurus gracilis*" by Masamitsu Ōshima 2 years later (Oshima 1920), and it was also given the common name of "Kikuchi's Habu" in order to honor the first collector. In 1941, 60 snake species including nine sea snakes were described in *Snakes of Taiwan* edited by Yasuichi Horikawa, in Japanese (Liu 2005).

Research on snake venom and antivenom began in the 1910s after the establishment of a pharmaceutical station in the Governmental-General of Taiwan (Shen 2012). In 1916, an official report showed that snake envenomation was one of the major research topics of this station. In 1925, the marketed antivenoms might have included those specific for *T. stejnegeri*, *P. mucrosquamatus*, *N. atra*, and *B. multicinctus*. In 1944, an antivenom specific for *B. multicinctus* and *P. mucrosquamatus* was also prepared; however, the quality and effectiveness of the above-noted antivenoms were unclear. In 1952, this station was reorganized into Vaccine Center, which belongs to Centers for Disease Control, Taiwan, and was responsible for systemic evaluation and production of snake venom and antivenom (Department of Information 1971; Liau 1999). During the 1960s, three types of equine-derived antivenom including bivalent for *T. stejnegeri* and *P. mucrosquamatus*, bivalent for *N. atra* and *B. multicinctus*, and monovalent for *D. acutus* were produced by the center and distributed to various healthcare facilities (Liau and Huang 1997; Sawai and Tseng 1969). In 1970, the annual production amounts of these antivenoms were 905, 361, and 527 vials, respectively (Department 1971). In the 1980s, the vaccine center further modified the

Table 1.1 The six venomous snakes of medical importance in Taiwan (Hsiang et al. 2009; Tu 2008)

Family	Subfamily	Species	Common name	Snake length (cm)	Fang length (mm)	Distance between fangs puncture (mm) (Mao 1993)[b]
Viperidae	Crotalinae	*Trimeresurus* (*Viridovipera*) *stejnegeri*	Taiwan bamboo viper	60–90	–	9.8–12.6
		Protobothrops (former: *Trimeresurus*) *mucrosquamatus*	Taiwan habu, turtle-designed snake, pointed-scaled pit viper	60–120	–	12.0–18.2
		Deinagkistrodon (former: *Agkistrodon*) *acutus*	100-pacer, 100-pace snake, five-pacer	80–155	–	18.0–22.0
	Viperinae	*Daboia russelli siamensis* (former: *Vipera russelli formosensis*)	Russell's viper, chain snake	70–110	–	13.0–19.0
Elapidae		*Naja atra* (former: *Naja naja atra*)	Chinese cobra, Taiwan cobra, common cobra	70–150	3–4 (Hung and Lin-Shiau 2001)[a]	11.0–13.5
		Bungarus multicinctus	Taiwan banded krait, banded krait	70–180	–	9.0–12.2

[a]Unpublished data
[b]Limited numbers of specimens
–: No data

immunization and manufacturing processes; hence the immunization period was shortened and the mortality of horses during immunization was eliminated. The potency of antivenom was enhanced and the shelf-life was also lengthened (Huang et al. 1985, 1986; Liau and Huang 1997; Liau et al. 1982). In the 1990s, antivenom production for *D. r. siamensis* was started and finally approved for human use in 2008 (Liau and Fuh 1991; Xu et al. 2013). Currently, there are four types of antivenom including 2 bivalent and 2 monovalent, all $F(ab')_2$ fragment, available for the treatment of six major venomous snakebites. In 2011, the number of bivalent antivenom for *T. stejnegeri* and *P. mucrosquamatus*, for *N. atra* and *B. multicinctus*, and monovalent for *D. acutus* and for *D. r. siamensis* purchased by the all healthcare facilities in Taiwan was 3,067, 1,186, 171, and 21 vials, respectively. On average, 2,000 units (or at least 1,000 Tanaka units) were contained in each vial of the above-noted antivenoms (Liau and Fuh 1991; Xu et al. 2013).

Epidemiology of Venomous Snakebites in Taiwan

Snakebite is an environmental and occupational disease, especially in tropical regions (Alirol et al. 2010; Warrell 2010b). Globally, more than 5,000,000 snakebites with 125,000 deaths might have occurred each year (Chippaux 1998). In the Asia-Pacific region, 237,379–1,184,550 snake envenomings with 15,385–57,636 deaths annually have been estimated (Kasturiratne et al. 2008). In Taiwan, only a few nationwide epidemiological studies can be retrieved from the literature. On the other hand, quite a few hospital-based, questionnaire-oriented, and consultation-driven studies, with limited case numbers, were published after the 1970s (Table 1.2). These studies are summarized according to their source population and/or sampling method into the following sections. The regional and monthly distributions of snakebite as well as the anatomic area of bitten wound from various studies are listed in Tables 1.3, 1.4, and 1.5.

Whole Island

Snakebite was once a compulsorily reported disease in Taiwan. Dr. To collected and analyzed data from the Department of Police Administration, reporting 12,645 cases with 839 deaths during 1904–1938 (To 1941). The average cumulative incidence of snakebite during this 35-year period was 361.3 cases annually or an incidence rate of 8.8 cases per 100,000 person-years, and the average cumulative incidence of death was 24 cases per year or 0.6 cases per 100,000 person-years. The victims bitten by *T. stejnegeri* was 5,987 cases (47.3 %), *P. mucrosquamatus* 3,283 (26.0 %), *B. multicinctus* 894 (7.1 %), *N. atra* 593 (4.7 %), *D. acutus* 240 (1.9 %), *D. r. siamensis* 45 (0.3 %), *Sinomicrurus macclellandi* 3 (0.02 %), and unidentified venomous snakes 1,600 (12.7 %). After 1910, statistics revealed that most snakebite occurred in the warm seasons between May and October, and those between July and September accounted for 42.6 % of total cases. Dr. To also found that the

Table 1.2 Epidemiological studies of snakebite cases and mortality in Taiwan

Authors, areas of study	Study period	TS		PM		DA		DrS		NA		BM		Unidentified or nontoxic		Total	
		Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
To, whole island (To 1941)	1904–1938	5,987	54	3,283	275	240	58	45	1	593	87	894	206	1,600	158	12,645[a]	839
Sawai and Tseng, whole island (Sawai and Tseng 1969)	1964–1968	165	0	393	6	37	6	4	0	100	0	152	6	66	1	891[b]	19
Sawai et al., Kaohsiung (Sawai et al. 1970)	1968–1969	58	0	72	0	4	2	0	0	3	0	5	2	8	0	150	4
Sawai et al., Pingtung (Sawai et al. 1972)	1965–1971	97	0	115	2	34	6	20	3	24	2	26	5	19	0	335	18
Kuo and Wu, southern Taiwan (Kuo and Wu 1972)	1962–1971	16	0	23	1	4	0	1	0	13	0	6	3	0	0	63	4
Liang et al., northern Taiwan (Liang et al. 1992)	1984–1991	26	0	23	0	0	0	0	0	3	0	3	0	45	0	100	0

Miao et al., Questionnaire (Miao et al. 1995)	1988–1991	54	0	66	0	2	0	1	0	17	0	6	0	43	0	189	0
Wu et al., whole island, PCC (Wu et al. 1999)	1986–1998	99	0	98	0	15	1	8	0	44	0	36	9	144	1	444	11
Chen et al., northern Taiwan (Chen et al. 2000)	1991–1994	68	0	31	0	0	0	0	0	0	0	0	0	31	0	130	0
Liao et al., southern Taiwan (Liao et al. 2000)	1986–1999	20	0	6	1	1	0	1	0	6	0	5	0	7	0	46	1
Hung et al., central Taiwan (Hung 2004)	1993–2000	37	0	38	0	4	0	1	0	102	1	25	0	75	0	282	1
Shih et al., northern Taiwan (Shih et al. 2006)	1999–2004	29	0	54	0	1	0	1	1	14	0	6	0	13	0	118	1
Chang et al., southern Taiwan (Chang et al. 2007)	2001–2005	11	0	5	0	0	0	10	0	8	0	0	0	3	0	37	0
Total cases		6,667	54	4,207	285	342	73	92	5	927	90	1,164	231	2,054	160	15,430	898

TS Trimeresurus (Viridovipera) *stejnegeri*, *PM Protobothrops mucrosquamatus*, *DA Deinagkistrodon acutus*, *DrS Daboia russelli siamensis*, *NA* naja atra, *BM Bungarus multicinctus*

[a]The total number includes three cases of bites by *Sinomicrurus macclellandi*

[b]There was an error in the original article

Table 1.3 Regional distribution of snakebite cases

Comments	Study period	The North	The Central	The South	The East	Total cases
To, whole island (To 1941)	1919–1938					
TS		1,804	262	852	1,182	4,100
PM		1,185	206	482	308	2,181
DA		56	21	59	78	214
DrS		3	3	3	33	42
NA		77	83	119	110	389
BM		271	118	152	97	638
Unidentified		307	116	214	362	999
						8,563
Sawai and Tseng, whole island (Sawai and Tseng 1969)	1964–1968					
TS		43	1	150	4	198
PM		125	51	147	12	335
DA		3	9	17	9	38
DrS		0	0	3	3	4
NA		10	27	49	10	96
BM		47	21	53	7	128
Unidentified		80	0	3	10	93
						891[a]

TS Trimeresurus (Viridovipera) stejnegeri, *PM Protobothrops mucrosquamatus*, *DA Deinagkistrodon acutus*, *DrS Daboia russelli siamensis*, *NA Naja atra*, *BM Bungarus multicinctus*. *North* including Taipei, Ilan, Taoyan, Hsinchu, and Miaoli counties; *Central* including Taichung, Changhua, and Nantou counties; *South* including Yunlin, Chiayi, Tainan, Kaohsiung, and Pingtung counties; *East* including Taitung and Hualien counties

[a]There was an error in the original article

majority of cases occurred in farmland (either dry or rice farms), forest, homes, or on roads, and there were some between-species differences in places where snakebite happened: *T. stejnegeri* bites usually occurred in the forest, bush, or on farmland; *P. mucrosquamatus* in homes, on roads, or farmland; *N. atra* in homes or on farmland; *B. multicinctus* on roads or in homes; and *D. acutus* bites in forest or bush. The case fatality rate from envenoming was 0.9 % for *T. stejnegeri* bites, 8.4 % for *P. mucrosquamatus* bites, 24.2 % for *D. acutus* bites, 2.2 % for *D. r. siamensis* bites, 14.7 % for *N. atra* bites, and 23.0 % for *B. multicinctus* bites. During 1922–1929, the ratio of male to female victims was 2.9:1. During 1932–1936, snakebite mainly involved younger age groups, with 39.2 % of cases in the age group of 0–15 years and 29.5 % in 20–35 years.

Table 1.4 Monthly distribution of venomous snakebite cases

Authors, areas of study/ months	To, whole island (To 1941)	Sawai and Tseng, whole island (Sawai and Tseng 1969)	Sawai et al., Kaohsiung (Sawai et al. 1970)	Sawai et al., Pingtung (Sawai et al. 1972)	Kuo and Wu, southern Taiwan (Kuo and Wu 1972)	Liao et al., southern Taiwan (Liao et al. 2000)	Hung et al., central Taiwan (Hung 2004)	Total cases
Study period	1910–1938	1964–1968	1968–1969	1965–1971	1962–1971	1986–1999	1993–2000	
January	160	4	3	11	3	1	2	184
February	166	10	5	15	0	2	1	199
March	320	19	5	13	2	1	8	368
April	643	52	5	10	4	3	24	741
May	1,126	75	15	20	3	5	28	1,272
June	1,403	142	30	25	7	9	24	1,640
July	1,493	162	36	23	12	5	40	1,771
August	1,616	202	32	30	12	6	48	1,946
September	1,547	101	10	27	7	5	45	1,742
October	1,332	51	5	25	2	3	38	1,456
November	808	25	3	14	8	5	11	874
December	317	5	1	11	3	1	13	351
Unknown				111				111

Table 1.5 Anatomic area of bitten wound in snakebite cases

Authors, areas of study/ bitten sites	Sawai and Tseng, whole island (Sawai and Tseng 1969)	Sawai et al., Kaohsiung (Sawai et al. 1970)	Sawai et al., Pingtung (Sawai et al. 1972)	Kuo and Wu, southern Taiwan (Kuo and Wu 1972)	Liang et al., northern Taiwan (Liang et al. 1992)	Miao et al., Questionnaire (Miao et al. 1995)	Liao et al., southern Taiwan (Liao et al. 2000)	Shih et al., northern Taiwan (Shih et al. 2006)	Chang et al., southern Taiwan (Chang et al. 2007)	Lin et al., southern Taiwan (Chen et al. 2009a)
Study period	1964–1968	1968–1969	1965–1971	1962–1971	1984–1991	1988–1991	1986–1999	1999–2004	2001–2005	1993–2002
Upper extremities	243	57	108	33	55	73	30	51	18	35
Finger	125	31	45	16		51	24		13	22
Hand	82	23	55	15	52	22	4		4	13
Forearm	27	3	6	1	3		2		1	
Upper arm	9		2	1						
Lower extremities	518	87	202[a]	28	44	50	16	67	15	19
Toe	93	14	20	4		18	1		1	6
Foot	365	62	163	17	41	32	13		10	
Lower leg	54	8	14	7	3		2		3	13
Femur	6	3	3	0					1	
Others	20	6	47	2	1				1	4
Head		4								
Face				1					1	
Neck				1						
Not specified	20	2	47		1					4

[a]There was an error in the original article

In the study by Sawai et al., four inspectors were dispatched to all districts of whole island to visit the Bureau of Hygiene, Health Stations, herbalists, village masters, snake shops, and snakebite victims from 1964 to 1968 (Sawai and Tseng 1969). A total of 891 cases were identified, including 393 cases (45.5 %) of *P. mucrosquamatus* bites, 165 (19.6 %) of *T. stejnegeri* bites, 152 (17 %) of *B. multicinctus* bites, 100 (11.2 %) of *N. atra* bites, 37 (4.2 %) of *D. acutus* bites, 4 (0.4 %) of *D. r. siamensis* bites, and 66 cases (7.4 %) of unidentified snakebites. With respect to the location of snakebites, 229 occurred in homes; 205 in the forest, bush, or mountains; 184 on farmland; and 124 on roads. Bites frequently occurred on the distal limbs including the toes, feet, fingers, or hands. Of note, most of these cases (748 of 891) were treated by herbalists, and only 136 (15.3 %) were treated with antivenom despite the availability of plentiful antivenoms in hospitals. Among the 136 cases, more than half had initially visited herbalists. Forty-six patients eventually experienced limb dysfunction (e.g., contracture or deformity) after the wound healed, and half of these cases were caused by *P. mucrosquamatus* bites. In this investigation, 19 fatalities (2.1 %; 6 from *P. mucrosquamatus* bites, 6 from *D. acutus* bites, 6 from *B. multicinctus* bites, 1 from an unidentified snakebite) were recorded. However, the incidence of snakebites might have been largely underestimated because 72 snakebite-related deaths were reported to the Department of Health during 1966–1967. Nevertheless, this study represents the first reliable report of snakebites in Taiwan after World War II (1945).

Specific Counties

Sawai et al. further studied the snakebites in southern Taiwan (Kaohsiung and Pingtung) during 1968–1969 and 1965–1971, respectively (Sawai et al. 1970, 1972). In Kaohsiung county, 150 snakebite cases were reported, including *P. mucrosquamatus* bites in 72 cases (48 %), *T. stejnegeri* in 58 (38.7 %), *B. multicinctus* in 5 (3.3 %), *D. acutus* in 4 (2.6 %), *N. atra* in 3 (2 %), and unidentified snakebites in 8 (5.3 %); whereas no cases of *D. r. siamensis* bites were recorded. Notably, almost all patients were treated by herbalists without antivenoms. Four deaths (2.6 %) were recorded, with 2 caused by *D. acutus* and 2 by *B. multicinctus*. Four patients had limb dysfunction, with 2 caused by *P. mucrosquamatus*, 1 by *T. stejnegeri*, and 1 by *N. atra*.

In Pingtung county, 335 snakebite cases were reported, including *P. mucrosquamatus* bites in 115 cases (34.3 %), *T. stejnegeri* in 97 (28.9 %), *D. acutus* in 34 (10.1 %), *B. multicinctus* in 26 (7.5 %), *N. atra* in 24 (7.2 %), *D. r. siamensis* in 20 (5.9 %), and unidentified snakebites in the remaining 19 (5.7 %). The overall mortality was 5 %, with the highest case fatality rate for *B. multicinctus* bites (19.2 %) followed by *D. acutus* bites (17.6 %). In the same study, a regional difference was observed in the distribution of venomous snakes: *N. atra* and *D. acutus* were found more frequently in the southern areas, whereas *D. r. siamensis* was confined to the south and east regions. By contrast, *N. atra* and *B. multicinctus* were frequently found in the plain areas, and *D. acutus* was found in

mountainous areas. The occurrence of snakebites correlated with the venomous snakes' geographic distribution: *N. atra* and *B. multicinctus* bites were reported more frequently in villages in plain areas; *D. acutus* in the aboriginal villages located in mountainous areas; and *D. r. siamensis* bites in open or dry grounds such as farmlands. According to the two studies, *P. mucrosquamatus* was the most common offending snake in southern Taiwan and more than half of snakebite incidents occurred in homes or farmlands.

Questionnaire-Based Study

Miao et al. used a questionnaire to survey snakebites during 1988–1991 (Miao et al. 1995). A total of 317 questionnaires were sent to hospitals and remote health stations, and 189 (59.6 %) were returned for analysis. Of the 189 cases, 162 were reported from five hospitals: three located in the eastern region, one in the southern region, and one in the central region of Taiwan. In this investigation, there were 66 cases (34.9 %) of *P. mucrosquamatus* bites, 54 (28.6 %) *T. stejnegeri* bites, 17 (8.9 %) *N. atra* bites, 6 (3.2 %) *B. multicinctus* bites, 2 (1.1 %) *D. acutus* bites, 1 (0.5 %) *D. r. siamensis* bite, and 43 (22.7 %) unidentified snakebites. No death was noted in that study. The ratio of male to female victims was 3:1, and 77 % snakebites occurred in individuals aged 21–70. The anatomic sites of bites included fingers in 51 cases, feet in 32, hands in 22, and toes in 18. The majority of patients (88 %) received medical treatment within 4 h; among them, more than half arrived at hospitals within 1 h. With respect to the timing of incidents, *P. mucrosquamatus* bites usually occurred at night, whereas *T. stejnegeri* and *N. atra* bites usually occurred in the daytime possibly related to their different living habits. Regarding the location of incidents, *P. mucrosquamatus* bites usually occurred inside homes or near firewood, whereas *T. stejnegeri* and *N. atra* bites usually occurred in the wild.

National Poison Control Center (PCC-Taiwan) Data

Taiwan's National Poison Control Center (PCC-Taiwan) was established in 1986 to provide a 24-h poison consultation service to both healthcare professionals and the general public. Most phone inquiries (80.2 %), however, are from clinicians or other healthcare professionals (Yang et al. 1996). Wu et al. studied 444 snakebite cases reported to the PCC-Taiwan during 1986–1998 (Wu et al. 1999). There were 99 cases of *T. stejnegeri* bites (22.3 %), 98 of *P. mucrosquamatus* bites (22.1 %), 44 of *N. atra* bites (9.9 %), 36 of *B. multicinctus* bites (8.1 %), 15 of *D. acutus* bites (3.4 %), 8 of *D. r. siamensis* bites (1.8 %), and 144 (32.4 %) of unidentified snakebites. Most bite incidents occurred between May and October. The ratio of male to female victims was 2.8:1. Of these cases, 65.8 % received antivenom therapy. Eleven deaths (2.5 %) were recorded including 9 caused by *B. multicinctus* bites, 1 by *D. acutus* bite, and 1 by an unidentified snakebite.

National Health Insurance (NHI) Claims Data

Taiwan launched a single-payer National Health Insurance (NHI) program in 1995, which has greatly influenced the healthcare-seeking behavior of patients and the physicians' daily practice. According to the Bureau of NHI, the coverage of NHI has surpassed 99 % of the national population and the number of cooperating medical facilities reached 93.7 % in 2013. Liu et al. investigated snakebite cases during 2002–2005 based on the NHI claims database (Liu et al. 2009). A total of 3,862 cases received antivenom therapy were identified, and 2,018 of them were hospitalized. Overall, the median age of snakebite victims was 50 years, with male to female ratio of 2.6:1. Most of the bites occurred during April to November, with the peak incidence in August. The majority of cases received single type of antivenom: 2,728 (70.6 %) received bivalent antivenom for *T. stejnegeri* and *P. mucrosquamatus*, 760 (19.7 %) received bivalent antivenom for *N. atra* and *B. multicinctus*, and 36 (0.9 %) received monovalent antivenom for *D. acutus*. A total of 337 cases (8.7 %) received more than one type of antivenom therapy. Three deaths were noted during the study period, with one in each of the bivalent antivenom groups and one in the monovalent antivenom group. The major limitation of this study was that the snake species could not be clearly determined through the antivenom prescription data. Furthermore, monovalent antivenom specific for *D. r. siamensis* was not included during the study period because it was under preclinical evaluation at that time.

Hospital-Based Study

Single hospital records of snakebites represented only a small proportion of snakebite cases in Taiwan. However, hospital-based studies contained more detailed information on clinical manifestations and treatments, as compared to the above-noted epidemiological studies. Kuo and Wu studied snakebite cases in a medical college hospital located in southern Taiwan during 1962–1971 (Kuo and Wu 1972). Among 63 snakebite cases, 23 were of *P. mucrosquamatus* bites, 16 of *T. stejnegeri* bites, 13 of *N. atra* bites, 6 of *B. multicinctus* bites, 4 of *D. acutus* bites, and 1 of *D. r. siamensis* bite. There were four reported deaths (6.4 %), 3 caused by *B. multicinctus* bites and 1 by *P. mucrosquamatus* bite. One died from acute renal failure 10 days after *P. mucrosquamatus* bite, whereas three cases were dead due to respiratory failure after *B. multicinctus* bites despite of antivenom use and intensive care. The distribution of inpatient age was predominately between 10 and 50 years, and the male to female ratio was 2.5:1. The anatomic areas of bites were as follows: 33 cases of bites on upper limbs, 28 on lower limbs, 1 on face, and 1 on neck.

Liang et al. studied snakebite cases in a military medial center in northern Taiwan, during 1984–1991 (Liang et al. 1992). Of 100 identified cases, 26 were of *T. stejnegeri* bites, 23 of *P. mucrosquamatus* bites, 3 of *N. atra* bites, 3 of *B. multicinctus* bites, and 45 of unidentified snakebites. All patients received antivenom therapy, and no death was reported in that study. The male to female

ratio was 3.3:1, with an age range of 8–75 years. Except for one case suffered from snakebite on the ear, all patients were bitten on their limbs. There were two patients developed respiratory failure and needed mechanical ventilation after neurotoxic-type snake envenoming. One patient suffered from acute renal failure and required hemodialysis after *P. mucrosquamatus* bite, and 19 cases received surgical operations for wound complications.

Chen et al. studied snakebite cases in another medical center in northern Taiwan during 1991–1994 (Chen et al. 2000). A total of 130 cases were reported (68 were of *T. stejnegeri* bites, 31 of *P. mucrosquamatus* bites, and 31 of unidentified snakebites). The data revealed a male predominance (2.3:1), with an age range of 3–80 years (mean age, 44.1 ± 20.1 years). All of the bites occurred on the limbs. Only 18 patients (13.8 %) needed to be hospitalized for further treatment after emergency department (ED) visit. The other 112 were discharged from the ED; however three of them were admitted at a later time because of wound infection. No death was recorded in that study.

Liao et al. conducted a study of snakebites in southern Taiwan during 1986–1999 (Liao et al. 2000). A total of 46 cases were reported, and 20 were of *T. stejnegeri* bites, 6 of *P. mucrosquamatus* bites, 6 of *N. atra* bites, 5 of *B. multicinctus* bites, 1 of *D. acutus* bite, 1 of *D. r. siamensis* bite, and 7 of unidentified snakebites. Death from multiple organ failure caused by *P. mucrosquamatus* envenoming was recorded in one patient. Patients' age ranged from 5 to 77 years (mean age, 43 years), with a male to female ratio of 3.2:1. The anatomic areas of bites were the upper limbs in 65 % and lower limbs in 35 % of the cases. All 46 cases received 1–4 vials of antivenoms. Four cases (3 cases of *P. mucrosquamatus* bites and 1 of *D. r. siamensis* bite) developed acute renal failure and needed renal replacement therapy. In addition, ten patients underwent surgery for wound necrosis; five of them were of *N. atra* bites.

Hung reviewed the medical charts of 282 snakebite cases during 1993–2000 in a referring center in central Taiwan (Hung 2004). Among these, there were 102 cases of *N. atra* bites, 38 of *P. mucrosquamatus* bites, 37 of *T. stejnegeri* bites, 25 of *B. multicinctus* bites, 4 of *D. acutus* bites, 1 of *D. r. siamensis* bite, 50 of unidentified venomous snakebites, and 25 of non-venomous snakebites. One death related to advanced tissue necrosis, and multiple organ failure after *N. atra* bite was identified in that study. Regarding monthly distribution, 88 % of cases occurred from April to October. The ratio of male to female victims was approximately 3:1. A total of 138 individuals were bitten on the upper limbs and 136 on the lower limbs. The location of incidents was recorded in 182 snakebite cases [75 in farmlands, 22 in homes, 33 in yards (surrounding homes), and 32 in the bush or forest]. Approximately 82 % of the cases that occurred in the home were of *N. atra* and *P. mucrosquamatus* bites. Four cases were bitten while on a tree by *T. stejnegeri*. Most *B. multicinctus* bites occurred in the farmland and forest area (72 %). Six patients presented with ophthalmologic injury due to venom spitting by *N. atra* while they tried to catch the snake, and all recovered with conservative treatment.

Shih et al. evaluated the surgical risk factors of venomous snakebites in a referring center in northern Taiwan during 1999–2004 (Shih et al. 2006). A total

of 118 cases were reported (54 of *P. mucrosquamatus* bites, 29 of *T. stejnegeri* bites, 14 of *N. atra* bites, 6 of *B. multicinctus* bites, 1 of *D. acutus* bite, 1 of *D. r. siamensis* bite, and 13 of unidentified snakebites). Surgical intervention was required in 16 patients, among which 7 were bitten by *N. atra*, 5 by *P. mucrosquamatus*, 1 by *V. stejnegeri*, 1 by *D. acutus*, and 2 by unidentified snakes. Envenomation caused by *N. atra* bite was thought to be a risk factor for subsequent surgical interventions. A patient, bitten by *D. r. siamensis*, died from disseminated intravascular coagulopathy including intracranial bleeding, and acute myocardial infarction was recorded.

Chang et al. investigated snakebite cases in a medical center in southern Taiwan during 2001–2005 (Chang et al. 2007). Of a total of 37 identified cases, 11 were of *T. stejnegeri* bites, 10 of *D. r. siamensis* bites, 8 of *N. atra* bites, 5 of *P. mucrosquamatus* bites, and 3 of unidentified snakebites. The majority of snakebites occurred between April and September, with a peak in June. Patients' age ranged from 20 to 71 years (mean age, 41 years), with a male predominance (73.5 %). In terms of anatomic areas of bites, most bites were on the hands and toes (44 %), while 38.2 % and 14.7 % were on the lower and upper limbs, respectively. Elapsed time between bites and arrival at the ED ranged from 30 min to 30 h. All patients received antivenom therapy. Only one patient was initially treated with unknown traditional medicine, and five patients underwent operations. No death was recorded in that study.

Discussion and Summary

Incidence of Venomous Snakebites

Nationwide epidemiological studies are scarce in Taiwan and only two studies utilizing governmental statistics are available; one published in Japanese and the other in Chinese (Liu et al. 2009; To 1941). In the past 70 years the average cumulative incidence of snakebites has raised 2.7-fold; however, the incidence rate has declined in half, possibly resulted from the rapid expansion of human population. Prior to 1938, the incidence rate was estimated to be 8.8 cases per 100,000 person-years, with 11.7 in the north, 4.0 in the central, 5.3 in the south, and 74.7 in the east of Taiwan (To 1941). In a recent study using the NHI claims database, an incidence rate of 4.3 cases per 100,000 person-years was calculated, with 3.9 in the north, 3.3 in the central, 3.5 in the south, and 27.6 in the east (Liu et al. 2009). Both studies indicated the highest incidence rate of snakebites in eastern Taiwan, possibly due to the smallest population size in that area.

T. stejnegeri is the most common offending snake among the six major venomous snakes in overall studies, and it is the only one of the six medically important species that is not under conservative protection by the law in Taiwan. *B. multicinctus* and *N. atra* rank as the third and fourth, whereas *D. acutus* and *D. r. siamensis* affect the least number of victims. Sawai et al., on the contrary, showed that *P. mucrosquamatus* bites were more common than *T. stejnegeri*

throughout Taiwan during 1964–1971 (Sawai et al. 1972; Sawai and Tseng 1969; Sawai et al. 1970). This might have resulted from human-induced environmental change, such as urban construction or natural disasters (e.g., floods) during that period. Of note, regardless of the fact that the photographs of the six major venomous snakes are available in most EDs, the offending snakes were not identified in a significant proportion of cases (32–45 %) in previous studies (Liang et al. 1992; Wu et al. 1999). Many medical staffs at the EDs are not familiar with the venomous snakes in Taiwan because snakebite cases remain relatively uncommon as compared with other medical diseases and there is a large number of snake species in Taiwan, which might lead to misidentification of the snake species (Hung et al. 2003).

After 1972, most of the epidemiological studies of snakebites arose from hospital statistics. Surveys in Sri Lanka have shown that hospital data record less than half of the deaths due to snakebite. A review of district hospital records in Nepal also revealed that national figures underestimated the incidence of snakebite by one order of magnitude (Alirol et al. 2010). Although a nationwide estimation of the epidemiology of snakebites is practical through the NHI claims database since 1995, there are several inherent limitations. First, cases recorded in the NHI claims database were sorted using an antivenom prescription code; therefore, patients who did not receive antivenom therapy would not be included. On the other hand, if an ICD-9-CM (989.5) or E code (E905.0) was used as the searching strategy, 17.9 % of the patients who received antivenom would be excluded because they were coded as open wounds rather than snakebites. Second, it is not possible to differentiate between *T. stejnegeri* and *P. mucrosquamatus*, and between *B. multicinctus* and *N. atra* based on antivenom prescription because bivalent antivenoms are used in patients bitten by these snakes. In addition, the monovalent antivenom for *D. r. siamensis* was not approved to use before 2008; therefore, data on *D. r. siamensis* antivenom prescription before 2008 were lacking. Finally and most importantly, the severity, complications, and causes of death in snakebites cannot be elucidated in the database. A data linkage system integrating the NHI claims database and the data derived from all healthcare facilities in Taiwan might be able to solve this problem in the future. Before that kind of study is available, the NHI claims database remains a good source to estimate the incidence and impacts of snakebites.

Regional Distribution of Venomous Snakebites

A regional pattern of snake distribution was previously reported in Taiwan, with *T. stejnegeri* and *P. mucrosquamatus* occurring widely throughout the country, especially in the northern and southern areas, *D. acutus* and *N. atra* more frequently in the southern, and *D. r. siamensis* being confined to the southern and eastern (Sawai et al. 1972). The occurrence of snakebite largely correlates with the geographic distribution of the snakes, and this is supported by a study that employed the antivenom prescription records derived from the NHI claims database between 2002 and 2005 (Liu et al. 2009). However, Hung et al. investigated the case records

of snakebites in a referring center during 1993–2000 and found that *N. atra* was the most common offending snake in central Taiwan (Hung 2004; Hung and Lin-Shiau 2001). The healthcare-seeking behaviors of Taiwanese and illegal capturing, killing, or releasing of snakes for religious reasons might have changed the pattern of snakebites in Taiwan (Lin 2001). Continuous surveillance of the epidemiology in various regions can be helpful in the better management of snakebites.

Season, Gender, and Age Distribution of Venomous Snakebites

In Southeast Asia, the risk of snakebite is strongly associated with occupations such as farming and plantation work, catching and handling snakes for food, or in the preparation of traditional medicines. The incidence of snakebites is higher during the rainy season and during periods of intense farming activities. The mean age of snakebite victims is approximately 30 years, with a male predominance (Alirol et al. 2010; Warrell 2010a). In Taiwan, snakebite generally occurs between April and October. Kuo and Wu identified another peak of incidence in November, a winter month, in southern Taiwan, which may be related to the fact that this is generally the last month in a year to harvest in that tropical region. There is also a clear preponderance of males among snakebite victims; however, the mean age of snakebite patients is growing. In fact, it was more than 40 years with a median age of 50 in recent reports (Chang et al. 2007; Chen et al. 2000; Liao et al. 2000; Liu et al. 2009; Shih et al. 2006). The increase in the mean age of the victims might herald the facts of aging population and shrinkage in agriculture manpower in Taiwan. Snakebite envenoming in the elderly may lead to higher medical expenses, which demand further investigations.

Anatomic Area of Bitten Wound and Locality of Venomous Snakebites

Similar to other agricultural countries in South Asia (Alirol et al. 2010), most of the snakebites involved the limbs in Taiwan. Overall, 57.1 % of the bitten sites were on the lower limbs, 38.4 % on the upper limbs, and 4.4 % on other sites including head, neck, or trunks. Although the snake species were frequently not specified in previous studies, bites on the head and trunk are likely due to species (e.g., *N. atra* or *B. multicinctus*) that enter home biting sleeping people (Warrell 2010a). Regarding the location of snakebites, most of the incidents occurred in farmlands during weeding, plowing, or watering. Other potential locations include homes and their surrounding areas, roads, forest, and bush. Hung et al. reinvestigated the locality of six venomous snakebites, showing that *N. atra* and *P. mucrosquamatus* bites could occur in homes, especially in kitchens or barns. *B. multicinctus* bites occurred more frequently in the farmland, likely due to its preference for dank and deforested areas. *T. stejnegeri*, a tree viper, is mostly found in groves (Hung 2004). In general, humans do not encounter *T. stejnegeri* unless they are engaged in plantation work beneath low-growing fruit trees or if they climb

trees. Incidentally, most bites from this species occur on the upper limbs (Chen et al. 2009b). These epidemiological data disclosed that venomous snakebites are most likely to occur where snake habitat overlaps with the human environment. Knowledge of snake habits and habitats is helpful in snakebite prevention, especially during the season of peak incidence. Adequate precautious measures, such as wearing gloves and rubber boots, might be useful in avoiding snakebites during intense farming activities.

Brief Review of the Management of Snake Envenomation in Taiwan

The management of snake envenomations includes snake identification, first aid, prompt transportation, administration of relevant antivenom, antibiotic therapy, and surgical intervention whenever indicated. In the prehospital settings, there is no role for the use of arterial tourniquet, incision and suction, electrotherapy, or cryotherapy for snakebites. There is also insufficient data regarding the routine use of pressure immobilization or a constriction band for snakebite cases in Taiwan. Given the availability of expeditious transportation and easy access to healthcare facilities in Taiwan (Hu et al. 1996; Lin et al. 1998), rest prior to EMS transportation is probably the only needed recommendation in the prehospital setting.

The Taiwan government produces four types of equine-derived antivenoms to treat the six medically important snakebites. According to the guideline set by the PCC-Taiwan, the recommended dosage of relevant antivenoms to treat a moderately severe case of envenomation is 1–2 vials for *T. stejnegeri*, 2–4 vials for *P. mucrosquamatus*, 2–4 vials for *D. acutus*, 2–4 vials for *D. r. siamensis*, 6–10 vials for *N. atra*, and 2–4 vials for *B. multicinctus*; all administered intravenously at an infusion rate of 1–2 ml/min. Prophylactic antibiotics are usually not required in crotalid snake envenomations (e.g., *T. stejnegeri* and *P. mucrosquamatus*) because of low incidence of wound infection. On the other hand, wound necrosis, abscess formation, gangrenous change of distal limbs, or necrotizing fasciitis is not uncommon in *N. atra* bites, which is also a significant risk factor for subsequent surgery. Judicious use of prophylactic and empirical antibiotics is thus recommended in *N. atra* bites according to bacteriologic findings. If the offending snake was not identified (e.g., snake escaped), management by or consultation with experienced experts is strongly recommended.

Conclusion and Future Directions

In the whole island of Taiwan, crotalid snakebites, including *T. stejnegeri* and *P. mucrosquamatus*, are more common than the elapid snakebites (*B. multicinctus* and *N. atra*). *D. acutus* and *D. r. siamensis*, on the other hand, are the least common snakes within the six medically important snake species to cause envenomations because they have a scattered distribution in the wild. Human activities, environmental changes, and altered healthcare-seeking behaviors of the

patients and the practicing pattern of healthcare professionals all might have influenced the epidemiology of snakebites in Taiwan. Furthermore, the growing in population size and the rapidly aging of population in Taiwan also could have significant impacts on the medical expenses related to snakebites. Nationwide studies on snakebites remain scant and fragmented in Taiwan. Continuous surveillance of this disease is warranted and will be helpful in the better planning for disease prevention and treatment in the future.

Cross-References

► Management of Snake Envenomation in Taiwan

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Management of Snake Envenomation in Taiwan

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Abstract

There are six venomous snakes of medical importance in Taiwan: three crotalids, *Trimeresurus (Viridovipera) stejnegeri*, *Protobothrops mucrosquamatus*, and *Deinagkistrodon acutus*; one viperid, *Daboia russelli siamensis*; and two elapids, *Naja atra* and *Bungarus multicinctus*. In the prehospital settings, there is no role for incision and suction, electrotherapy, and cryotherapy for snakebite wounds. Routine use of a constriction band or pressure immobilization device is not indicated. The Taiwan government produces four types of equine-derived antivenoms to treat the above-noted snakebites, namely, bivalent antivenom for *T. stejnegeri* and *P. mucrosquamatus*, bivalent antivenom for *N. atra* and *B. multicinctus*, and two monovalent antivenoms for *D. acutus* and *D. r. siamensis*, respectively. These antivenoms are $F(ab')_2$ fragment in the lyophilized form containing 2,000 units per vial (or at least 1,000 Tanaka units). The Taiwan Poison Control Center formulated a flowchart for the management of six major venomous snakebites based on animal studies, clinical observation, and expert opinion in 1999. The recommended dosage of relevant antivenoms is 1–2 vials for *T. stejnegeri* snakebites, 2–4 vials for *P. mucrosquamatus*, 2–4 vials for *D. acutus*, 2–4 vials for *D. r. siamensis*, 6–10 vials for *N. atra*, and 2–4 vials for *B. multicinctus*. The use of antibiotics is suggested when secondary wound infection has developed, whereas surgical indications include wound necrosis, abscess formation, distal limb gangrenous change, necrotizing fasciitis, or, in rare cases, compartment syndrome. Further studies on the severity assessment (e.g., severity score), risk factors for the development of severe disease, optimal dosing of antivenom, effect of prophylactic antibiotics, and timing of surgery in cases of venomous snakebites in Taiwan are warranted.

Introduction

Taiwan is an island that lies at the junction of the tropical and subtropical zone and is heavily forested with dense undergrowth, mountainous and hilly terrains, and seasons of high rainfall. Taiwan's terrains and warm climate provide a highly suitable habitat for snakes. There are 61 (52) snake species that inhabit the island, of which 29 (22) are venomous, including 13 (7) sea snakes and 16 (15) land snakes (Hsiang et al. 2009; Tu 2008). These numbers are variable (shown in parentheses) according to different reports because some of the snake species are extremely rare or may even be exotic. Among the indigenous venomous species, only six land snakes are considered medically important. They are *Trimeresurus (Viridovipera) stejnegeri*, *Protobothrops mucrosquamatus*, *Deinagkistrodon acutus*, *Daboia russelli siamensis*, *Naja atra*, and *Bungarus multicinctus*. The former three belong to the subfamily Crotalinae; *D. r. siamensis* belongs to Viperinae, whereas the remaining two belong to the family Elapidae. The managements of snake

envenomations include snake identification, first aids, prompt transportation to healthcare facilities, timely administration of relevant antivenom, antibiotic therapy, and/or surgical interventions whenever indicated. The Taiwan government developed endemic antivenoms quite early. In the 1980s, the Vaccine Center further modified the manufacturing processes to improve the quality and speed of production of antivenoms (Huang et al. 1985; Huang et al. 1986; Liau and Huang 1997; Liau et al. 1982). In 1995, Taiwan established a National Health Insurance system, and in 1999, the Taiwan Poison Control Center (PCC-Taiwan) formulated a flowchart on the management of the six medically important venomous snakebites (Hung et al. 1999). The good quality of antivenoms, easy accessibility to modern medical care, and standardization of management protocol have jointly improved the outcome of snake envenomations in Taiwan. The case-fatality rate declined from 24 deaths per year before 1940 to 1 death out of 286 cases during 1993–2000 (Hung 2004; To 1941) and 3 deaths out of 3,862 cases during 2002–2005 (Liu et al. 2009).

Brief Review of the Epidemiology of Snake Envenomation in Taiwan

In Taiwan, systemic evaluations of snakebites started during the Japanese colonial period (1895–1945). Snakebite was once a compulsorily reported disease. Dr. To analyzed the statistics archived by the government in Taiwan during 1904–1938 and estimated that on average there were 361.3 cases of snakebite with 24 deaths per year (To 1941). After World War II (1945), the recording of snakebite statistics by the government was discontinued. Sawai et al. reinvestigated the epidemiology of snakebites, reporting a total of 891 cases and 19 deaths in the 1960s (Sawai and Tseng 1969). In a recent study utilizing the National Health Insurance claims database, 3,862 snakebite cases with 3 deaths were identified during 2002–2005 (Liu et al. 2009). Overall, *T. stejnegeri* is the most common offending snake among the six major venomous snakes, followed by *P. mucrosquamatus*, *B. multicinctus*, *N. atra*, *D. acutus*, and *D. r. siamensis*. Snakebites generally occur during April to October, the warm and rainy seasons in Taiwan. Kuo and Wu identified another peak of incidence in November, a winter month, in the southern part of Taiwan, which may be related to the fact that this is generally the last month in a year to harvest in that tropical region. There is also a clear preponderance of males among snakebite victims; however, the mean age of snakebite patients is increasing. In recent reports, the mean age of snakebite victims was more than 40 years, while the median age was around 50 years (Chang et al. 2007; Chen et al. 2000b; Liao et al. 2000; Liu et al. 2009; Shih et al. 2006). The increase in age of snakebite victims probably heralds the aging of population and the shrinkage in agriculture manpower in Taiwan. Snake envenomation in the elderly (Warrell 2010) may lead to higher medical expenses, which demands further investigation.

Characteristics of Six Major Venomous Snakes and Snakebites

Except for *T. stejnegeri*, five of the six medically important venomous snakes are endangered in Taiwan and are under protection according to the Wildlife Conservation Act. All six snakes have distinct biological features (Fig. 2.1); thus, capturing or killing them for identification is not necessary. Nevertheless, it can be helpful

Fig. 2.1 Features of six medically important venomous snakes in Taiwan (Pictures were provided and used with the permission from Mr. Chih-Ming Lai)

Viperidae: *Ovophis monticola makazayazaya*

Viperidae: *Trimeresurus gracilis*

Elapidae: *Sinomicrurus hatori*

Elapidae: *Sinomicrurus sauteri*

Elapidae: *Sinomicrurus macclellandi swinhoei*

Colubridae: *Rhabdophis tigrinus formosanus*

Fig. 2.2 (continued)

in the management of snakebites if the victim or their companions remember the color pattern of the offending snake or if the snake can be identified by medical staff in case of captured snake. For comparative purposes, the features of 10 less venomous land snakes are also shown here (Fig. 2.2).

Fig. 2.2 Ten less venomous land snakes are shown with family and scientific names (Pictures are provided and used with the permission from Mr. Chih-Ming Lai)

Snake envenomations can result in various categories of toxic effects, including coagulopathy, neurotoxicity, myonecrosis, renal injury, cardiotoxicity, and severe local tissue damage at bitten site. Any single species of snake may show activity in one or more of the above-noted categories (White 2005). A summary of the distribution (Hsiang et al. 2009; Lin et al. 1990; Tu 2008), venoms, and clinical manifestations of the six medically important venomous snakes and related bites in Taiwan is presented in this chapter.

Trimeresurus (Viridovipera) stejnegeri

(a) **Distribution**

T. stejnegeri is found in India, Nepal, Myanmar, Thailand, Vietnam, Laos, and southern China. In Taiwan, it occurs more commonly at lower altitudes of wooded, shrub, and mountainous areas.

(b) **Venom**

Ouyang had demonstrated that the venoms of *T. stejnegeri* and *D. acutus* had anticoagulant effect in low concentration and procoagulant in high concentration, though this effect was less prominent in the former (Ouyang 1957). Venom of *T. stejnegeri* contains several proteins and peptides including thrombin-like enzyme (TLE), prothrombin activation inhibitor (acidic phospholipase A), fibrinogenases, platelet aggregation inhibitor, platelet aggregation inducer, and hemorrhagins (e.g., metalloproteinases), (Huang et al. 1984; Ouyang and Huang 1983a; Ouyang et al. 1982a). TLE acts on fibrinogen similar to thrombin, producing the procoagulant effect. On the other hand, TLE differs from thrombin: it digests fibrin and the fibrin formed by this enzyme is more susceptible to plasmin degradation than fibrin formed by thrombin. TLE also does not activate coagulation factor VIII and is not inhibited by heparin (Ouyang and Yang 1974). Prothrombin activation inhibitor interferes with prothrombin and its activation factors through reversible binding to these factors (Ouyang and Yang 1975). Fibrinogenases cause fibrinogenolysis and fibrinolysis. The degradation products of fibrinogen might further polymerize with fibrin and prolong the reaction time of thrombin to fibrinogen.

A potent platelet aggregation inhibitor and an inducer both were isolated from the *T. stejnegeri* venom. The inhibitor, with 5′-nucleotidase, which cleaves adenosine diphosphate (ADP), inhibits ADP- or collagen-induced platelet aggregation without platelet lysis. It also inhibits the thrombin-induced clot stabilization (Huang and Ouyang 1984) and causes indirect hemolysis in the presence of phosphatidylcholine (Ouyang and Huang 1983b). In experimental models, the crude venom elicits platelet aggregation in low concentration (<100 μg/ml); however, this action declines in higher concentrations (Ouyang and Huang 1983a).

Hemorrhagins, α-fibrinogenase, and hemorrhagin II are metalloproteinases and may be the key factor causing systemic injury, local damage, hemorrhage, edema, and necrosis (Markland and Swenson 2013). Their relative hemorrhagic activity compared to crude venom was 1:2:8 (Huang et al. 1984). Crude venom contains phospholipase A_2, which may potentiate the hemorrhagic activity of hemorrhagins. The actions of hemorrhagins in *T. stejnegeri* envenomation might be reverse by relevant antivenom (Huang et al. 1984).

(c) **Clinical manifestations**

Clinical manifestations and treatment of *T. stejnegeri* and *P. mucrosquamatus* envenomations are quite similar although they are biologically distinct. Chen et al. studied 50 cases of *T. stejnegeri* bites and reported the following symptoms and signs in decreasing frequency: local pain (100 %), inflammation (100 %), bruising (51 %), transient bleeding from fang marks (22 %), mild thrombocytopenia (10 %), cellulitis (6 %), renal dysfunction (4 %), rhabdomyolysis (2 %), and

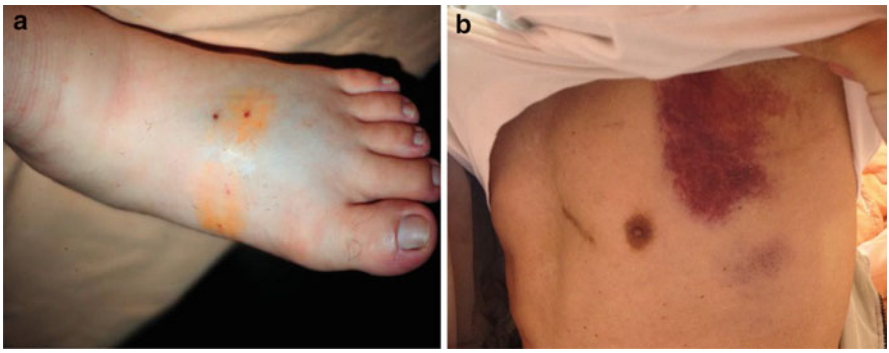

Fig. 2.3 (**a**) Patient was bitten by *T. stejnegeri* on the foot. (**b**) Patient was bitten by *T. stejnegeri* on the left hand and distant ecchymosis developed over the chest wall 1 day later

compartment syndrome (2 %) (Chen et al. 2009). Occasionally, there was bruising distant away from the bitten area (Fig. 2.3); however, significant wound bleeding or bleeding from the vital organs was not observed.

Protobothrops mucrosquamatus

(a) **Distribution**

P. mucrosquamatus is widely distributed in northeastern India, Bangladesh, Myanmar, southern China, and Taiwan.

(b) **Venom**

The venom of *P. mucrosquamatus* contains prothrombin activation inhibitor (basic phospholipase A), fibrinogenases, platelet aggregation inducer, and platelet aggregation inhibitors (Ouyang and Teng 1978; Ouyang et al. 1982b). The anticoagulant effect of prothrombin activation inhibitor, which could be reversed by the antivenom, may be due to both enzymatic and strong binding activities, inhibiting prothrombin and factor X through the inactivation of the procoagulant activity of phospholipids. Fibrinogenases isolated from *P. mucrosquamatus* venom, similar to that from *T. stejnegeri*, cause fibrinogenolysis and fibrinolysis and possess hemorrhagic activity. They are also weak anticoagulants and inhibit platelet aggregation (α-fibrinogenase). In vitro studies, the venom has two opposite actions on the platelet: an increase in platelet aggregation activity in venom concentrations ranged from 1 to 30 μg/ml, while the activity gradually declined at concentration of 30–1,000 μg/ml, which might be secondary to the coexistence of platelet aggregation inhibitors in the venom (e.g., fibrinogenases, 5′-nuecleotidase, phospholipase A)

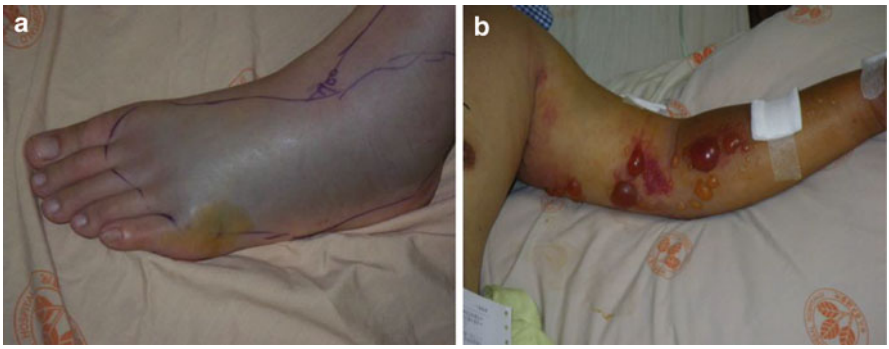

Fig. 2.4 (**a**) Patient was bitten by *P. mucrosquamatus* on the foot 8 h earlier. (**b**) Patient was bitten by *P. mucrosquamatus* on the left hand; bullae formation and marked tissue swelling were observed within 24 h

(Ouyang and Teng 1978). In animal studies, the venom causes a drop in platelet counts (Ouyang and Teng 1978), possibly due to platelet aggregation.

For better understanding of hematological derangement, Li et al. measured the maximum platelet aggregation rate (MAR) and antithrombin III and α2-plasmin inhibitor activities, both sensitive parameters for disseminated intravascular coagulopathy (DIC), as well as the fibrinogen level and fibrinogen degradation products (FDPs) in three cases of *T. stejnegeri* and one case of *P. mucrosquamatus* envenoming (Li et al. 2000). The study revealed that MAR was unchanged in *T. stejnegeri* envenoming but lowered in *P. mucrosquamatus* envenoming; antithrombin III activity, α2-plasmin inhibitor activity, and the fibrinogen level were lower in all four cases; and FDPs were elevated in *T. stejnegeri* envenoming. Therefore, low-grade DIC may be present in both snake envenomations.

(c) **Clinical manifestations**

Although the clinical manifestations of *T. stejnegeri* and *P. mucrosquamatus* bites are similar, envenomations caused by *P. mucrosquamatus* bite are usually more severe (Fig. 2.4) because the latter is bigger in size and the venom contents are higher (Liau and Huang 1997). The symptoms and signs of envenoming of both snakes include local swelling, pain, bruising, wound bleeding, cellulitis, necrosis, coagulopathy, rhabdomyolysis, acute renal failure, or compartment syndrome. Chen et al. studied 149 cases of *P. mucrosquamatus* bites; the frequency of bruising (75 %), cellulitis (26 %), necrosis (11 %), and rhabdomyolysis (11 %) was significantly higher than that in *T. stejnegeri* bites. The incidence of coagulopathy (6 %) was also higher but did not reach statistical significance (Chen et al. 2009). Similar to *T. stejnegeri* bite, there was occasional occurrence of bruising distant away from the bitten area; however, significant wound bleeding or bleeding into vital organs was neither observed.

Deinagkistrodon acutus

(a) **Distribution**

D. acutus is found in southern China, northern Vietnam, and possibly Laos. In Taiwan, it is distributed in mountainous or forested areas, in covered rocky or hilled regions.

(b) **Venom**

Both procoagulants and anticoagulants had been isolated from the venom of *D. acutus*, including thrombin-like enzyme (TLE), anticoagulant principles, prothrombin activation inhibitor, α-fibrinogenase (Cheng and Ouyang 1967; Ouyang et al. 1982a), as well as platelet aggregation inhibitor and hemorrhagins (Mori et al. 1984; Ouyang and Huang 1986; Xu et al. 1981). The procoagulant effect was caused by a thrombin-like action on fibrinogen (Ouyang 1957). TLE can also induce fibrinolysis in addition to fibrinogenase (Ouyang et al. 1972, 1982b). The anticoagulant principles, on the other hand, inactivate prothrombin, tissue factor, and coagulation factor V (Ouyang and Teng 1973; Ouyang et al. 1982b). The combination of the above-noted effects results in retardation of blood clotting in vivo studies (Ouyang and Teng 1976). Moreover, some other proteins (e.g., hemorrhagins, metalloproteinase, protease, and hyaluronidase) play crucial roles in vascular injury and local toxic effects. All of the actions lead to clinically significant bleeding and tissue damage in *D. acutus* envenomations.

(c) **Clinical manifestations**

D. acutus bites have been rarely observed in Taiwan, with only a few cases being reported in the English literature. A 10-year-old girl bitten by *D. acutus* on her left hand was reported in 1969 (Kuo and Wu 1972). The patient developed fever, bleeding tendency, and multiple dark reddish vesicles on the left hand in spite of treatment with traditional medicine. She was transferred to a medical center 27 h later. On presentation, continuous oozing from the wound, bleeding from the nose, gingival, and eponychium were noticed. The left hand was rigid and could not be moved due to painful swelling. Laboratory examination revealed blood hemoglobin (Hb) 7.9 g/dl, and the bleeding time was prolonged for more than an hour without clotting. The patient received a vial of monovalent antivenom for *D. acutus* with good response. The bleeding time soon normalized, and oozing from the wound discontinued 12 h after the infusion of antivenom. The patient received local wound debridement and skin grafting 3 weeks later. The pathological findings of the wound at 40 h post-bite revealed epidermal layer fragmentation and cleavage by edema, infiltration of the deeper dermis by neutrophils and lymphocytes, and extravasation with engorged blood vessels. The patient recovered with limited movement of left thumb.

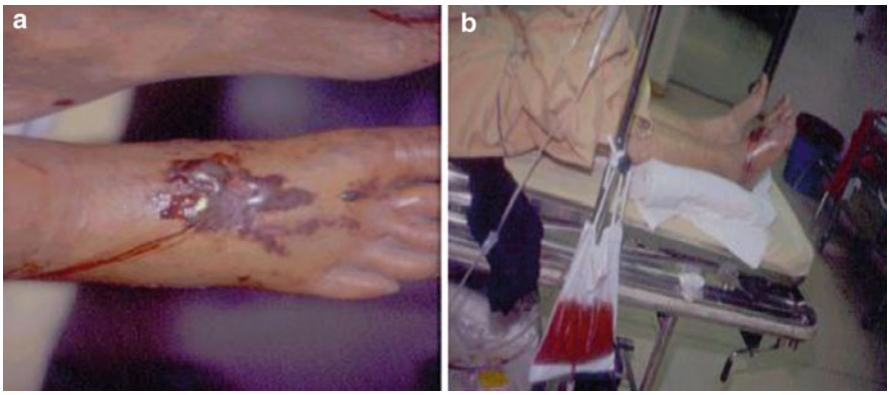

Fig. 2.5 (**a**) Patient was bitten by *D. acutus* on right foot. Continuous bleeding from the fang marks and hemorrhagic bullae formation were observed 5 h post-bite. (**b**) Continuous bleeding from the wound and gross hematuria 12 h after the bite (the same case as Fig. 2.5a)

The second case was a 16-year-old girl bitten by *D. acutus* on right palm in 1977 (Shen 1983). The patient developed generalized petechia and persistent oozing from the bite wound, had near syncope, and was sent to a hospital 28 h post-bite. On examination, the swelling had extended to the shoulder. Her blood was incoagulable with prothrombin time (PT) and activated partial thromboplastin time (aPTT) both exceeding 300 s, and the fibrinogen level was immeasurable. For unknown reasons, the patient received 4 vials of bivalent antivenom for *T. stejnegeri* and *P. mucrosquamatus* rather than monovalent antivenom for *D. acutus* and whole blood transfusion of 1,500 ml in the following 3 days. On day 6, the patient's Hb level was 7.2 g/dl, platelet count was 169,430/mm^3, and bleeding time was 4 min. On day 8, the patient received 10 units of plasma and then went home against medical advice. The patient was still alive after 6 years at a medical follow-up.

The third case was a 44-year-old male bitten by *D. acutus* on the dorsal aspect of the left middle finger in 1991 (Hung et al. 1997). Swelling, subcutaneous ecchymosis, hemorrhagic blisters, and oozing from the wound developed a few minutes later. The patient was sent to a hospital where two vials of bivalent antivenom for *T. stejnegeri* and *P. mucrosquamatus* were administered due to the unavailability of monovalent antivenom for *D. acutus*. After 44 h, the patient went to another hospital because of persistent pain, progressive swelling, ecchymotic change in left forearm, and continuous bleeding from left hand. In the emergency department, severe thrombocytopenia (blood platelet count 2,000/mm^3) was noted, and bivalent antivenom was administered again in addition to fresh frozen plasma and platelet replacement. Monovalent antivenom for *D. acutus* was administered 59 h post-bite after consulting PCC-Taiwan. The blood platelet count rose to 10,000/mm^3 6 h after the antivenom was administered and returned to a normal level 2 days later. Unfortunately, the compartment syndrome of left forearm and gangrenous change in left middle finger developed. The patient later received left forearm fasciotomy and amputation of left middle finger with fair wound healing. The typical findings of *D. acutus* envenoming are shown in Fig. 2.5.

Zhao and Rao studied 111 cases of *D. acutus* bite during 1974–1980 in southern China. Envenomation caused various degrees of hemorrhagic symptoms, local tissue swelling, and pain, skin ulceration, necrosis, or even shock (Zhao and Rao 1982). Hemorrhagic diathesis included bleeding from the wound (64.9 %), mouth, nose (41.4 %), and subcutaneous tissues (61.3 %). In addition, skin or deep tissue necrosis might develop 3–5 days later. Li et al. reported a case of *D. acutus* envenomation with hemostatic disturbance 22 h after snakebite and found that antithrombin III and α2-plasmin inhibitor activities were depressed, while undetectable fibrinogen and elevated FDPs were noted in blood. *D. acutus* envenomation causes significant DIC (Li et al. 2000).

Daboia russelli siamensis

(a) **Distribution**

D. r. siamensis, a true viper, is found in Myanmar, Thailand, Cambodia, southern China, and Indonesia. In Taiwan, it has a scattered distribution in the southern part of the country and in the eastern side of the central mountain range, at lower altitudes below 1,500 ft. It is a nocturnal species and is found mainly in open or dry habitat.

(b) **Venom**

Wuster et al. examined the morphology of *Daboia russelli* (referred to as *Vipera russelli*) and reclassified them into two subspecies: a western subspecies (*D. r. russelli*), which includes populations formerly known as *D. r. russelli*, *D. r. nordicus*, and *D. r. pulchella*, and an eastern subspecies (*D. r. siamensis*), which includes the populations formerly assigned to *D. r. siamensis*, *D. r. formosensis*, *D. r. limitis*, and *D. r. sublimitis* (Wuster et al. 1997). There is much variation in venom composition and clinical effects both between and within the subspecies. The venom of *D. r. siamensis* contains several toxic components including procoagulants, which can activate coagulation factors V and X, phospholipase A_2 (PLA_2), proteinases (e.g., metalloproteinase), anticoagulants, and others (Risch et al. 2009). The procoagulants can induce widespread intravascular fibrin formation and consumptive coagulopathy. They also adversely affect the renal hemodynamics in studied animals in addition to inducing fibrin deposition in the renal microvasculature (Suntravat et al. 2011). The major lethal component, PLA_2, produces presynaptic neuromuscular blocking activity, edema-inducing activity, myonecrotic activity, and indirect hemolytic activity (Lee 1948; Maung Maung et al. 1995; Wang et al. 1992). The isoenzymes also cause unfavorable renal hemodynamics and platelet aggregation (Mitrmoonpitak et al. 2013; Suwansrinon et al. 2007). The metalloproteinases might degrade the extracellular matrix proteins, damage the integrity of blood vessels, and induce local wound bleeding (Mitrmoonpitak et al. 2013). In animal studies, rapid cardiac arrest resulted from intravascular clotting occurred when the venom was injected

intravenously. On the contrary, the animals usually died from neuromuscular blockade or consumptive coagulopathy when the venom was administered subcutaneously (Aung-Khin et al. 1977; Lee 1948). The overall pathological effects of the venom depend on the amount of each component, the route of administration, and individual susceptibility (Aung-Khin et al. 1977).

(c) **Clinical manifestations**

There is fascinating geographical variation in the clinical manifestations of Russell's viper bites. Conjunctival edema is unique to Myanmar, acute pituitary infarction to Myanmar and south India, and rhabdomyolysis and neurotoxicity to Sri Lanka and south India (Warrell 1989). Hung et al. studied 18 cases of *D. r. siamensis* bites during 1987–1999 and concluded that the most prominent effects of *D. r. siamensis* envenoming in Taiwan were coagulopathy and renal dysfunction (Hung et al. 2002). The clinical manifestations of *D. r. siamensis* envenoming included local pain, ecchymosis, and/or bleeding at the bitten site in 17 cases; mild swelling limited to 1 joint area in 15 cases; systemic bleeding in 13 cases, including gastrointestinal tract (10), genitourinary tract (11), lung (5), or central nervous system hemorrhage (1); thrombocytopenia, acute renal failure, and hemolysis in 13 cases; ecchymosis at distant site in 11 cases; coagulopathy in 10 cases; rhabdomyolysis in 9 cases; wound necrosis in 3 cases; and arterial thrombosis in 2 cases. There was a case of a dry bite and 3 out of the 18 cases died (17 %). Local effects caused by *D. r. siamensis* bites were less severe compared with those caused by other venomous snakebites in Taiwan. After systemic envenomation, coagulopathy, including PT and aPTT prolongation, usually developed within 1.8–4.6 h post-bite. Coagulopathy subsided 3 h to 2 days after the administration of specific antivenom. Thrombocytopenia usually developed within 2–30 h and platelet level returned to normal 1–3 days after the administration of antivenom (Chen et al. 1997). Acute renal failure usually developed within 3 h to 6 days post-bite; however, most of these cases had oliguria on the first day after envenoming. Nine of 13 cases of acute renal failure necessitated renal replacement therapy, with gradual recovery of renal function in 13–61 days. Notably, eight cases were unconsciousness on the first day after a bite. Four of these cases spontaneously recovered in the successive days; however, the other two had cerebral infarction and the remaining two died without determination of the central nervous system pathology. There was no neuromuscular blocking effect, except for slight dizziness or local numbness after envenoming in human cases.

Hung further investigated renal pathology after *D. r. siamensis* envenoming in dogs. Renal injury was observed as early as 30 min after envenoming (Hung and Lin-Shiau 2001). In a phase 2 study involving 13 patients, renal dysfunction was milder in cases that received monovalent antivenom within 6 h of envenoming than those who received antivenom after 6 h (Hung et al. 2006). Based on these findings, Hung et al. suggested that 2–4 vials of monovalent specific antivenom should be administered as soon as possible after envenomation (Hung et al. 2006). The typical findings of *D. r. siamensis* envenoming are shown in Fig. 2.6.

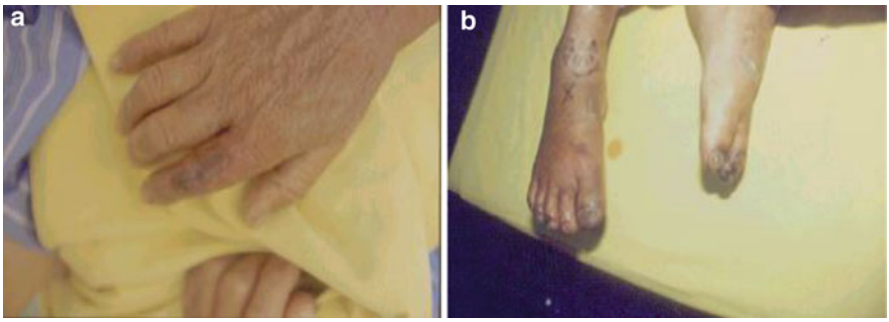

Fig. 2.6 (**a**) Patient was bitten by *D. r. siamensis* on right index finger, and local necrosis was noted on the bitten site. (**b**) Patient developed gangrenous change over toes of both lower legs 36 h later (the same case as Fig. 2.6a)

Naja atra

(a) **Distribution**

N. atra is found in southeastern China, northern Laos, and northern Vietnam. It is distributed throughout Taiwan at low altitudes and is more common in the west central and southern parts of the country.

(b) **Venom**

The venom of the *N. atra* consists of at least 100 proteins and peptides including cardiotoxins (cytotoxin), neurotoxins (cobrotoxin), hemotoxins, phospholipase A_2 (PLA_2), and other proteins (Li et al. 2004). The cardiotoxins and neurotoxins are the major components, which account for 55 % and 10 % of the dry weight of crude venom, respectively (Hung et al. 2003). The most toxic fraction to small animals was shown to be neurotoxins, which caused neuromuscular blockade and respiratory failure (Lee 1995). However, the most prominent effect of *N. atra* envenoming in humans is bite wound necrosis. The cardiotoxins may act individually or synergistically with other proteins (e.g., PLA_2) to induce local tissue necrosis through complex and poorly understood mechanisms (Fletcher and Jiang 1993; Kao et al. 2009a, b), whereas neurotoxins or PLA_2 alone do not (Lee 1995). In rabbits, local tissue necrosis was visible within 1 h after subcutaneous injection of 0.3–1 mg crude venom (Fukuyama and Sawai 1972) or 4 h in humans after a bite (Hung et al. 2003).

(c) **Clinical manifestations**

In a PCC-based study during 1986–1998, 43 cases of *N. atra* injury, including 36 bites and 7 spit ophthalmia, were analyzed (Lee et al. 2000). The clinical effects were local swelling and pain in 94.4 % of patients around the bitten area, wound necrosis in 25 %, fever in 22.2 %, and limb numbness in 13.9 %; miscellaneous

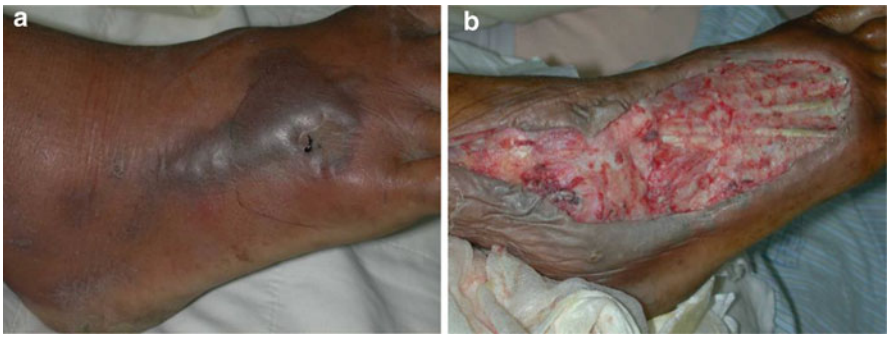

Fig. 2.7 (**a**) *N. atra* snakebite on right foot, local tissue necrosis and bullae developed 12 h later. (**b**) Debridement was performed 5 days after the bite (the same case as Fig. 2.7a)

effects such as transient hypotension, headache, dizziness, throat ache, dyspnea, ecchymosis, and blister or bullae formation around the wound or wound bleeding were less frequently reported (2.8 % –8.3 %). In the seven spit ophthalmia cases, all developed conjunctivitis and one case had headache and vomiting. All of them recovered well after conservative and local treatment.

Mao and Yang studied 119 *N. atra* bites from two medical centers (Mao and Yang 2013). Swelling and cellulitis were the most commonly observed clinical effects (94.1 % and 72.2 %, respectively), followed by wound necrosis (63 %), fever (43.7 %), necrotizing fasciitis (39.5 %), local numbness (28.6 %), and blisters or bullae formation (17.6 %) around the wound or compartment syndrome of the bitten limb (1.7 %). A few cases (32/52) developed temporary gastrointestinal effects such as nausea, vomiting, abdominal upset, or diarrhea shortly after envenoming. Systemic neurotoxicity manifested as transient/mild weakness or ptosis was only observed in only 5 of 115 cases (4.3 %; 2 weakness, 3 ptosis). Dry bite was recorded in 6.3 % of cases. No death occurred during the study period.

The typical findings of *N. atra* envenoming are shown in Fig. 2.7.

Bungarus multicinctus

(a) **Distribution**

B. multicinctus is found in mainland China, Myanmar, Laos, and northern Vietnam. It is very common in Taiwan. It is distributed at lower altitudes throughout the country, possibly below 2,000 ft.

(b) **Venom**

The venom of *B. multicinctus* contains several neurotoxins, including α- and β-bungarotoxins and muscarinic toxin-like proteins. α-Bungarotoxin binds to post-synaptic acetylcholine receptor in the motor end plates with high affinity, producing

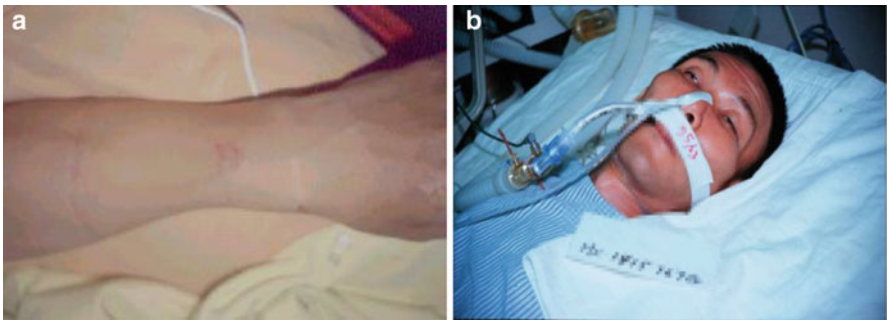

Fig. 2.8 (**a**) Patient was bitten by *B. multicinctus* on the forearm without overt local effects. (**b**) Respiratory paralysis occurred 5 h after the bite necessitating intubation and mechanical ventilation. Ptosis is also present (the same case as Fig. 2.8a)

essentially irreversible neuromuscular blockade; on the other hand, β-bungarotoxin acts presynaptically to depress acetylcholine release from the nerve endings (Pe et al. 1997). β-Bungarotoxin is a basic protein, consisting two subunits named A-chain, the active subunit, with phospholipase A_2 activity, and B-chain, which may function as an affinity probe to guide the toxin to its target on nerve terminals (Rowen 2001). β-Bungarotoxin causes damage of motor nerve terminals, change in the numbers of synaptic vesicle, and mitochondrial uncoupling and synergistically with other bungarotoxins, leading to neuromuscular blocking effect. Its toxicity seems to parallel the phospholipase A_2 activity (Abe et al. 1977). Although κ-bungarotoxin and γ-bungarotoxin have been described, their toxicological activities and clinical significance are less extensively studied (Chiappinelli 1991; Endo and Tamiya 1991).

(c) **Clinical manifestations**

The victims of *B. multicinctus* envenomations usually have typical neurological manifestations, including ptosis; ophthalmoplegia; paralysis of jaw, tongue, and deglutition; respiratory paralysis; quadriparesis; and parasympathetic abnormalities upon envenomings (Chan et al. 1995; Pe et al. 1997). Paralytic symptoms usually develop within a few hours (0.5–4 h) and the local symptoms were minimal (Kuo and Wu 1972; Pe et al. 1997). In the literature, the intervals between *B. multicinctus* bite and death of victims not treated with antivenom ranged from 6 to 30 h, while natural recovery from paralysis ranged from 8 to 30 days when mechanical ventilation was initiated in a timely manner (Chan et al. 1995; Pe et al. 1997).

Apart from the typical neurological effects, a life-threatening hyponatremia syndrome was recently described in a case of *B. multicinctus* envenoming in Vietnam, the mechanisms of which remain unclear (Hojer et al. 2010). Therefore, electrolytes should be carefully monitored in cases of *B. multicinctus* bites in addition to serial neurological examinations.

The typical findings of *B. multicinctus* envenoming are shown in Fig. 2.8.

Diagnosis

Snakebite is an occupational and environmental disease. In Taiwan, this health problem is complicated by the fact that snakes from different genera and species are commonly found in the same geographic area. Although there are typical findings of the six medically important venomous snakebites, either by clinical or laboratory tests (e.g., PT, aPTT, renal function), it may not be easy to distinguish between them upon bites instantly due to the similarity of early local manifestations. Currently, there is no commercialized rapid venom detection kit available in Taiwan. Misdiagnosis or inability to identify the culprit snake species thus frequently occurs in the emergency department (ED), accounting up to 45 % of all venomous snakebites (Liang et al. 1992). Hung et al. developed a sandwich-type enzyme-linked immunosorbent assay for the measurement of *N. atra* venom in biological samples in the early 2000s and obtained 1 ng/ml of detection limit in both urine and serum specimens (Huang et al. 2002, 2003). Using this method could quickly and successfully confirm the diagnosis of *N. atra* snakebites initially mistaken to be *P. mucrosquamatus* snakebites. The authors also developed a quick test for *N. atra* venom detection in 20 min by using immunochromatographic method. However, further validation of the applicability of this immunoassay in human cases is still needed. Moreover, the development of similar assays for other medically important venomous snake species is desirable.

Management

First Aids

Several measures have been described in the management of snakebites; however, many of them are controversial or even harmful. Arterial tourniquet, incision and suction, electrotherapy, and cryotherapy are no longer recommended (McKinney 2001). The effectiveness of constriction band and pressure immobilization also necessitates further evaluations in Taiwan.

Constriction Band or Pressure Immobilization

A constriction band, designed for crotalid snakebites in North America, is an elastic bandage, thick rope, or piece of clothing circumferentially wrapped above the site of snakebite to exert a pressure great enough to occlude venous or lymphatic return (McKinney 2001). It is typically recommended when the victim is more than 2 h driving distance away from a hospital and less than 30 min has elapsed since the bite. Once the patient arrives at the hospital, if there is no sign of envenomation, the constriction band should be removed. If the patient has evidence of systemic toxicity on arrival at the hospital, antivenom should be administered before the constriction band is loosened.

The pressure immobilization (PI) method, which originated in Australia mainly used in elapid envenomations, has two components: the affected extremity is firmly

wrapped with an elastic bandage, and equally important, the entire extremity is then splinted (Pearn et al. 1981). The tightness of the wrap is defined as a pressure between 40 and 70 mmHg in the upper extremity and 55–70 mmHg in the lower extremity. However, there is insufficient evidence of their efficacy in the management of various types of snake envenomations in other countries. In 2011, six toxicological organizations have made a position statement: PI is not recommended for prehospital treatment of North America Crotalinae snakebites (ACMT et al. 2011). In Taiwan, given the fact that the prehospital transportation time is fairly short (e.g., within 30 min) (Hu et al. 1996; Lin et al. 1998) and trapping the highly cytotoxic venom of snakes at the bitten site using these measures may even worsen local necrosis (McKinney 2001), rest prior to transportation is probably the only management that is recommended in the prehospital setting.

Incision and Suction

The recommendation of incision and suction is accompanied by multiple qualifications: incisions 3 mm deep, 1 in. long, only if more than 30–40 min from reaching a healthcare facility, within 5 min of the snakebite with clear signs of envenomation, and with incisions made longitudinally on the extremities (Hall 2001). Evidence from animal models suggests that incision and suction can remove 1 % to >50 % of injected venom if applied within minutes of injection and may improve survival in some cases (McKinney 2001). In humans, however, laceration of tendons, nerves, and arteries, wound deterioration, and an increased infection rate from these attempts have been reported (Hall 2001). Given the fact that mortality from snakebites in Taiwan is uncommon and the most important principle of first aids is "do no harm," this technique is not recommended.

Electrotherapy or Cryotherapy

Guderian et al. reported local application of high-voltage (25 kV), low-amperage (<1 mA) direct electric current to human cases, with the bitten area electrically grounded, for the treatment of venomous snakebites in Ecuador in the 1980s (in particular, *Bothrops atrox*, *B. bilineatus*, *B. nasutus*, *B. schlegelii*, *B. castelnaudi*, and *Lachesis muta*) (Guderian et al. 1986). When applied to a reconstituted *Crotalus atrox* venom solution, direct electric current at low voltage showed neutralizing properties against venom phospholipase A_2 and metalloproteinases (Panfoli et al. 2007). However, it has been found to be ineffective in animal models of envenomation. Complications of this therapy, including burns, myocardial infarction, and seizures, have been reported in humans. As such, electrotherapy is contraindicated in the treatment of venomous snakebites (McKinney 2001).

Cryotherapy, which involves packing or immersion of the bitten limb in ice or ice water, was thought to be beneficial by slowing the spread of the venom, lowering enzymes activity, and thus decreasing the severity of envenomation (Mullins and Naylor 1960). This approach has become less popular over the past 40–50 years because experimental models have failed to demonstrate its effectiveness. There were some cases of sustained tissue loss, amputations, or permanent disability after prolonged cryotherapy. An ice pack intermittently placed on a bite

for pain control, similar to that used in case of an ankle sprain, is less likely to be harmful; however, more aggressive cryotherapy or ice therapy is contraindicated (McKinney 2001).

Summary of First Aids

There are no definitive data on prehospital management of snakebites in Taiwan. Given the fact that prehospital transportation is expeditious and the most effective therapy for snake envenomation is the timely administration of antivenom, any first aid procedure leading to delayed transportation should be scrutinized. At present, the following recommendations, described by Seifert et al. in 2011, are encouraged: (1) remove jewelry (e.g., rings on the bitten finger) and loosen tight-fitted clothing on the bitten limb; (2) loosely splint or immobilize the limb in a functional position, while the other potential actions should be guided by an experienced clinician; (3) maintain the bitten limb in a neutral position with regard to the heart; (4) get to a hospital, preferably transported by an EMS provider (in general, supine positioning will aid providers in managing possible effects such as hypotension or vomiting); and (5) avoid useless or potential harmful interventions such as arterial tourniquet, incision and suction, electrotherapy, or cryotherapy (Seifert et al. 2011).

In-Hospital Management

Antivenom and Its Side Effects

Four types of antivenom, all $F(ab')_2$ fragment in the lyophilized form, are available in Taiwan, namely, a bivalent antivenom against *T. stejnegeri* and *P. mucrosquamatus*, a bivalent antivenom against *N. atra* and *B. multicinctus*, a monovalent antivenom against *D. acutus*, and a monovalent antivenom against *D. r. siamensis*. Liau and Huang studied the dry weight of venom milked from the six medically important venomous snakes, calculated the median lethal dose (LD_{50}) of different snake venoms, and estimated the average dosage of antivenom required for neutralization (Table 2.1) (Liau and Huang 1997). Based on previously published studies and clinical observations, PCC-Taiwan formulated a flowchart for the management of snakebites (Fig. 2.9). The recommended dosage of relevant antivenom to treat a moderate-to-severe envenoming is 1–2 vials for *T. stejnegeri,* 2–4 vials for *P. mucrosquamatus,* 2–4 vials for *D. acutus,* 2–4 vials for *D. r. siamensis*, 6–10 vials for *N. atra*, and 2–4 vials for *B. multicinctus* bites (Hung et al. 1999). Moreover, PCC-Taiwan recommends completely filling each vial with 20 ml diluent and suggests all the antivenom should be administered intravenously at an infusion rate of 1–2 ml/min, which is based on pharmacokinetic studies on antivenom (Hung et al. 1999).

Antivenom was once used reluctantly due to the fear of developing adverse reactions and the uncertainty about its effectiveness (Sawai and Tseng 1969). Although the incidence of adverse reactions was not comprehensively evaluated in early studies, it is quite uncommon nowadays. In the past, the Vaccine Center in Taiwan adopted the Tanaka method to obtain sera from horses; however, two-thirds

Table 2.1 Amount of venom extracted by milking of 6 medically important venomous snakes and recommended doses of antivenom (Hung et al. 1999; Liau and Fuh 1991; Liau and Huang 1997)

Snake species	No. of specimen	Amount of envenoming of each snake (mg)		LD_{50} (μg/g mice)	Antivenom			
		Mean ± SE	Range		Neutralizing activity (mg/unit)	Average dose of antivenom (vials)	Type (2,000 units/vial)	Recommended dose (vials)
T. stejnegeri	115	6.9 ± 3.1	0.6–30.3	2.0 ± 0.23	0.013	0.3	Bivalent	1–2
P. mucrosquamatus	124	33.4 ± 15.5	6.6–125	2.9 ± 0.61	0.0195	0.9	Bivalent	2–4
D. acutus	103	105 ± 52	16.7–460	4.9 ± 0.41	0.026	2	Monovalent	2–4
D. r. siamensis	53	18.4 ± 10.3	4.7–83.2	0.29 ± 0.05	0.00247	NA	Monovalent	2–4
N. atra								
Eastern origin	45	217 ± 88	76.5–574	0.67 ± 0.02	0.00871	12.5	Bivalent	6–10
Western origin	107	48.0 ± 19.6	23.4–120	0.33 ± 0.01	0.00429	5.6	Bivalent	
B. multicinctus	118	4.4 ± 2.3	0.5–13.6	0.1 ± 0.02	0.001	2.2	Bivalent	2–4

1 unit antivenom neutralizes 1 LD_{50} in a 13 ± 0.5 g mouse/I.P.; LD_{50}: median lethal dose
NA not available

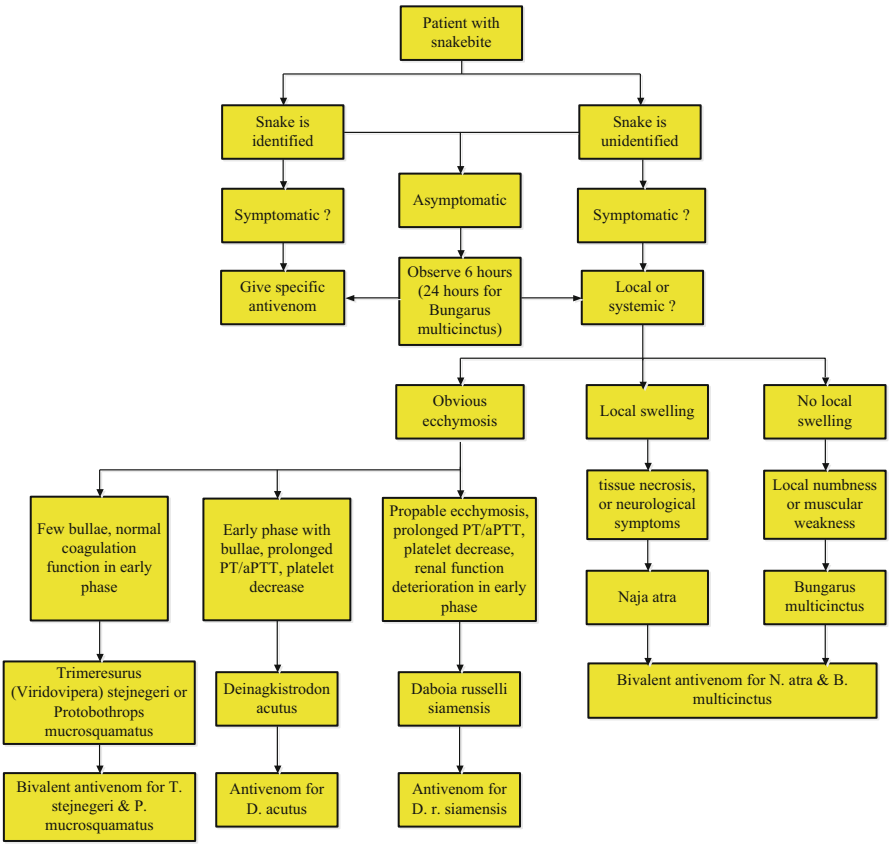

Fig. 2.9 Flowchart of the management of six medically important venomous snakebites in Taiwan (Hung et al. 1999)

of the horses failed to produce a satisfactory titer of neutralizing antibody. Thus, the manufacturing process has undergone several modifications since the 1980s (Huang et al. 1985, 1986). At present, horses are immunized with glutaraldehyde-attenuated venom toxoid and Freund's adjuvant (Liau and Huang 1997). When the plasma antibody titer reaches a plateau, the horse blood is withdrawn into a container with sodium citrate as an anticoagulant. The plasma is then processed by sedimentation and digestion with pepsin. Ammonium sulfate is repeatedly added during the purification process to precipitate the nonimmune protein. Finally, the mixture is filtered and the immune protein pellet is collected and press-dried on a filter paper. The obtained immunoglobulin fragment $F(ab')_2$ is redissolved in a buffer containing 0.01 % thimerosal and 2 % glycine. After sterilization, the antivenom is lyophilized, sealed in vacuum, and stored in 20-ml serum vial. The antivenom powder, with a shelf life of 5 years, is best stored at 4 °C before use (Huang et al. 1985, 1986). The newer immunization protocol is highly effective and safer

for horses compared with the conventional Tanaka method. Potent antivenom is obtained after an immunization period of 2 months instead of 6 months. There are 2,000 units per vial of antivenom (or at least 1,000 Tanaka units), and 1 unit neutralizes 1 LD_{50} of specific venom intraperitoneally injected in a mouse weighting 13 g (Liau and Fuh 1991).

Several molecular mechanisms account for the development of adverse reactions during antivenom infusion, including anaphylaxis mediated by IgE, anaphylactoid reaction caused by complement system activation, or pyrogenic response to contaminated endotoxins. The reported incidence of early adverse reactions secondary to the administration of ammonium sulfate-precipitated whole IgG or $F(ab')_2$ antivenom ranged from 10 % to 87 % (Otero et al. 1999). In Taiwan, studies on antivenom-related adverse reactions are sparse. Chen et al. examined 130 cases of snakebites and found that 32.3 % developed a positive antivenom skin test (Chen et al. 2000a). Following pretreatment with antihistamine and hydrocortisone, one patient (0.7 %) eventually had a skin rash amenable to conservative treatment. In a different study, Chen et al. investigated 179 cases of crotalid snakebites (*T. stejnegeri* and *P. mucrosquamatus*). Seventeen percent of the patients developed positive antivenom skin tests; however, allergy responses occurred only in 3 % of the patients with negative skin tests (Chen et al. 2007a). Otherwise, no serious antivenom reactions were recorded in either study.

There is hardly any information about the incidence of delayed allergic response. Two cases of serum sickness after receiving bivalent antivenom for *T. stejnegeri* and *P. mucrosquamatus* had been reported in the English literature (Huang et al. 2010; Ko and Chung 2013). The survey conducted by Shih et al. revealed that a patient had anaphylaxis after receiving bivalent antivenom for *N. atra* and *B. multicinctus* injection and two patients possibly had serum sickness. However, the relevant medical history was lacking (Shih et al. 2006). In Taiwan, the antivenom skin test is still advocated by the manufacturer because of medicolegal issues, and it is a common practice in hospitals regardless of plentiful evidence that suggests the low probability of adverse reactions of antivenom and the unreliability of skin test.

For more than 100 years, horses were used in the production of antivenoms; therefore, many people are sensitive to horse sera and may develop anaphylaxis after a second contact. The development of an avian-derived yolk immunoglobulin (IgY or truncated version of IgY) against snake venom has been evaluated in Taiwan. Lian et al. preliminarily studied the egg yolk IgY production in "big Kaiya" ducks immunized with *N. atra* venom toxoid (Lian et al. 2004). The duck was immunized with venom toxoid every 2 weeks, and the antibody titer reached a stable level between 5 and 21 weeks. The antibody amount harvested from 8 eggs during this period is comparable with that present in one vial of antivenom derived from horses. Chiou further estimated the antibody productivity from duck egg yolk to be more efficient than that from horses (egg yolk, $>$500 mg/kg body weight/month; horse serum, $<$200 mg/kg body weight/month) (Chiou 2008). Considering workers' safety, animal welfare, the cost of antivenom production, and possible allergy to horse sera, the development of avian-derived immunoglobulin against

snake venom seems to be attractive. However, all antivenoms derived from foreign proteins are capable of producing acute and delayed hypersensitivity responses. Healthcare providers must be aware of the possibility of life-threatening reactions resulting from the use of these products and be prepared to effectively treat a reaction should one occur.

Antivenom in Special Population

Chen et al. reported three pregnant women bitten by *T. stejnegeri* at 8, 17, and 28 weeks of gestation (Chen et al. 2007b). One of them received 1 vial of bivalent specific antivenom and 1 received 13 vials, while the other did not receive antivenom because her symptoms were mild. Fetal monitoring of these cases did not reveal evidence of fetal distress, and there was no maternal vaginal spotting. After delivery, follow-up of the children at 6, 8, and 10 years of age revealed no growth abnormalities. The author speculated that the safety of antivenom for pregnant patients is similar to that for other adults.

Wang et al. investigated snakebites in children and adolescents during 1994–2007 based on the database of Taichung Veterans General Hospital (Wang et al. 2009). There were 35 envenoming cases in patients aged between 2 and 18 years, with a median age of 9.5 years. The offending snake was *N. atra* in 11 cases, *T. stejnegeri* in 7, *P. mucrosquamatus* in 5, *D. acutus* in 1, and unknown venomous snakes in 11. In the study, the dose of bivalent antivenom for *T. stejnegeri* and *P. mucrosquamatus* and that for *N. atra* and *B. multicinctus* administered to snakebite patients ranged from 1 to 11 and 2 to 12 vials, respectively. No cases developed anaphylaxis in that study, whereas 3 patients had skin rashes amenable to treatment with antihistamine and steroids. Although none of the patients died, 5 of them underwent surgical intervention for wound necrosis caused by *N. atra* envenoming. The author speculated that the antivenom dosage recommended for adults may be equally safe for children and adolescent patients.

Antibiotic Therapy

Routine use of prophylactic antibiotics in snakebite cases is generally not recommended unless wound infection has occurred (Kerrigan et al. 1997). However, the venom itself may cause local reactions (e.g., tenderness, heat, swelling), fever, or elevated white blood cell counts that resemble wound infection during a bite (Blaylock 1999). Thus, the differentiation between wound infection and envenoming is difficult or even impossible in the early stages post-bite. In Taiwan, prophylactic antibiotic prescription is still a common practice in the management of snakebites. In Chen's study, 63 % *T. stejnegeri* and 76 % *P. mucrosquamatus* bites received prophylactic antibiotics; however, only 6 % and 26 %, respectively, developed clinically suspected cellulitis or wound infection (Chen et al. 2009). Chen et al. evaluated the bacteriology of snakebites in 21 patients with positive wound cultures during 2001–2010 (Chen et al. 2011). The most common pathogens isolated from infected snakebite wounds were *Morganella morganii* and *Enterococcus* species. Among these cases, 17 were bitten by *N. atra,* 1 by *P. mucrosquamatus,* 1 by *T. stejnegeri*, and 2 by unknown snake species. Huang

et al. studied 17 cases of snakebites with positive bacterial culture during 2005–2007 (Huang et al. 2012). The most common 3 pathogens were *M. morganii*, *Aeromonas hydrophila*, and *Enterococcus* species. Among these, 16 cases were bitten by *N. atra* and 1 by *T. stejnegeri*. The authors concluded that most of the wound infections were caused by *N. atra* bites; thus, empirical antibiotics with quinolones, third generation of cephalosporins, piperacillin/tazobactam, and/or aminoglycosides for gram-negative pathogens, vancomycin or ampicillin for gram-positive pathogens, and metronidazole or clindamycin for anaerobic pathogens should be considered for the management of an infected wound caused by *N. atra* bite.

Mao and Yang analyzed 112 cases of *N. atra* envenomings from 2 medical centers and found that 86 (76.8 %) patients developed wound infection or cellulitis, 75 (67.0 %) had wound necrosis, and 47 (42.0 %) had necrotizing fasciitis (Mao and Yang 2013). In their study, bacterial cultures of wound discharge, necrotic tissues, or blood were obtained from 59 of the 86 cases. Fifty of the patients (84.7 %) had positive results, and more than 2 organisms (polymicrobial) were isolated from 32 (54.2 %) patients. Twenty-three organisms were recognized, and the most common pathogens were gram-negative rods, followed by gram-positive cocci. The species of bacterium isolated is *M. morganii* in 32 cases, *Enterococcus* spp. in 21, *Proteus* spp. in 8, *A. hydrophila* in 7, and the anaerobe *Bacteroides* spp. in 7. Although the choice of empiric antibiotics necessitates the bacteriology of snakebite to be determined first, there is no prospective evaluation of the optimal timing and choice of prophylactic antibiotics in the management of snakebites in Taiwan. Based on currently available data, we therefore suggest that antibiotics should be withheld for crotalid snakebite treatment unless wound infection has developed. Further studies of the effects of prophylactic antibiotic administration on the outcome of snakebites, especially *N. atra*, in Taiwan are warranted.

Surgery

The local effects of snake envenomation could result in significant tissue destruction such as local necrosis, necrotizing fasciitis, or even compartment syndrome. Shih et al. studied 118 cases of snakebites during 1999–2004. Among them, 16 required surgery, including 7 (of 14) *N. atra* bites, 5 (of 54) *P. mucrosquamatus* bites, 1 (of 29) *T. stejnegeri* bite, 1 *D. acutus* bite, and 2 (of 13) unknown snakes bites (Shih et al. 2006). The procedures performed were debridement in 11 cases, incision and drainage in 4, amputation of digit in 1, split-thickness skin graft in 4, and local or distant flap in 5. The surgical indications included wound necrosis, abscess formation, gangrene of digits, or necrotizing fasciitis. Early excision of the bitten wound was abandoned, and there was no prophylactic fasciotomy for crotalid snakebites. In the study, *N. atra* was significantly associated with a risk of surgery. Moreover, hospital stay was significantly longer in the surgical group than in the nonsurgical group (19.06 vs. 5.43 days, statistic significance was not specified).

Liao et al. evaluated 46 cases of snakebite during 1986–1999; 10 patients received necrotic tissue debridement and 3 of 5 ultimately requiring skin grafts

were bitten by *N. atra* (Liao et al. 2000). In a study of 112 cases of *N. atra* envenomation by Mao and Yang, surgery was performed in 61 patients (54.5 %) at a median of 4 days post-bite (interquartile range of 3–7 days) due to wound necrosis, abscess formation, necrotizing fasciitis, or, in rare cases, compartment syndrome (Mao and Yang 2013). Based on these limited case studies, most snakebite patients in Taiwan that underwent surgery were bitten by *N. atra*. Although early excisional therapy and prophylactic fasciotomy in the management of snakebites were no longer recommended (Cumpston 2011), the optimal timing of surgery, partly depending on the severity of local effects and secondary infectious complications, remains unknown, which warrants better-designed prospective studies in the future.

Summary of in-Hospital Management

According to the guideline proposed by the PCC-Taiwan, the recommended dosage of relevant antivenoms to treat a moderate-to-severe case of envenoming is 1–2 vials for *T. stejnegeri*, 2–4 vials for *P. mucrosquamatus*, 2–4 vials for *D. acutus*, 2–4 vials for *D. r. siamensis*, 6–10 vials for *N. atra*, and 2–4 vials for *B. multicinctus*. The antivenoms should be administered intravenously at an infusion rate of 1–2 ml/min. Prophylactic antibiotics are generally not required in crotalid snake envenomings (e.g., *T. stejnegeri* and *P. mucrosquamatus*) because of low incidence of wound infection, whereas they may be needed in patients with *N. atra* bite given that wound necrosis, abscess formation, gangrenous change of distal limbs, and necrotizing fasciitis are relatively common in such patients. The use of prophylactic and empirical antibiotics in *N. atra* bites should judiciously follow the results of bacteriologic studies. If the offending snake was not identified (e.g., snake escaped), management by or consultation with experienced experts is advised.

Conclusion and Future Directions

Snakebite is an environmental and occupational disease. A general knowledge of management against snake envenomations is necessary for the citizens, EMS providers, and clinicians. More researches on the severity assessment (e.g., severity score), risk factors for the development of severe disease, optimal dosing intervals of antivenom, effect of prophylactic antibiotics on the outcomes, and the timing of surgery of venomous snakebites are warranted in Taiwan. Further development of specific antivenom toward certain toxic venom fractions instead of polyvalent antivenom should be considered; however, it may not be cost-effective and analysis of more cases to determine the major effects of various snake venoms in humans should be established first.

Cross-References

► Epidemiology of Snake Envenomation in Taiwan

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Venomous Terrestrial Snakes of Malaysia: Their Identity and Biology

3

Indraneil Das, Norhayati Ahmed, and Lim Boo Liat

Contents

Abstract

This article presents an overview of the identity and biology of the venomous terrestrial snakes of Malaysia, from Peninsular Malaysia and the Bornean states of Sabah and Sarawak. Two families account for a majority of venomous snakes that are of medical significance – the Elapidae (cobras, kraits, and coral snakes) and Viperidae (vipers and pit vipers). Certain members of the Colubridae are capable of giving life-threatening bites to humans (especially species of *Rhabdophis*), but little is known of the Malaysian species of the genus.

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A number of other species in the family have been implicated with human envenomation, although little objective evaluation appears to have been published. This article synthesizes data on the identification, distribution, and conservation of these snakes; provide colored images of every recognized species and subspecies of venomous terrestrial snakes of the families Elapidae and Viperidae known to occur in the country; and conclude with strategies to improve knowledge of the snakes of the country.

Introduction

The political unit that comprises modern-day Malaysia composes of the southern-most tip of the Southeast Asian continental landmass (referred to as Peninsular Malaysia); its smaller, offshore islands; as well as two large areas on the northern portion of the island of Borneo (namely, Sabah and Sarawak). The total land area is 328,657 sq km, and the extent of surface area underwater is 1,190 sq km. The country is bounded by southern Thailand to the north of the Peninsular, and across the Straits of Melaka lies the island state of Singapore. Malaysia's Bornean possessions share its southern and southeastern boundaries with several provinces of Kalimantan, belonging to the Republic of Indonesia. The independent Sultanate of Brunei Darussalam is wedged within the state of Sarawak, as two discontinuous land masses, both with coastlines. Affected by its location and position, the northern Peninsula shows weak but distinct seasonality, with a recognizable dry season, while on the island of Borneo, which straddles the equator, rainfall is more or less spread year-round, albeit with higher precipitation coinciding with the (winter) Northeast Monsoons.

Native vegetation of the region, at least prior to extensive habitat alteration for development of agroindustries, timber industry, and urbanization, comprised mostly of tropical forests. The vegetation range currently extant includes mangrove forests, peat swamps, heath forests, lowland and mixed dipterocarp forests, and various types of highland forests, climaxing in stunted, montane forests. It is therefore not difficult to understand why Malaysia ranks among the 17 megadiverse countries of the world. The vertebrate fauna, in particular, is rich, with many distinct lineages (or autochthonous elements), often linked to particular mountain systems or have ranges demarcated by river valleys or marine boundaries, in addition to elements from the Indo-Chinese and Indian subregions, plus widespread Indo-Malayan lineages.

The bulk of the venomous terrestrial snakes of Malaysia can be accommodated into two families, the Elapidae and the Viperidae. The obvious character that distinguishes members of the Elapidae from that of the harmless snakes of other families is the short and erect fangs set anteriorly, on the maxillary bone. Further, Asian species are smooth scaled, have forehead covered with large scales, show rounded pupils, and are oviparous. Members of the second major family, the Viperidae, are differentiated from those of the Elapidae and other snake families

Fig. 3.1 *Bungarus candidus* (Linnaeus 1758), Malayan krait (Photo: Norhayati Ahmed)

Fig. 3.2 *Calliophis bivirgatus flaviceps* (Cantor 1839), Malayan blue coral snake (Photo: Indraneil Das)

in having large triangular heads, retractile hollow fangs, as well as short, moveable maxillary bone adapted for deep penetration of prey. Additionally, vipers tend to be relatively stocky, have keeled scales, and typically show vertically elliptical pupils. While most species are ovoviviparous (or live-bearing), oviparity has risen independently in several lineages of vipers.

In Malaysia, the family Elapidae is represented by cobras, kraits, and coral snakes, the venom of which shows neurotoxic properties (Figs. 3.1, 3.2, and 3.3). Coral snakes are ground dwellers or even burrowing. Cobras and kraits, on the other hand, while being mostly terrestrial, are also accomplished swimmers, often venturing into forest streams and ponds to prey on amphibians, fish, and other snakes. All elapids are oviparous. Sea snakes are currently classified as Elapidae but are excluded from this discussion. All species have strong neurotoxic venom, which attacks the nervous system, but some possess other effects including swelling, necrosis, and even cardiotoxic effects.

The Viperidae is traditionally divided into two subfamilies, distinguished by the presence or absence of a loreal pit on each side of the head between the eye and

Fig. 3.3 *Naja sumatrana* (Müller 1890), Sumatran spitting cobra (from Borneo) (Photo: Indraneil Das)

Fig. 3.4 *Calloselasma rhodostoma* (Kuhl 1824), Malayan pit viper (Photo: Chan Kin Onn)

nostril and both possessing hemotoxic venom (Figs. 3.4, 3.5, and 3.6). All the Malaysian species are characterized by the presence of the loreal pit and are referred to as pit viper of the subfamily Crotalinae. The loreal pit is a thermosensitive organ, enabling the snake to detect its warm-blooded prey at night. All Malaysian species of pit vipers are ovoviviparous. The Russell's vipers (currently including two *Daboia* species, subfamily Viperinae) are large, pitless vipers found in South Asia, Myanmar, Thailand, and Cambodia and also in Java, Sumatra, the Lesser Sundas, and Eastern Asia (see Belt et al. 1997). It does not naturally occur in either Peninsular Malaysia or Borneo (perhaps owing to the year-round moist conditions in these areas). It is not unusual to occasionally find these species in suburban and rural areas in Malaysian states bordering Thailand, such as Perlis and Kedah, that are presumably escapees or released by animal dealers and hobbyists. Most of Malaysian pit vipers are arboreal, and a few strictly terrestrial. The abundance of proteases (protein-degrading enzymes) in vipers is associated with intense pain suffered as a result of its bite, in addition to local swelling, blood loss from disruption of the blood-clotting system, and necrosis, and death is typically caused by collapse in blood pressure and shock.

Fig. 3.5 *Garthius chaseni* (Smith 1931), Kinabalu brown pit viper (Photo: Indraneil Das)

Fig. 3.6 *Tropidolaemus wagleri* (Boie 1827), male, Wagler's pit viper (Photo: Indraneil Das)

A third group, the so-called back-fanged snakes (Colubridae of some authors, classified as Natricidae by Pyron et al. (2013), including the genus *Rhabdophis*, comprises certain members that are capable of giving life-threatening bites to humans (Weinstein et al. 2013a). Four species of the genus (*chrysargos, conspicillatus, murudensis*, and *subminiatus*) are known from Malaysia, although no records of envenomation from the bites of these species in the country are available. The bite of *R. subminiatus* is known to show signs of envenomation (see Nivattayakul 2001; Smeets et al. 1991). A highly venomous congeneric species from Eastern Asia, *R. tigrinus*, has been shown to sequester toxins from toads ingested (Hutchinson et al. 2007).

Other species of colubrids have been suggested to pose some danger to humans. Large-growing species of cat snakes, especially the mangrove cat snake (*Boiga dendrophila*), has been linked to mild envenomation (see Monk 1991), and a three-finger toxin (denmotoxin) isolated from the species has been shown to display potent postsynaptic neuromuscular activity (Lumsden et al. 2004; Pawlak et al. 2006). Within the Colubridae, neurotoxic activity has been demonstrated from secretions of two congeneric species (*Boiga blandingi* and *B. irregularis*;

see Weinstein and Kardong 1994). Several other species of Colubridae (such as *Macropisthodon rhodomelas*; see Subaraj 2008) have been linked to symptoms of envenomation, but many published records lack rigor in their documentation, including qualified clinical assessment and sound conclusion from objective information (further arguments in Weinstein et al. 2013b). For others (e.g., Ashton 1963), rapid nomenclatural changes and lack of voucher specimens or images render identification of species uncertain.

In the last two decades, improved analytical approaches such as multivariate morphometrics (Wüster and Thorpe 1992a) and the use of mtDNA sequences (Slowinski and Wüster 2000; Broadley and Wüster 2004) have been instrumental in both revealing cryptic species and refining understanding of higher-level systematics within the venomous snakes of the region. In the case of the venomous land snakes of Malaysia, the species numbers for both elapid snakes and vipers have increased, with more genera and species recognized at present, particularly that of the vipers. Venomous snakes and snake bite in adjacent Brunei Darussalam have been treated to a review in this volume by Das and Charles (2014).

The revised nomenclature of the venomous land snakes (Elapidae and Viperidae) is documented in a recent work for Southeast Asia by Das (2010). As every species of these families has been described, a dichotomous key to their identification is presented in Table 3.1 instead.

Elapidae

Within the Elapidae, the number of species has increased from 9 to 10 since 1983 (Table 3.2). The species added to the fauna is the monocled cobra (*Naja kaouthia*), which was formerly treated as a subspecies of the Indian cobra (*N. naja*) by Tweedie (1961). Studies carried out by Wüster and Thorpe (1989, 1992a, b) on the Asiatic *Naja* complex showed that *N. naja* is not found in Southeast Asia; rather, the species encountered is the equatorial spitting cobra (*Naja sumatrana*). Sometimes referred to as the golden or Sumatran spitting cobra, *N. sumatrana* is the most common elapid among the rest of the nine species known in Malaysia (Table 3.2). Its geographical distribution includes extreme southern Thailand, Peninsular Malaysia, Singapore, Indonesia (Sumatra, Kalimantan, Bangka, Belitung, Riau, and Lingga), East Malaysia (Sabah and Sarawak), and Brunei (Wüster and Thorpe 1989), as well as the southern Philippines. It preys on vertebrate animals, primarily rodents and frogs, and inhabits many habitat types, ranging from human surroundings, fields, and plantations to forests at low altitudes throughout the country. This cobra appears catholic in its diet.

The monocled cobra (*Naja kaouthia*), a non-spitting species, is distributed from eastern India to south to northern Peninsular Malaysia, while being abundant in southern Thailand. In Malaysia, this species is confined to the northern parts of the Peninsula and has a feeding habit similar to that of *N. sumatrana*. It inhabits more open environments, such as rice fields, plantations, and other human-modified environments, and also lowland forest habitats. Bites by both these species are

Table 3.1 Dichotomous identification key to the venomous terrestrial snakes of Malaysia

1.	Head broad and flat, covered with small irregularly arranged scales of which six or more lie along a line between eyes	
	Viperidae	11
	Head variously shaped, usually covered with symmetrically arranged shields, three lie in a line between eyes	
	Elapidae	2
2.	Third upper labial large, touching the eye and nostril	3
	Third upper labial normal, not touching the eye and nostril	5
3.	Paired occipitals contact with each other behind parietals	*Ophiophagus hannah*
	No occipital behind parietals	4
4.	Body uniformly black, belly bluish gray, white markings on throat	*Naja sumatrana*
	Body brown to grayish brown, belly paler, a white circle centrally on the back of hood	*Naja kaouthia*
5.	Subcaudals of underside of the tail single anteriorly and paired posteriorly or entirely single	6
	Subcaudals of underside of the tail paired throughout	8
6.	Subcaudals single behind vent, paired posteriorly, body bluish black with the head, neck, and tail bright red	*Bungarus flaviceps*
	Subcaudals single throughout	7
7.	Body banded with alternate black and white bands, black bands encircling body; tail with blunt end	*Bungarus fasciatus*
	Body is banded with alternate black and white bands, black bands confined to the back and sides; tail tapering to point	*Bungarus candidus*
8.	Anal paired	9
	Anal single	10
9.	Body brown, scales dark edged; narrow black vertebral stripe connects series of small black spots on each side; belly with alternate black and yellow; underside of the tail banded black and red	*Calliophis gracilis*
	Body brown above with small black spots longitudinally arranged along each side of the back or with black vertebral stripe and no spots, belly red, underside of the tail blue or gray	*Calliophis maculiceps*
10.	Body brown with red and orange stripes enclosed between two black lines and white stripes below each side. Belly banded with alternate black and white pattern and underside of the tail band black and red	*Calliophis intestinalis*
	Body dark blue or blue black; head, belly, and tail bright red	*Calliophis bivirgatus*
11.	Species confined to Peninsular Malaysia	12
	Species confined to Borneo and/or Peninsular Malaysia and Borneo	22
12.	Top of the head with shields systematically arranged; body reddish or purplish brown; throat, belly, and tail pinkish white	*Calloselasma rhodostoma*
	Top of the head scales small	13
13.	Body predominantly brown	14
	Body predominantly green	17

(*continued*)

Table 3.1 (continued)

14.	Snout flat and projected	*Trimeresurus wiroti*
	Snout rounded and not projecting	15
15.	Body brown with a series of large dark square spots along each side of the back	*Ovophis convictus*
	Body without square-shaped spots	16
16.	Body dull olive or bluish green in males and grass green in females, scales on body keeled rusty or dull brown, belly dull brown with dark edges	*Popeia venustus*
	Body blackish variegated with brown or olive, belly dark brown or grayish	*Cryptelytrops purpureomaculatus*
17.	Tail mainly reddish throughout length	18
	Green banded with brown	19
18.	Body greenish black with scales black bordered, green above and yellow on sides. Belly greenish white with irregular yellow patches, bordered with black or black spotted	*Tropidolaemus wagleri*
	Tail entirely pinkish of posterior half	20
19.	Body olive green to bluish, with spots arranged to form transverse maroon bands and brownish bands on tail	*Popeia buniana*
	Tail rusty or reddish brown	21
20.	Nasal usually in contact, body green with two rows of blackish spots and a white line along the lowest dorsals bordered with black or by a row of black spots	*Parias hageni*
	Nasal not in contact	
21.	Body green in both sexes, with irregular rusty or reddish brown crossbands; tail rusty or reddish brown, sometimes mottled	*Popeia fucata*
	Body green above, pale green below; juveniles green, white stripe, bordered below with red, along the lowest row of dorsals	*Popeia nebularis*
22.	Body brown	23
	Body green	24
23.	Body brown with black-edged saddles across the back and row of light spots low on the sides of tail and the nose formed into a leaflike projection	*Trimeresurus borneensis*
	Body brown with irregular dark blotches in paired rows down center of back, tail brown with dark blotches	*Garthius chaseni*
24.	Anal entire	25
	Anal divided	26
25.	Body with striking pattern of bright green dots on black background, tail with parallel red dots with dark green scales	*Parias malcolmi*
	Body green with dark crossbands at intervals of 4–5 scales along body, a white line running along the lowest two rows of dorsal with a green line below it	*Popeia sumatranus*
26.	Body bright green, flanks with white or red spots or stripes in males, white or yellow in female	*Popeia sabahi*
	Body green or greenish blue with white or red spots or stripes in males, bluish-green and red crossbands in females	*Tropidolaemus subannulatus*

Table 3.2 The elapid and viperid snakes of Malaysia (Peninsular Malaysia, Sabah and Sarawak, and Borneo)

No.	Species	Common name	Locality
	Elapidae		
1.	*Bungarus candidus* (Linnaeus 1758)	Malayan krait	PM-P-C
2.	*Bungarus fasciatus* (Schneider 1801)	Banded krait	PM-P-C, SW-P-C
3.	*Bungarus flaviceps* Reinhardt 1843	Red-headed krait	PM-P- NC, SW-P-NC
4.	*Calliophis bivirgatus* (Boie 1827)	Blue coral snake	PM-P-C, SW-P-C
5.	*Calliophis gracilis* Gray 1835	Spotted coral snake	PM-P-NC
6.	*Calliophis intestinalis* (Laurenti 1768)	Striped coral snake	PM-P-C, SW-P-C
7.	*Calliophis maculiceps* (Günther 1858)	Speckled coral snake	PM-P-NC
8.	*Naja kaouthia* Lesson 1831	Monocled cobra	PM-P-C
9.	*Naja sumatrana* Müller 1890	Sumatran spitting cobra	PM-P-C, SW-P-C
10.	*Ophiophagus hannah* (Cantor 1836)	King cobra	PM-P-C, SW-P-C
	Viperidae		
11.	*Calloselasma rhodostoma* (Kuhl 1824)	Malayan pit viper	PM, northern states-C
12.	*Cryptelytrops venustus* (Vogel 1991)	Beautiful pit viper	PM, northern states-NC
13.	*Cryptelytrops purpureomaculatus* (Gray 1832)	Mangrove pit viper	PM-C
14.	*Garthius chaseni* (Smith 1931)	Kinabalu brown pit viper	S-HA, endemic to Borneo
15.	*Ovophis convictus* (Günther 1864)	Malayan brown pit viper	PM-C
16.	*Parias hageni* (Lidth de Jeude 1886)	Hagen's green pit viper	PM-C
17.	*Parias malcolmi* (Loveridge 1938)	Kinabalu green pit viper	S-HA, endemic to Sabah
18.	*Parias sumatranus* (Raffles 1822)	Sumatran pit viper	PM-C, SW-C
19.	*Popeia buniana* (Grismer et al. 2006)	Pulau Tioman pit viper	PM, endemic to Pulau Tioman
20.	*Popeia fucata* (Vogel et al. 2004)	Thai Peninsular pit viper	PM-C
21.	*Popeia nebularis* (Vogel et al. 2004)	Cameron Highlands pit viper	PM-C, endemic
22.	*Popeia sabahi* (Regenass and Kramer 1981)	Sabah green pit viper	SW-HA, endemic to Borneo
23.	*Trimeresurus borneensis* (Peters 1872)	Bornean palm pit viper	SW-C, endemic to Borneo

(*continued*)

Table 3.2 (continued)

No.	Species	Common name	Locality
24.	*Trimeresurus wiroti* Trutnau 1981	Wirot's palm pit viper	PM-C
25.	*Tropidolaemus wagleri* (Boie 1827)	Wagler's pit viper	PM-C
26.	*Tropidolaemus subannulatus* (Gray 1842)	Bornean pit viper	SB-C

PM Peninsular Malaysia, *SW* Sarawak, *S* Sabah, *P* present, *C* common, *NC* not common, *HA* high altitude. Species showing neurotoxic venom, nos. 1–10; species showing hemotoxic venom, nos. 11–26

common due to its close association with humans. A recent medical report indicates that cobra bites at a hospital in Penang were significantly more likely to result in severe envenomation, compared to bites by other species (Chew et al. 2011). Unfortunately, no identities of species were provided in this study, and given that both species are present in the hinterland of Penang State, it would have been of interest to know the identities of species, as their venom constituents as well as effects on humans are different (Yap et al. 2011). The behavior of spitting is well documented in *N. sumatrana* (see Wüster and Thorpe 1992b), thereby introducing additional medical complications among emergency physicians of the country.

The king cobra (*Ophiophagus hannah*) is the most feared elapid because of its size, and when encountered, it could raise the forepart of its body to about 1.2–1.5 m high at "eye level" with its hood extended. Its distribution extends from India to Hong Kong, Indochina, Peninsular Malaysia, Singapore, Sumatra, the Philippines, Borneo, Java, Sulawesi, and Bali (Grismer 2011). The behavioral response presumably depends on the extent of provocation it receives, and a brooding female guarding its eggs or young in its nest is ready to aggressively defend when confronted. Although *O. hannah* is common in oil palm plantations and forested areas and around human habituated areas in urban and suburban areas, bites by this snake are rarely recorded. Human mortality from its bite has been documented for Sabah and Sarawak (Haile 1958, 1963; Sawai 1972).

The kraits in Malaysia are represented by a single genus (*Bungarus*), with three species (Table 3.2). They are of moderate size and are conspicuously marked and colored, two with alternating black and yellow or white bands and one with a red head, making them easily recognizable. They are sluggish in behavior upon encounter but need to be considered extremely dangerous, and bites cause fatalities in humans. Among them, the red-headed krait (*Bungarus flaviceps*) is the least common, occurring mostly in primary forests in the highlands (Grismer 2011), although on Borneo this species is also encountered in the lowlands and along the foothills (Das, pers. obs.). The Malayan krait (*Bungarus candidus*) is common and is more of a forest snake, although it is occasionally encountered within human habitation. The banded krait (*Bungarus fasciatus*), the largest of the three species, is also the most common. This snake inhabits many habitat types, ranging from light forests, swamps, and near villages, under 2,500 m above mean sea level, asl (Das 2012). Within human habitations, this species is frequently sighted near mangrove

forests and often found as roadkills in rural areas. Bites from this snake are rarely reported, but there were occasions when the Orang Asli were bitten while handling *B. flaviceps* and *B. candidus* in Gombak and Ulu Langat Forest Reserve, Selangor. According to them, they survived using herbal remedies from the forest.

The Asian coral snakes are represented by the genus *Calliophis*, with four local species, *C. gracilis*, *C. maculiceps*, *C. bivirgatus*, and *C. intestinalis*. The last two were, till recently allocated to *Maticora*, a genus synonymized under *Calliophis* by Slowinski et al. (2001). Coral snakes are small- to medium-sized snakes, slender, and brightly colored, with very small heads. Among them, the longest is *C. bivirgatus*, which reaches 185 cm (Das 2012). Both *C. intestinalis* and *C. bivirgatus* have been encountered from lowland to forest fringes, such as in agricultural areas, to submontane forests (<1,200 m asl). *C. gracilis* and *C. maculiceps* have been found in lowland forests but can also occur in submontane forests and plantations (<1,300 m asl). Bites by coral snakes are rare. Tweedie (1961) reported two isolated cases of coral snake bites in Java in Indonesia and Melaka in Peninsular Malaysia. The first case was an adult bitten by *C. intestinalis*, which survived after suffering severe pain and vomiting (Jacobson 1937). The second case was a 2-year-old child, who was bitten by *C. bivirgatus* and died two hours after envenomation (Harrison 1957).

Viperidae

Intense interest in the study of the Malaysian vipers in recent years by several independent (and often competing) investigators has generated an increase in species diversity to 16 species of eight genera, as opposed to nine species from three genera since the end of the twentieth century (Tweedie 1983; Stuebing and Inger 1999). The new additions include species described on the basis of new material, such as *Popeia buniana* from Pulau Tioman, Pahang, by Grismer et al. (2006); application of different species concepts and/or discovery of new characters, as in the case of *Parias malcolmi* (cf. Stuebing and Inger 1998); as well as recognition of cryptic diversity within larger complexes during the course of faunal revisions, such as *Popeia popeiorum* and *Trimeresurus puniceus* (see Vogel et al. 2004; David and Vogel 2006). After a prolonged period of retention of the large number of the so-called green pit vipers within the genus *Trimeresurus*, Malhotra and Thorpe (2004) suggested a new taxonomy, including the recognition of seven genera. David et al. (2011) discussed the new taxonomy, choosing to retain the names proposed at subgeneric, rather than generic, levels; throwing in a caveat that the allocation to genera or subgenera remains open to discussion; and advancing merits and demerits of both decisions. In this essay, these novel generic names are retained, as they reflect distinct evolutionary lineages, the placement of which into a single genus would obscure their relationships. The latter authors' philosophy that "...recognizing 'genera' that cannot be diagnosed morphologically is not helpful to practicing taxonomists, especially when they do not have access to molecular facilities" is not followed, as the role of systematics should not be

facilitation, but recovering evolutionary relationships. Certainly, combining morphological with molecular and ecological data (see example in Sanders et al. 2006) has greatly enriched our knowledge of these interesting species.

Among the vipers, the Malayan pit viper (*Calloselasma rhodostoma*) is the most common among the 16 species known. This species is confined to the northern states of Perlis and Kedah in Peninsular Malaysia. It is terrestrial, its diet comprising rats, birds, and, more occasionally, fish and frogs. Radiotelemetric studies on the species, one of few snake species to be thus studied in the region, show a relationship between ambient relative humidity (rather than temperature, precipitation, or lunar cycle) and local movement (Daltry et al. 1998).

The mangrove pit viper (*Cryptelytrops purpureomaculatus*) is another common species of pit viper. It primarily inhabits mangrove forests and has also been found in peat swamp forests throughout Peninsular Malaysia. It is semiarboreal, its diet similar to that of the aforementioned species.

The other Malaysian species of pit vipers are patchily distributed throughout the country. They primarily feed on warm-blooded prey species, such as rats and birds. Stomach contents of *Parias hageni* and *P. sumatranus* and *Ovophis convictus* have shown remnants of the slender tree squirrel (*Sundasciurus* sp.), as well as gekkonid lizards.

Apart from the venomous land snakes of the families Elapidae and Viperidae and a few species of the genus *Rhabdophis*, there are several species of nonvenomous land snakes of the family Colubridae that have been reported to be mildly venomous. These are the mangrove snake (*Boiga dendrophila*) and mock viper (*Psammodynastes pulverulentus*). According to the Orang Asli (introduction to these indigenous people of the Malay Peninsula in Knox et al. 1996) and local snake handlers, bites by these snakes have caused severe pain and, in some cases, are accompanied by vomiting and headaches. It is, thus, possible that there are other seemingly harmless snakes but are actually mildly venomous.

Distribution and Status

Among the elapids, *Naja sumatrana* and *N. kaouthia* are not habitat specific. The former species is widely distributed throughout Malaysia, while the latter species is restricted to the northern parts of Peninsular Malaysia. They appear adaptable to local environmental conditions and can persist in changed habitats, where prey species may be more easily assessable. In recent years, *N. kaouthia* has been found further south of the country, from urban, suburban, and rural areas in the west and east coasts of Peninsular Malaysia. However, this species is yet to be encountered in forest habitats of these areas. The dispersal of this northern inhabitant species is suspected to be due to escapees from the trade, which obtains the snake from either the northern parts of the country or from southern Thailand to be traded as food for the local and overseas market. With time, this species is likely to be established throughout the peninsular portion of the country.

The rest of the elapid species, *Ophiophagus hannah* as well as species of *Bungarus* and *Calliophis*, are more habitat specific, being primarily forest inhabitants. Although common and widely distributed throughout the country, the density of each of these species may fluctuate corresponding to deforestation.

Calloselasma rhodostoma is the most common viper in Malaysia. In the last 15 years, this northern species has been collected on Bukit Larut, in the state of Perak. In 1990, individuals were collected around human habitations in Cheras, Kuala Lumpur, and in secondary and disturbed patch of forest surrounding the headquarter building complex of the Department of Wildlife and National Parks (DWNP) (Jasmi and Lim 1991). In 2010, roadkills of four juveniles and two adults were collected along the road toward DWNP. Its presence in Kuala Lumpur could derive from dealers who transported this species from the northern states for sale as food, for transhipment overseas, and for breeding or sale to zoos. However, the finding of this snake in Kuala Lumpur, especially the juveniles, indicated that this species has probably established itself in the forested areas around the country's capital. Significantly, in the last 10 years, the density of this species has dwindled within its natural range, due to the many plots of rice fields being developed into monoculture plantations.

Another common species of viper, *Cryptelytrops purpureomaculatus*, is restricted to mangrove and peat swamp forest. The density of this viper appears to be affected by the harvest of mangrove forest trees for commercial purposes; on the other hand, its density may be higher in peat swamp forests that are protected from deforestation. This species has also been reported from many offshore islands, such as Pulau Sembilan (Norhayati pers. obs.), Pulau Jarak (Daicus pers. obs.), and Pulau Langkawi (Lim et al. 2010).

The other vipers are forest inhabitants, and some are associated with highlands, with endemics of specific areas, while others are more widely distributed throughout the country. Those species that are inhabitants of low and hill forest up to 800 m asl. may be threatened by transformation of habitat, caused by logging and urbanization.

Conservation

There might be some disagreement in some quarters where conservation of the snake fauna, particularly of venomous land snakes, is mentioned. This appears due to reputation of snakes themselves, perhaps derived from irrational fear and societal conditioning. However, if knowledge held by a few of the economic importance of snakes, as predators of crop pests and as essential parts of a working ecosystem, in addition to their great diversity, is disseminated more widely, perhaps the rationale for conserving these maligned animals would be better appreciated. Snakes also offer excellent examples as models to test ecological and evolutionary theories (see, for instance, Sanders et al. 2004).

The association between many snakes, venomous as well as harmless, and rats is well known. Among the venomous land snakes, 19 species (three members of the

Elapidae and 16 of the Viperidae), out of a total of 26 species, feed primarily on rodents. It must be borne in mind that this is more than economic importance (rodents destroy significant amount of standing and stored grains), since rodents of various species are dangerous reservoirs of diseases. The potential rate of increase of jungle and field rats exceeds that of most mammals, and if it were not for natural predators to help control their numbers, the rat problem would be much more severe. In turn, snakes themselves may be responsible for eliminating their own excessive numbers by feeding on one another, as do *Ophiophagus hannah*, as well as species of *Bungarus* and *Calliophis*. It is quite obvious that snakes (venomous and harmless snakes) do more good than harm in nature. Unfortunately, in spite of the valuable assistance they render and the small danger they constitute, they are widely perceived as dangerous and the good they do remains unknown. This ignorance can be overcome in large measure by producing programs on the natural history of snakes, emphasizing their great beauty and diversity, as well as integral role in many ecosystems, for school children in their natural history lessons. At the same time, the general public and medical doctors involved in the treatment of snake bite should be made aware of the differences between the venomous and harmless snakes.

Conclusion and Future Direction

Life-threatening bites to humans can be given by at least 26 species of terrestrial snakes, belonging to two families (the elapids and vipers) in Malaysia. Additionally, some members of an otherwise nonvenomous group (the natricids) may be dangerously venomous, particularly a few species of the genus *Rhabdophis*.

Bites by several species produce extremely serious and potentially fatal results in human beings. These are the three species each of the cobras (*Ophiophagus hannah*, *Naja kaouthia*, and *N. sumatrana*) and kraits (*Bungarus candidus*, *B. fasciatus*, and *B. flaviceps*) of the family Elapidae and one species of the viper (*Calloselasma rhodostoma*) of the family Viperidae. The four species of coral snakes (*Calliophis bivirgatus*, *C. gracilis*, *C. intestinalis*, and *C. maculiceps*) among the elapids, based on two cases of bites, one each of *C. bivirgatus* and *C. intestinalis*, are known also to be of importance for human health. Bites have resulted in death and excruciating discomfort, respectively.

Human mortality associated with the bite of most arboreal vipers (with the exception of the relatively toxic *Cryptelytrops purpureomaculatus* and *Tropidolaemus wagleri*) may be primarily due to clinical complication of individual cases during treatment. Little is known of the effects of envenomation by most local pit viper species or their potency.

With the widespread use of contemporary analytical approaches (such as multivariate morphometrics and their combined use with mtDNA sequences), it is envisaged that the systematics of cryptic species would be resolved further in the future, including the recognition of cryptic species and stability of higher classification among the venomous snakes of Peninsular Malaysia and of Sarawak and

Sabah. Parallel to such advanced approaches, it is equally important that basic research be augmented in fields as diverse as ecology, ethology, population biology, evolution, and many others.

In biological research, an elemental understanding of the subject is critical. In this case, the ecology of the snake necessitates the acquisition of masterful knowledge of functions, interactions, and relationship of the concerned organism with its natural environment, as well as awareness of the utility of such knowledge for human welfare. In this context, some suggestions on future direction for research have been made:

1. To increase the knowledge of species diversity, the curation of specimens from the field becomes essential. It is through the establishment of natural history museums and specimen acquisition that information on habitats, interaction with their prey, and their relative abundance, and many other topics can be gathered.
2. Venom should be extracted from live specimens for studies on toxicity and for other medical usage especially antivenom production.
3. Tissues from freshly euthanized specimens can provide material for systematic research and should be added to the protocol for all material being acquired. For instance, blood direct from the heart and tissues of specific organs can be used for studies of relationships within species (phylogeography), recognition of cryptic species or higher taxonomic relationships. All specimens used for research need to be preserved as permanent vouchers, with all relevant documentation.
4. The need of the day is the creation of an institute of toxicology, perhaps established as a regional center for Malaysia and including expertise for species occurring both in Peninsular Malaysia and in Sabah and Sarawak. Apart from the study of venom of vertebrate and invertebrate species, such a center can synergize with other existing institutes in the region and perhaps in the future produce vaccine for the treatment of noxious venomous animals, including Malaysia's venomous snakes.

Cross-References

► Venomous Snakes and Envenomation in Brunei

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Snakebite and Envenomation Management in Malaysia

4

Ahmad Khaldun Ismail

Contents

Abstract

Malaysia is a tropical country and snakes are an essential component of its many ecosystems. A number of medically significant venomous land and marine species have been recorded from Malaysia. Humans are exposed to bites and envenoming from these snakes during their engagement in various activities that bring them into the animal's natural habitat. Snakebite is an important medical emergency and one of the common causes of hospital admission. There is a clear association between the knowledge and confidence level of healthcare providers managing snakebite with the quality of patient care, the provision of appropriate clinical management, the selection of appropriate antivenom, and the outcome of such treatment. The clinical management of snake bites and envenoming may

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still be suboptimal due to neglect of this issue and negligence at various levels of medical care. The true scale of mortality and morbidity from snakebite remains uncertain as a result of inadequate documentation. To overcome these deficiencies, snake bite envenoming must be recognized as an important notifiable disease. Awareness programs for the public and specially tailored educational programs for healthcare providers should be encouraged and supported. An appropriate clinical management guideline should be established and the inappropriate ones removed. The establishment of an easily accessible qualified clinical expert assistance in managing snakebites and envenomation is also necessary.

Introduction

Snakes are an essential component of the many ecosystems in Malaysia. A large number of species have been recorded, with approximately 25 % being categorized as medically important species (Das 2010, 2012; Ismail et al. 2013). Malaysians and visitors to Malaysia are exposed to the risk of snakebite each year. Some may seek treatment in a healthcare facility, but it is believed that many cases are still not appropriately documented or reported. Snakebite is not categorized as a notifiable disease in Malaysia, even though reporting death from snakebite is mandatory. Consequently, snakebite data are not exact, nor reflect their exact incidence in the country. Therefore, it is a flawed notion that snakebite is uncommon causing it to be regarded as an unimportant medical issue in Malaysia despite the significant burden of snake bites and envenomation.

As in other parts of Southeast Asia, snakebite-related injuries generally affect agricultural workers (Chippaux 1998; Kasturiratne et al. 2008; Lim and Abu Bakar 1970; Stephen and Lim 1998; Bawaskar et al. 2002; Alirol et al. 2010). Snakebite is, thus, categorized as an occupational hazard (Gutierrez et al. 2006, 2010). There appears to be a variety of situations of snakebite in Malaysia, not just related to occupation. The situations and frequency of incidents may fluctuate with the diversity of human activities and the changing of lifestyles. These associations are dynamic, yet with the modernization of the agricultural sector, the chance for unfavorable contact is expected to reduce. Unfortunately, as the human population increases, so do the size of residential and industrial areas. These expansions encroach into and directly decrease the continued existence and quality of natural habitats. Humans contribute to the multiplication of rodents and certain other animals that may attract snakes. Keeping and/or breeding snakes as pets, and activities that bring humans closer to nature, such as ecotourism, are becoming more popular. The seasonal and climatic changes may also influence the distribution of snakes. These may indirectly increase the chances for unfavorable human contacts with medically significant snakes. It is also important to note that poor monitoring of the exotic pet trade by concerned enforcement departments and irresponsible importation of exotic venomous snakes by dealers may introduce medically important species that are not indigenous to Malaysia. This may add to the complexity of managing envenoming.

It is estimated that Malaysia has 400–650 snakebites per 100,000 populations per year and carries a mortality rate of 0.2 per 100,000 populations per year (Chippaux 1998; Kasturiratne et al. 2008). The northern states of Peninsular Malaysia recorded a higher number of mortality and morbidity compared to the southern states (Lim and Abu Bakar 1970; Stephen and Lim 1998). In 2011, the state of Kedah recorded the highest incidents with 836 cases, and the state of Perak recorded the second highest with 576 cases. There are no published data on the incidence or prevalence of snakebite in Sabah and Sarawak. This estimation is based on the data obtained from hospital admission records. In a recent press release by the Ministry of Health (MOH), the total number of admissions appears to be declining, from 4,024 cases in 2009 to 3,658 cases in 2011. However, the existing data itself is limiting, as often the identity of snakes involved, the nature of bite, and the outcomes cannot be generated directly from the inpatient database. Therefore, continuous research and documentation on snake bite envenoming are required, as the circumstances that places human and serpent into conflict changes over time.

Venom and Its Effects

Venom is defined as a complex substance produced in a specialized gland of a living organism. It is deleterious in a certain amount when injected through a specialized apparatus into other organisms. It is actively used to subjugate and digest prey or for defence (Minton 1974; Minton and Minton 1980; Russell 1980; Mebs 2002). Snake venoms contain a diverse group of proteins, many with enzymatic activity. The major toxic components of venom have a molecular weight that are too large to cross through intact capillaries into the bloodstream; therefore, a lymphatic drainage is believed to be the main route for its spread into the blood circulation (Mebs 2002; Chippaux 2006; Mackessy 2009). However, damaged vasculature inflicted by the mechanical injury from the fangs may provide a faster route for spread. Some enzymes contribute to the lethal properties of venoms. Components such as procoagulant enzymes activate the coagulation cascade. Phospholipases A2 may have multiple or varied actions (e.g., cytotoxic, myotoxic, and/or neurotoxic). Cardiotoxin, also known as direct lytic factor (DLF), may cause hemolysis and increased vascular permeability. Proteases hydrolyzes supportive tissue structures especially of small blood vessels. Polypeptide toxins may disrupt neuromuscular transmission, and some other components may aid the spread of venom. Some bites may cause secondary damage or complication such as hypovolemic shock, renal failure, and sepsis. These clinical features are the manifestations of various toxic components in the venom. Toxins can vary within the same biological species. Ontogenetic progression, geographical distribution, and prey specificity factors may influence the compositions of venom toxins (Barlow et al. 2009). Therefore, the same snake species from distant geographical regions may manifest different physiological effects. Ignorance of this important fact has resulted in the inappropriate purchase and use of antivenom (Warrell 2008).

Fig. 4.1 (**a**) King cobra, *Ophiophagus hannah*, is found in Peninsular Malaysia, Sabah, and Sarawak (Photo courtesy of Zainal Abidin Mohamed@Ismail & Ahmad Khaldun Ismail). (**b**) Fasciotomy of the forearm displaying extensive local necrosis with severe tissue damage and edema following a king cobra, *Ophiophagus hannah*, envenoming (Photo courtesy of Hasniza Ahmad Zakaria)

The venom of Malaysian cobras, kraits, and coral snakes mainly affects the neuromuscular junction (NMJ) as pre- or postsynaptic blockade (Mebs 2002; Chippaux 2006; Mackessy 2009). Cobra envenoming may cause acute neurological dysfunction with ptosis, ophthalmoplegia, dysphagia, aphasia, hypersalivation, and respiratory paralysis. Patients with these conditions may not respond to antivenom instantly and may require intubation and ventilation, as clinically appropriate. Local tissue necrosis, along with local pain and edema of the bite site, may manifest without any neurological or cardiovascular dysfunctions (Mackessy 2009; Barlow et al. 2009; Wuster 1996; Ismail et al. 2012). All postsynaptic envenoming can also cause fixed dilated pupils, which can be mistaken as loss of higher brain function and lead to discontinuation of ventilation of a "locked in" syndrome patient. Envenomation from cobra species, particularly the King cobra, *Ophiophagus hannah*, may also cause local envenoming with severe tissue damage that often results in infected necrotic wounds (Figs. 4.1–4.3). In such circumstances, an early broad-spectrum antibiotic should be considered. Krait envenoming may cause a sudden or delayed onset of rapidly progressive respiratory paralysis with minimal local manifestation. The venom does not affect the central nervous system, as widely thought. The main issue with the Krait, *Bungarus* species envenoming is neuromuscular junction (NMJ) beta-presynaptic blockade. Beta-neurotoxins, presynaptic PLA2-basic subunit neurotoxins, binding to the presynaptic receptors

Fig. 4.2 (**a**) Monocled cobra, *Naja kaouthia*, is found in Peninsula Malaysia but not indigenous to Borneo (Photo courtesy of Ahmad Khaldun Ismail). (**b**) A hand which received skin grafting following a severe local necrosis and systemic envenomation from a bite on the middle finger by a monocled cobra, *Naja kaouthia* (Photo courtesy of Ahmad Khaldun Ismail)

cause a rapid hydrolysis resulting in a rapid presynaptic blockade that causes the motor end plate to degenerate, and in some cases, it undergoes partial myolysis. Depending on the proportion of beta toxins injected and patient's physiological response, neuroparalysis sets in from one to a few hours. Giving antivenom at this stage does not significantly reverse it, but can stop its progression. In such neuroparalysis, the patient will require ventilation until the motor end plate regenerates, requiring between 24 hours to a couple of months. This is different from the NMJ postsynaptic blockade that follows cobra envenoming.

Sea snakes spend some or all of their life in the sea. Most are fish eaters. They may be inquisitive but are not usually aggressive unless threatened. In some parts of Malaysia, sea snakes have been found swimming quite a distance up river. This

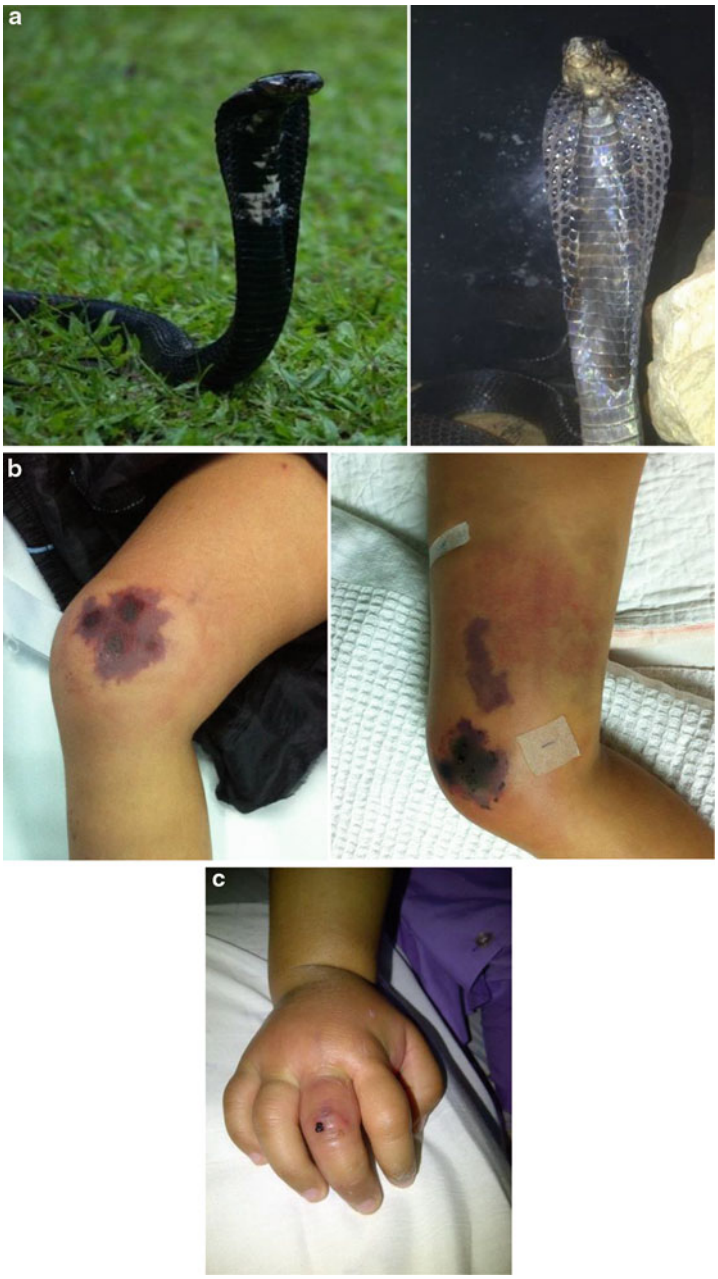

Fig. 4.3 (**a**) Equatorial spitting cobra, *Naja sumatrana*, is widely distributed in Peninsula Malaysia, Sabah, and Sarawak (Photo courtesy of Ahmad Khaldun Ismail). (**b**) Proximally spreading local necrosis following multiple bite by equatorial spitting cobra, *Naja sumatrana* (Photo courtesy of Mohd Shukruddeen Salleh). (**c**) Progressive painful swelling of the hand following a bite from a single-fanged equatorial spitting cobra, *Naja sumatrana* (Photo courtesy of Ahmad Khaldun Ismail)

presents a risk of human-snake contact beyond the boundaries of the sea or shores. From the early 1950s to late 1970s, Reid had played a significant role in sea snake research from specimens collected in and around Malaysian waters. These efforts have paved the way in the study of sea snake taxonomy, anatomy, and toxinology (Reid 1956a, b, 1961, 1975b). Sea snake bite is relatively painless, with small but distinct teeth marks or multiple scratch marks, which are mostly from non-fang teeth (Reid 1956a, 1961; Pickwell 1994). There could be an edema but otherwise not very obvious. Sea snake envenoming is relatively uncommon. The signs and symptoms of systemic envenoming are expected to appear within 6 h after a bite. Myolysis may dominate the clinical feature of a sea snake envenoming (Reid 1956a, 1961; Pickwell 1994). The destruction of striated muscles frequently manifests as myalgia, muscle stiffness, dark-colored urine, or myoglobinuria, though myoglobinuria is not an immediate sign of severe rhabdomyolysis. Acute kidney injury and severe hyperkalemia may occur secondary to major myolysis (Reid 1956a, 1961; Pickwell 1994). However, some geographic races of several species inflict predominantly postsynaptic NMJ blockade with early paralytic effects such as ptosis, ophthalmoplegia, and limb or respiratory weakness (Reid 1956a, 1961; Pickwell 1994). Coagulopathy is not seen in sea snake envenoming.

The venom of pit vipers generally affects homeostasis and often causes extensive local effects (Mebs 2002; Chippaux 2006; Mackessy 2009). Progressively worsening pain, swelling, blisters, and subsequent necrosis are common (Figs. 4.4–4.6). Vascular effects such as precipitous hypotension, bleeding, and indirect hemolysis could also happen. Life-threatening coagulopathy can cause bleeding from the bite site, gingival sulci, and venepuncture sites as well as from visceral organs. Some venom components alter capillary permeability causing extravasations of erythrocytes, electrolytes, and albumin through the vessel wall causing significant third-space loss that leads to hypovolemic shock. Extensive local swelling with bleeding tendencies is also true for other pit vipers and not exclusively for Malayan pit viper, *Calloselasma rhodostoma*. An exception to this is for the Wagler's pit viper, *Tropidolaemus wagleri*. There is a marked sexual dimorphism in *T. wagleri* as the males are much smaller and differently patterned than the females (Fig. 4.7). The primary lethal waglerin toxins are highly ontogenetically specific for the epsilon subunit of adult mice (Weinstein et al. 1991; Aiken et al. 1991; Schmidt and Weinstein 1995; McArdle et al. 1999). These potent postsynaptic peptides have no activity in neonatal rats, human, or avian. Envenoming were limited to local effects and moderate pain, and there was no documented systemic envenoming resulting in coagulopathy.

Snakebite Management Flow

Humans are not the natural prey of snakes. Some of the effects manifested in the natural prey may not have the same manifestations in humans. Therefore, a careful

Fig. 4.4 (**a**) Malayan pit viper, *Calloselasma rhodostoma*, is found in Peninsular Malaysia but not in Borneo (Photos courtesy of Taksa Vasaruchapong and Ahmad Khaldun Ismail). (**b**) Envenoming from a Malayan pit viper, *Calloselasma rhodostoma*, resulting in coagulopathy, spreading edema, local necrosis, and amputation of the finger (Photos courtesy of Kwanthai Darin Wong)

documentation of progression of a bite is crucial to a successful management regiment. A patient may first arrive at a basic primary care facility with or without a doctor at hand. This is especially true in remotely located community health facilities such as in some parts of Sabah, Sarawak, and Peninsula Malaysia. Transportation to a more appropriate medical facility may take hours or even days. Appropriate first aid is essential for all snakebites, and no time should be wasted to transport patient to an appropriate healthcare facility. An open and clear communication is required between these facilities. A standardized management flow may assist healthcare providers in providing optimal care (Fig. 4.8). In situations where expert consultation is required, the Remote Envenomation Consultation Services (RECS) is available. Remote Envenomation Consultation Services is a 24-hour voluntary "on-call" consultation service for healthcare providers in Malaysia, established in 2012 by a group of emergency physicians with special interest in clinical toxinology and members of the Malaysian Society on Toxinology (MST). The objective of RECS is to provide support to those in need, especially clinicians and medical professionals managing envenoming, but not exclusively. A favorable outcome is achieved by optimizing and advocating appropriate and safe treatment modalities to all snakebite patients.

Fig. 4.5 (**a**) Mangrove pit viper, *Cryptelytrops purpureomaculatus*, is widely distributed along the mangrove forests along the coast of Peninsula Malaysia, Sabah, and Sarawak (Photos courtesy of Ahmad Khaldun Ismail). (**b**) A bite by a mangrove pit viper, *Cryptelytrops purpureomaculatus*, resulting in coagulopathy and local necrosis with painful progressive edema proximally (Photos courtesy of Anisah Adnan)

Fig. 4.6 (**a**) Hagen's pit viper, *Parias hageni*, belonging to the green pit vipers group *Trimeresurus* complex (Photo courtesy of Ahmad Khaldun Ismail & Taksa Vasaruchapong). (**b**) Systemic coagulopathy and an extensive necrosis of the middle finger resulting in amputation following a bite by a Hagen's pit viper, *Parias hageni* (Photo courtesy of Siti Hafizah Ismail)

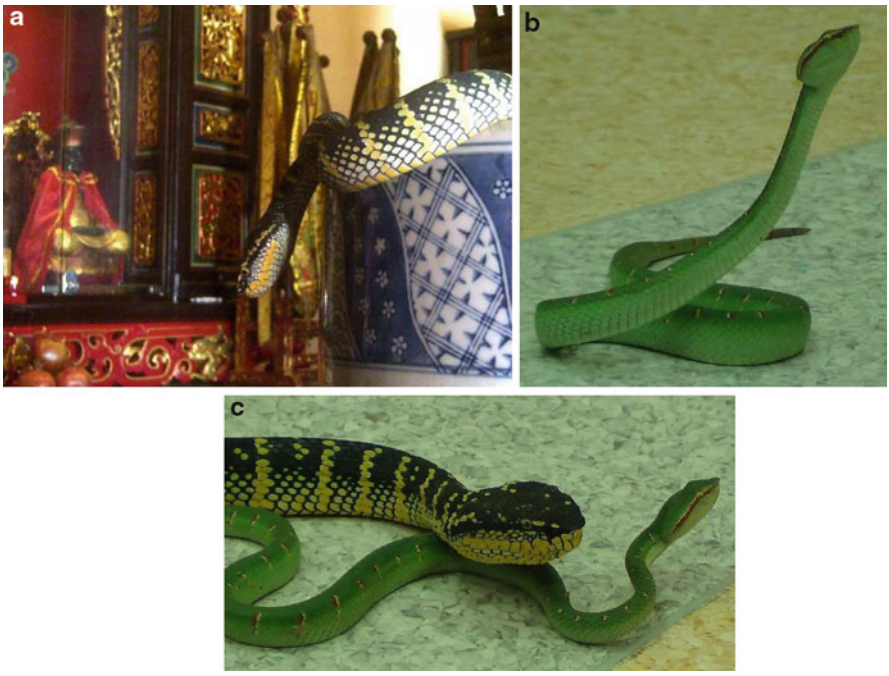

Fig. 4.7 The morphological and size differences between the adult female (**a**) and male (**b**) temple pit viper, *Tropidolaemus wagleri* (Photos courtesy of Ahmad Khaldun Ismail)

Identification

The taxonomy of the snakes in Malaysia and Southeast Asia is undergoing extensive revision and change. This is a continuous process of refining knowledge through new tools or via explorations of previously unexplored areas. This process may directly or indirectly affect the clinical management of envenoming. The capacity to identify the species of medical significance and their venom effects or symptomatology is key to optimal management of snake bite. Snakes of medical significance also include some of the non-front-fanged snakes (Das 2012; Ismail et al. 2013; World Health Organization 2010). The two snake families, Elapidae and Viperidae, are referred to as front-fanged snakes (Fig. 4.9). The Elapidae, fixed-front-fanged snakes, are considered highly dangerous. These includes the cobras, the king cobra, kraits, coral snakes, and all sea snakes. The Viperidae, retractable-front-fanged snakes, may cause significant local and systemic envenoming syndrome. These includes vipers and pit vipers. A non-confirmed number of rear-fanged Colubridae snakes occur locally, of which two or three species are able to cause significant systemic and local envenoming syndrome, while others could probably cause limited local reactions of variable severity. Pythonidae (the giant constricting snakes) include pythons, all large-growing individuals being potentially dangerous to humans and may inflict significant local injuries (Fig. 4.10).

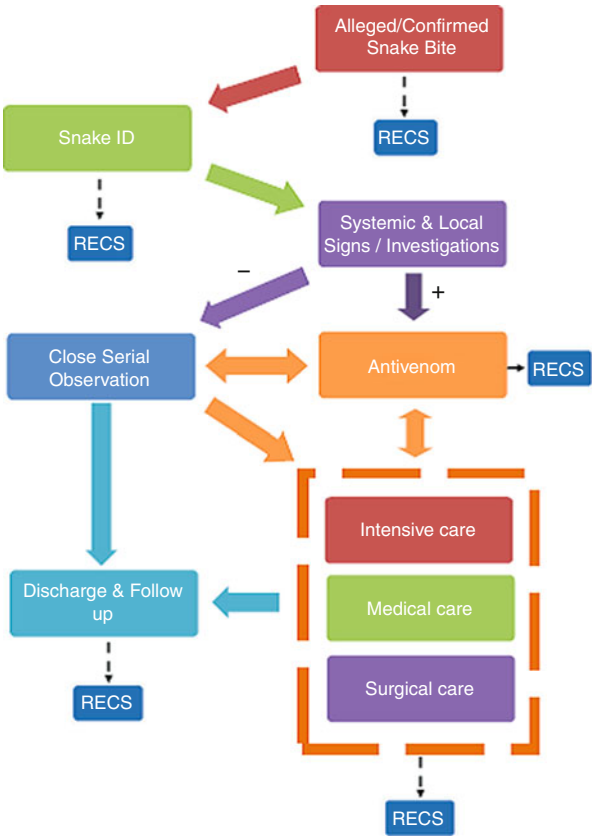

Fig. 4.8 Snakebite management flow from the Emergency Department or other primary care setting to the hospital (Copyright © Ahmad Khaldun Ismail)

One species, the reticulated python, *Python reticulatus*, may even constrict and consume adult humans.

There is no simple way of differentiating a potentially dangerous snake from a non-dangerous one. Healthcare providers are constantly faced with the challenge to identify snake specimens or the pictures brought in by patients following a bite. They may have the preconceived idea on how a "dangerous" snake should look like, based on an often limited information from various media, including resources describing nonindigenous venomous species to Malaysia. For instance, a triangular head shape is not restricted to the pit vipers in Malaysia. A raised hood-like structure is not necessarily always a cobra. Therefore, the notion that these distinctive morphological features or characteristics can be used universally is misleading. It is also important to identify and differentiate the two *Naja* and one *Ophiophagus* species indigenous to Malaysia from other parts of the world, similarly with the many pit vipers species in Malaysia. Therefore, reliable reference is invaluable in helping healthcare providers to identify snakes (Das 2010, 2012; Ismail et al. 2013; World Health Organization 2010).

Reliable identification can be used to guide the most appropriate patient management. There is a need for a more careful and qualified identification of a snake

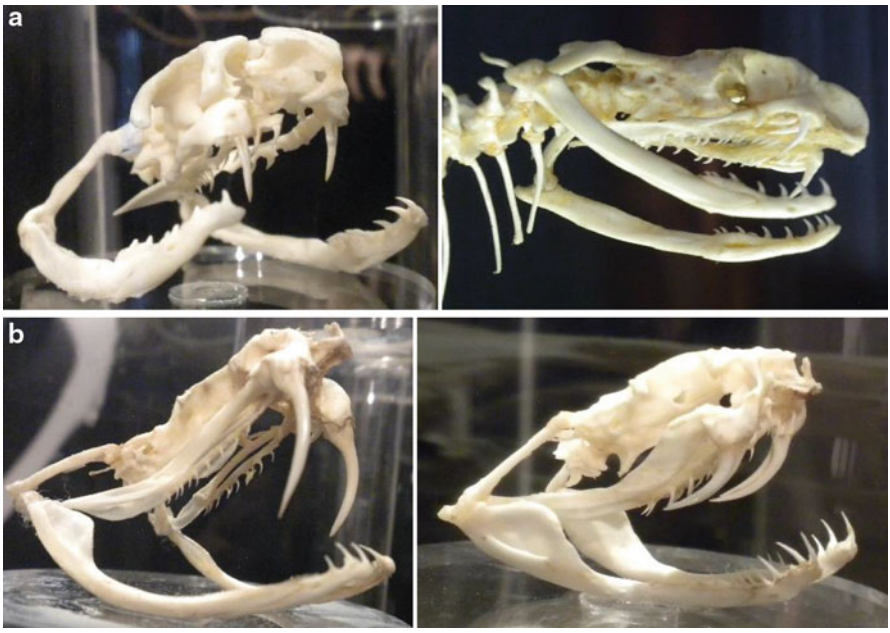

Fig. 4.9 The distinctive features of the fixed-front-fanged cobra, *Naja* species, and king cobra, *Ophiophagus Hannah* (**a**), and the retractable-front-fanged pit vipers (**b**) (Photos courtesy of Ahmad Khaldun Ismail)

involved in a snake bite incident. Determining whether a snake is venomous or not, must be correctly done through identification of the species with the help from snake systematists. In their absence, a close examination of the snake or good quality pictures, perhaps taken with a mobile phone camera and using authoritative references about the snakes of the particular geographical region, will help to identify the species. Consultation with RECS specialists will greatly minimize misidentification. There are several characteristics which can be conveyed during such consultation. The most noticeable characteristic about any snake, at first glance, will be its color (Ismail et al. 2013). This can help to identify some snakes that have distinctive coloration. However, snakes from the same species do vary in coloration, and several nonvenomous snakes are known to mimic venomous ones. Within the same species, the sexes and growth stages may display variations in coloration, pattern, and size. Another appropriate character for the identification of snake species is the scales. The shape, texture, and number of scales are often unique to each species. Knowledge of scale morphology is useful if a dead snake was found or a shed skin was brought along for identification. However, for safety reasons, scale count is not always useful or even safe, if a live snake was brought along to the medical center. The biological traits, including general habits (e.g., terrestrial, arboreal, fossorial, or aquatic), and patterns of activity (diurnal, nocturnal, or crepuscular) are useful for making a positive identification.

Fig. 4.10 (**a**) Reticulated python, *Python reticulatus*, the longest snake in the world, is widely found in Malaysia and hunted for skin trade (Photos courtesy of Ahmad Khaldun Ismail). (**b**) Significant soft tissue injury requiring extensive surgical intervention following a bite from a 4-m-long reticulated python, *Python reticulatus* (Photos courtesy of Ahmad Khaldun Ismail)

Several snakes are widespread in the country, while others have a more limited range and may be further restricted to specific altitudes. It is also helpful for identification by noting the exact locality where a snake is found. Identifying the geographical distribution of these medically significant snakes will determine the need for appropriate antivenom for that state or region (World Health Organization 2010; WHO Expert Committee 2010; Ismail 2011). The list of medically significant snakes for Malaysia can be obtained from the WHO Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins 2010 (WHO Expert Committee 2010). The most important elapids are the monocled cobra, *Naja kaouthia*; the Equatorial spitting cobra, *Naja sumatrana*; the king cobra, *Ophiophagus hannah*; Malayan krait, *Bungarus candidus*; and banded krait, *Bungarus fasciatus*. The most important pit vipers are the Malayan pit viper *Calloselasma rhodostoma*, the mangrove pit viper *Cryptelytrops purpureomaculatus*, and other arboreal "green" pit vipers of the *Trimeresurus* complex (Fig. 4.11). The word "important" here refers to the medically important species that justify the production or supply of antivenom for this region.

Currently there is no diagnostic test for snake species identification in Malaysia. Syndromic review with laboratory tests could be helpful in differentiating systemic envenomation from unidentified snake species of medical importance (Ariaratnam et al. 2009). Syndromic approach for treatment is important as even the general identification into probable elapids or viperids will help optimize management of

Fig. 4.11 Some examples of the arboreal "green" pit vipers of the *Trimeresurus* complex: (**a**) mangrove pit viper, *Cryptelytrops purpureomaculatus*; (**b**) white-lipped green pit viper, *Cryptelytrops albolabris*; (**c**) big-eyed pit viper, *Cryptelytrops macrops*; (**d**) Cameron Highlands green pit viper, *Popeia nebularis*; (**e**) Hagen's pit viper, *Parias hageni*; (f) Kinabalu Mountain pit viper, *Parias malcomi* (Photos courtesy of Taksa Vasaruchapong, Ahmad Khaldun Ismail and Steven Wong)

Table 4.1 Recommended first aid for snakebite in Malaysia (Ismail 2011)

Step	Action
1	Reassure and calm the victim who may be anxious
2	Move to safety
3	Reduce physical movements and rest
4	Immobilize the bitten area/limb or with a splint sling[a]
5	Irrigate eyes with copious amount of water if venom entered the eyes
6	Remove jewelry and loosen tight-fitting clothing
7	Urgent transport to the appropriate medical facility
8	Maintain immobilization throughout the patient's transport

[a]Note: Consider PBI if the transport time is prolonged and the snake is positively identified as krait, coral snake, sea snake, or the identity is unknown. Avoid PBI in pit viper bites

the patient. There are five distinctive syndromes that are associated with snake envenomation in Southeast Asia (World Health Organization 2010). Knowing what signs and symptoms to observe will help healthcare providers determine the appropriate treatment modalities and antivenom administration. However, the WHO guidelines note the limitations of this syndromic approach. Envenoming from even the same snake species demonstrates slight variations from the classical syndromes. Inadequacy of obtaining all the details necessary associated with the bite incident also limits the use of this methodology. Certainly, the practice of administering various types of antivenom to the same patient for the purpose of identifying the species of snake causing the syndromes is not justified and reflects poor clinical practice.

Prehospital Care Management of Snakebites

Public awareness is fundamental to the success of snakebite management. A management protocol should also be tailored to the healthcare provider's perspective (World Health Organization 2010; WHO Expert Committee 2010; Ismail 2011). The recommendations for treatment can be made simpler by rearranging the must-do first aid procedure in a stepwise manner (Table 4.1). Washing snakebite wound is not encouraged in Australia because of the availability of a venom detection kit. Snake species is detected from swabbing the venom around the wound or from the bandage wrapped over and in contact with the wound. However, similar detection kit is not available for identifying Malaysian snake species of medical significance. Therefore, generous irrigation of the bite site with clean water while avoiding vigorous scrubbing or massaging and covering with a clean piece of cloth or gauze is deemed appropriate. The next step in slowing down the spread of venom is immobilization. Simple immobilization of a joint above and below the bite site on a limb or an arm sling is sufficient. The pressure bandaging and immobilization (PBI) technique is recommended following envenomation by snakes with neurotoxic venom (Fig. 4.12). This technique was developed in

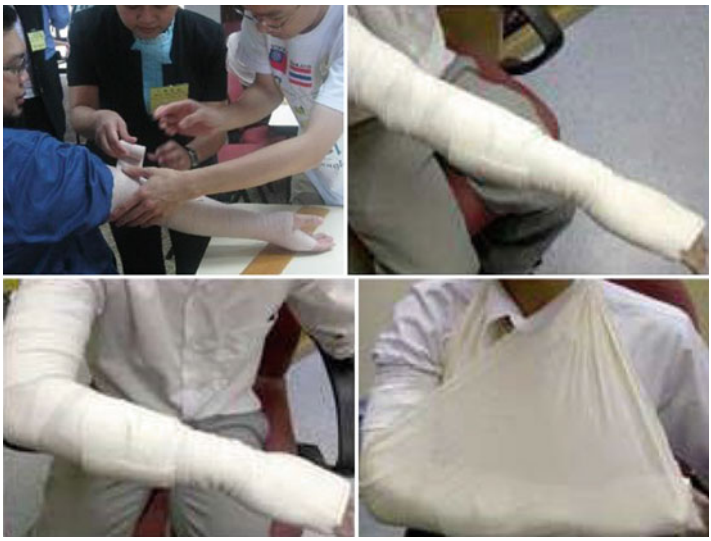

Fig. 4.12 The pressure bandaging and immobilization (*PBI*) technique of the upper limb using an elasticized bandage to achieve sufficient and sustained pressure on the bitten limb (Photos courtesy of Ahmad Khaldun Ismail)

Australia and may provide further benefit in slowing the spread of venom. It is not advised for bites from snakes which could potentially cause local necrosis, which in the Malaysian context are bites from the pit vipers and cobras (Seifert et al. 2011; American College of Medical Toxicology et al. 2011; Norris et al. 2005; Simpson et al. 2008; Weinstein et al. 2009). Pressure bandaging and immobilization is only considered in situations where the transport time to the nearest appropriate medical care is expected to be prolonged or the snake responsible is positively identified as a krait, coral snake, sea snake, or unknown identity. Several studies have shown the importance of training first aiders in performing PBI in order to achieve the desired pressure level using the appropriate elasticized bandage (Seifert et al. 2011; American College of Medical Toxicology et al. 2011; Norris et al. 2005; Simpson et al. 2008; Weinstein et al. 2009).

One should avoid any unnecessary interference of the wound. Harmful practices and disproved methods of first aids must be discouraged. These includes the use of tourniquet, cut and suck, various types of suction devices, electric shocks, herbal rubs, "magic" snake stones, drinking special herbal brew, mutilating skin nicks, vigorous massages, and various others. The main objective of first aid is to keep the patient calm and prevent further harm. This includes reducing unnecessary movements while preparing for transport to the appropriate medical facility. If a snakebite patient arrived to a medical facility with a bandage, tourniquet, or suction device over the bitten area, it is advisable to remove it only when the patient is in the resuscitation bay with the vital signs, cardiac rhythm monitored, and the "crash" trolley and equipment prepared. If the snake is positively identified, preferably the

infusion of the first dose of antivenom is on the ready to be administered prior to releasing the restrictive bands or equipment.

Definitive Management of Snakebite

Healthcare providers who encounter and manage more snakebite cases may have more experience and confidence in managing these cases. However, this may not reflect their knowledge on current and correct practice of managing snake bite envenoming relevant to the country of practice. Admission, referral, or disposition of patients should be decided by a physician or clinical toxinologist who are familiar and experienced with snakebite and envenomation management appropriate for Malaysia. Confirmation of a nonvenomous snake must be determined through a trustworthy identification by a qualified person. Any consultation for clinical management and identification assistance can be obtained from RECS Malaysia or an emergency physician familiar with managing snakebite.

Snakebite patient who arrives at a medical centre must be reviewed in the critical area. There is a specific list of information to be obtained (World Health Organization 2010; Ismail 2011; Weinstein et al. 2009). It is important to find out about the anatomical area or areas that was or were bitten, the time of incident, the activity at the time of the incident, the geolocation or address of the incident, the identity or description of the snake, intervention done after the bite, eyewitness to the incident, and any signs and symptoms felt by the patient since the incident. History of previous contact with snakes (nonvenomous and venomous), previous bite and envenoming incident, and history of allergy and comorbidities should also be obtained. Similar to history taking, there is a standard set of actions for physical examination (World Health Organization 2010; Ismail 2011; Weinstein et al. 2009). It is generally divided into (i) general examination, (ii) wound examination, and (iii) examination for specific signs of envenoming. Therefore, all unidentified snakebite patients, especially those without symptoms, must be admitted for serial monitoring and observation for at least 24 h (World Health Organization 2010; Ismail 2011; Weinstein et al. 2009). Some emergency department provides an observation ward for this purpose.

Grading the severity of envenomation is a dynamic process. The snakebite severity score (SSS) for rattlesnake and copperhead bites used in the United States (USA) works reasonably well in combination with overall clinical impressions derived from all available information used in order to assess the clinical progression or lack thereof from an envenoming by a pit viper. The SSS provides a navigable reference point for the patient's status, but obviously cannot be used without qualification. The SSS may have a definite merit for American crotaline envenoming but requires further research on its clinical applicability for Southeast Asian pit vipers envenoming. Perhaps with some adjustments to the local population of snake species, it may be possible to adapt the SSS for elapids and viperids envenoming in Malaysia.

Table 4.2 Recommended serial bedside and laboratory investigations following a snakebite (Ismail 2011)

A. 20-min whole-blood clotting test (20WBCT)			
1.	Place 2 ml of freshly sampled venous blood in a small, new or heat cleaned, dry **glass** vessel/tube		
2.	Leave undisturbed for 20 min at ambient temperature, then tip the vessel once		
Note	It is a quick bedside test (but with a questionable sensitivity) for unidentified bite or when a pit viper bite is suspected. If initial test was negative (fully clotted), repeat test every 30 min for the first 3 h, then hourly as necessary. If the blood remain liquid (unclotted), this is suggestive of a positive test for coagulopathy or defibrination syndrome secondary to systemic envenomation from a pit viper bite		
B. Other laboratory investigations			
1.	Coagulation profile[a]	4.	Creatine kinase (CPK)
2.	Full blood count & picture	5.	Urinalysis (myoglobinuria)
3.	Renal profile & electrolyte	6.	Liver function
Note	[a]Include **d-dimer** and **directly measured fibrinogen** level. Repeat serially		

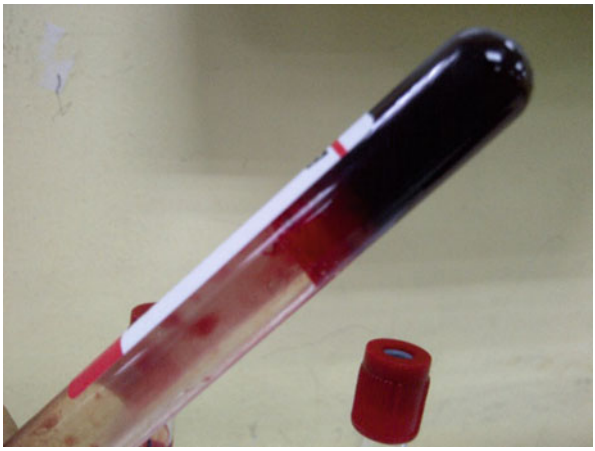

Fig. 4.13 The 20-min whole-blood clotting test (20WBCT) in a clean glass tube showing a negative result (Photo courtesy of Ahmad Khaldun Ismail)

Important investigations to be considered for snakebites should be done serially at regular intervals (Table 4.2). The 20-min whole-blood clotting test (20WBCT) has limited utility and should be used only if there are no reasonably rapid lab facilities available (World Health Organization 2010; Ismail 2011; Weinstein et al. 2009). It is used in unidentified snakebites or suspected pit viper bites. The use of glass tube or container is critical, as it is the micro-imperfections in the glass that allow tissue factor activation of the cascade to occur (Fig. 4.13). One negative test does not exclude a pit viper bite with systemic envenomation. Therefore, it must be performed serially (Weinstein et al. 1991). It only confirms systemic coagulopathy from envenomation by a pit viper, but does not identify the species of the pit viper. It can also be used to review the response to antivenom therapy from a pit viper envenoming. Laboratory investigations and surveillance of red cell

morphology, e.g., identification of schistocytes, spherocytes, etc., may help identify the rare case of possible microangiopathic hemolytic anemia (MAHA) (World Health Organization 2010; Simpson et al. 2008; Weinstein et al. 2009). This is more commonly observed in Australian elapid envenomation, but it can occur in other snakebite victims.

Antivenom

Antivenom is the cornerstone for managing envenomation. There are specific systemic and local indications and strict protocol for antivenom administration (World Health Organization 2010; WHO Expert Committee 2010; Weinstein et al. 2009; Gold et al. 2002). Antivenom administration should be based on the clinical and laboratory evidence and the severity of systemic and local envenomation. The choice of antivenom will depend on the snake identity. If the snake species is positively identified, the monovalent or monospecific antivenom is preferable, and if the snake species is not identified, the polyvalent antivenom is recommended. A positive 20WBCT, or other laboratory blood tests, reflects the presence of coagulopathy suggestive of systemic envenoming from a pit viper and indicates the administration of appropriate antivenom. Determining significant local envenoming can be subjective and open to individual speculation. This reflects the importance for a close and thorough examination and observation of patients. Perhaps a consultation with experts in clinical toxinology would be preferable.

Antivenom is administered intravenously (IV). Intramuscular (IM) injection of antivenom will cause pain, delayed absorption into the circulation, and bleeding if the patient is envenomed by a pit viper. Intramuscular route may have its use in very limited circumstances (World Health Organization 2010). However, with the improvement of prehospital care in Malaysia and widely available intraosseous cannulation devises, the need for IM injection of antivenom is not appropriate.

The dose of antivenom for envenomed patients in both children and adult is the same because snakes inject the same amount of venom into one's body despite age difference (World Health Organization 2010; WHO Expert Committee 2010; Weinstein et al. 2009; Gold et al. 2002). In practice, the choice of an initial dose of antivenom is based on the severity of clinical presentation. It is a reflection of the amount of venom injected by the snake. The first dose and the safety levels of antivenom however are largely based on manufacturer's recommendation. However, this is probably not a proportional relationship in some patients with significant preexisting medical comorbidities, malnutrition, significant parasite burdens, or substance dependencies. It needs to be emphatically stressed that the manufacturer's suggestions for initial volumes must be balanced with the clinical presentation. For example, one would consider administering a higher starting dose for any significant *Naja* species bite as there is a narrow therapeutic window for any efficacy that might be seen as systemic paralytic effects and possibly on the local effect progression. The time interval prior to administering more antivenom for

unresolved signs of envenomation is well explained in the WHO guideline. These criteria are important to ensure that patients receive the appropriate antivenom in a timely manner and may prevent delay in antivenom administration or wastage.

With advancement of technology, better manufacturing techniques, and stringent quality control of antivenom production, the risk of anaphylaxis from antivenom has markedly reduced (WHO Expert Committee 2010; Weinstein et al. 2009; Gold et al. 2002). Figuring the risk and benefit of antivenom administration is subjective. Few safety issues, however, need to be considered; (1) the need for anaphylaxis protocol in place prior to the provision of antivenom and (2) in any facility that provides it, ventilation support should be made available in the setting of paroxysmal respiratory paresis following possible scenarios of delayed or unavailability of appropriate antivenom, delayed presentation of envenoming, and ineffective antivenom therapy. Antivenom reactions are variable and depending on the origin and region. Since the antivenom from QSMI is prepared from horse serum, sensitization to hetero-logous protein may occur in some individuals. Skin test is no longer to be performed prior to the administration of antivenom (World Health Organization 2010; WHO Expert Committee 2010; Weinstein et al. 2009; Gold et al. 2002). Not only does it poorly predict anaphylactic reactions, it may also sensitize a patient to antivenom and delay antivenom administration.

There is no contraindication for antivenom unless the patient is known to be hypersensitive to the constituents of the product. The indications for pre-treatment are for those with previous reaction to antivenom and to horse serum. In this situation, pre-treatment with combinations of steroids or antihistamines may be considered. Early hypersensitivity to antivenom is mostly a rate-dependent anaphylactoid reaction. Occasionally, transient tenderness at the injection site, cutaneous reaction, and alterations in temperature may occur. In some cases this reaction is limited to nausea, vomiting, and circulatory reactions such as tachycardia, bradycardia, hypotension, sweating, and vertigo. Allergic reactions such as urticaria and dyspnea have also been observed extending in isolated cases to anaphylactic shock. In this situation, where antivenom is potentially lifesaving but the patient developed anaphylaxis, the antivenom should be withheld until the reaction is treated and resolved. Following that, antivenom infusion should be resumed at a slower rate with close vigilance for further reactions. If the adverse reaction was just a rash, without hypotension or bronchospasm, one may just reduce the rate of infusion and treat the reaction. Depending on the nature and severity of side effects, therapeutic measures such as antihistamines, adrenaline, corticosteroids, volume replacement, and oxygen should be considered. The patient should be closely monitored for an extended period of time. Serum sickness is a type III immune complex disease, which can develop from 4 to 5 days after the completion of therapy, especially following a high dose and repeated antivenom administration (World Health Organization 2010; WHO Expert Committee 2010; Weinstein et al. 2009; Gold et al. 2002). Oral prednisolone is almost always needed to treat such cases. The non-tapered regimen should preferably be for at least 5 days.

There are clear signs that will be observed when the amount of antivenom given is adequate. There is always a danger of discontinuing the antivenom too early

because of poor assessment of what is considered satisfactory clinical improvement. Most of the signs for reversal or satisfactory improvement are observed after the completion of the initial dose of antivenom infusion. There is also the possibility of delayed venom effects which can occur even after the completion of antivenom and disappearance of clinical signs and symptoms. The half-life of antivenom in the body, the amount of venom injected into the tissue, and the variable sizes of toxin molecules may reflect on this phenomenon.

The term "polyvalent" has been misunderstood by many healthcare providers and pharmacists in Malaysia. It is thought to be a universal therapy for treating envenoming from all venomous snakes despite their species or geographical variations. Poor understanding of this topic may have contributed to the purchasing and administration of inappropriate antivenom. An antivenom is selected only if its stated range of specificity and paraspecific neutralization capacity includes the species known or highly suspected to have been responsible for that locality. Not all manufacturers produce antivenom appropriate for Malaysian venomous snakes. Antivenom specifically raised against Southeast Asian snakes is made from species indigenous to Southeast Asia. Since Malaysia does not produce its own antivenom, they have to be imported from countries such as Thailand and Australia (Table 4.3). Queen Saovabha Memorial Institute (QSMI), Thailand, provides a selection of seven monovalent antivenoms, a neuro-polyvalent antivenom, and a hemato-polyvalent antivenom. There are also a preliminary evidence to show that the new polyvalent antivenoms from QSMI have a higher neutralization power and more effective than the monovalent antivenom in treating envenoming from certain species (Leong et al. 2012). Commonwealth Serum Laboratories (CSL) Ltd. Australia provides sea snake antivenom.

Envenoming from the *Bungarus* species (Fig. 4.14) in Malaysia may not be as frequent as from the *Naja* species, but it is usually anything but trivial. Dangerous statement like this requires careful and appropriate advice by the experts in the field. There are monovalent antivenoms available from QSMI for Malayan krait, *Bungarus candidus*, and Banded krait, *B. fasciatus*. The neuro-polyvalent from QSMI also covers these two species of kraits with good cross neutrality for venoms of the red-headed krait, *Bungarus flaviceps* (Gold et al. 2002). Envenoming from Coral snakes, *Calliophis* species (Fig. 4.15), could be lethal. Currently, there is no antivenom for *Calliophis* species envenoming. According to the Department of Health, Centre of Disease Control in Taipei, the venom toxins of *Calliophis bivirgatus* have a LD_{50} approx 0.8 μg/g and may be similar to *B. multicinctus* venom. There is a possibility that the *C. bivirgatus* venom could be neutralized by the bivalent *Naja atra-Bungarus multicinctus* antivenom produced in Taiwan. This, however, requires further analysis.

The monovalent antivenom for the white-lipped green pit viper, *Cryptelytrops albolabris*, from QSMI shows good cross neutrality with other *Trimeresurus* complex species, except for the Wagler's pit viper, *Tropidolaemus wagleri*. The hemato-polyvalent from Thailand is raised from the venom of the green pit viper, *Cryptelytrops albolabris*; the Malayan pit viper, *Calloselasma rhodostoma*; and the Thai Russell's viper, *Daboia siamensis*. This hemato-polyvalent is also found to be

Table 4.3 Recommended selection of antivenom appropriate for use in Malaysia (Ismail 2011)

	Species raised from and manufacturer	Coverage area	First dose/ vial
1.	Monocle cobra, *Naja kaouthia*	Peninsular Malaysia, Sabah, & Sarawak	100 ml/10 vials
	QSMI Thai Red Cross: cobra antivenin to neutralize 0.6 mg/ml of venom		Subsequent dose 1–2 h
2.	King Cobra, *Ophiophagus hannah*	Peninsular Malaysia, Sabah, & Sarawak	100 ml/10 vials
	QSMI Thai Red Cross: king cobra antivenin to neutralize 0.8 mg/ml of venom		Subsequent dose 1–2 h
3.	Malayan krait, *Bungarus candidus*	Peninsular Malaysia	50 ml/5 vials
	QSMI Thai Red Cross: Malayan krait antivenin to neutralize 0.4 mg/ml of venom		Subsequent dose 1–2 h
4.	Banded krait, *Bungarus fasciatus*	Peninsular Malaysia, Sabah, & Sarawak	50 ml/5 vials
	QSMI Thai Red Cross: banded krait antivenin to neutralize 0.6 mg/ml of venom		Subsequent dose 1–2 h
5.	Malayan pit viper, *Calloselasma rhodostoma*	Peninsular Malaysia	40 ml/4 vials
	QSMI Thai Red Cross: Malayan pit viper antivenin to neutralize 1.6 mg/ml of venom		Subsequent dose 6 h
6.	Green pit viper, *Cryptelytrops albolabris*	Peninsular Malaysia, Sabah, & Sarawak	30 ml/3 vials
	QSMI Thai Red Cross: green pit viper antivenin to neutralize 0.7 mg/ml of venom		Subsequent dose 6 h
7.	Malayan pit viper, *Calloselasma rhodostoma*; green pit viper, *Cryptelytrops albolabris*; SEA Russell's viper, *Daboia siamensis*	Peninsular Malaysia	30 ml/3 vials
	QSMI Thai Red Cross: hemato-polyvalent snake antivenom		Subsequent dose 6 h
8.	Monocled cobra, *Naja kaouthia*; king cobra, *Ophiophagus hannah*; banded krait, *Bungarus fasciatus*; Malayan krait, *Bungarus candidus*	Peninsular Malaysia, Sabah, & Sarawak	50–100 ml/5–10 vials
	QSMI Thai Red Cross: neuro-polyvalent snake antivenom		Subsequent dose 1–2 h
9.	Sea snakes, *Hydrophiinae*	Peninsular Malaysia, Sabah, & Sarawak	10–30 ml/1–3 vials
	CSL, Australia: polyvalent sea snake antivenom		Subsequent dose 1–2 h

Note: Subsequent repeat doses are according to the clinical signs and symptoms. Antivenom for monocled cobra, *Naja kaouthia*, has paraspecific properties and provides good cross neutralization to the venom of equatorial spitting cobra, *Naja sumatrana*. Malayan pit viper, *Calloselasma rhodostoma*; Malayan krait, *Bungarus candidus*; and monocled cobra, *Naja kaouthia* are not indigenous to Borneo. Southeast Asian Russell's viper, *Daboia siamensis*, is not indigenous to Malaysia

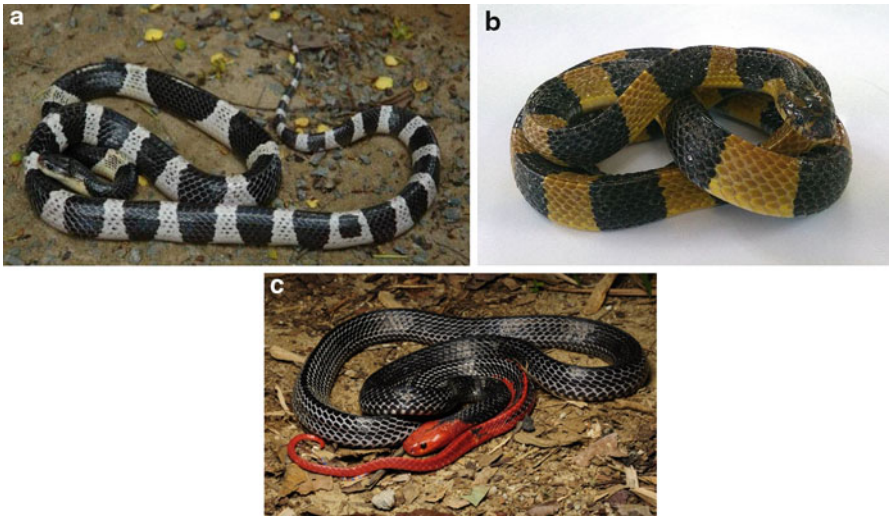

Fig. 4.14 Kraits, *Bungarus*, species indigenous to Malaysia: (**a**) Malayan krait, *Bungarus candidus*; (**b**) banded krait, *Bungarus fasciatus* (Photos courtesy of Taksa Vasaruchapong); (**c**) red-headed krait, *Bungarus flaviceps* (Photos courtesy of Taksa Vasaruchapong)

Fig. 4.15 Coral snakes of Malaysia. An attempt to extract venom from a banded coral snake, *Calliophis intestinalis lineata* (**a**). The Malayan blue coral snake, *Calliophis bivirgata flaviceps* (**b**) (Photos courtesy of Ahmad Khaldun Ismail)

effective against a wide range of pit viper venom in Malaysia. To date, the red-necked keelback, *Rhabdophis subminiatus* (Fig. 4.16), is the only colubrid in Malaysia known for its ability to inflict bites that may cause severe systemic coagulopathy (Iddon and Theakston 1986). There are no recorded fatality from *R. subminiatus* bites in Malaysia. However, a bite may be life threatening and should be treated with anti-yamakagashi antivenom available from Japan, when indicated.

Fig. 4.16 A bite from the red-necked keelback, *Rhabdophis subminiatus*, a non-front-fanged colubroid, may inflict significant coagulopathy (Photos courtesy of Taksa Vasaruchapong)

Fig. 4.17 Clinical presentation of ptosis due to post-synaptic neurotoxic envenoming and dark colored urine (myoglobinuria) due to rhabdomyolysis following Beaked sea snake, E. *schistosus* envenoming (Photo courtesy of Heng Yik Shan, Vinod Kumar Gunushakran, Tjen Jhung and Zamri Mahfudz)

Sea snake envenomation requires appropriate first aid and timely arrival to a medical facility. Similar to other snake bites, antivenom is indicated if there is clear evidence of envenoming (Fig. 4.17). Timely administration of antivenom is crucial, but this may be a difficult task to achieve as fulminant rhabdomyolysis and acute kidney injury may have already developed secondary to the delay in seeking medical attention while at sea. Delays in antivenom therapy have resulted in deaths especially among Malaysian fishermen. It appears that not all medical facilities located in coastal areas hold sea snake antivenom. The CSL SSAV will only be dispatched from the main referral hospital to other medical facilities when

requested. This obviously contributes to prolonging the "envenoming to antivenom time" with poor patient outcome. Sea snake antivenom is relatively expensive. Even though it is raised from one species of sea snake, it is used to neutralize systemic envenoming by a wide range of sea snake species. The starting dose for CSL SSAV is one vial, but significant envenoming usually requires three vials and rarely more than 10 vials. In the past, some Malaysian healthcare professionals had considered CSL tiger snake antivenom to be a cheaper and better alternative to SSAV (Pickwell 1994; Reid 1975a). It is very important to point out that in the past there was a clear reason why tiger snake antivenom might also work against sea snake venom. The SSAV was raised from the venom of the beaked sea snake, *Enhydrina schistosus*. *Enhydrina schistosus* venom was also used in the immunizing mixture for the production of CSL tiger snake, *Notechis scutatus*, antivenom and CSL polyvalent antivenom for Australian and Papua. The plasma from the same host was also used in the production of CSL tiger snake antivenom and CSL polyvalent antivenom. Due to the paraspecific protection from a neutralizing titer of anti-*E. schistosus* antibody in the anti-*Notechis* monovalent, as well as polyvalent, a higher volume of antivenom was normally required if these were to be used as alternatives to the SSAV. The recommended choice of antivenom for sea snake envenoming was first the CSL SSAV, followed by the CSL tiger snake antivenom, and lastly the CSL polyvalent antivenom. However, in recent years, manufacturing processes have changed. The production of sea snake antivenom is now a separate process. *Enhydrina schistosus* venom has not been included in the manufacturing of the tiger snake antivenom, and the immunized horses have been separated, rotated, as well as in some instances replaced. Therefore, the Australian CSL tiger snake antivenom and the CSL polyvalent antivenom are no longer reliable alternative to SSAV and should not be considered for this purpose.

The frequency and severity of snake envenoming determines the selection and stocking of antivenom for a particular healthcare centre. The amount and type of antivenom to be ordered and stocked should be based on the requirement or burden of snake bite for the individual healthcare centre or medical facility. It is unwise to practice blanket ruling for stocking antivenom without looking at the statistics of snakebite incidence and species of snake involved. This is to avoid irresponsible purchasing and stocking of inappropriate antivenom of inappropriate origin or manufacturer.

The antivenom raised from venomous snakes of Indian origin is not suitable for use in Southeast Asia (Warrell 2008). Indian antivenom manufacturers have been utilizing the venoms from snake species, which are geographically and antigenically dissimilar to the Malaysian species. Therefore, Indian polyvalent antivenoms should not be marketed in Malaysia (Warrell 2008; World Health Organization 2010; WHO Expert Committee 2010). Unfortunately, these antivenoms were found to be unscrupulously marketed to those responsible for purchasing antivenom. It is disturbing that government regulation had allowed the purchase of Indian antivenom serum by licensed importers. Several factors may have contributed to this confusion. First, it is possibly due to the widespread belief or misunderstanding among healthcare providers and pharmacist regarding the term "polyvalent"

antivenom. Second, it is possibly due to the labeling of antivenom with ambiguous genus or name such as "cobra" or "krait" that fails to distinguish the various Asian species which venoms are used in their production. Antivenom bearing general names that seem relevant to national needs may prove ineffective in clinical use (Warrell 2008; WHO Expert Committee 2010; Weinstein et al. 2009). This is a major concern. Therefore, importation of inappropriate antivenom not fulfilling the species' specificity of a country should be prevented. A more stringent government regulation should be put in place to prevent importation of inappropriate antivenom for use in any region. This reflects the need for the comprehensive snake antivenom protocol to be adhered to by all parties including pharmacists and licensed importers. The situation is slowly improving in Malaysia as awareness among healthcare providers improves and appropriate antivenoms are being stocked in major government hospitals. At times, limited resources can also be mobilized relatively quickly and shared via close communication between treating physicians, pharmacist, and RECS consultants. Close ties have also been forged between neighboring Singapore and Thailand where access to exotic antivenom is possible.

Intensive and Supportive Care

Not all snakebite patients require admission to the intensive care unit (ICU). If a patient has received sufficient appropriate antivenom and the envenomation is reversing, ICU admission can be averted. A high dependency unit (HDU), general ward, or a short stay ward would be sufficient for close serial monitoring of a non-intubated and ventilated patient. Envenomation from a pit viper may require a longer period of observation because of venom depot effects causing prolonged or relapsing coagulopathy and bleeding.

Anticholinesterase test is may be useful, especially in competitively reversing signs and symptoms of postsynaptic blockade from cobra envenoming (Seifert et al. 2011; Lam et al. 2011; Tanen et al. 2004). Kraits and sea snakes envenoming may not respond to a similar extent. It can be used as a test to confirm neurological envenomation or treatment regime when the appropriate antivenom is not available. As mentioned earlier, Malaysian hospitals have access to Malayan krait antivenom, in the form of both monovalent and polyvalent. There are two anticholinesterase drugs that can be utilized. Edrophonium chloride and neostigmine methylsulfate can be utilized separately for testing neuromuscular blockade. Both can be delivered either intramuscularly or intravenously. Flexibility in the method of administering these reversal agents is encouraged. Edrophonium chloride may not be widely available in all hospitals in Malaysia. Neostigmine methylsulfate, however, is more widely available as a reversal agent for a muscle-relaxing drug used in general anesthesia and for the diagnosis of myasthenia gravis. This can be an alternative drug to edrophonium, although its clinical usage may be limited in children.

Prophylactic antibiotics are no longer an accepted practice in snakebite management. Antibiotics are only administered in selected cases where there are higher

chances of necrosis and infection, such as bites from the Malayan pit viper or the Equatorial spitting cobra, or if there has been non-sterile interference with the bite site (World Health Organization 2010). Interference with the wound (incisions with an unsterilized razor blade or knife) and suction of the wound only create a higher risk of secondary bacterial infection and should be avoided. These circumstances justify the use of immediate prophylactic broad-spectrum antibiotics. The oral cavity of snakes may harbor a wide range of gram-positive, gram-negative, and anaerobic bacteria (Weinstein et al. 2009; Gold et al. 2002; Lam et al. 2011). Anaerobic and aerobic cultures (blood and wound site) should be obtained for culture and sensitivities prior to administering the appropriate antibiotics. When indicated by the culture results, the antibiotics of choice should then be provided. Locally uncomplicated snakebites do not require prophylactic antibiotics. Tetanus prophylaxis follows the routine wound management guide. It is considered only for significant bites or puncture marks with no history of completion of primary tetanus diphtheria immunization series or if the most recent immunization is more than 5 years. Extra caution must be taken when administering the IM injection. It is advisable to withhold the IM tetanus toxoid if the causative snake was identified as a pit viper and the blood coagulation test was normal. One may consider administering a drug via the IM route once the trend of the serial coagulation test is satisfactorily within normal range over an extended period of time.

In the clinical setting of severe coagulopathy and the appropriate antivenom is not available, transfusions of fresh frozen plasma (FFP) or cryoprecipitate may be considered. Transfusing FFP and platelets must not be a routine practice for correcting coagulopathy from pit viper envenoming. Providing FFP or cryoprecipitate in this instance will not correct the coagulopathy as the causative venom is still present in the circulation and tissue. In most pit viper venom-induced thrombocytopenia cases, it takes about 3–7 days for the coagulation profile to return to pre-envenoming levels. However, some cases can be so severe that they may require platelet transfusion following optimal antivenom administration (World Health Organization 2010; Gold et al. 2002). This is not commonly the case, and the possible use of FFP and platelet transfusion in snake bite envenoming is strictly on a case-by-case basis and only when decided clinically necessary by a qualified physician.

Dialysis may be required, especially in severe cases of renal failure due to rhabdomyolysis following a sea snake envenoming. Hemodialysis is probably superior to peritoneal dialysis (Reid 1975a; World Health Organization 2010).

Surgical Consideration

Physicians unfamiliar with the marked myositis that can occur after bites from a few pit viper and cobra species are quick to perform fasciotomy. This most commonly results in long-term disability well beyond that might have occurred from the envenoming alone. There are likely that a few cases may eventually develop a measurable and clinically supported elevated compartment pressure;

however, the majority of these can be managed medically (Tanen et al. 2004; Dart et al. 2001; Lavonas et al. 2004; Corneille et al. 2006; Sotelo 2008; Warrell 2010). The few that require surgical intervention should be demonstrably causing neuromuscular compromise on clinical assessment and by obtaining intracompartmental pressure measurements. It is reasonable and clinically responsible to consider compartment syndrome from distal pulses, but this may be interpreted by some with surgical tendencies to inappropriately or prematurely perform a fasciotomy when severe edema is observed. For a majority of cases, this is unnecessary and is considered a malpractice as those unfamiliar with the local effects of many snake bites mistake myositis and the interstitial edema as compartment syndrome (Gold et al. 2002). In the rare cases of occurrence, compartment syndrome must be confirmed (World Health Organization 2010; Weinstein et al. 2009; Gold et al. 2002; Warrell 2010). Surgical intervention with fasciotomy for venomous snakebite is currently reserved for rare cases of severe envenoming resulting in severe local circulatory and compromise. Fasciotomy should never be performed without directly measuring the compartment pressure with Stryker or Wick catheter measurements and should be considered if it is beyond the consensus level of 35–40 mmHg (World Health Organization 2010; Weinstein et al. 2009; Tanen et al. 2004; Warrell 2010). It should not be performed prophylactically because the local effects of venom may closely resemble sign and symptoms of true compartment syndrome. Various studies have shown that this venom effect resolves with sufficient ASV administration alone (Tanen et al. 2004; Dart et al. 2001; Lavonas et al. 2004; Corneille et al. 2006; Sotelo 2008; Warrell 2010). There is very limited evidence of the efficacy of fasciotomy in managing envenomation. Therefore, this procedure should not be indiscriminately employed. There is no evidence that indicates any decrease in local effects from surgical intervention of any kind (Tanen et al. 2004; Dart et al. 2001; Lavonas et al. 2004; Corneille et al. 2006; Sotelo 2008). The notion that fasciotomy will allow the venom to be diluted by wound irrigation and when serous fluid flow away from the wound is unfounded and may even worsen the situation.

Once the primary concern with coagulopathy has been resolved, the next issue to be reviewed is local tissue damage. Tissue destruction can be extensive as seen in pit viper, king cobra, and cobra envenoming in Malaysia especially if the appropriate antivenom is delayed (World Health Organization 2010; Weinstein et al. 2009; Tanen et al. 2004; Warrell 2010). The size of local necrosis may still be increasing even without the presence of significant systemic envenoming. It is not certain if ecchymosis is due to local coagulopathy effect or destructive effect of the venom on the capillaries or tissues. Manipulation of the affected limb and tissue may or may not cause a "surge" of residual venom from the tissue into the systemic circulation. Debridement of necrotic tissues should be carefully performed as required by an experienced surgeon. Sometimes ecchymosis may be confused with skin necrosis. Routine debridement of snakebite wounds is a case-by-case determination. The universal approach to surgical debridement and for wounds to be left open for all animal and human bites needs to be reviewed. There is a markedly variable case-by-case response to the tissue compromising effects of

cytotoxic-necrotic venom-induced pathology. The approach to the complication of local envenoming is determined by the existence of comorbidities affecting the peripheral microcirculation such as in diabetes mellitus and the efficacy of management plan for those preexisting conditions such as good glucose control. Thus, the time to intervene with surgical debridement and its extent will be determined by the condition and integrity of the affected tissue, maintenance and facilitation of good perfusion, and associated comorbidities (World Health Organization 2010; Weinstein et al. 2009; Warrell 2010). It is likely that early debridement of affected tissues that exhibit early signs of necrotic change may result in better prognosis. This may partly be due to the removal of a medium hospitable for bacterial multiplication and infection. Therefore, clinically significant envenoming from species likely to produce necrotic change and envenoming that show early changes suggestive of such should be considered for early prophylactic broad-spectrum antibiotic coverage, following the procurement of culture specimens.

Observation, Follow-Up, and Rehabilitation

Acknowledging the limitations in conclusively identifying snake species, it is advisable that all unidentified bites or alleged bites without local reaction or significant effects are to be closely observed for 24 h in a well-equipped medical facility (World Health Organization 2010; Ismail 2011; Warrell 2010). Those who are bitten by a snake of medical significance, without symptoms of local or systemic envenomation, must be admitted to the hospital and observed for as long as necessary with serial bedside or laboratory tests. Vital signs, cardiac rhythm, pain score, edema progression, bleeding tendencies, and any paralytic features such as ptosis, weakness, and dyspnea need to be closely and serially monitored. In the event a patient remains asymptomatic following a significant period of observation, he or she may be discharged with the advice to return to the hospital for any abnormal manifestation. Direct communication with the treating doctor who is familiar with the case is advisable. Early liaison with other physician experienced in clinical toxinology and managing envenomation is recommended (Ismail 2011; Weinstein et al. 2009; Warrell 2010). Such action will expedite a safe and appropriate management of envenomed patients. Severe snakebite injury, especially from pit vipers and some cobras, may require a long and meticulous follow-up, with no promise of full return of function. Occupational therapy and rehabilitation with social and financial support may be required.

Conclusion and Future Direction

Public awareness on snakebite prevention and safety and training in snakebite first aid are important to reduce the incidence and complication of snakebite injury. Animal bites and stings reporting should be made mandatory. Snakebite management may require the development of special interest group of doctors that

subspecialize in clinical toxinology and able to provide guidance to other healthcare providers who are likely to treat snakebite patients. These doctors will also be able to advise and produce the most updated version of clinical practice guideline relevant for the country. Appropriate and sufficient antivenom supply with more stringent regulation by the governing bodies is needed. Providing snakebite and envenomation management programs to healthcare providers at all levels may turn out to be a more cost-effective and lasting practical solution. Medical schools should consider revising their curriculum to include clinical toxinology or snakebite and envenomation management during clinical postings in relevant departments, for example, Emergency Medicine and Family Medicine. A standardized and updated curriculum should be developed and monitored by relevant bodies such as the Medical Education Departments and the Malaysian Society on Toxinology. Closer interdisciplinary cooperation and contact between healthcare providers with toxinologists, pharmacists, herpetologists, veterinarians, and wildlife department personnel through dialogue, forums, and the like are necessary. This is to enhance knowledge sharing and provision of mutual support for the betterment of patient's outcome. Networking and research collaborations between local and international experts on snake venom and clinical toxinology should be encouraged. Following the recent incidents of envenoming from nonindigenous crotaline obtained through the pet trade, it is also prudent to consider the setting up of a regional snakebite and antivenom support network. Transnational cooperation between healthcare providers, wildlife department, and national zoological societies with regard to supplies of exotic antivenom should be encouraged. Reducing red tapes to obtain and transport these antivenoms will expedite treatment, reduce complications, and improve outcome.

Cross-References

- ▶ Clinical Uses of Snake Antivenoms
- ▶ Venomous Snakes and Envenomation in Brunei
- ▶ Venomous Terrestrial Snakes of Malaysia: Their Identity and Biology

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Venomous Snakes and Envenomation in Brunei

5

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Contents

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Abstract

The venomous snakes recorded from Brunei Darussalam are enumerated. A total of 19 species, representing two families (Elapidae, 15 species, and Viperidae, four species), have been recorded in the country. For each species, there is a brief description of biology, localities, and references. Antivenom sera available at RIPAS Hospital are listed and annotated with their potential use. Apart from bites from venomous snakes, the presence of one "spitter," the equatorial cobra, *Naja sumatrana*, increases the risk of humans to venom ophthalmia. Finally, future directions for research and management of snake envenomation, and for enhancing knowledge of the country's snakes for conservation and improving health care, are discussed.

Introduction

Brunei Darussalam (total land area: 5,765 km^2) lies along the northwest coast of Borneo, where its two disconnected portions are separated by the East Malaysian state of Sarawak. It shows great contrasts, with vast tracts of unbroken lowland dipterocarp forests, peat, and freshwater swamps, on one hand, to a large and rapidly expanding capital city (Bandar Seri Begawan), connected to other urban centers and to the nearby cities of Malaysia via an extensive system of highways, on the other hand. The small human population (2013 estimate: 415,717), coupled with the dependence on oil and gas, has resulted in the lowland forests being left relatively intact. This is in stark contrast to the situation in the nearby states in Borneo.

The relatively pristine forests and matchless biodiversity of Brunei have been the subject of numerous popular and review articles (Das 1994; Eaton and Ibrahim 1995; Slik et al. 2003), and forestry practices have been compared favorably relative to those in neighboring states (Bryan et al. 2013). The herpetofauna, as expected from the pristine nature of forests and the diversity of forest types represented, is exceptional. Comprehensive inventories, though, are available only for a few areas (see Das 1995; Das et al. 2008), and new species records continue to be reported (e.g., Dehling and Das 2006; Keller 2011). Snakes figure prominently in biotic and herpetofaunal inventories. For instance, the early work of Motley and Dillwyn (1853) includes the snake fauna of the island of Labuan, situated off Brunei (Fig. 5.1).

This essay provides an inventory of the venomous snake fauna of Brunei, including both the terrestrial and marine species, based on museum records and the published literature, providing locality records. Notes on emergencies brought

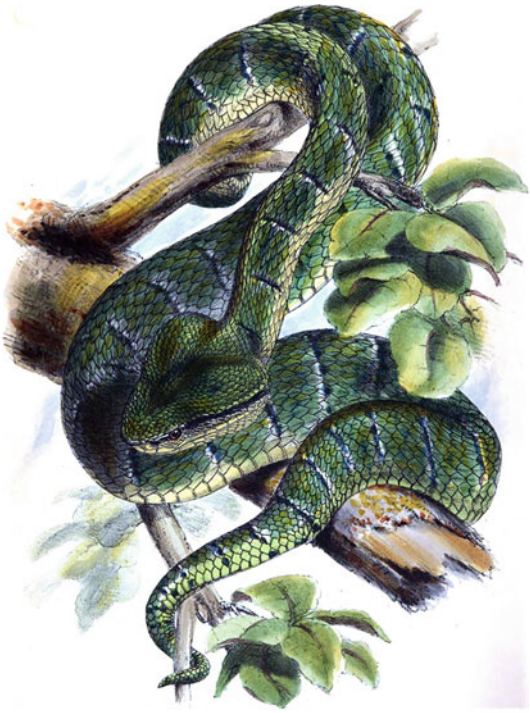

Fig. 5.1 Reproduction of a watercolor showing *Tropidolaemus subannulatus* from Motley and Dillwyn (1855) "*Contributions to the natural history of Labuan and adjacent coasts of Borneo*"

about by venomous snakes and on conservation measures specific for Brunei Darussalam are provided.

Institutional abbreviations used are as follows: BM = Brunei Museum, Bandar Seri Begawan, Brunei Darussalam; LSUMZ = Museum of Zoology, Louisiana State University, Baton Rouge, Louisiana, USA; and UBD = Zoological Museum, Universiti Brunei Darussalam, Bandar Seri Begawan, Brunei Darussalam.

The Venomous Snake Fauna

Brunei's major venomous snakes fall into two families, the Elapidae (comprising 15 species of cobras, kraits, coral snakes, and sea snakes) and Viperidae (comprising four species of pit vipers). Several venomous representatives of an otherwise nonvenomous family (Colubridae) are also known from Borneo, belonging to the genus *Rhabdophis*. One member of this genus (*R. chrysargos*) is known from Brunei, but shall be not considered further for lack of reports of human envenomation. In general, the snake fauna of the country is a subset of that described from Malaysia (see "▶ Chap. 3, Venomous Terrestrial Snakes of Malaysia: Their Identity and Biology," for this volume).

Elapidae

Banded Krait, *Bungarus fasciatus* (Schneider, 1801)

The banded krait is arguably the most common krait in Brunei and known from no less than five localities, including several from within the capital city of Bandar Seri Begawan. This nocturnal species is typically active at night and associated with swamp forests and, within its range, often encountered as roadkills. Although bites are rare, it should be treated as dangerous, its venom being neurotoxic.

Localities in Brunei are listed below:

BM 56.1995 Kampung Melilas, Belait District
BM 36.2001 Jalan Selayun, Brunei-Muara District
BM 117.1985; BM 178.1985 Kampung Anggrek Desa, Brunei-Muara District
BM 185.1989 Makam Sultan Bolkiah, Bandar Seri Begawan, Brunei-Muara District
BM 19.1994 Kampung Tamoi Tengah, Brunei-Muara District
BM 200.1992 Jalan Labu, Temburong District
BM 88.2006 near Tasek Merimbun, Tutong District

Red-Headed Krait, *Bungarus flaviceps* (Reinhardt, 1843)

The red-headed krait is known from a single specimen in Brunei, suggesting its rarity. Also nocturnal, it inhabits lowland dipterocarp forests. Like the previous species, the venom of this krait is believed to be neurotoxic, although bites on humans have not been recorded.

The sole locality in Brunei is:

BM 5.1987 Kampung Bukit Puan, Belait District

Blue Coral Snake, *Calliophis bivirgatus* (Boie, 1827)

The blue coral snake is a strikingly colored venomous snake and may be more common than suggested by data from Brunei (represented by a single specimen). It is nocturnal and associated with lowland forests.

The only Brunei record is:

UBD 431 Batu Apoi Forest Reserve, Temburong District

Malayan Striped Coral Snake, *Calliophis intestinalis* (Laurenti, 1768)

The striped coral snake bears a distinct coloration on the ventral surface, which is brightly banded, and has a red tail venter. A subfossorial feeder of small snakes, it is

known to cause mild envenomation in human adults. A bite from this species was reported at RIPAS Hospital. No further details are available.

The coral snake is known from three sites:

BM 189.1983 near Sungei Tilong, Muara, Brunei-Muara District
UBD 662 Kampung Mata Mata, Brunei-Muara District
UBD 639 Batu Apoi Forest Reserve, Temburong District

Equatorial Spitting Cobra, *Naja sumatrana* (Müller, 1890)

The equatorial or Sumatran cobra is familiar in Brunei, but reports of cobras by laypersons in Brunei tend to turn out to be other species of mostly nonvenomous snakes. The Bornean population is blue-black dorsally in adults, while juveniles bear distinct pale bands. This is a dangerously venomous species, capable of defending itself aggressively via biting and spraying its venom in the direction of the aggressor, causing ophthalmia.

BM 04.1999 Kampung Sabun, Brunei-Muara District
BM 28.1972 Jalan Muara, Brunei-Muara District
BM 13.1981 Kampung Delima, Brunei-Muara District
BM 315.1984 Sungei Jambu, Tungku, Brunei-Muara District
BM 11.1986 McFarm Limited, Brunei-Muara District
BM 23.1986 Jalan Manggis Dua, Brunei-Muara District
BM 114.1986 Berakas, Brunei-Muara District
BM 87.1988 Jalan McArthur, Bandar Seri Begawan, Brunei-Muara District
BM 59.1990 Padang Golf, Mentiri, Brunei-Muara District
BM 09.1996 Lambak Kanan, Brunei-Muara District
BM 4.1999 Kampung Sabun, Brunei-Muara District
UBD 430 Jerudong, Bandar Seri Begawan, Brunei-Muara District
UBD 528 Berakas Army Camp, Brunei-Muara District
UBD 606 Kampung Sungei Damit, Brunei-Muara District
BM 86–87.2006 near Tasek Merimbun, Tutong District

This species has also been recorded from Batu Apoi Forest Reserve, Temburong District, by Rader and Hemens (2002).

King Cobra, *Ophiophagus hannah* (Cantor, 1836)

The king cobra is a large, diurnal, and dangerously venomous snake that, prior to extensive development of Bandar, appears to have been common locally. Currently, it may be mostly associated with hill dipterocarp forests and is ophiophagous in its diet, although monitor lizards are also consumed. The venom of the species is neurotoxic, with massive amounts of venom discharged during a typical bite.

BM 03.1992 Anduki, Belait District
BM 7.1975 Jalan Tutong, Brunei-Muara District
BM 2.1979 Dewan Museum, Bandar Seri Begawan, Brunei-Muara District
BM 224.1991 Kampung Pintu Halim, Bandar Seri Begawan, Brunei-Muara District
BM 6.1998 Kampung Beribi, Gadong, Bandar Seri Begawan, Brunei-Muara District
BM 1984.80 Kota Batu, Bandar Seri Begawan, Brunei-Muara District
LSUMZ 55839 Forest Hill, Jalan Muara, Bandar Seri Begawan, Brunei-Muara District
BM 12.1995 Bukit Udal, Kampung Sungei Damit, Tutong District

Beaded Sea Snake, *Aipysurus eydouxii* (Gray, 1849)

This unusual sea snake is from shallow coastal waters. It has a specialized diet, comprising fish eggs, that may have resulted in the 50–100-fold decrease in venom toxicity compared to related species (Li et al. 2005). The Brunei record is by Elkin (1992) and apparently not backed with voucher specimens or images.

Annulated Sea Snake, *Hydrophis cyanocinctus* Daudin, 1803

The annulated sea snakes inhabit shallow coastal waters and are often stranded on beaches. Its venom has been shown to cause myonecrosis in lab animals. Hemolysis and respiratory and renal failures have been reported from its bites on humans.

The Brunei record is:

BM 11.1992 Belait District, which has also been cited by Elkin (1992)

Lesser Dusky Sea Snake, *Hydrophis melanosoma* Günther, 1864

The lesser dusky sea snake is found off the coast and may also travel some distance upriver. A poorly known species, its venom has not been investigated. The Brunei record is from a published report by Elkin (1992), apparently without voucher specimens or images.

Ornate Sea Snake, *Hydrophis ornatus* (Gray, 1842)

The ornate sea snake inhabits shallow seas with coral reefs and has also been recorded from turbid waters near estuaries. Its venom has not been studied. The Brunei record is from a published report by Elkin (1992), apparently without voucher specimens or images.

Spiral Sea Snake, *Hydrophis spiralis* (Shaw, 1802)

The spiral sea snake is found in deep waters, typically over 10 m deep. Its bite and venom are not well understood at present. The Brunei record (BM 130.1993) is cited by Elkin (1992) and without a precise locality.

Annandale's Sea Snake, *Kolpophis annandalei* (Laidlaw, 1901)

Annandale's or bigheaded sea snake is a poorly known, monotypic sea snake from coastal waters of Southeast Asia, especially in the eastern Indian Ocean. Nothing is known of its venom or envenomation.

The sole record from Brunei and from Borneo is listed below:

UBD 655 Tungku Beach, Brunei-Muara District (cited by Das 1993)

Short Sea Snake, *Lapemis curtus* Shaw, 1802

The short sea snake inhabits shallow seas, such as off coasts with muddy bottoms and also coral reefs. Its venom has neurotoxic properties.

The Brunei record is listed below:

BM 17.1994 Pantai Penaga, Belait District (cited by Elkin 1992: as *Lapemis hardwickii*)

Yellow-Lipped Sea Krait, *Laticauda colubrina* (Linnaeus, 1758)

The sea krait is associated with coral islands and, on Brunei, has been recorded from the rocky islet of Pulau Punyit, where it is known to ascend trees (Booth et al. 1997; Das 1992). This sea snake is more terrestrial than others occurring in Brunei waters, and although its venom is neurotoxic, it does not pose a particular danger to humans.

BM 04.1992 Pantai Kuala Belait, Belait District
BM 17.1973; BM 87.1993 Tungku, Pulau Punyit, Brunei-Muara District
BM 14.1976 Pantai Muara, Brunei-Muara District
BM 50.1992 Pelong Rocks, Brunei-Muara District
UBD 328, 329 Pulau Punyit, Brunei-Muara District

This species has also been reported from Brunei by Elkin (1992).

Yellow-Bellied Sea Snake, *Pelamis platura* (Linnaeus, 1766)

The yellow-bellied sea snake is the most pelagic of all marine snakes, and the two Brunei records (a specimen and a sighting record) are suspected of being stranded specimens. Mild envenomation and deaths have been reported from its bite elsewhere.

The Brunei record is represented by:

BM 314.1984 Bandar Seri Begawan, Brunei-Muara District

This species has also been reported from Brunei by Elkin (1992).

Viperidae

Sumatran Pit Viper, *Parias sumatranus* (Raffles, 1822)

This large and strikingly patterned pit viper is known from Batu Apoi, Temburong District (Keller 2008). It is arboreal and restricted to the low hills. Its diet comprises small mammals, birds, and frogs, and its venom is neurotoxic.

Sabah Green Pit Viper, *Popeia sabahi* (Regenass and Kramer, 1981)

The Sabah green pit viper is known from the country (Das 2007), based on an unlabelled museum specimen, and is likely to occur in the upper ranges of mountains, such as Gunung Pagon in the Temburong District. It is arboreal and feeds on small mammals. Nothing is known of its venom, which is believed to be neurotoxic.

Bornean Pit Viper, *Trimeresurus borneensis* (Peters, 1871)

This is a terrestrial and semiarboreal (low vegetation) pit viper from hill dipterocarp forests of Borneo. Its venom is believed to be hemotoxic, although no bites have been recorded.

UBD 489, 638, 681, 682 Batu Apoi Forest Reserve, Temburong District

Bornean Keeled Green Pit Viper, *Tropidolaemus subannulatus* (Wagler, 1830)

The Bornean keeled green pit viper was previously confused with Wagler's pit viper, *Tropidolaemus wagleri*, a species now known to be restricted to the Malay

Peninsula, Sumatra, and adjacent islands. This is arguably the most common pit viper in Borneo and associated with lowland dipterocarp forests and other habitats in the plains, especially in the vicinity of water bodies. It is a sit-and-wait predator of small mammals and birds, and its venom is hemotoxic and known to cause pain, bleeding, local swelling, and occasional necrosis, but more serious effects of envenomation, including human mortalities, are unknown.

The records from Brunei include:

BM 02.1992 Penaga, Seria, Belait District
BM 76.1993 Kampung Melilas, Ulu Belait, Belait District
BM 257–258.1992 Jalan Labi, Kuala Belait, Belait District
BM 54.1973 Kota Batu, Brunei-Muara District
BM 67.1973 Maktab Perguruan Gadong, Brunei-Muara District
BM 88.1973 Kampung Subok, Brunei-Muara District
BM 10.1986 Jalan Mudang, Brunei-Muara District
BM 43.1988 Kampung Pulori, Brunei-Muara District
UBD 131 Jalan Muara and Jalan Manggis Dua, Brunei-Muara District
BM 188.1992 Jalan Bukok, Temburong District
BM 265.1992 Jalan Temada, Temburong District
BM 95.1993 Pulau Labi, Tutong District

Snake Envenomation and Ophthalmia

The low number of cases involving snakebites in Brunei (from none to about 12 annually, with no recorded mortalities) may be attributed to the low population density that is mostly concentrated in the urban centers.

The Raja Isteri Pengiran Anak Saleha Hospital (RIPAS Hospital; Fig. 5.2), in Brunei's capital, Bandar Seri Begawan, the country's national hospital (established 28 August 1984), has emergency physicians attending to snakebite and ophthalmia cases. Other primary health centers that stock antivenom serum include the Tutong Hospital, Kuala Belait Hospital, and several special clinics. The country imports six Thai-manufactured antivenom serum, including two polyvalent ones, as follows, from the Thai Red Cross Society:

1. Banded krait antivenom (equine, monovalent), manufactured from *Bungarus fasciatus* that is found in Brunei
2. Russell's viper antivenom (equine, monovalent), manufactured from the Thai population of *Daboia siamensis*. This species (and the genus itself) is not found in Brunei or Borneo.
3. Thai cobra antivenom (equine), manufactured from the Thai population of *Naja kaouthia*, a species not found in Brunei or Borneo. This may have some effect in neutralizing the bites of the congeneric *N. sumatrana*.
4. Green pit viper antivenom (equine, monovalent), manufactured from *Cryptelytrops albolabris*, a species (and genus) that is not found in Brunei.

Fig. 5.2 A view of the entrance to the Accident and Emergency Section of Raja Isteri Pengiran Anak Saleha (RIPAS) Hospital at Bandar Seri Begawan (Photo: Joseph K. Charles)

However, the serum is sometimes recommended for the bite of *Popeia sabahi* as well as *Tropidolaemus subannulatus*, being the most likely nonspecific antivenom to use for bites from these species.

5. Hemato antivenom (equine, polyvalent), manufactured from three viper species – *Daboia siamensis*, *Cryptelytrops albolabris*, and *Calloselasma rhodostoma*, none of which is found in Brunei or in Borneo. This antivenom may be used, in the lack of a monovalent serum, for treating bites from vipers in Brunei.
6. Neuro antivenom (equine, polyvalent), manufactured from three species – *Ophiophagus hannah* (venom extracted from the northeastern, central, as well as southern lowland Thai populations; these populations represent independent lineages that are non-conspecific with the species in Brunei), *Bungarus fasciatus*, and *B. candidus* (this last mentioned species is not found in Borneo). Nonetheless, the serum may be indicated in bites from kraits, other than *B. fasciatus* (for which a monovalent serum is available; see above) and coral snakes (*Calliophis* sp.).

Several venomous species are capable of orally ejecting their venom for a distance of over a meter, targeting the eyes of potential or perceived enemies (see Chu et al. 2010). While most famously recorded from African spitting cobras (Ridley 1944), several Asian species of *Naja* are known to spit. Ophthalmia, as a result of snake venom in the eyes of humans and pets such as dogs and cats, if left untreated, can result in severe ocular injury, leading to potential blindness. Venom ophthalmia has been recorded from Borneo and results from interactions with *Naja sumatrana*. Published records are from Sabah (Garrett 1911) and there are two records from Brunei Darussalam. Attributed to *Naja siamensis* (a Thai endemic), based on nonspecialist literature examined by Raja and Kok (2011) and unidentified to species by Siraj and Joshi (2012), treatment for all spitting species of cobras may be similar (copious ocular irrigation and topical antibiotics).

Conclusions and Future Direction

It is essential to emphasize here of the need to develop antivenom specific to different parts of a geographic region, particularly as the snake fauna, from the rapidly accumulating knowledge of systematics and distribution shows, change with distance. In the case of Brunei, imported Thai antivenom sera are manufactured by the Thai Red Cross Society, from species that were once thought to be conspecific. Taxonomic literature from the past two decades has continued to delimit species boundaries, restricting species names one nearly pan-regional, to more localized populations. Within venomous snake populations, phylogenetically close species and even different populations of conspecifics are known to show significant difference in venom chemistry, including action on humans, further emphasizing the need for local antivenom sera for Bornean venomous snakes.

Better distributional data are needed for Brunei herpetofauna, particularly its venomous snakes, and updated maps of their distribution will aid the treatment of snakebites. Confirmed records for a large number of species, especially the sea snakes, are required, via voucher specimens that are lodged at a museum or unequivocal secondary evidence, such as digital images. Also essential is knowledge of their systematics and natural history. For instance, the population of *Naja sumatrana* in Borneo shows a dramatic ontogenetic shift, from a dark-light banded juvenile to a unicolored adult (Fig. 5.3). Such knowledge is essential for species identification, the first step for species inventories, taxonomy, ecological studies, and, especially, snakebite treatment. Public education on snakes, targeting school children and the general public, within the context of biodiversity conservation, has the potential to dispel fear and promote positive perception of snakes. Such activities, which can be through a medium via radio and television, as well as public talks and more formal inputs into academic curriculum, can enhance appreciation of a valuable part of Brunei's biological diversity.

With the forest resource management practices in Brunei Darussalam being highlighted as one of the best in the region, it can be inferred that there is adequate protection for its biota that is restricted to several habitats in the country. This enlightened approach needs to be emulated regionally and globally for more effective protection of tropical biodiversity of Southeast Asia.

Fig. 5.3 Ontogenetic color change in Bornean *Naja sumatrana*, as shown by a juvenile (**a**) and an adult (**b**) (Photo: Indraneil Das)

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Diversity and Distribution of Medically Important Snakes of India

6

Romulus Whitaker and Gerard Martin

Contents

Abstract

Of the 285 species of snakes found in India, only four are thought to be responsible for the majority of life-threatening bites, spectacled cobra (*Naja naja*), Russell's viper (*Daboia russelii*), common krait (*Bungarus caeruleus*), and saw-scaled viper (*Echis carinatus*), the so-called Big Four. The only available antivenom in India targets the bites of these four species. While it is true that

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these four species are the most medically important Indian snakes, the challenge of saving lives from snakebite is much more complex.

India has four species of cobras, eight species of kraits, and two subspecies of saw-scaled vipers, all of which must be considered medically important. Additionally, several other species like king cobra, some sea snakes, and at least two pit viper species can be classed as medically important based on clinical records of (sometimes single) fatalities from their bites. Further studies are required to determine if there are other species of Indian snakes that can be so classified. It is not known whether the existing Big Four antivenom is effective against the venom of these other or related species, a neglected research area that urgently needs addressing. The venom of Russell's viper is reported to vary significantly in its clinical effects in different parts of its range which has implications for the production of regionally specific antivenom (Jayanthi and Gowda 1988).

To compound the problem, the distribution of most of these less well-known species is still poorly known. Further, in a country as vast as India, there are different assemblages of venomous snakes in different parts of the country. For example, there are no true vipers in the northeast, and in Eastern India, the spectacled cobra is largely replaced by the monocled cobra. Local anecdotal accounts (particularly in the northeast where large pit vipers occur) suggest several pit viper species that may cause significant morbidity and occasional mortality. The kraits are all known to possess very toxic venoms, but so far, antivenom is only made for the widely distributed common krait, *Bungarus caeruleus*.

Introduction

Of the over 285 known species of snakes in India, over 50 species have the venom toxicity and capability of delivering a harmful or fatal bite to a human in defense (Whitaker and Captain 2004). Snakes typically bite when they are stepped upon or otherwise constrained, injured, or grabbed. An exception to this (which has yet to be satisfactorily explained) is the frequency of krait bites on sleeping humans (many people sleep on the floor/ground in rural India) when there is seemingly no threat to the snake but it bites nonetheless. Bites from Russell's and saw-scaled vipers generally occur when the snake is trod upon, usually after dark, the period of maximum activity of these species of snakes. Many cobra bites seem to occur in agricultural fields during various activities like weeding or harvesting. The following is a discussion of the main snakes responsible for serious bites in India, their distribution, and in some cases, their relative abundance. In addition to the Big Four, venomous species of India, the other members of the cobra, krait, and saw-scaled viper families, are mentioned and discussed, pointing to the need for more work on distribution, epidemiology, and differentiation from their "sister" species (Whitaker and Whitaker 2012).

Cobras of India

Spectacled cobra (*Naja naja*) is the most widespread of all the venomous snakes in India. It is a species commonly associated with agricultural crops (typically rice and other grains), targeting as they do one of man's commonest commensal field rodents, the lesser bandicoot or mole rat (*Bandicota bengalensis*) (Whitaker and Advani 1983). Spectacled cobras typically dwell in rodent burrows in rice field *bunds* (embankments between fields), termite mounds, piles of rubble, building foundations, heaps of straw and firewood, and brush piles. In addition to mole rats, cobras commonly feed on other species of small rodents, other snakes, frogs, and notably toads which they seem to particularly target. Spectacled cobras are found throughout most of India except the far northeast, altitudes above 2,000 m, and the Andaman and Nicobar Islands. Cobras reach 2,200 mm in total length.

Monocled cobra (*Naja kaouthia*) is the common cobra in the northeastern part of India starting in Orissa and extending east into parts of Uttar Pradesh. This is a more aquatic snake than the spectacled cobra and commonly includes fish in its diet, along with frogs, toads, and rodents of several species. Like *Naja naja*, monocled cobras are also strongly associated with rice fields with their abundance of prey species. There are locations in West Bengal with exceedingly high concentrations of this species. From conversations with various physicians and snake rescue groups in the region, bites are fairly common and fatalities frequent. There are several reports of this species "spitting" venom in defense.

Central Asian cobra (*Naja oxiana*) has been recorded from only a few localities in Northwest India, including Jammu and Kashmir and may occur in Punjab and parts of Himachal Pradesh. The species often referred to as the "black cobra" found throughout the northwest of India (particularly Gujarat) is apparently (until further taxonomic work is done) a black color morph of the spectacled cobra (*Naja naja*) and has long been mistaken for the Central Asian cobra. This snake of dry rocky and sandy habitats feeds primarily on rodents, no doubt including amphibians during the short rainy period of the region. The efficacy of Indian antivenom serum on the venom of this cobra is unknown.

Andaman cobra (*Naja sagittifera*) is a rarely seen, little-known species found on the main islands in the Andaman group where king cobras (*Ophiophagus hannah*) are quite common. There is no information on the feeding or other habits of the Andaman cobra, no bites have been recorded, and no studies of their venom have ever been undertaken. No tests have been done to check the efficacy of Indian antivenom serum against the venom of this species.

Kraits of India

Common krait (*Bungarus caeruleus*) is the most widely distributed of the eight species of kraits found in India and except for the far northeast and Andaman and

Nicobar Islands is likely to be found in every Indian state. The common krait has long been acknowledged as the species of snake with among the most toxic of venoms and causing considerable mortality. The most perplexing aspect of krait bite is the high percentage of bites which occur on humans sleeping on the floor or ground. Snakes typically avoid humans yet clinicians in some regions report that the majority of krait bites happen when the victim is asleep. It is suspected that there is an olfactory cue which causes the krait to bite a sleeping human (is the snake mistaking the human for a rat?).

Kraits are abundant near agricultural fields with their ready supply of rodents. The favored prey of the common krait in Tamil Nadu is the field mouse (*Mus booduga*), and they are well-known snake-eaters. Common kraits reach 1,750 mm in total length; males are larger than females in all the krait species. Like all kraits, this is a nocturnal snake which hides by day, usually in a rat or mouse burrow, inside a termite mound, or under debris.

Banded krait (*Bungarus fasciatus*) is a familiar snake found from Northern Andhra Pradesh up through West Bengal and the northeastern states and westward into Bihar and Chhattisgarh. Though the venom is highly toxic, this gentle snake is most unlikely to bite, even when trod upon and handled roughly. It is often said that this snake will only bite at night. It is not known whether Indian polyvalent antivenom has a positive neutralizing effect for banded krait bite. Banded kraits are well-known snake-eaters but also prey on rodents. They grow to the largest size of any of the kraits, reaching 2,250 mm in total length. Very few bites are known from this species, but fatalities have been recorded.

Wall's krait (*Bungarus sindanus walli*) and the **Sind krait (*Bungarus sindanus sindanus*)** are taxonomically clubbed as a single species, but it seems that they are two distinct species, the Sind krait being found in West India (from Maharashtra to Rajasthan) and Wall's krait in the western, sub-Himalayan region (Northern Uttar Pradesh, Bihar, West Bengal). Very little is known about their habits and no studies have been done on their venoms, but fatalities have been recorded for the bites of both (Dr. Tanwar, P. D. pers. comm). Maximum total length is 1,518 mm.

Black krait (*Bungarus niger*) is found in North Bengal and apparently throughout Assam and other states of the far northeast. Very little is known about this snake's habits. Its venom can produce both neurotoxic and myotoxic envenoming, and fatalities have been recorded (Ulrich Kuch pers. comm.; Faiz et al. 2010). As with the other kraits, the black krait is likely to feed on rodents and other snakes. It grows to a total length of 1,295 mm. This species is now being found with fair regularity in locations both within and outside of its known distribution range including Uttarakhand. There are reports from some doctors located within the range of this species that antivenom works erratically with snake bites pointing to the urgent need to test antivenoms against venoms of these lesser known species.

Lesser black krait (*Bungarus lividus*) is another little-known snake from the northeastern part of India for which fatalities have been recorded in India and in the neighboring countries of Nepal and Bangladesh (Ahmed et al. 2009).

Himalayan krait (*Bungarus bungaroides*), for which no human fatalities are reported, is also found in the hills of the northeast, and as with most of the other krait species, no studies have been done on their venoms.

Andaman krait (*Bungarus andamanensis*) is another little-known snake for which no fatalities have been recorded, and nothing is known of its venom. Being a *Bungarus* species, however, infers that its venom is highly toxic, and though consistent with other krait venoms, it is unknown whether Indian polyvalent antivenom can neutralize its effects. The Andaman krait is fairly common throughout the main Andaman Islands and probably feeds primarily on other snakes, skinks, and possibly rodents. It grows to 1,000 mm total length.

Vipers of India

Russell's viper (*Daboia russelii*) is by far the most medically important viper in India. It is a heavyset snake with a large head, long fangs, and very destructive venom. It grows to 1,800 mm in length. Russell's vipers are common throughout much of India, often associated with agricultural fields with their abundance of rodents, the primary prey of this species. It is absent from the desert areas of Rajasthan and from Assam and the other far northeastern states. This snake is less often found in holes, where kraits and cobras are typically found, and prefers the cover of thick, thorny vegetation, leaf litter, and thick, tall grass. Russell's vipers are primarily nocturnal, ambush predators, and their habit of lying in wait for prey makes them more likely to be trod upon by humans than other, more active snakes. Disturbingly, the venom of Russell's vipers varies greatly with their geographic range, again with implications for making regionally specific antivenoms.

Saw-scaled viper (*Echis carinatus carinatus*) ranges widely over India but is restricted to pockets of dry, open country such as the laterite plateau country along the western coast and open tracts of wasteland in South India. Its average maximum length is 300 mm. **Sochurek's saw-scaled viper (*Echis carinatus sochureki*)** is much larger, reaching 800 mm, and is common in the desert areas of Gujarat, Punjab, and Rajasthan. There is speculation among some herpetologists that this is a separate species. In general, *Echis* is a dry country snake, avoiding wet and heavily forested parts of the country. Saw-scaled vipers are not found in the northeast nor the Andaman and Nicobar Islands. Being small snakes, they feed on a wide range of smaller prey including arthropods such as scorpions, spiders, crickets, and grasshoppers as well as mice, geckos, skinks, and small frogs. Saw-scaled vipers typically reside under rocks and in earthen and stony crevices and are primarily nocturnal. They climb well and commonly hide behind bark on palmyra trees. Most bites happen when they are trod upon by barefoot people who are not carrying a torch while walking at night. There has been little work carried out comparing the venoms of these two saw-scaled vipers, though preliminary results indicate venom of the southern form to be more toxic than the northern subspecies. It is unknown

how effective Indian polyvalent antivenom is for the bite of the northern form (Indian antivenom is all made using venom from the South Indian *Echis carinatus carinatus*).

Levantine viper (*Macrovipera lebetina*) is a large mountain viper living in parts of the mountains of Kashmir about which very little is known. It is the third so-called true viper of India (as opposed to "pit" vipers, of which India has a rich fauna of 21 species). A fatality from the bite of an allied species of *Macrovipera* was reported in Iran, but none have been reported in India. There is no antivenom available for this species in India, and no tests have been done to determine whether Indian polyvalent antivenom would be effective. The Levantine viper feeds on rodents, birds, and lizards and is a forest snake, found among rocks along streams and hillsides. Bites from this species are very rarely reported, but it is reported to have a strong hemotoxic venom and grows to over 2 m in length so must be considered "medically important" within the small region that it occurs in India.

Pit Vipers of India

There are 21 species of pit vipers in India. They are mainly hill and forest snakes, concentrated in the Western Ghats, Northeast India, and Andaman and Nicobar Islands, with a tremendous variation in size, abundance, and venom toxicity, though little is known about the latter for most species. Though pit viper bite symptoms of swelling and necrosis can be severe, there is only one fatality record in India in recent years: one from a Cantor's pit viper (*Cryptelytrops cantori*) in the Nicobar Islands (Whitaker 1991). It is very unlikely that Indian polyvalent antivenom will have any neutralizing effect on pit viper venoms. Antivenom for allied pit viper species is made in Thailand and Formosa, and it would be worthwhile to test them for effectiveness against Indian pit viper venoms. Since the most common pit viper in the Western Ghats, the Malabar pit viper (*Trimeresurus malabaricus*), is known to cause severe cytotoxic effects, and some of the pit vipers of the northeast, including Pope's pit viper (*Popeia popeiorum*), Kaulback's pit viper (*Protobothrops kaulbacki*), and Jerdon's pit viper (*Protobothrops jerdonii*), reach large sizes of well over 1,000 mm in length, it would be wise to include some of the Indian pit vipers in the classification of "medically important" snakes of India and take appropriate steps to make specific antivenoms available.

Medically Important Snakes of India

List of Confirmed Medically Important Snakes in India*

1. Spectacled cobra (*Naja naja*)
2. Monocled cobra (*Naja kaouthia*)

3. King cobra (*Ophiophagus hannah*)
4. Common krait (*Bungarus caeruleus*)
5. Banded krait (*Bungarus fasciatus*)
6. Sind krait (*Bungarus sindanus sindanus*)
7. Black krait (*Bungarus niger*)
8. Lesser black krait (*Bungarus lividus*)
9. Wall's krait (*Bungarus sindanus walli*)
10. Hook-nosed sea snake (*Enhydrina schistosa*)
11. Russell's viper (*Daboia russelii*)
12. South Indian saw-scaled viper (*Echis carinatus carinatus*)
13. Sochurek's saw-scaled viper (*Echis carinatus sochureki*)
14. Hump-nosed pit viper (*Hypnale hypnale*)
15. Cantor's pit viper (*Cryptelytrops cantori*)

*Indian snake species confirmed to have been responsible for human fatality

1. **The Big Four dangerous snakes of India** (for which indigenously produced antivenom serum has been available for 100 years). These are the four most widely distributed and relatively common species apparently responsible for most serious and fatal snakebites in India.
   Spectacled (binocellate) cobra (*Naja naja*) – common, widely distributed snake, responsible for high percentage of fatal bites. This species is the most commonly found one in urban and suburban areas. Snake rescuers around the country claim that between 60 % and 80 % of the snakes they are called to remove from residential and commercial areas are spectacled cobras (Fig. 6.1).
   Common krait (*Bungarus caeruleus*) – common, widely distributed snake, responsible for high percentage of fatal bites. Nocturnal by habit, a significant number of bites occur while the victim is asleep. Village folk, sleeping on the ground, are at particular risk. Patients are presented in hospitals, often with advanced symptoms (Fig. 6.2).
   Russell's viper (*Daboia russelii*) – common, widely distributed, probably responsible for highest percentage of fatal bites. This species is responsible for the most visible morbidity across its range. Coconut, rubber, and areca nut plantation workers are at particular risk as they need to walk through thick mulch and leaf litter to get to the plants they tend. The Russell's viper relies on camouflage and is often not spotted until stepped upon (Fig. 6.3).
   Saw-scaled viper (*Echis carinatus*) – common, patchy distribution, responsible for an unknown percentage of fatal bites. In South India, the subspecies (*Echis carinatus carinatus*) grows to a maximum of around 45 cm, and its bite is not often fatal. However, the subspecies in Rajasthan and Northwest India (*Echis carinatus sochureki*) grows to twice the size and is known to cause fatal bites (Fig. 6.4).

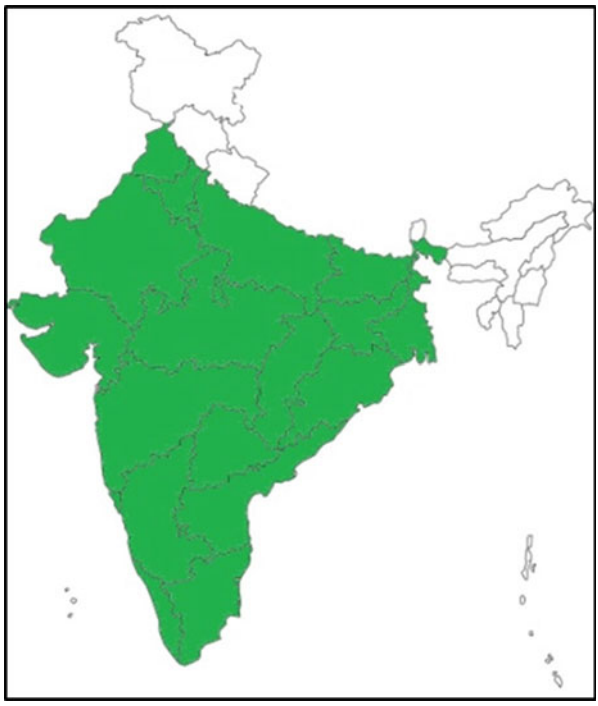

Fig. 6.1 Indian Mainland. The colored area refers to the known distribution of the snake species spectacled (binocellate) cobra (*Naja naja*)

2. **Snakes within the same genera of the Big Four** (all of which have the capacity to deliver a serious or fatal bite)

Cobras:

Monocled (monocellate) cobra (*Naja kaouthia*) – Common in Northeast India and northeast states, responsible for large but unknown percentage of fatal bites (Fig. 6.5).

Central Asian cobra (*Naja oxiana*) – Rarely seen in India, the "black cobra" found in most of Northwest India is apparently the black color morph of *Naja naja* (Fig. 6.6).

Andaman cobra (*Naja sagittifera*) – A rare snake in the Andaman Islands, possibly because of the fair abundance of king cobras (*Ophiophagus hannah*), a top cobra predator (Fig. 6.7).

Kraits:

Banded krait (*Bungarus fasciatus*) – A common snake in Northeast India, there are few recorded bites nor fatalities from this large krait which seems reluctant to bite, especially when compared with the common krait (Fig. 6.8).

Sind krait (*Bungarus sindanus sindanus*) – This may be a common species in some areas. Fatalities have recently been recorded from the bite of this species, previously referred to as the common krait. The distribution of the Sind krait needs to be ascertained (Fig. 6.9).

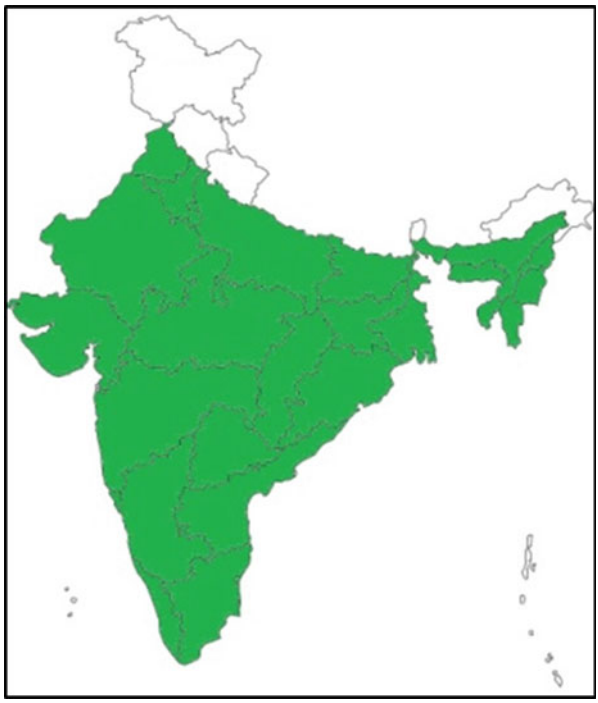

Fig. 6.2 Indian Mainland. The colored area refers to the known distribution of the snake species common krait (*Bungarus caeruleus*)

Fig. 6.3 Indian Mainland. The colored area refers to the known distribution of the snake species Russell's viper (*Daboia russelii*)

Fig. 6.4 Indian Mainland. The colored area refers to the known distribution of the snake species Saw-scaled viper (*Echis carinatus*)

Fig. 6.5 Indian Mainland. The colored area refers to the known distribution of the snake species monocled (monocellate) cobra (*Naja kaouthia*)

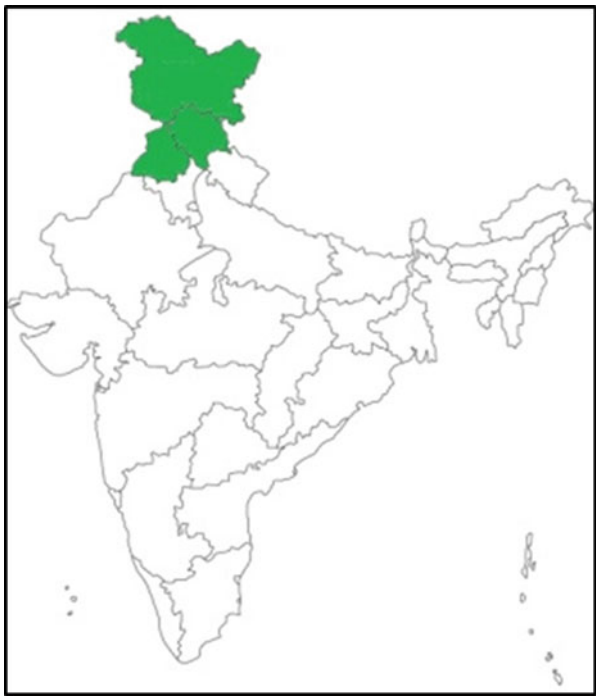

Fig. 6.6 Indian Mainland. The colored area refers to the known distribution of the snake species Central Asian cobra (*Naja oxiana*)

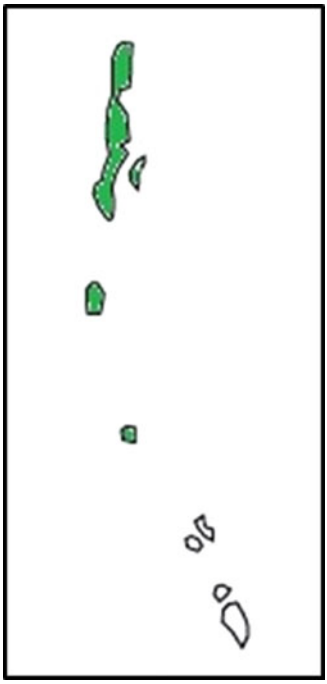

Fig. 6.7 Andaman and Nicobar Islands (India). The coloured area refers to the known distribution of the snake species Andaman cobra (*Naja sagittifera*)

Fig. 6.8 Indian Mainland. The colored area refers to the known distribution of the snake species banded krait (*Bungarus fasciatus*)

Fig. 6.9 Indian Mainland. The colored area refers to the known distribution of the snake species Sind krait (*Bungarus sindanus sindanus*)

Fig. 6.10 Indian Mainland. The colored area refers to the known distribution of the snake species Wall's krait (*Bungarus sindanus walli*)

Wall's krait (*Bungarus sindanus walli*) – This appears to be an uncommon species. Fatalities have also been recently recorded from Wall's krait bite; again, it has long been confused with the common krait and its exact distribution is not known (Fig. 6.10).

Black krait (*Bungarus niger*) -Fairly common in some parts of the northeast and recently reported from Uttarakhand. Recent fatalities from this krait confirm its medical importance (Fig. 6.11).

Lesser black krait (*Bungarus lividus*) – Little is known about this krait found in Northeast India, parts of Nepal, and Bangladesh, fatalities from lesser black krait bite have been recorded in all three countries.

Himalayan krait (*Bungarus bungaroides*) – Another krait species from the northeast (Arunachal Pradesh) about which very little is known but predictably has a toxic venom capable of killing a human.

Andaman krait (*Bungarus andamanensis*) – There are no known bites from this species which is fairly common on the main islands of the Andamans. Consistent with other kraits, the venom is likely to be highly toxic (Fig. 6.12).

Vipers:

Sochurek's saw-scaled viper (*Echis carinatus sochureki*) – Growing to twice the size of the South Indian *Echis*, this snake causes the majority of venomous bites in the desert areas of Gujarat, Rajasthan, and Punjab (Fig. 6.13).

Fig. 6.11 Indian Mainland. The colored area refers to the known distribution of the snake species black krait (*Bungarus niger*)

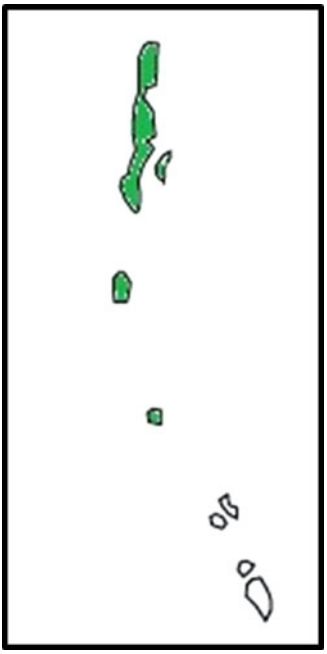

Fig. 6.12 Andaman and Nicobar Islands (India). The colored area refers to the known distribution of the snake species Andaman krait (*Bungarus andamanensis*)

Fig. 6.13 Indian Mainland. The colored area refers to the known distribution of the snake species Sochurek's saw-scaled viper (*Echis carinatus sochureki*)

3. Venomous snakes outside of the genera of the "Big Four"
   Levantine viper (*Macrovipera lebetina*) – Very little is known about either the habits or venom of this viper restricted to some localities in Kashmir. No antivenom available (Fig. 6.14).

Pit Vipers of Known Medical Importance

These are the only two species of Indian pit vipers which are known to have caused fatalities:

Hump-nosed pit viper (*Hypnale hypnale*) – For a long time, this small snake was misidentified by medical professionals as the saw-scaled viper (*Echis carinatus carinatus*). Several human fatalities are recorded from this species in Sri Lanka (Ariaratnam et al. 2008). Life-threatening symptoms have been recorded in India (Joseph et al. 2007). Bites from the hump-nosed pit viper occur in rubber, coffee, and cardamom plantations in its range states of Kerala, Tamil Nadu, Karnataka, and Goa. It is found from sea level up to about 700mASL (Fig. 6.15).

Fig. 6.14 Indian Mainland. The colored area refers to the known distribution of the snake species Levantine viper (*Macrovipera lebetina*)

Cantor's pit viper (*Cryptelytrops cantori*) – One fatality from Camorta Island in the Nicobar Islands. There is no further data on bites from this species (Fig. 6.16).

Pit Vipers of Possible Medical Importance

Studies are needed on venoms and clinical symptoms of bites of the below listed species of Indian pit vipers to determine if bites of any of these species cause fatal or seriously debilitating symptoms. Most of the serious sequelae to pit viper bites, such as necrosis, sloughing of skin, toxicemia, and gangrene, seem to be due to or at least aggravated by inappropriate first aid such as tight tourniquets, cut and suction with dirty implements, and use of herbal or even cow-dung poultices. The effects of venom of only one Indian species, the Malabar pit viper (*Trimeresurus malabaricus*) have been studied in some detail, showing a fairly high incidence of necrosis and cytotoxic effects in mice (Gowda et al. 2006a, b).

Recent documentation of pit viper bites in India includes the Medo pit viper (*Viridovipera medoensis*) (Gerry Martin, pers.comm., 2011), Himalayan white-lipped pit viper (*Cryptelytrops septentrionalis*) (Chandna 2007), and large-scaled green pit viper (*Peltopelor macrolepis*) (Whitaker 1973).

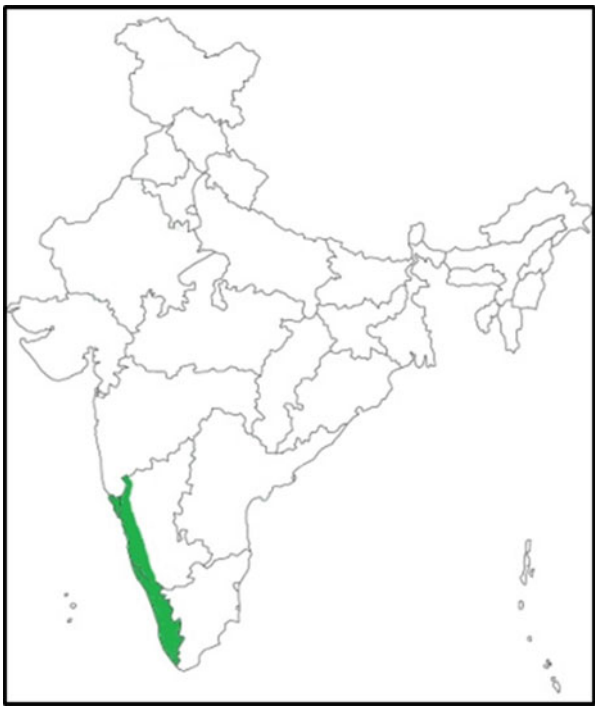

Fig. 6.15 Indian Mainland. The colored area refers to the known distribution of the snake species hump-nosed pit viper (*Hypnale hypnale*)

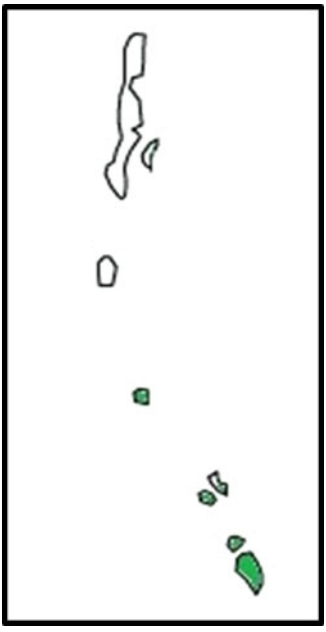

Fig. 6.16 Andaman and Nicobar Islands (India). The colored area refers to the known distribution of the snake species Cantor's pit viper (*Cryptelytrops cantori*)

Indian Pit Vipers

Species	Known distribution[a]
Himalayan pit viper (*Gloydius himalayanus*)	The Western Himalayas-Kashmir, Himachal Pradesh, Northern Punjab, Uttar Pradesh, and Uttarakhand. Records from Sikkim need confirmation. Also Pakistan and Central Nepal. Records from Eastern Afghanistan and Bangladesh need confirmation. Recorded between 1,500 and 4,877 m
Large-scaled pit viper (*Peltopelor macrolepis*)	Endemic to India. Known from a few localities in South India: Tamil Nadu (Nilgiri, Palani, Ashambu, High Wavy, and Anamalai Hills); also Central Kerala (Nelliampatti Hills) at altitudes ranging from 610 to 2,134 m
Brown-spotted pit viper (*Protobothrops mucrosquamatus*)	Recorded from several states in Northern and Northeast India: Bihar, Jharkhand, parts of Uttar Pradesh, West Bengal, Manipur, Nagaland, Meghalaya, Mizoram, Tripura, and Assam
Western mountain pit viper (*Ovophis monticola monticola*)	India: Arunachal Pradesh, Assam, Mizoram, Manipur, Meghalaya, Nagaland, Sikkim, Uttarakhand, West Bengal. Also Bangladesh, Tibet, China, Myanmar, and Nepal
Jerdon's pit viper (*Protobothrops jerdonii*)	India: the northeast from Meghalaya to Arunachal Pradesh from about 1,370 to 2,700 m. Also China, Tibet, and Myanmar. Record from Nepal needs confirmation
Malabar pit viper (*Trimeresurus malabaricus*)	The Western Ghats from Maharashtra (Mahabaleshwar) south to Kanyakumari in suitably wet and cool forests at altitudes varying from 610 to 2,134 m
Horseshoe pit viper (*Trimeresurus strigatus*)	Southern Western Ghats (Nilgiri, Anamalai, Palani, and Ashambu Hills). Precise localities include: Coonoor, Udhagamandalam (Ooty), Mukurthi Peak in Tamil Nadu; Silent Valley and Ponmudi in Kerala. Found between 915 and 2,400 m
Bamboo pit viper (*Trimeresurus gramineus*)	Western Ghats – the northernmost limit probably being the Dangs in Gujarat. Occurs in the Eastern Ghats, including the Shevaroy and Javadi Hills. Found up to about 450 m. Also recorded from near sea level at Tamil Nadu (Gingee) and Maharashtra (Phansad, Sanjay Gandhi National Park)
Yunnan pit viper (*Viridovipera yunnanensis*)	Reported from Khasi Hills, Meghalaya. Other reports from Northeast India need confirmation. Also Southern China (Yunnan and Sichuan)
Medo pit viper (*Viridovipera medoensis*)	India: known from two locations in Arunachal Pradesh – near Gandhigram Village, Changlang District, and Leporiang Village, Papum Pare District. Also Southern China and Upper Myanmar. Found between 1,000 and 1,400 m

(*continued*)

Species	Known distribution[a]
Pope's pit viper (*Popeia popeiorum*)	India: Sikkim, Meghalaya (Khasi Hills), West Bengal (Darjeeling, Jalpaiguri District), Arunachal Pradesh. Also Myanmar, Thailand, Malaysia, Singapore, Laos
Andaman pit vipers (*Trimeresurus andersoni*)	Only found on the Andaman Islands (common);one unconfirmed record for Car Nicobar Island (1937)
Spot-tailed pit viper (*Cryptelytrops erythrurus*)	India: Sikkim, Nagaland, West Bengal, and probably more states in the northeast. Records from Mizoram and Meghalaya and also Nepal and Bhutan need confirmation
White-lipped pit viper (*Cryptelytrops albolabris*)	India: West Bengal (Darjeeling); An old record from Nagpur is doubtful and needs confirmation. Also Southeast Asia
Nicobar pit viper (*Trimeresurus labialis*)	Found only on the Nicobar Islands. A single record from the Andaman Islands needs confirmation
Hutton's pit viper (*Tropidolaemus huttoni*)	Found in Theni District, Tamil Nadu. Known from 2 specimens, from the type locality – High Wavy Mountains. elevation 1,590 m
Himalayan white-lipped pit viper (*Trimeresurus septentrionalis*)	Known from Simla in Himachal Pradesh and Mori, Tons River, Uttarakhand. Reports from Jalpaiguri District in North Bengal need confirmation
Gumprecht's pit viper (*Viridovipera gumprechti*)	Reported from Jalpaiguri District in North Bengal. Reports from Nagaland and Manipur need confirmation
Kaulback's pit viper (*Protobothrops kaulbacki*)	First reported in India in 2007 at Leporiang Village, Papum Pare District, Arunachal Pradesh. Also in Myanmar
Hump-nosed pit viper (*Hypnale hypnale*)	Throughout Western Ghats as far north as Goa. Occurs from 300 to 600 m. Also in Sri Lanka from sea level to 1,525 m
Cantor's pit viper (*Cryptelytrops cantori*)	Only found on the Central Nicobar Group of Islands. A single record from the Andaman Islands needs confirmation

[a]The distribution and taxonomic status of the Indian pit viper species are under review

Other Venomous Snakes in India

In addition to the above species, there are several others that are potentially of medical importance but are either very rare, reluctant to bite, or for which there is little or no data about their venom. Some of these species are closely allied to those that are established as snakes of medical importance.

King Cobra

Ophiophagus hannah is the world's largest venomous snake and has a wide distribution in the Western and Eastern Ghats plus the Western, Central, and Eastern Himalayas up to over 2,000 m. It is found in the mangrove swamps of Odisha and in the Andaman Islands. It has been suggested that this is a species

complex. This is an alert snake which usually knows how to stay out of the way of humans. Bites are very infrequent, with very few records in most parts of its Indian range. There is no king cobra antivenom in India.

Coral Snakes

Belonging to the family **Elapidae**, coral snakes are close relatives of cobras and kraits and may have venoms of similar potency. No work has been done on the venom of any species of Indian coral snake. However, there is ongoing research being done on species from Southeast Asia. (Fry et al., pers comm). These snakes are largely fossorial (living underground) and not often seen. Some species, like the striped coral snake (*Calliophis nigrescens*), grow to over a meter in length and could possibly cause serious bites. However, no bites are recorded from any of these species in India.

Sea Snakes

Several species of sea snakes come close to India's shores and all are venomous. The one species which is most often implicated in the few cases of sea snake bite on record is the hook-nosed sea snake (*Enhydrina schistosa*), found on both coasts. Bites occur when the snake gets entangled in fish nets or is roughly handled by fishermen. There is no antivenom for sea snake bite in India.

Rear-Fanged Snakes

The family **Colubridae** has numerous species of "rear-fanged" snakes. They do not possess well-developed fangs nor venom-injecting systems and need to chew the venom into their prey. Most of these have venom that does not affect humans. However, there are a few species that could be a threat.

Red-necked keelback (*Rhabdophis subminiatus*) – This species is common in Northeast India, and though no fatalities have been recorded in India, its bite induces coagulopathy and has caused at least one human fatality in Southeast Asia. Local people in the areas where the species is common do not generally regard it as a dangerous species.

Large species of cat snakes such as Forsten's cat snake (*Boiga forsteni*), which grows to 2.3 m in length, might also be capable of dangerous envenomation. However, there are no case records or venom research data to confirm this.

Conclusion and Future Directions

Snakebite mortality in India has recently been estimated at 46,000 per year (Mohapatra et al. 2010). While the Big Four (cobra, krait, and Russell's and saw-scaled vipers) are clearly responsible for most serious and fatal snakebite cases and for which polyvalent antivenoms are manufactured by several companies, India has an additional dozen species of medically important venomous snakes besides the Big Four. Distribution and status of most of these snakes is poorly known and studies on most of their venoms have yet to be done. Identification aids

such as field guides and charts to encourage better knowledge of the venomous snakes in the region for both the public and clinicians who deal with snakebite would be of tremendous value.

There are growing indications from medical clinicians that antivenom produced from venoms of the Big Four, mainly sourced from the South India-based Irula Cooperative, may not effectively neutralize envenomation by the Big Four and related species in other parts of the country (Warrell et al. 2013). Whether this is due to regional venom variation, bites by other species, low antivenom potency, or a combination of these factors needs to be urgently determined and rectified by research and action to improve Indian antivenom.

Cross-References

- ▶ Clinical Uses of Snake Antivenoms
- ▶ Complications of Hemotoxic Snakebite in India
- ▶ Snake Envenomation in Bangladesh
- ▶ Snakebites in Tamil Nadu, India
- ▶ Venomous Snakes and Snakebites in India
- ▶ Viperidae Envenomation in India

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Venomous Snakes and Snakebites in India

7

B. Vijayaraghavan and S. R. Ganesh

Contents

Abstract

An overview of the venomous snake fauna of India is presented. Regions having high diversity of venomous snakes that are consequent on the presence of distinct ecoregions are projected. Annual statistics from India as to the number of snakebites and deaths from snakebite are reviewed. The lack of a consensual estimate of snakebite statistics from India is highlighted. Differences in methods of data collection, time span used for the study, and venomous snake diversity among the Indian states are presumed to be the causal factors. The concept of "Big Four venomous snakes" in the current context is reviewed in light of the venomous snake diversity of the country. Among the "other venomous snakes," identification of lethal (e.g., sea snakes, king cobra, banded krait) and nonlethal though venomous ones (most species of Indian pit vipers) is highlighted for snakebite management. Lastly, for those that do not necessitate the use of antivenom for treatment, acquisition of knowledge on effects and appropriate medical intervention is highlighted.

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Introduction

India is one of the tropical countries having three biodiversity hotspots, namely, the Western Ghats, the Eastern Himalayas, and the Andaman and Nicobar Islands, and is bestowed with a great biological diversity (Fig. 7.1). Snakes are no exception. About 285 described species of snakes occur in India. Of these, 66 species are "venomous" (including one "probably venomous"), 42 are "mildly venomous," and 178 are "nonvenomous." India's venomous snakes occupy a variety of biotopes from warm tropical coastlines to arid sandy areas and deserts, to evergreen rainforests and montane hill-forests, and to villages, countryside, farmlands, and even cities and towns. Being the second most populous country in the world, the burgeoning human population has to live side by side with the numerous snakes living here. Hence, human-snake interactions are inevitable. Further, considering

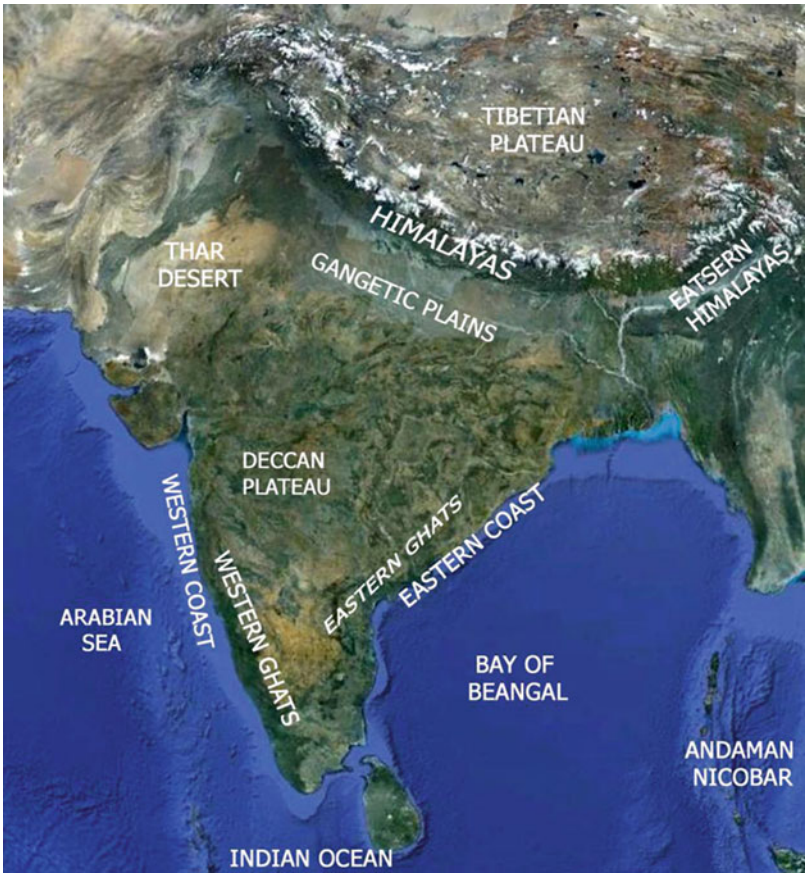

Fig. 7.1 Physical map of Indian subcontinent showing important relief features (Map rendered from Google Earth)

the general reputation of snakes and the prejudices against them, such interactions, unlike in the case of other fauna, are almost wholly adversarial.

An account on the venomous snake diversity of India, including both important medically recognized and medically obscure ones, nonconsensual Indian snakebite estimates, and antivenom production centers in India, both in the past and at present is presented here.

A total of 66 species of venomous snakes, distributed in four families, namely, Colubridae, Elapidae, Hydrophiidae, and Viperidae, are currently known from India. Each family has subfamilies – Colubridae contains Colubrinae (not represented here) and Natricinae (keelbacks), family Elapidae consists of Bungarinae (kraits, cobras) and Elapinae (coral snakes), Hydrophiinae (true sea snakes) and Laticaudinae (sea kraits) in the family Hydrophiidae, and Viperinae (true vipers) and Crotalinae (pit vipers) in the family Viperidae. Some authorities recognize keelbacks and sea kraits not as subfamilies Natricinae and Laticaudinae, respectively, but as belonging to families Natricidae and Laticaudidae, respectively.

Among the venomous snakes in India, the principal, common, dangerously venomous snakes are:

(i) Spectacled cobra (*Naja naja*)
(ii) Common krait (*Bungarus caeruleus*)
(iii) Russell's viper (*Daboia russelii*)
(iv) Saw-scaled viper (*Echis carinatus*)

These four are often together described as the "Big Four " venomous snakes of India (Figs. 7.2–7.5). However, some (see Simpson 2007) have objected to this terminology on the ground that it has misled the public into thinking that there are no other equally or more venomous snakes in India. This apprehension has no basis. The sobriquet "Big Four" is for the convenience of the lay persons since, in terms of the spread of distribution; abundance, particularly in areas largely frequented by humans; potency of venom; and frequency of bites, these four take the pride of place. This does not mean that there are no other Indian species which are equally or, perhaps, even more dangerous.

Another argument is that the use of the term "Big Four" has misled laboratories to believe that there is no need to manufacture antivenom for any other species. This again is fallacious. The reason why the laboratories have not gone beyond the "Big Four" is that in view of the rarity of bites or categorical finding as to the species responsible and the very high cost of manufacture of antivenom for the other species, they have not found the manufacture of antivenom for other species a commercially attractive proposition.

In India, apart from the front-fanged venomous snakes, there are two species of rear-fanged, venomous colubrids (*Rhabdophis* spp.) in the Himalayan and north-eastern regions.

Elapid diversity in India is comprised of three types of snakes: the cobras (*Naja* spp.; *Ophiophagus hannah*), the kraits (*Bungarus* spp.), and the coral snakes (Fig. 7.6. *Calliophis* spp. and *Sinomicrurus* sp.). A total of four endemic species of

Fig. 7.2 Spectacled cobra (*Naja naja*)

Fig. 7.3 Common krait (*Bungarus caeruleus*)

coral snakes occur in the hills of southwestern India, one throughout most of peninsular India, and one in the Eastern Himalayas. Contrary to this, the Eastern Himalayas dominate the krait diversity with no less than five species of kraits. The Andaman Islands have one endemic krait. Apart from the king cobra, there are four species of true cobras in India, one found throughout the country, one restricted to Western Himalayas, one restricted to Northeast India, and one endemic to the Andaman Islands. Majority of the true sea snakes (Fig. 7.7) (*Hydrophis, Praescutata, Astrotia, Kerilia, Enhydrina, Lapemis, Pelamis*) occur in the warm tropical coastal waters, all over most of the Indian coast, while the sea kraits (Fig. 7.8. *Laticauda*) are distributed in Sundarbans, Andaman, and Nicobar coastlines.

Vipers in India are represented by three species of true vipers. One of them, the saw-scaled viper (*Echis carinatus*), is believed to be a species complex. Pit vipers (Fig. 7.9) are one of the most diverse groups of venomous snakes in India. Four

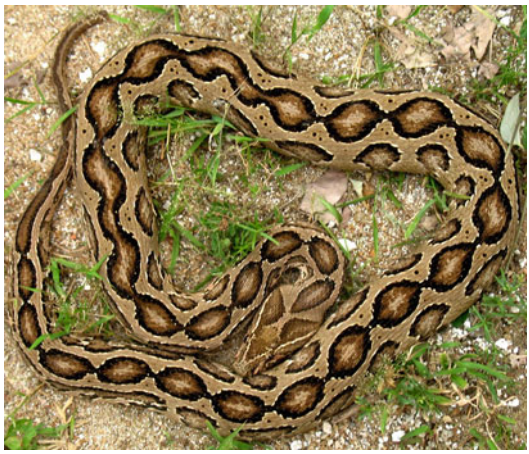

Fig. 7.4 Russell's viper (*Daboia russelii*)

Fig. 7.5 Saw-scaled viper (*Echis carinatus*)

Fig. 7.6 Striped coral snake (*Calliophis nigrescens*)

Fig. 7.7 Hook-nosed sea snake (*Enhydrina schistosa*)

Fig. 7.8 Yellow-lipped sea krait (*Laticauda colubrina*)

genera of pit vipers (*Gloydius, Ovophis, Protobothrops*, and *Trimeresurus*) are found in the Himalayas and Northeast India, whereas three (*Hypnale, Trimeresurus*, and *Tropidolaemus*) occur in the Western Ghats. The islands of Andaman and, more importantly, the Nicobar are also home to endemic species of pit vipers (*Trimeresurus* spp.). Thirteen species of pit vipers are found in the Himalayan region, six species occur in the Western Ghats, and four are found in the Andaman and Nicobar Islands. It is noteworthy that some species such as the Hutton's pit viper (*Tropidolaemus huttoni*) rank as enigmatic and poorly known snakes of tropical Asia.

Taxonomic obscurity imperils the venomous snake fauna of India. This is true even of the medically important ones. Examples include the Russell's viper *Daboia russelli* (Shaw and Nodder, 1797) sensu stricto now separated from *D. siamensis*

Fig. 7.9 Hump-nosed pit viper (*Hypnale hypnale*)

(Smith 1917) (*vide* Thorpe et al. 2007); the saw-scaled viper *Echis carinatus* (Schneider 1799) now separated from *E. c. sochureki* (Stemmler 1969) (*vide* Pook et al. 2009); the Indian cobra *Naja naja* (Linnaeus 1758) that was reviewed recently (*vide* Wüster 1998); the Sind krait species-complex *Bungarus sindanus walli* (Wall 1907) (*vide* Khan 1985); the hump-nosed pit viper *Hypnale hypnale* (Merrem 1820) that was revised recently (*vide* Maduwage et al. 2009); the Pope's pit viper *Trimeresurus popeorum* (Smith 1937) which was recently revised (Vogel et al. 2004); and the striped coral snake species-complex *Calliophis nigrescens* (Gunther 1862) (*vide* Whitaker and Captain 2008). The noteworthy generic reallocation of the enigmatic Hutton's pit viper *Tropidolaemus huttoni* (Smith 1949) by David and Vogel (1998) also deserves special mention here. The recent new descriptions like Gumprecht's pit viper *Trimeresurus gumprechti*; David, Vogel, Pauwels, and Vidal, 2002, Castoe's coral snake *Calliophis castoe*; and Smith, Ogale, Deepak, and Giri, 2012, and Protobothros himalayanus Pan, Chettri, Yang, Jiang, Wang, Zhang & Vogel, 2013 also underscore our incomplete knowledge of India's venomous snakes. Additionally, the recent "first records from India" of venomous snake species such as the Medo pit viper *Trimeresurus medoensis* Djao in Djao and Jaing, 1977 (see David et al. 2001), red-spotted Jerdon's pit viper *Protobothrops jerdonii xanthomelas* (Günther 1889) (see Zambre et al. 2009), and Kaulback's pit viper *Protobothrops kaulbacki* (Smith 1940) (see Bhide et al. 2008) also speak to our need for more precise knowledge on the venomous snake fauna of India (see checklist below, Table 7.1).

As may be seen from the above table, most species of venomous snakes in India are found in high rainfall areas with hilly tracts and evergreen forests. First, the Eastern Himalayas with several species of kraits and pit vipers, followed by both the Western Ghats Mountains and the Andaman and Nicobar Islands that harbor endemic pit vipers and elapids. As already mentioned, of the venomous land snakes, only four species are common and widespread across mainland India. These are the spectacled cobra (*Naja naja*), the common krait (*Bungarus caeruleus*), the Russell's viper (*Daboia russelii*), and the saw-scaled viper (*Echis*

Table 7.1 Checklist of venomous snakes of India (Sourced mostly form Whitaker and Captain (2008) and partly from others)

Sl. No.	Common name and *scientific name*	Distribution range within India	Status within its distribution range	Potency of venom	Incidence of bites	Effect of bites
1	Himalayan keelback *(Rhabdophis himalayanus)*	The Eastern Himalayas from Sikkim to Arunachal Pradesh	Common	It has no venom but has highly toxic saliva	Not many cases known	Severe reactions known from one case
2	Red-necked keelback *(Rhabdophis subminiatus)*	Eastern Himalayas from Sikkim and Assam to Arunachal Pradesh	Common	It has no venom but has highly toxic saliva	Not many cases known	Severe symptoms
3	Himalayan krait *(Bungarus bungaroides)*	Eastern Himalayas (Darjeeling district; Sikkim); Assam (Khasi Hills); Cachar	Rare	N.A. But kraits generally have potent venom	N.A.	N.A.
4	Banded krait *(Bungarus fasciatus)*	West Bengal, Bihar, Orissa, Assam upwards to Arunachal Pradesh, parts of Madhya Pradesh, Maharashtra, Northern Andhra Pradesh and UP	Common	Potent but not as potent as No. 5	Rare Reluctant to bite	One case of death known
5	Common krait *(Bungarus caeruleus)*	Most of mainland India	Common	Highly potent	Many cases	Many fatalities
6	Andaman krait *(Bungarus andamanensis)*	Andaman Islands	Uncommon	N.A. But kraits generally have potent venom	Not many known	No fatalities known
7	Black krait *(Bungarus niger)*	Sikkim, West Bengal, Assam, Meghalaya, and Arunachal Pradesh	Rare	N.A. But kraits generally have potent venom	N.A.	N.A.

8	Wall's Sind krait *(Bungarus sindanus walli)*	Gangetic plain, Central and Western India – Uttar Pradesh, Maharashtra, Bihar, and Bengal	Rare	N.A. But may be as toxic as No. 5	N.A.	N.A.
9	Lesser black krait *(Bungarus lividus)*	West Bengal, Assam, Eastern Himalayas	Rare	N.A.	N.A.	N.A.
10	Slender coral snake *(Calliophis melanurus)*	Probably most of Peninsular India. Definite records from Gujarat, Maharashtra, Karnataka, and Tamil Nadu. Also West Bengal	Rare	Mild	A few known	Slight swelling and itching
11	Striped coral snake *(Calliophis nigrescens)*	Western Ghats	Rare	N.A.	N.A.	N.A.
12	Beddome's coral snake *(Calliophis beddomei)*	Shevaroy, Nilgiri hills (Tamil Nadu), and Koppa (Karnataka)	Rare	N.A.	N.A.	N.A.
13	Castoe's coral snake *(Calliophis castoe)*	Western Ghats in South Maharashtra, Goa, and North Karnataka	N.A.	N.A.	N.A.	N.A.
14	Bibron's coral snake *(Calliophis bibroni)*	Western Ghats as far north as Agumbe (South Karnataka)	Rare	N.A.	N.A.	N.A.
15	MacClelland's Coral Snake *(Sinomicrurus macclellandi)*	Northeast India and Kasauli (Himachal Pradesh)	Rare	N.A.	N.A.	N.A.
16	Spectacled cobra *(Naja naja)*	Throughout mainland India	Common	Highly toxic	Many cases	Severe. Only a small % of bites fatal

(*continued*)

Table 7.1 (continued)

Sl. No.	Common name and *scientific name*	Distribution range within India	Status within its distribution range	Potency of venom	Incidence of bites	Effect of bites
17	Monocled cobra *(Naja kaouthia)*	North and Eastern India from Haryana, most of the Gangetic plains, West Bengal, Orissa, Sikkim, and Assam to Arunachal Pradesh. Probably in Uttar Pradesh and Bihar also	Common	Highly toxic	Many cases	Severe. Only a small % of bites fatal
18	Andaman cobra *(Naja sagittifera)*	Andaman Islands	Rare	N.A.	N.A.	N.A.
19	Central Asian cobra *(Naja oxiana)*	Jammu and Kashmir, Himachal Pradesh. Probably in Punjab, Rajasthan, and Gujarat also	Rare	N.A.	No record of bites from India	N.A.
20	King cobra *(Ophiophagus hannah)*	Western Ghats south of Goa, UP, Bihar, Orissa, West Bengal, the northeast to Arunachal Pradesh, and the Andaman Islands	Rare	Less toxic than Nos.16 and 17. But capable of injecting a large dose of venom	Rare	Four deaths in the last 30 years, all from South India
21	Common sea krait *(Laticauda laticauda)*	Off Kolkata coast and Nicobar Islands	Rare	N.A.	N.A.	N.A.
22	Yellow-lipped sea krait *(Laticauda colubrina)*	Andaman and Nicobar Islands	Common	Very potent	A few known	One or two deaths reported
23	Jerdon's sea snake *(Kerilia jerdonii)*	West coast (Kerala) and East Coast from Chennai to Puri	Uncommon	N.A.	N.A.	No fatalities reported

24	Viperine sea snake *(Praescutata viperina)*	Indian coasts	Rare	N.A.	N.A.	N.A.
25	Hook-nosed sea snake *(Enhydrina schistosa)*	Indian coasts	Common	Highly potent	Rare	Deaths reported
26	Black-banded sea snake *(Hydrophis nigrocinctus)*	Bay of Bengal (Sundarbans)	Rare	N.A.	N.A.	N.A.
27	Yellow sea snake *(Hydrophis spiralis)*	East Coast and occasionally on West Coast	Common	N.A.	Fatalities reported from outside India	N.A.
28	Annulated sea snake *(Hydrophis cyanocinctus)*	Indian coasts	Common	N.A.	N.A.	Fatalities reported
30	Bengal sea snake *(Hydrophis stricticollis)*	East Coast of India, north of Orissa	N.A.	N.A.	N.A.	Fatalities reported
31	Cochin banded sea snake *(Hydrophis ornatus)*	Coasts of India	Rare	High	A few known	Fatalities reported
32	Persian Gulf sea snake *(Hydrophis lapemoides)*	Coasts of India	Rare	N.A.	N.A.	N.A.
33	Bombay Gulf sea snake *(Hydrophis mamillaris)*	Coasts of India	Rare	N.A.	N.A.	N.A.
34	Malacca sea snake *(Hydrophis caerulescens)*	Between Mumbai and Karwar on the West Coast and from Chennai northwards to mouth of the Ganges on East Coast	Uncommon	N.A.	Generally inoffensive but will bite if provoked	Fatalities reported

(*continued*)

Table 7.1 (continued)

Sl. No.	Common name and *scientific name*	Distribution range within India	Status within its distribution range	Potency of venom	Incidence of bites	Effect of bites
35	Banded sea snake *(Hydrophis fasciatus)*	Coasts of India	Common	N.A.	N.A.	N.A.
36	Short sea snake *(Lapemis curtus)*	Coastal waters (more common on the West Coast)	Uncommon	Venom very toxic	Fatalities reported	Fatalities reported
37	Large-headed sea snake *(Astrotia stokesii)*	Bay of Bengal	Rare	High	N.A.	N.A.
38	Common small-headed sea snake *(Hydrophis gracilis)*	Gujarat and coasts of India	Common	N.A.	N.A.	N.A.
39	Cantor's narrow-headed sea snake *(Hydrophis cantoris)*	Western Coast of India	Common	N.A.	N.A.	N.A.
40	Black and yellow sea snake *(Pelamis platura)*	Indian coastal waters and Andaman and Nicobar Islands	Uncommon	N.A.	Usually inoffensive; bites if handled	No fatalities reported
41	Russell's viper *(Daboia russelii)*	Throughout India	Common	Potent	Cause as many, or more, snakebites than cobras	Usually not fatal but life-threatening

42	Levantine viper *(Macrovipera lebetina)*	A few localities in Kashmir including Srinagar and Dachigam	Uncommon	Toxic venom but not much known	N.A.	No known fatalities from India
43	Saw-scaled viper *(Echis carinatus)*	Throughout mainland India except West Bengal and the northeast	Common	Very toxic	Many cases	Many fatalities
44	Himalayan pit viper *(Gloydius himalayanus)*	The Western Himalayas – Kashmir, Himachal Pradesh, Northern Punjab, Uttar Pradesh, and Uttaranchal	Common in parts of range	Not particularly toxic	A few known	Localized pain and swelling; no fatalities reported
45	Hump-nosed pit viper *(Hypnale hypnale)*	Western Ghats south of Belgaum	Common	Quite toxic	A few known	Considerable swelling and pain. Life-threatening symptoms. But no fatalities reported from India
46	Mountain pit viper *(Ovophis monticola)*	Uttaranchal, Sikkim, West Bengal, Assam, Manipur, Meghalaya, and Nagaland to East Arunachal Pradesh	Common	Little is known	A few known	Much pain, swelling, and continuous bleeding. No fatalities recorded in India
47	Brown-spotted pit viper *(Protobothrops mucrosquamatus)*	Assam (the Naga Hills)	Rare	N.A.	N.A.	N.A.
48	Jerdon's pit viper *(Protobothrops jerdonii jerdonii)*	Northeast from Meghalaya to Arunachal Pradesh	Rare	N.A.	N.A.	N.A.
49	Jerdon's red-spotted pit viper *(Protobothrops jerdonii xanthomelas)*	West Kameng district, Arunachal Pradesh	N.A.	N.A.	N.A.	N.A.

(*continued*)

Table 7.1 (continued)

Sl. No.	Common name and *scientific name*	Distribution range within India	Status within its distribution range	Potency of venom	Incidence of bites	Effect of bites
50	Kaulback's pit viper *(Protobothrops kaulbacki)*	Papum Pare district, Arunachal Pradesh	N.A.	N.A.	N.A.	N.A.
51	Himalayan spotted-pit viper *(Protobothrops himalayanus)*	Chungthang, northern Sikkim	N.A.	N.A.	N.A.	N.A.
52	Large-scaled pit viper *(Trimeresurus macrolepis)*	Southern Western Ghats	Common	Not very toxic	N.A.	Local pain and swelling. No fatalities reported
53	Malabar pit viper *(Trimeresurus malabaricus)*	Western Ghats from Maharashtra to Kanyakumari	Common	Low	A few known	Pain and swelling
54	Horseshoe pit viper *(Trimeresurus strigatus)*	Western Ghats south of Nilgiris	Uncommon	Low	A few known	Pain and swelling
55	Bamboo pit viper *(Trimeresurus gramineus)*	Northern Western Ghats, Eastern Ghats, and central Indian ranges	Common	Low	A few known	Pain and swelling
56	Yunnan pit viper *(Trimeresurus yunnanensis)*	Uttaranchal, Assam, and Himalayas	N.A.	N.A.	N.A.	N.A.
57	Medo pit viper *(Trimeresurus medoensis)*	Arunachal Pradesh	Rare	Low	A few known	Pain and swelling

58	Pope's pit viper *(Trimeresurus popeorum)*	Sikkim, Meghalaya, West Bengal	Uncommon	Low	A few known	Pain and swelling Bite from a large snake could be lethal
59	Cantor's pit viper *(Trimeresurus cantori)*	Central Nicobar group of islands	Common	Low	A few known	Not serious. One fatality reported from Central Nicobar
60	Andaman pit viper *(Trimeresurus andersoni)*	Andaman Islands	Common	Low	A few known	Local pain, swelling, and necrosis. No deaths reported
61	Spot-tailed pit viper *(Trimeresurus erythrurus)*	Sikkim, Nagaland, West Bengal, and probably more states in the northeast	Uncommon	N.A.	N.A.	Effects unknown, but a bite from a large snake could be serious
62	White-lipped pit viper *(Trimeresurus albolabris)*	West Bengal and Assam	Uncommon	Low	A few known	Local pain and swelling. Bite from a large snake could be serious or even fatal
63	Nicobar pit viper *(Trimeresurus labialis)*	Nicobar Islands	Common	N.A.	N.A.	N.A.
64	Himalayan white-lipped pit viper *(Trimeresurus septentrionalis)*	Lower Himalayas in Himachal Pradesh, UP, Uttarakhand, and Bihar	N.A.	N.A.	N.A.	N.A.
65	Gumprecht's green pit viper *(Trimeresurus gumprechti)*	Northeast Indian hill ranges	N.A.	N.A.	N.A.	N.A.
66	Hutton's pit viper *(Tropidolaemus huttoni)*	High Wavys (southeast of Madurai, Tamil Nadu)	N.A.	N.A.	N.A.	N.A.

N.A. information not available

carinatus), commonly called the "Big Four." At present, antivenom is commercially available only for these four species and, that too, only as a polyvalent serum that is used for the bites of all these four species. These four species due to their commonness, occurrence even in and around human habitations, highly toxic venom, and propensity to bite humans have gained utmost medical importance. But it is now increasingly realized that spice-plantation workers in India's evergreen hill forest tracts and fishermen along the coasts encounter snakebite hazard from other lesser-known species of venomous snakes like pit vipers, coral snakes, and sea snakes that have a restricted range within India (Simpson 2007).

Snakebite Symptoms: The Problems and Paradoxes

Discussed below are the symptoms of bites from India's Big Four venomous snakes. Cobras and kraits have neurotoxic venom, while vipers have hemotoxic venom. Apart from antivenom therapy, neurotoxic envenomation may require artificial respiration, while hemotoxic envenomation may require blood transfusion, hemodialysis, and tissue transplant. These aside, antivenom therapy itself may require administration of steroids (antihistamines, adrenalin, dexamethasone) to combat possible anaphylactic shock. Cobra venom contains postsynaptic neurotoxin, but krait venom is a presynaptic neurotoxin and hence much more lethal as the paralysis is difficult to reverse (Murphy 2010). A cobra bite may or may not be painful and causes inflammation, edema, necrosis, drowsiness, ptosis, nausea and vomiting, frothing at the mouth, paralysis, asphyxia, and ataxia. Krait bite is similar to cobra bite except that pain at the bite site and frothing at the mouth and necrosis are absent but, unlike in cobra bite, violent abdominal pain will be present, typically a few hours after the bite. Death is due to respiratory or cardiorespiratory failure. Russell's viper and saw-scaled viper venom causes fierce, burning pain at the bite site; inflammation; edema; necrosis; internal and external hemorrhages, often also from body orifices; and renal failure. Death is due to heavy blood loss. Rarely, in chronic cases, weakness, secondary sexual hair loss, menstruation abnormalities, testicular atrophy, hypothyroidism, and hypopituitarism may manifest themselves even months or years after the snakebite (Eapen et al. 1976; Gopalakrishnakone et al. 1990; Gundgurthi et al. 2012; Uberoi et al. 1991; Warrell 2005; Whitaker 1978).

Several Indian pit viper bites have been reported to be serious. Examples include the hump-nosed pit viper (Ariaratnam et al. 2008; Karunathilaka et al. 2001; Maduwage et al. 2011; Menon et al. 2007; Premawardena et al. 1998; Sunanda et al. 2010) found in the Western Ghats of Southwestern India; the purple-spotted, white-lipped, and Stejneger's pit vipers (Chan et al. 1993; Chen et al. 2007; Rojnuckarin et al. 1998; Soogarun et al. 2006; Soogarun et al. 2007; Yang et al. 2007) found in Northeastern India; and the Cantor's pit viper found in the Nicobar Islands. The main symptoms of pit viper envenomation reported (*op. cit.*) in human beings are coagulopathy, fibrinolysis, necrosis, blistering, regional

lymphadenopathy, disseminated intravascular coagulation, retroperitoneal hemorrhage, encephalopathy, mucosal and cutaneous bleeding, hypofibrinogenemia, and thrombocytopenia.

Sea snake bites are also being intensively studied and consensually accepted as being potentially lethal to human beings (Chetty et al. 2004; Marsden and Reid 1961; Sitprija et al. 1971; Reid 1957, 1961, 1962; Reid and Lim 1957). The main symptoms of systemic sea snake envenomation reported (*op. cit.*) in human beings are postsynaptic neurotoxic activity, attenuated twitch blockade, degenerative changes in the central nervous system, petechiae and ecchymoses throughout the viscera, distal tubular necrosis in the kidney, lung emphysema and patchy edema, slight endocardial fibrosis, coronary sclerosis, centrilobular degeneration and necrosis in the liver, porter round-cell infiltration in the liver, and myoglobinuria.

Snakebite Statistics

Even leaving aside the lesser-known venomous snake species that have a restricted distribution, there does not exist clear statistics of snakebite in India, even concerning those presumably related to the Big Four. The following account illustrates this scenario.

Mohapatra et al. (2011) after conducting exhaustive interviews mention the annual snakebite deaths in India to be 45,900, with the states of Uttar Pradesh (8,700), Andhra Pradesh (5,200), and Bihar (4,500) in the lead.

On the other hand, a recent statement by India's Health Minister, on this subject, in the Indian Parliament in April 2012, based on statistics reported from govt. hospitals mentions the annual snakebite deaths in India to be only 1,440, with West Bengal (380), Orissa (296), and Andhra Pradesh (258) having the highest incidence. However, notwithstanding that the information was given in Parliament, the fact is that this is a highly imperfect compilation of data collected from govt. hospitals in various states, since it is well known that hospital records, the only available source for raw data in regard to snakebites, are often incomplete or misleading. The reasons for this are as follows:

(a) Most of the snakebite deaths occur in the rural areas, and the immediate response of the friends or relatives of the victim is to take the patient to the so-called native doctor who has no competence to treat snakebite; or, they try various native remedies. These cases are not reflected in the statistics collected by official agencies.
(b) Even when the victim is taken to a qualified doctor, it need not be a regular hospital but a private clinic or the doctor's house where there may be no arrangement for documentation of such cases.

In the result, only a minority of incidents are accounted for by the hospital records accessed by the official agencies. It is not also clear whether the hospitals which had furnished the data compiled by the Minister are only govt. hospitals or all hospitals, which can make a significant difference. Also,

it can well happen that many of the hospitals, particularly nongovernment hospitals would not have responded to the government queries at all.

(c) Then again, these records may not be reliable because, as experience shows, neither the victim nor his minders nor the doctors would have been in a position to clearly identify whether it is a snakebite at all and, if a snakebite, whether it is a venomous or nonvenomous snake or what species.

(d) There are no foolproof procedures for collection of data even from such hospitals which maintain such records. These are reflected in the following features of statistics furnished in the Parliament. The following states have reported nil figures which are unlikely to be factually correct: Andhra Pradesh, Manipur, Mizoram, Nagaland, Sikkim, Jharkhand, D & N Haveli, Daman and Diu, and Lakshadweep. The following states have reported single-digit figures: Assam (6), Goa (2), Haryana (7), Jammu and Kashmir (3), Meghalaya (1), Punjab (4), Rajasthan (8), Tripura (1), Uttarakhand (1), Andaman and Nicobar (3), and Delhi (3). No reports have been furnished by Bihar and Chandigarh. This too is of doubtful validity. The unacceptability of nil or single-digit figures is confirmed by the proximity of the states concerned to other ecologically similar states that have featured high snakebite incidence in the details furnished by the Minister.

Unfortunately, Indian annual snakebite statistics is bedevilled by many complications, and there is no consensual estimate of snakebites and snakebite deaths in India. A quote from Vijayaraghavan (2008) will be in order here: "Quoting the National Crime Record Bureau, Ministry of Home Affairs, Govt. of India, 1998, the '*Statistical Abstract India – 1998*' published by the Central Statistical Organisation gives a figure of 18,907 for deaths from 'poisoning' in India in 1998 but that includes not only snakebite but also bites from other animals and food poisoning, consumption of spurious liquor, intake of poisonous gases and so on. Deoras (1971) gives a figure of 15,000 for 1953 though how the figure was arrived at is not known. In the course of a book review, Whitaker (2007) says: "Admittedly, we have no reliable statistics for incidence of snakebite and mortality for India (the country alleged to have the highest snakebite mortality worldwide), but informed guesstimates have rarely exceeded 20,000 deaths." A disturbing estimate of "between 35,000 and 50,000" is given by Warrell (2005). Here again, no source is indicated and the para itself begins with the admission that there are "no reliable national statistics."

Later, in 2008, Whitaker himself along with Ashok Captain mentions the figure of snakebite deaths in India to be 10,000–50,000 although no supporting data as to how they so concluded was present (Whitaker and Captain 2008). Mohapatra et al. (2011) reported 45,900 Indian annual snakebite deaths, after conducting "a nationally representative study of 123,000 deaths from 6,671 randomly selected areas in 2001–03."

All in all, like someone said about something else, snakebite in India (as elsewhere) is a leading cause of statistics".

If the conflicting figures of Indian annual snakebite statistics are a problem, then the question as to which species among the "Big Four" could potentially be the

principal cause of snakebite is another. While the spectacled cobra (*Naja naja*) is considered to be the most common in all of India, except perhaps the northwest where the saw-scaled viper (*Echis carinatus*) abounds, the Russell's viper (*Daboia russelii*) is consensually believed to be the snake that causes the most number of bites among the "Big Four," and the common krait (*Bungarus caeruleus*), because of its habit of living around houses and its powerful presynaptic neurotoxin and its bite being painless, rarely attracting the notice of the sleeping victim until it is too late, is believed to cause the most of snakebite deaths. This stands so despite the subject being debated by some (Simpson 2007). The Big Four's snakebites have received medical attention dating back to Wall (1883). Unfortunately, none of the aforesaid information is based on sound, quantitative, scientific evidence. The probable reasons for this situation could possibly be that most snakebite victims just do not tend to identify the responsible snake, either because of lack of opportunity to observe, and hence with only envenomation symptoms to guide them, most doctors are left helpless to zoom in any further than elapid or viper envenomation. Recent advancements such as enzyme-linked immunosorbent assay (ELISA) must be implemented to improve the current situation. To add to these, although local geo-climatic variations do apparently shape the patchy and skewed Indian distribution of the widespread "Big Four," there is a serious lack of convincing data to objectively demonstrate this.

Mildly Venomous Snakes

Apart from the better-studied front-fanged venomous snakes (Fig. 7.10 elapids and Fig. 7.11 vipers), there are many rear-fanged venomous snakes (Figs. 7.12 and 7.13, colubrids) whose venom, its composition, toxinology, and snakebite symptoms and consequences remain poorly known. In India, several genera of rear-fanged snakes are reported to be "mildly venomous" and two species even "potentially harmful to humans" (Whitaker and Captain 2008). "Mildly venomous" snakes are those

Fig. 7.10 Short, fixed fang of cobra (elapid)

Fig. 7.11 Curved, hinged fang of Russell's viper

Fig. 7.12 Indian cat snake (*Boiga trigonata*)

Fig. 7.13 Rear fang of vine snake (colubrid)

rear-fanged ones whose Duvernoy's gland secretions kill or paralyze their intended prey species (like lizards, frogs, and mice) but seldom harm humans beyond causing mild local swelling and bleeding.

The "mildly venomous" Indian snakes comprise the following genera: *Ahaetulla*, *Chrysopelea*, *Platyceps*, *Psammodynastes* (Colubrinae), *Amphiesma*, *Pseudoxenodon* (Natricinae), *Boiga*, *Cerberus*, *Diurostus*, *Ferania*, *Fordonia*, *Gerarda* (Homalopsinae), and *Psammophis* (Psammophinae) (Whitaker and Captain 2008; Weinstein et al. 2011). The three potentially harmful Indian natricine snakes are *Rhabdophis subminiatus*, *R. nuchalis*, and *R. himalayanus*. Many bites from the mildly venomous snakes have caused considerable local envenomation symptoms in humans, including swelling and prolonged bleeding at the bite site.

Moreover, recent studies have shown that even species such as *Amphiesma stolatum* and *Coelognathus radiata* that have long been regarded as nonvenomous do have toxic saliva (Hill and Mackessy 2000; Mackessy 2002; Lumsden et al. 2004). Hill and Mackessy (2000) caution us that *Amphiesma stolatum* has to be handled with "extreme care," stating that its Duvernoy's gland secretion shows marked endoproteolytic activities. Mackessy (2002) remarks that *A. stolatum* could potentially cause hemorrhage due to the presence of at least six metalloproteases in its venom. Interestingly, native belief has it that this is the most harmless snake. Similarly, in the case of *Coelognathus radiata*, a study by Lumsden et al. (2004) reports the presence in it of "potent three finger neurotoxins, a toxin type previously thought to be unique to elapid venom." However, as detailed as these studies are, the two aforementioned colubrids have not produced any serious bites to humans, warranting further studies.

In India, the only potentially venomous rear-fanged snakes that have been proved to cause severe envenomation in humans are the two *Rhabdophis* species (*R. himalayanus* and *R. subminiatus*) found in northeastern hill states (Whitaker and Captain 2008; Weinstein et al. 2011; vide Table; sp. 1 & 2). *Rhabdophis* venom secreted by their Duvernoy's glands are largely hemotoxic causing hemorrhage and blood damage, so much so that *R. tigrinus* (a related, Japanese, species) bite symptoms were neutralized by saw-scaled viper antivenom (Mackessy 2002). Monovalent antivenom from *R. tigrinus* venom effective against its bite has also been more recently prepared. A toxin named "tigrin" which is characterized as a "CRISP 25 kD protein" has also been isolated from *R. tigrinus* venom (Mackessy 2002). It has also been discovered that these polyclonal antibodies cross-reacted with similar-sized proteins from several elapid and viper venoms (Mackessy 2002).

Curiously, *Rhabdophis* species have a remarkable feature shared among Asian natricine snakes. They have a series of glands on their neck that secrete toxin. These glands, called the "dorsonuchal glands," are a series of paired glands separated by an intermediary groove, the entire structure being embedded under the skin along the dorsum of the snake's neck. These glands have neither lumina nor ducts but are reported to easily rupture under pressure releasing a secretion (Stuebing and Lian 2002). The primary toxin present in these glands is a type of cardiac steroid named

gamma-bufotalin or bufadienolides, which, as the name suggests, is a toxin secreted by toads – the keelback's staple prey. Recent studies (Hutchinson et al. 2007; Mori et al. 2011) on a Japanese congener, *R. tigrinus*, have shown that the toxin is not secreted per se by the dorsonuchal glands but is sequestered from its main prey – the toads. Other species such as *Macropisthodon*, although reported to have these dorsonuchal glands (Weldon et al. 2008), are not considered dangerous to humans (Weinstein et al. 2011).

Indian Snake Antivenom Centers

Whitaker and Whitaker (2012) reported the following antivenom production centers in India, belonging to both public and private sectors. These are either active at present or were in the past. The list below gives their names, year of establishment, location, website (accessed in March 2013), and information regarding their antivenin production:

1. Haffkine Institute (http://www.haffkineinstitute.org) estd. 1899 in Mumbai, Maharashtra. Public Sector.
2. King Institute (http://www.tnhealth.org) estd. 1899 in Chennai, Tamil Nadu. Public sector. In 1970 antivenom production started.
3. Bengal Chemicals & Pharmaceuticals Ltd. (http://www.bengalchemicals.com) estd. 1901 in Kolkata, West Bengal as a private company later taken over by Govt.
4. Central Research Institute estd. 1904 in Kasauli, Himachal Pradesh. Public sector. Antivenom production stopped in 2007.
5. Biological E Limited (http://www.biologicale.com) estd. 1953 in Hyderabad, Andhra Pradesh. Private sector.
6. Serum Institute of India (http://www.seruminstitute.com) estd. 1966 in Pune, Maharashtra. Private sector. Antivenom production stopped in 2008.
7. Bharat Serums and Vaccines (http://www.bharatserums.com) estd. 1971 in Mumbai, Maharashtra. Private sector.
8. Mediclone Biotech (http://mediclonebiotech.com) estd. 1995 in Chennai, Tamil Nadu. Private sector. Recently started antivenin production.
9. VINS Bioproducts Limited (http://www.vinsbio.in/) estd. 2000 in Hyderabad, Andhra Pradesh. Private sector.

Conclusion and Future Directions

From the present account, it is clear, considering India's venomous snake diversity and the high human population in parts of the biodiversity hotspots such as Northeast India and the Western Ghats, that snakebite is a major health hazard. Additionally, the practical difficulties faced in arriving at a consensual estimate of

snakebites and snakebite mortalities in India must be investigated and rectified. Probable causal factors such as the methodology of collection and compilation of primary data, the time span for the study, and the presence of distinct ecoregion (s) and the consequent additional venomous snake species in a state must be considered. Some words of caution are in order here. It must be borne in mind that states having the highest number of species of venomous snakes or human population need not necessarily top the annual Indian snakebite statistics. More important are the population of rural sector where man-snake interaction is more frequent and the extent of natural or anthropogenic habitat(s) occasioning man-snake interactions (e.g., paddy fields), vulnerable occupations (e.g., plantation work, sea fishing), and finally the correct follow-up actions of quantitative documentation of snakebites and snakebite deaths in every state.

As regards snake venom and/or antivenom production in India, firstly, the intraspecific variations in venom composition and the consequent need to distribute at least the venom-harvesting centers regionally must be understood and necessary action taken. Secondly, the other venomous snake species that have largely been overlooked till recent years need to receive attention, considering the human population in such ecoregions and the occupational hazards they encounter, e.g., fishermen along the coastlines with respect to sea snakes and plantation workers of the hills with respect to pit vipers, king cobra, coral snakes, and other elapids (if any). Although some venomous snake species may not necessitate antivenom for the treatment of their bites, at least the precise consequences of their bites and the associated medical interventions necessary need to be studied.

Some major issues for improving the present scenario as regards snakebite and treatment in India are summarized as follows (see Vijayaraghavan 2008 for details). In snakebite, there are severe limitations to first aid. The only reliable treatment is antivenom and this must be administered preferably $<$24 h. when there is an indication. Child mortalities caused by impure vaccine used against microbial diseases should also alert us as to the precautions necessary while preparing antivenom. Rigorous public awareness campaigns, adequate stock of good quality antivenom, requisite life-support systems, and well-trained doctors are imperative to combat India's snakebite problem.

The cost of antivenom should be brought down as much as possible, without compromising its quality and quantity. One vial (10 ml.) of polyvalent snake antivenom of equine origin presently costs INR 300–500 (US$ 6.50–11.00), while it is supplied by antivenom producers to govt. hospitals for INR 115 (US$ 2.50) (Whitaker and Whitaker 2012).

The World Health Organization (WHO), which prescribes the guidelines for snakebite treatment, should ensure a comprehensive coverage of snake farms for venom extraction to ensure proper procedures, collection of snakes from as large a population as possible within its geographic range, determination of pool size for venom, quality control, selection and care of the horses for immunization, etc. The concerned government authorities must scrupulously enforce the WHO guidelines.

Cross-References

- ▶ Complications of Hemotoxic Snakebite in India
- ▶ Diversity and Distribution of Medically Important Snakes of India
- ▶ Snakebites in Tamil Nadu, India

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Snakebites in Tamil Nadu, India

8

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Abstract

Snakebite is prevalent all over India including the state of Tamil Nadu. It is observed in all ages and more among men and women involved in agricultural and plantation works. Often encountered snakebites in Tamil Nadu are in the order of viper group, krait, and cobra. Sea snake bites are extremely rare.

The challenges faced by the state toward the management of snakebite were related to community, policy, transport, governance, and therapy. The government has overcome these challenges through the Tamil Nadu Health Systems Project. The health-care professionals and providers were trained to provide appropriate treatment and care. The success was evidenced by early health-seeking behavior; provision of free transport facilities and specific treatment at primary, secondary, and tertiary health institutions as per guidelines; reduced bite-to-needle time, referral rate, morbidity, and mortality; and increased consumption of anti-snake venom. During the period April 2011 to March 2013, the overall referral rate and

death rate were 3–5 and 0.6 % respectively in non-teaching hospitals/health centers, and consumption of anti-snake venom of the state during that period was 435,815 vials (10 ml). The services and supply of materials toward treatment are monitored at district and state level, and difficulties or deficiencies encountered were rectified.

The symptoms, signs, complications, diagnostic aspects, treatment, snakebite in special situations including pregnancy, prevention, community aspects, and futurology are discussed. The political commitment, effective bureaucracies, and good governance at health department have saved the lives of many snakebite victims.

Introduction

Snake is well known to human race, as it has been associated with many epics, myths, superstitions, folklore, tales, and so on. Snakes are also considered as sacred, and certain rituals are followed while the snakes are found or killed. The biological aspects of snake are a source of wonder and admiration. On the contrary, snakebite is devastating and often affects the poor and downtrodden, more so those living in Africa and South Asia especially India. Historically, Alexander the Great appreciated the efficiency of earlier Indian physicians treating snakebite victims. Snakebite kindled interest among many in India and is debated over centuries (Vijayaraghavan 2008). However, it does not receive due attention as it deserves from policy makers, professionals, pharmaceuticals, and public. India has achieved a lot in health but has to do much more in snakebites.

Looking into the status of snakebite in tropical countries, the World Health Organization (WHO) has declared snakebite as a neglected tropical disease. To get true data on snakebite, WHO recommends that snakebite should be formally recognized as an important occupational disease and should be made as a notifiable disease in all countries in the Southeast Asian (SEA) region (Warrell 2010a). Snake envenoming warrants urgent medical attention and has to be considered as an important health issue. Considering the gravity of snakebite issues in the Southeast Asian (SEA) region (Alirol et al. 2010), the SEA regional office of WHO released guidelines (Warrell 2010b) to improve the management of snakebite cases and to save human lives and mitigate misery due to it. Still, snakebite remains an underestimated cause of accidental death in modern India (Mohapatra et al. 2011). Global burden of snakebite with special reference to regional estimates on envenoming and death has been reviewed by Kasturiratne et al. (2008). Considering the status, global initiatives for snakebite were mooted by Williams et al. (2010).

Tamil Nadu

Tamil Nadu is one of the states of India, located on the southernmost part of India. It includes a wide range of and has a rich variety of flora and fauna. Agriculture is the main source of living. It has an area of 130,058 km^2 with a total population of 72,138,958 as per census 2011 and males constitute 50.1 %. The rural urban ratio is

3.7:3.4 with literates more among males than females (86 % and 73 % respectively) and more in urban than rural areas. There are 32 revenue districts in the state of Tamil Nadu.

Snakes and Snakebite in Tamil Literature

Tamil language is an ancient language. The poems and proverbs of the Tamil language describe the status of living at that time, highlight their knowledge, express talents, reflect culture, bring out traditions, and reveal their beliefs and practices, though the place of origin of many is not available. One can also appreciate the changes that had happened over a period of time through literature. Good amount of information related to snakes and snakebite (Jeganathan 1983) is available in Tamil literature and Tamil medicine (Siddha). Descriptions on the use of venom to reduce the pain of wounded soldiers were made available in Tamil epigraphy. Proverbs irrespective of the language are thought provoking and carry rich information. These proverbs help to transfer relevant facts with beauty and brevity between the speaker and the audience or readers. Moreover, poems and proverbs act as a bridge between the health-care professional and the patients or public to convey health messages convincingly and clearly within few minutes. In fact, the descriptions may be used to educate the community and considered for research. However, all these need further exploration.

Snakes

Of the 2,968 species of snakes in the world, nearly 560 (19 %) are venomous (Vijayaraghavan 2008, 2010). Most of the venomous species are in families of Elapidae and Viperidae, all front fanged. Elapids and vipers constitute some 535 venomous species. In India, there are nearly 276 species of snakes. Among them, 62 (26 %) are venomous, 42 mildly venomous, and 172 are nonvenomous. Thus, India has rich assortment of snake fauna. The details of 62 snakes such as their common name, scientific name, distribution range, venom potency, and bite aspects (incidence and effect) are well described by Vijayaraghavan (2010). Of the 62 venomous snakes, 20 are sea snakes.

In India, venomous snakes belong to three broad families such as Elapidae (cobra, kraits, mambas, and tiger snake), Viperidae (Viperinae-Russell's viper and saw-scaled viper and Crotalinae-pit viper), and Hydrophiidae (sea snakes). The four various snakes widely distributed in India are the Indian cobra (i.e., the spectacled cobra and the monocled cobra), the common krait (*Bungarus caeruleus*), the Russell's viper (*Daboia russelii*), and the saw-scaled viper (*Echis carinatus*). These four are called as the big four, and hence anti-snake venom (ASV) is raised against these four. Among them, there are four species of cobra, eight species of krait, and two distinct subspecies of saw-scaled viper (Whitaker and Whitaker 2012). Apart from the four, envenoming following *Hypnale hypnale* has been reported from Southern (Joseph et al. 2007; TNHSP 2008) than Northern India.

The newborns and the young ones of venomous snakes are dangerous as these have venom glands that are fully functional. In fact, the venom of newborns and juveniles is more potent than adults of the same species. Mildly venomous means they produce local symptoms but no serious complication or death. Dry bite is a bite wherein little or no venom is injected. In Indian conditions, nearly 50 % of bites are dry bites, and in sea snakes, dry bites are as high as 70 %. In saw-scaled viper, dry bite constitutes 10 %. However, the dry bites vary with species and circumstances (Vijayaraghavan 2008).

Some of the warning signals of snakes are hissing sound by cobra and Russell's viper, rasping sound by rubbing its scales together by saw-scaled viper, spreading the hood by cobra, vibrating the tail by keeping itself in coiled position by some of the vipers such as saw-scaled viper, bamboo pit viper, hump-nosed pit viper, etc. (Vijayaraghavan 2008).

Snakebites are more during monsoon and post-monsoon times. The ratio between venomous and nonvenomous bites is 3 or 4:1. The fang marks are visible in about 90–95 % of occasions. Occasionally, scratch marks are noticed. Absence of fang marks does not exclude snakebite. The location of bite is invariably outdoor in about 80–85 % of times. The anatomical site of bite is in the order of lower limb (70–90 %), upper limb (15–25 %), others (1–5 %), and multiple (less than 1 %).

Chemical Components of Venom

The snake venom contains numerous enzymatic and nonenzymatic proteins and toxins (Mukherjee 2013) which are responsible for imparting toxicity in victims. Some of the enzymes are phospholipases, phosphodiesterases, amino acid oxidases, acetylcholinesterases, proteolytic enzymes, arginine esterases, nucleosidases, hyaluronidases, etc., and peptides such as neurotoxins, cytotoxins, myotoxin, cardiotoxins, disintegrins, etc., so the systemic manifestations of snakebite depend upon various polypeptide toxins present in the venom. In view of the multiple chemical components in snake venom, its use for other diseases is a potential area for research (Meenakshisundaram et al. 2009).

Risks, Severity, and Fatality in Snakebite

Rural agricultural workers are at risk for snakebite. Thus, the people involved in cutting grass, working in plantation activities such as rubber, areca nut, coconut, tea, coffee, etc., harvesting, picking vegetables or fruits, clearing weeds, walking at night with barefoot and without torch or along the edge of waterways, etc. are prone for snakebite (Warrell 2010b; TNHSP 2008).

Severity of snakebite (Vijayaraghavan 2008; Warrell 2010a) differs from case to case and snake to snake even when the snakes belong to the same species. In fact, it may be considered under factors related to individuals, snakes, and health-care systems. Factors related to individuals are age, gender, body mass index, habits

(alcohol), physical activity after snakebite, delay in health-seeking behavior, types of first aid adopted, coexisting health status, etc. Those related to snakes are quantity and quality of venom, nature, age, sex, health status, season, time of bite (before or after meals), intention, number and nature of bite, bite over the cloth, and distance while striking. Health-care system-related issues are availability of doctors and their confidence to treat, consistent supply of anti-snake venom and supportive measures, and delay in administration of antivenom.

Most fatalities in snakebite are due to any one of the following or a combination of them (David et al. 2012; Patil et al. 2011; Punde 2005; Saravu et al. 2012), and these are adherence to traditional practices, influence of elders and neighbors in decision making, bias against modern health care, delay in health-seeking behavior, non-availability of transport of victims, not reaching the hospital in time, not providing adequate quantity of ASV, non-availability of ASV and other required medicines in hospitals, lack of confidence among health-care providers to treat such cases, etc.

Deaths Following Snakebites

Mohapatra et al. (2011), based on their snakebite mortality study in India, have stated that death due to snakebite remains underestimated in India. However, they have estimated the age-standardized death rate due to snakebite per 100,000 per year was 4.1(99 %, CI 3.6–4.5) with higher rates, i.e., 5.4 (99 %, CI 4.8–6.0) in rural areas of India. It was clear that victims were males more than females and peaking at ages 15–29 years. Annual death was greatest in the state of Uttar Pradesh, Andhra Pradesh, and Bihar wherein the accessibility and availability of health care is far less. In Tamil Nadu, the estimated death rate due to snakebite is three per 100,000 per annum in contrast to nine in Uttar Pradesh, five in Andhra Pradesh, and four in Bihar. Death occurs more among farmers/laborers and during monsoon season. Thus the Million Death Study (Mohapatra et al. 2011) estimated that nearly 50,000 people die of snakebite every year in India. The risks involved and factors contributing to severity and fatality in snakebite are described vide supra.

Challenges Related To Snakebite in Tamil Nadu

Many challenges related to the management of snakebite in the state of Tamil Nadu before 2007 were broadly grouped under community, policy, transport, governance, and therapy. Community followed various traditional methods (Vijayaraghavan 2008) such as biting the body of snakes, eating the body parts of snake, or crushing the head of snake; applying goat's cheese, ash, olive oil, snakestone, herbal extract or paste, chicken blood, or animal excreta; immersing the bitten part in goat's milk; drinking fresh urine (human) of opposite sex or alcohol; applying tourniquet, ethylenediaminetetraacetic acid (EDTA), or potassium permanganate; slashing the site of bite by knife or razor; cutting the part of bitten finger or toe; sucking the blood from the site of bite by mouth or using extractor; and application of

electro- or cryotherapy. Thus the risks involved for snakebite and factors contributing to severity and fatality were high.

The doctors and nurses working in various government hospitals/health centers were not confident to treat snakebite victims or did not follow standard methods to treat or refer to higher centers. ASV was repeatedly given at 6-h interval based on symptoms, and they have used ASV to the tune of 100 or more vials at times to victims of snakebite. The state government did not have any policy on teaching and training in-service doctors and nurses on snakebite or supply of injection of ASV and other drugs required to treat snakebite cases uninterruptedly. Also, the government did not make any provisions or policy to transport the snakebite cases from the place of bite or living to the nearest hospital/health center. The health department did not have any guidelines or measurable methods to govern the supply of injection ASV and other medicines required to manage snakebite or monitor the treatment aspects and outcome of snakebites at different hospitals/health centers.

Government's Initiatives

The existing status of snakebite, health needs, and the challenges were realized by the government, and the government wanted to reform them. Hence, Tamil Nadu Health Systems Project (TNHSP) was designed, and health reforms were implemented. The Health and Family Welfare (H&FW) Department of the state government of Tamil Nadu in late 2007 issued orders (Tamil Nadu Government order (2D). No125. Health and Family Welfare EAPI/1) Department, dated 02.11.2007) to train doctors and nurses on snakebite management and educate the community. As a result, pictorial charts on identification of snakes, details of important venomous snakes, snakebite treatment guidelines for primary, secondary, and tertiary health centers, signs and symptoms of snakebite, first aid, clinical aspects, toxic manifestations, and prevention of snakebite were prepared. These materials were disseminated by the Health and Family Welfare Department to all government hospitals and primary health centers; education department for school students; rural development department for villages' administrative officers and employees; and agricultural department for the benefit of vast number of agricultural workers who are susceptible to snakebite. Equally, first aid aspects and prevention measures were brought to the notice of common persons through various audiovisual media. Government has also taken a policy to supply ASV and other supportive measures to treat snakebite consistently at all government hospitals and health centers.

Tamil Nadu Health Systems Project (TNHSP)

The Tamil Nadu Health Systems Project (TNHSP) is a World Bank-funded project, initiated to improve the health outcome of the people of Tamil Nadu especially the poor and disadvantaged. Funds were also allotted adequately to train doctors, nurses, and other health-care workers. Its activities include improving health

service delivery, health work force, health financing, health governance, and health management information systems, introduction of newer technologies, and provision of equipments and special drugs.

The Indian state government of Tamil Nadu through TNHSP established a poison treatment center with adequate infrastructure in 34 centers spread across the state. These 34 centers, i.e., 29 revenue district headquarters hospitals (tertiary) and five taluk-level (secondary) hospitals, were upgraded to diagnose and treat snakebite cases. This is in addition to the teaching hospitals affiliated to 18 government medical college hospitals wherein snakebite victims are treated free of cost.

At this juncture, it is worth mentioning that the Indian state of Tamil Nadu provides health care free of cost to all those attending these hospitals. All together, there are 46 hospitals (tertiary) attached to 18 medical colleges, 29 revenue district headquarters hospitals (tertiary), 241 sub-district hospitals (secondary), and 1,306 primary health centers. In addition, there are 8,706 subcenters, manned by health-care workers under the supervision of a medical officer.

These centers were constantly monitored, and data generated were transferred via health management information system to the office of Tamil Nadu Health Systems Project (TNHSP) at Chennai where analysis was made and functioning status was reviewed. The goal of the TNHSP is to provide easy access to quality health for all. The TNHSP has provided ambulances to all district headquarters and sub-taluk hospitals, and the services are free for the sick people and carried out via public private partnership.

Training

The training was given in two phases during 2008. In phase I, 270 medical officers spread all over the state of Tamil Nadu were trained in 18 batches on snakebite management, and another set of 128 medical officers were trained during phase II. The medical officers were given educational modules and trained to train other medical officers, nurses, and other hospital workers in their hospital and nearby hospitals and health centers. They were also tuned to conduct community health education programs which included recognition of snakes, identification of envenoming, provision of first aid to snakebite victims, and prevention aspects. In addition, 400 nurses were also trained. Thus the standard treatment guidelines to treat snakebite were disseminated among the health-care workers (TNHSP 2008). Another training module was prepared for staff nurse and auxiliary nurse midwife (Training module 2007) and was distributed to all trainees and health centers and hospitals.

Welfare Measures

During training, the medical officers were informed about welfare measures offered to the family members of the deceased snakebite victims, as different state

governments in India provide solatium to them (family members of the deceased victim). However, the amount offered and the procedures vary from state to state. The treating medical officers were motivated to issue a medical certificate mentioning the cause of death as "complications following snakebite" in a clear manner to the family members of the deceased victim. Treating doctor shall guide the family members regarding the ways and means of getting the welfare measures provided by the government. Farmers registered under farmers' security scheme get the benefit much easily.

Statistics on Snakebites and Anti-Snake Venom (ASV)

In non-teaching health centers/hospitals, a total of 39,998 snakebite cases were treated from April 2005 to March 2007, with an annual death rate of 0.4 % and referral rate of 6 % before training. They have used 188,536 vials of ASV during that period. In addition, each government medical college hospital in Tamil Nadu treats 800–1,200 cases of snakebites per annum.

The poison treatment centers provide better attention to snakebite victims, make early diagnosis, administer ASV, observe cases, and refer them if required to higher centers. To avoid delay in getting appropriate care, ASV and supportive measures are made available in all government health centers/hospitals. Overall, the public utilizes the services of the poison treatment centers and ambulances, and utilization is increasing every year. During the period April 2011 to March 2013, the overall referral rate and death rate at these centers were 3–5 and 0.6 % respectively. In contrast, the death rate of snakebite in teaching hospitals varied from 3 % to 7 % at different medical college hospitals, as medical college hospitals dealt with complicated and referred cases.

The government of Tamil Nadu is spending huge amount of money on ASV, and it is supplied in 10 ml vials/ampoules, and its use has increased from 188, 536 (from April 2005 to March 2007) to 435,815 (from April 2011 to March 2013) after training. Based on the geographical location of the revenue districts, the Tamil Nadu state was divided into five zones, viz., north, east, west, central, and south, and the ASV utilization was analyzed. The details are provided in Table 8.1. There was no change in percentage of use of ASV to the total quantity in north, east, and west zones. On the contrary, there was a reduction by 8.4 % in central and an increase by 8.7 % in south zone.

Overall, variations in the use of ASV by medical officers were attributable to the training given, selection of cases, confidence to treat snakebites, availability of antivenom, improved health-seeking behavior of snakebite victims, geographical variation in snakes, and nature of envenoming. The use of ASV varied from one geographical area to another. One or other adverse effects were noticed with ASV and varied from 3 % to 12 %.

Table 8.1 Utilization of anti-snake venom in Tamil Nadu

No	Zone[a]	Inj ASV vials used from April 2005 to March 2007		Inj ASV vials used from April 2011 to March 2013		Difference	
		Vials	%	Vials	%	Vials	%
1	North	19,161	10.2	46,777	10.7	27,616	(+)0.5
2	East	40,192	21.3	87,018	19.9	46,826	(−)1.4
3	West	58,619	31.1	137,905	31.6	79,286	(+)0.5
4	Central	43,641	23.1	64,181	14.7	20,540	(−)8.4
5	South	26,923	14.2	99,934	22.9	73,011	(+)8.7
	Total	188,536		435,815		+247,279	

(+) indicate increase, (−) indicate decrease, *ASV* Anti-snake venom, *Inj* Injection
[a]*North Zone* includes revenue districts of Chennai, Kanchipuram, Thiruvallur, Vellore, and Tiruvannamalai; *East Zone* includes revenue districts of Villupuram, Cuddalore, Thanjavur, Thiruvarur, and Nagapattinam; *West Zone* includes revenue districts of Salem, Namakkal, Dharmapuri, Krishnagiri, Coimbatore, The Nilgiris, and Theni; *Central Zone* includes revenue districts of Erode, Karur, Dindigul, Tiruchirapalli, Pudukkottai, Perambalur, and Ariyalur; and *South Zone* includes revenue districts of Madurai, Sivaganga, Ramanathapuram, Virudhunagar, Tirunelveli, Tuticorin, and Kanyakumari

Achievements

Handbook on treatment guidelines for snakebite and scorpion sting (TNHSP 2008) was released by the then state minister for Health and Family Welfare Department of Tamil Nadu. Doctors and nurses were trained. Required medicines to treat snakebite were supplied to all health centers, and services were monitored during monthly review. Difficulties encountered were rectified. One such example was training on ventilator support, which was also carried out. The Poison Control Training and Research Centre (PCT&RC) of Rajiv Gandhi Government General Hospital affiliated to Madras Medical College, Chennai, played a vital role to bring this into reality. The Indian telephone department, Bharath Sanchar Nigam Limited, Chennai, in appreciation of the services rendered by the center (PCT&RC), gave a toll-free number to the PCT&RC, for the benefit of public and professionals to get more information on all 24 h and all 7 days a week.

Doctors working in various government health centers/hospitals of the Indian state of Tamil Nadu were trained to manage snakebite cases at primary, secondary, and tertiary care level. As a result, they have gained knowledge (cognitive skills) on the principles involved in the treatment and acquired psychomotor and affective skills to manage the cases confidently with the available resources and materials. They have also learned to recognize and refer the deserving cases in time to higher center or specialists whenever required. In addition, these doctors were trained to maintain records at their respective institution and primed to carry out clinical audit of snakebite case records. Snakebite cases are treated free of cost at Tamil Nadu Government's health institutions. Patients are transferred from one health institution to another free of cost by the ambulance. Thus the attributes of success

included good governance, political commitment, effective bureaucracies, and willingness of medical officers to work and adapt to resource limitations (Balabanova et al. 2013).

Yardsticks to Measure Achievements

Some of the yardsticks used to measure the services are reduction (a) in bite-to-needle time (i.e., the time interval between snakebite and antivenom therapy), (b) case fatality rate, and (c) referral rate (TNHSP 2008).

Clinical Aspects

Syndromic Approach to Snakebite

The management decisions in cases of suspected snakebites are difficult or problematic, as identification of snake is uncertain and about 40 % of patients do not see the offending snake. So, an alternative approach is necessary to categorize snakebite cases based on clinical manifestations for the purpose of treatment and follow-up. This type of syndromic classification of snakebites (Ariaratnam et al. 2009; DG AFMS: Warrell 2010b) is logical and effective and also enables identification of snakebites (Kumar et al. 2006) without relying solely on the live or killed snake or victim's description of the snake.

Syndrome 1

- Local envenoming (swelling, etc.) with bleeding/clotting disturbances = **Viperidae**

Syndrome 2

- Local envenoming with bleeding/clotting disturbances, shock, or renal failure = **Russell's viper** (and possibly saw-scaled viper – *Echis* species – in some areas)
- With conjunctival edema (chemosis) and acute pituitary insufficiency = **Russell's viper**
- With ptosis, external ophthalmoplegia, facial paralysis, etc. and dark-brown urine = **Russell's viper**

Syndrome 3

- Local envenoming (swelling, etc.) with paralysis = **cobra or king cobra**

Syndrome 4

- Paralysis with minimal or no local envenoming
- Early-morning abdominal pain
- Bite on land while sleeping = **krait**
- Bite in the sea = **sea snake**

Syndrome 5

- Paralysis with dark-brown urine and renal failure:
- Bite on land (with bleeding/clotting disturbance) = **Russell's viper**
- Bite in the sea (no bleeding/clotting disturbances) = **sea snake**

Limitations of Syndromic Approach

The range of activities of particular venom is variable. Asian cobras may cause severe local envenoming which was earlier considered to be an effect only of viper venoms. In Sri Lanka and Southern India, Russell's viper venom causes paralytic signs (ptosis etc.), suggesting elapid neurotoxicity, and muscle pains and dark-brown urine suggesting sea snake rhabdomyolysis (DG AFMS). There may be considerable overlap of clinical features caused by venoms of different species of snake, and hence, treating doctors have to carefully consider different causes and treat the cases as required.

Clinical Features

Clinical aspects have been described in many of the earlier publications (Ahmed et al. 2008; DG AFMS; Indian national snake bite protocol 2007; Interventions for snake bite 2012; Mahadevan and Jacobsen 2009; Simpsom 2007; Snake bite management in Asia and Africa; TNHSP 2008; Warrell 2010a, b). An overview of clinical features and management aspects are described in the ensuing paragraphs.

General Symptoms and Signs

Some people may develop quite striking symptoms and signs, even when no venom has been injected. Fear of the consequences of a real venomous bite and anxiety may lead to hyperventilation, paresthesia, and even tetany. At times, vasovagal reactions, panic reactions, agitation, and a wide range of misleading symptoms may be seen after the bite or suspected bite in some individuals. Symptoms and signs vary with the species of snakes responsible for the bite and the amount of venom injected. However, variations are attributable to composition of venom, which is not always totally species specific.

Early symptoms and signs when venom has been injected are increasing local pain (burning, bursting, and throbbing) at the site of the bite, local swelling that gradually extends proximally up the bitten limb, and tender, painful enlargement of the regional lymph nodes draining the site of the bite. Some other features are fang marks, blistering, necrosis, local bleeding, bruising, local infection, abscess formation, etc.; however, bites by kraits and sea snakes may be painless and may cause negligible local swelling.

Systemic Symptoms and Signs

General symptoms are malaise, weakness, and prostration.

Gastrointestinal features are nausea, vomiting, and abdominal pain.

Cardiovascular (Viperidae) symptoms and signs are dizziness, faintness, collapse, shock, hypotension, cardiac arrhythmias, conduction disturbances (Nayak et al. 1990), pulmonary edema, chest pain, ischemic manifestations, and cardiac arrest.

Bleeding and clotting disorders(Viperidae) manifest as bleeding from recent wounds (including fang marks, venipunctures, etc.) and from old partly healed wounds, spontaneous systemic bleeding – from gums, epistaxis, hemoptysis, hematemesis, rectal bleeding or melena, hematuria, vaginal bleeding, bleeding into the skin (petechiae, purpura, ecchymoses) and mucosae [e.g., conjunctivae], intracranial hemorrhage (meningism from subarachnoid hemorrhage, lateralizing signs) and/or coma from cerebral hemorrhage. Sometimes coagulopathy may continue for over 3 weeks.

Neurological (Elapidae and Russell's viper) manifestations are drowsiness, paresthesia, abnormalities of taste (Senthilkuman et al. 2011) and smell, "heavy" eyelids, ptosis, external ophthalmoplegia, paralysis of facial muscles, difficulty in opening mouth and showing tongue and weakness of other muscles innervated by the cranial nerves, aphonia, difficulty in swallowing secretions, and respiratory and generalized flaccid paralysis.

Skeletal muscle breakdown (sea snakes and Russell's viper) may present as generalized pain, stiffness and tenderness of muscles, trismus, myoglobinuria, hyperkalemia, cardiac arrest, and acute renal failure.

Renal (Viperidae and sea snakes) features are loin (lower back) pain, hematuria, hemoglobinuria, myoglobinuria, oliguria/anuria, and symptoms and signs of uremia (acidotic breathing, hiccups, nausea, pleuritic chest pain, etc.).

Endocrine (acute pituitary/adrenal insufficiency) (Russell's viper). Patients during acute phase may have shock and hypoglycemia (Jeevagan et al. 2013) and in c*hronic phase* (months to year after bite) may come for weakness, loss of secondary sexual hair, amenorrhea, testicular atrophy, hypothyroidism and hypopituitarism, etc. (Golay et al. 2013).

Elapidae Bites (Kraits and Cobras)

Indian cobra results in severe local pain, edema, blistering, and necrosis which may result in early wet gangrene with a distinguishing decomposed odor due to the direct cytolytic action of the venom, whereas krait causes little or no pain and tiny skin bleed (Kularatna 2002, Ariaratnam et al. 2008) without subsequent local tissue damage. The manifestations of krait bite invariably have three components, and these are early-morning abdominal pain, slow progressive paralysis, and hypokalemia.

Ptosis is the earliest neuroparalytic manifestation followed closely by ophthalmoplegia. Paralysis then progresses to involve muscles of palate, jaw,

Table 8.2 Comparative analysis of clinical manifestations of krait and cobra (Bawaskar and Bawaskar 2004)

Clinical manifestations	Krait	Cobra
Respiratory paralysis	Delayed	Early
Neurological manifestations	Delayed 30 min to 16 h	Early 15 min to 7 h
Recovery range (mean)	4–72 h (23 h)	1–24 (4 h)
Mechanism of action: (neuromuscular block)	Presynaptic	Postsynaptic
Response to anticholinergic drug	Poor/nil	Yes

tongue, larynx, neck, and muscles of deglutition but not strictly in that order. Generally, muscles innervated by cranial nerves are involved earlier. However, pupils are reactive to light till terminal stages. Intercostal muscles and diaphragm are involved relatively late, but due to the obstruction of the upper airway, death hastens (Table 8.2).

Viperidae Bites (Russell's Viper and Saw-Scaled Viper)

It is predominantly hematotoxic. Local bleeding including petechial and/or purpuric rash is seen most commonly with this family. It causes rapid swelling of the bitten part and may become devascularized. Viper bite is primarily vasculotoxic. Local necrosis is mainly ischemic, and thrombosis blocks the local blood vessels and leads to the development of dry gangrene. Systemic absorption occurs via the lymphatics, and regional lymphadenopathy has been reported as an early and reliable sign of systemic envenoming. Acute renal failure complicates the course in victims of severe viper envenoming.

Hump-Nosed Pit Vipers

Hump-nosed pit vipers (genus *Hypnale*) are venomous snakes found in Sri Lanka and the Western Ghats mountains that border the west coast of Indian peninsula. Interspecies variation among hump-nosed pit vipers contributes to different types of clinical manifestations. The details of venom composition are not clear. Available clinical data are related to single species *Hypnale hypnale*. Hump-nosed pit viper bite manifests as local pain, swelling, local hemorrhage, blistering, regional lymphadenopathy, and severe local necrosis and gangrene (Joseph et al. 2007; TNSHP 2008; Ariaratnam et al. 2009). Systemic effects are nonspecific, and these are headache, nausea, vomiting and abdominal pain, nephrotoxicity, coagulopathy, thrombocytopenia, and spontaneous hemorrhage. Though there are no reports on myotoxicity, there is a report on ptosis (Maduwage et al. 2011). Though locally available polyvalent antivenom does not neutralize any of the manifestations or effects produced by *Hypnale*, patients recover with appropriate supportive measures and care.

Hydrophiidae (Sea Snakes)

India has a long coastline and also has about 20 of 60 total world species of sea snakes. Most of the sea snakes are helpless on land as it can crawl slowly except yellow-lipped sea krait which can crawl well on land (Vijayaraghavan 2008). Most of the sea snake bites only if provoked. Some of them are aggressive and bite readily. Accidents happen mostly while fisherman handles the sea snake caught in the fishing net or while wading in the shallow water. Myalgic features are the common presenting feature of sea snake bite. Muscle necrosis occurs and patient develops myoglobinuria, rhabdomyolysis, hyperkalemia, and acute kidney injury (AKI). Currently available polyvalent antivenom in India is not effective for sea snake bite. Hence, patients require supportive care and conservative treatment. Reid observed fatality rate of 3.2–37 % following sea snake bite in different countries, and the death rate is influenced by species.

Assessment on Arrival

When the snakebite victim arrives at the emergency room (ER), the initial management includes basic resuscitation and assessment of airway, breathing, and circulation. After stabilizing the case, the diagnosis shall be made, and specific management will be provided. The current recommendation is that patients shall be observed for a period of at least 24 h, even when the patient is stable. The history shall be focused on the time of bite, circumstances for the bite, type of snake, and nature of first aid or traditional medicines given; determination of the snake involved may be made if possible on geographical and clinical grounds.

Investigations

The 20-min whole blood clotting test shall be carried out at the bedside in order to assess coagulopathy. If available other investigations such as full blood count with platelet, coagulation studies including d-dimer, FDP, PT, APTT, blood grouping, and Rh typing, and biochemical tests including creatine kinase shall be carried out. A urine analysis is helpful for detecting blood or myoglobin. ECG and other relevant investigations shall be carried out as and when required.

Management

Management includes admission to hospital, assessment of the case, administration of medications such as tetanus toxoid, anti-snake venom, and other supportive measures including antimicrobials if needed. A detail for repetition of ASV is furnished in Annexure-II. Snakebite wound shall be meticulously attended.

The surgical issues in general to be watched in snakebite cases are ulcer at the site of bite, necrosis, gangrene, and compartment syndrome. All these have to be carefully handled in order to minimize complications and avoid disability. The other measures adopted in the management of snakebite under special circumstances are fresh blood transfusion, fresh frozen plasma, platelets, clotting factors, and plasmapheresis.

Rare Manifestations of Snakebite

Rare manifestation of snake envenoming during recovery is acute acalculous cholecystitis (Senthilkumaran et al. 2013a). This has to be considered, evaluated, and managed, especially if the patient starts complaining of upper abdominal pain on the right hypochondrial region. Hypersensitivity and Kounis syndrome following viper bite were reported earlier (Frangides et al. 2006). Unusually, snakebite victims may develop hypertension (Meenakshisundram et al. 2013), pancreatitis, features of brain death (Senthilkumaran et al. 2013b), cardiac tamponade due to pericardial hemorrhage (Senthilkumaran et al. 2012), adrenal hemorrhage, vaginal bleeding or bleeding from unusual sites, etc., and an astute physician can recognize these and many others.

Predictors of Outcome in Snakebite

The outcome is poor in snakebite victims, if the patient has any of the following or a combination of these (David et al. 2012; Halesha et al. 2013) such as early onset of paralysis, severe envenoming, nonadministration or in adequate administration of ASV, lack of health-care support, and complications such as disseminated intravascular coagulation, low platelet count (David et al. 2012), pulmonary edema, cardiac involvement, renal failure, intracerebral hemorrhage, coma, or organ damage.

Snakebite in Special Situations

Ophthalmic Manifestations in Snakebite

The commonest ophthalmic manifestations (Nayak et al. 2007) observed in elapid group of snakebite are ptosis and ophthalmoplegia. Cobra venom leading to blindness due to retinal cell damage causing bilateral optic neuritis, and recorded cases of optic neuritis (Menon et al. 1997) are extremely rare. The other manifestations seen with viper envenoming are subconjunctival hemorrhage, hyphema, angle-closure glaucoma, uveitis, iritis, retinal and vitreous hemorrhage, central artery occlusion, optic neuritis, optic atrophy, and cortical blindness. In view of the

multiple ophthalmic manifestations, physicians and ophthalmologists shall consider the possibilities and search for the same, either at the time of presentation and during hospital course or while cases come for follow-up. One has to remember that some of them may be a manifestation of serum sickness and not necessarily due to snake envenoming.

Pregnancy and Lactation

If a pregnant woman is envenomed by snakebite (Sarkar et al. 2008), the fetus has to be followed up by ultrasound with special focus on its vital parameters, movement pattern, normal growth, and organ differentiation. Thus venomous snakebite during pregnancy may result in fetal wastage and may cause maternal mortality, vaginal bleeding, abruptio placentae, and uterine contraction. ASV may be administered to a lactating woman if bitten by a venomous snake and be treated like any other person. Breastfeeding is not contraindicated.

Snakebites Again

If a patient has been bitten by a venomous snake and received ASV earlier and comes back with features of repeat snakebite, he/she may be considered as a fresh case and treated accordingly (TNHSP 2008; Indian national snake bite protocol 2007). However, care shall be taken while administering ASV, since he/she has been sensitized.

Patients with Comorbid Illnesses

Even if the patient has any comorbid illness, viz., **a**utoimmune disorders, debilitating status, endocrine disorders, immunosuppressed status, HIV/AIDS, cancer, asthma and allergic disorders, or any other illness, and arrives with features of snake envenoming, they shall be given ASV in the same manner like any other case of venomous snakebite.

Immunity

Theoretically, immunity may be possible by repeated injection of snake venom, but this is unlikely. Moreover, slow accumulation can have serious consequences in course of time (Vijayaraghavan 2010), even it does not prove fatal. Repeated exposure to snakebite for pleasure effect is becoming a new culture among youngsters (Senthilkumaran et al. 2013c). Difficulties were encountered sometimes during blood grouping of snakebite cases who received antivenom (Shasty et al. 2009).

Chronic Complications

Chronic complications are rarely reported and not discussed much. As a result, patients are not motivated for periodical follow-up. Patients who had hypotension, coagulation abnormalities, and severe envenoming are prone for acute kidney injury (AKI), and some of them progress to chronic kidney disease (Herath et al. 2012; Golay et al. 2013). These are likely to happen among victims of viper bites.

Vasculotoxic snakebite envenoming leading to hypopituitarism (HP) has been highlighted by Golay et al. (2013). In their series, all those cases had AKI induced by vasculotoxic snakebite. The authors also suggested suspecting HP in survivors of snakebite, if they come for one or other symptoms of HP as timely recognition, investigation, and intervention significantly improve their quality of life.

Prehospital Care

Effective prehospital care is crucial and plays a major role in the management of a venomous snakebite. The goal of the first aid is to slow the dissemination of venom by slowing lymph flow which prevents the systemic absorption and transport the victim to nearest treatment facility center within the shortest possible time in the best possible condition so as to minimize the risks of disability and mortality associated with snakebite. Various traditional methods such as application of tight tourniquet, cutting and sucking venom out of the wound, washing the wound, and local application of ice packs, snakestone, or other methods have consequences, and hence, they have to be discouraged and discarded. The four main steps involved in sequence in the correct first aid can be remembered by the mnemonic, do it "**R.I.G.H.T**."

R = **Reassure the patient**: 75 % of snakebites are from a venomous species and that, even if it is a venomous snakebite, on average only 50 % of such bites actually envenomed, the rest are called "dry" bites.

I = **Immobilize without compression**: the bitten part shall be immobilized in the same way as a fractured limb. The victim shall not be allowed to walk and be carried. There is no need for any compression in the form of tight ligatures as their use contributes to the development of necrosis.

G. H. = **Get to hospital**: fast, as traditional remedies have no proven benefit in the treatment of snakebite.

T= **Tell the doctor**: the patient may be encouraged to tell the doctor if he/she develops any systemic symptoms such as drooping of eyelids, double vision, dribbling and any change in taste or any manifestations of bleeding while taken to hospital/center.

In most occasions, the first responders are layman or primary health-care workers who assist the snakebite victim. The community and the health-care workers are made aware of the sequences and the importance of the first aid.

Anti-Snake Venom

Albert Calmette in 1890 for the first time at the Institut Pasteur introduced antivenom treatment for snakebite. Antivenom is classified as monovalent and polyvalent. In India, polyvalent anti-snake venom is used. Antibodies raised against the venom of one species may have cross-neutralizing activity against other venoms to some extent but not necessarily always. Limitations of antivenom treatment shall be remembered and recognized. Antivenom should be given only to patients who are likely be benefited, and if indicated, it shall be given immediately. It may reverse systemic envenoming especially in case of hemostatic abnormalities; even this has persisted for several days (Warrell 2010a, b; Al-Hashaykeh and Al Jundi 2011). Practitioners shall remember that ASV sometimes may not work well (Jacob 2006) for various other reasons.

There are currently seven pharmaceutical laboratories in India which produce anti-snake venom (ASV) against four medically important Indian snake species. The issues related to Indian anti-snake venom highlighted by Whitaker and Whitaker (2012) are poor manufacturing standards, minimal efficiency, interspecies variations, and higher adverse reaction rates (Alirol et al. 2010). To ensure quality, WHO has recently published guidelines for the production, control, and regulation of snake antivenom immunoglobulin (WHO 2010).

Reactions to Antivenom

Antivenom reactions are classified as early (within minutes to few hours) and late (5 days or more) after antivenom is given (Warrell 2010b). Early anaphylactic reactions appear within 10–180 min of starting antivenom and manifest as itch (often over the scalp) and develop urticaria, dry cough, fever, nausea, vomiting, abdominal colic, diarrhea, and tachycardia. Few patients may develop severe life-threatening anaphylaxis and manifest hypotension, bronchospasm, and angioedema. Fatal reactions have been underreported. So, every snakebite receiving antivenom has to be carefully monitored while on antivenom therapy and after treatment. Pyrogenic reactions develop 1–2 h after treatment in the form of shaking chills (rigors), fever, vasodilatation, and fall in blood pressure. Febrile convulsions may be precipitated in children. To prevent acute reactions to antivenom, low-dose adrenaline (Premawardhena et al. 1999), promethazine, and hydrocortisone were tried (de Silva et al. 2011).

Late reactions [serum sickness type] develop 1–12 [mean 7] days after treatment. Clinical features include fever, itching, recurrent urticaria, arthralgia, periarticular swellings, myalgia, nausea, vomiting, diarrhea, lymphadenopathy, mononeuritis multiplex, proteinuria with immune complex nephritis, and rarely encephalopathy. By and large, patients who suffer early reactions and are treated with antihistamines and corticosteroid are less likely to develop late reactions (Warrell 2010b).

As far as ASV is considered, there is a need to improve the **product** (quality, efficacy and formula - mono- or polyvalent), consider **promotional aspects** (liquid or lyophilized form, storage and supply), concentrate on **protocol** (low or high dose and uniformity in education and training of students of health sciences), identify

problems (adverse reactions to ASV, non-supply of ASV, nonattendance of snakebite victims on time, lack of research activities related to snakes, antivenom, therapy, follow-up, sociocultural areas, and getting welfare measures by the family of deceased, etc.), and enhance **policies** (surveillance, price control on medication, allocation of funds for research, social support, public private partnership, etc.). There is no absolute contraindication to antivenom treatment, as there are no other alternatives available by and large for selected snakes. However, patients who had reacted earlier to horse or sheep serum and those with the history of atopic diseases are at high risk for severe reactions. In the absence of any prophylactic regimen, these high-risk patients may be pretreated empirically with subcutaneous adrenaline (epinephrine), intravenous antihistamine (both anti-H1 and anti-H2), and corticosteroid. In asthmatic patients, prophylactic use of salbutamol (inhalational form) may prevent bronchospasm. If any patient develops anaphylaxis, adrenaline may be given intramuscularly, and other drugs may be administered as per guidelines (Medical management of severe anaphylactoid and anaphylaxis reactions 2001). Late reactions may respond to oral antihistamine, and if they fail to respond in 24–48 h, patients shall be given 5-day course of prednisolone (Warrell 2010b).

Administration of Anti-Snake Venom (ASV)

Anti-snake venom is available in liquid and freeze-dried (lyophilized) forms. If the liquid form becomes opaque, it should not be used, as it indicates loss of potency and has an increased risk for reactions. Polyvalent antivenoms are preferred in many countries, as it is difficult to identify the species responsible for bites. Freeze-dried antivenoms are reconstituted using 10 ml of sterile water for injection per ampoule. Antivenom is administrated as intravenous infusion (diluted in 5–10 ml of isonomic fluid per kg body weight of isotonic saline or 5 % dextrose water) at a constant rate over a period of about 1 h. Antivenom should not be administered at the site of the bite. Patients with respiratory, circulatory, and renal failure need urgent resuscitation as well as antivenom (Warrell 2010b).

Intramuscular administration of antivenom has to be avoided as absorption is slow, and hence, bioavailability is poor. Also, blood levels of antivenom will never reach as observed in intravenous method. Intramuscular route causes pain at site and hematoma in patients with hemostatic abnormalities. There are few exceptions for intramuscular administration of antivenom (Warrell 2010b).

Children require the same dose of antivenom as adults, as snakes inject same dose of venom into children and adults. The response to antivenom are sense of feeling better; disappearance of nausea, vomiting, and headache; stoppage of spontaneous bleeding within 15–30 min; restoration of blood coagulability within 3–9 h; raise in blood pressure within 30–60 min and resolution of heart rate and/or arrhythmia; improvement of neurological manifestations as early as 30 min; and cessation of hemolysis and rhabdomyolysis within few hours. Recurrence of systemic envenoming after antivenom therapy was noticed 24–48 h later among some cases of viper bites, and this has been attributed to continuing absorption from

"depot" at the site of bite or redistribution of venom from tissues into vascular space. Recurrence has been described in neurotoxic envenoming also.

The criteria for repeating the initial dose of antivenom are persistence or recurrence of blood coagulability after 6 h or bleeding after 1–2 h or deterioration of neurotoxicity or cardiovascular signs.

Referral Aspects

The medical officers treating the snakebite cases at primary and secondary health-care centers may have to refer certain cases only after providing first aid (Annexure-I) as well as supportive measures due to technical constraints. Before referring the case to higher center/specialist, they have to look into respiratory status/failure, deteriorating neurological manifestations, spontaneous persistent bleeding, acute impending kidney failure, or other unusual manifestations. The medical officers while referring a case to a higher center shall follow the standard methods/procedures as per their region or state.

Once the ASV is completed for hemotoxic envenoming, and after dealing with the adverse reactions if any, the patient shall be referred to a higher center if found to have any systemic bleeding or renal impairment. The 6-h rule ensures that a 6-h window is now available in which patient shall be transported. In neurotoxic envenoming, if after 1 h from the end of the first dose of ASV, the patient's symptoms have worsened, i.e., paralysis has descended further, a second full dose of ASV is given over 1 h. ASV is then completed for this patient. If after 2 h, the patient has not shown worsening symptoms but has not improved shall be considered for referral to a higher center.

Responsibilities of Health-Care Provider

The responsibilities of the health-care providers/professionals are to **recognize** snakebite cases, **remember** the principles of management, **resuscitate** the patients, **relieve** the symptoms with appropriate medicines, **reassess** the status after anti-snake venom (ASV) and other supportive measures, **refer** to higher center if required without delay, **review** snakebite-related activities and health educational programs periodically, **retrain** their health-care team, and **revise** the strategies to suit the local needs.

Prevention

Various preventive methods are creating awareness on recognition of snakebite and early health-seeking behavior; education on hazards and use of personal protective measures; exercising caution while picking up leaves or sticks from forest or materials in construction site; carrying a stick and tapping on the floor while walking at night; avoidance of sleeping on floor (or) agricultural field, walking on

barefoot especially along the edge of waterways, growing plants and weeds on windows, and handling dead or live venomous snakes; keeping the environment around the house and the working place clean; using pest control to eliminate rodents from home or working area; taking adequate care while picking up cow dung cakes or firewood; checking shoes, coat packets, or trousers before use; etc. These are likely to be achieved if one implements community education, recognizes vulnerable groups and focuses on them at periodical intervals, undertakes regular review of snakebite cases and outcome, and maintains medical surveillance. Community shall be informed that venomous dead snakes have to be handled carefully, as wrong handling may lead to venomous bite if the handlers' body parts are caught inside the mouth. Such things may happen while handling even severed heads.

What Patients and Caregivers Shall Know?

While the details of snakes, snakebites, and clinical aspects including treatment and prevention are discussed, it is worth recalling certain information that shall be known to victims of snakebites and/or caregivers:

(a) Non-measurability of plasma or serum venom levels (quality and quantity), body's response to venom, effectiveness of ASV, and supportive care.
(b) Absence of envenoming shall not be construed based on non-visibility of bite or fang marks.
(c) Non-availability of biomarkers to predict clinical course, complications, and outcome.
(d) Limitations to diagnose the severity of envenoming.
(e) Non-availability of snake-specific antivenom(or) mono-component ASV in India.
(f) Ineffectiveness of ASV of one group against subgroups or other groups.
(g) Non-predictability of adverse effects of ASV in a given case.
(h) Need for follow-up to assess for long-term complications after snakebite and/or ASV.
(i) Disclosure of procedures to get solatium given by state governments in India to family members of the deceased due to snakebite.
(j) Non-availability of effective vaccine against snakebite.

Conclusions and Future Directions

Conclusions

Snake envenoming has professional, social, economical, educational, ethical, and legal challenges. Ethically, the treating doctor shall realize his/her multidimensional responsibilities and communicate well to patients and caregivers on the nature of envenoming, clinical course and outcome, anticipated

complications, limitations in the treatment, and referral to higher center if required and provide appropriate treatment and supportive care at the health center. Doctors shall also realize that victims of venomous snakebite survive with appropriate care and conservative treatment, even though snake antivenom is not available for very many. Venomous snake bite shall be remembered and considered in emergency room and excluded, as snakebite may present with protean manifestations at times.

Since neurotoxic manifestations develop as early as 3 min and may be delayed up to 19 h, medical attention is essential to distinguish venomous from nonvenomous snake bite. Moreover, systemic manifestations of snakebite depend upon various polypeptide toxins present in the venom. Blood coagulation gets restored between one and 25 h with a mean of 8 h after the first dose of ASV. Despite all care, death has been reported within 15 min in king cobra, 8 h in cobra, 18 h in krait, 3 days in viper, and 5 days in *Echis carinatus*. Doctors shall realize the therapeutic aspects of ASV and its limitations too.

Snakebite is a neglected tropical disease, and we must make all efforts to overcome difficulties encountered toward diagnostics, drugs and devices, and inherent failures or deficiencies of health-care system. Careful documentation of constraints and measures undertaken to overcome them by the treating doctors are paramount important, to defend oneself against a system which seeks prosecution and compensation.

Future Directions

It is suggested that the Ministry of Health and Family Welfare, government of India, has to form a national snakebite forum (NSBF), which shall work at district, state, and national level to monitor the treatment, clinical audit and prevention aspects; and maintain surveillance activities on snakebites. There is an urgent need to address on the quality of ASV along with potency and cross-reactivity of ASV among species, and preparation of antivenom for other venomous snakes in India as well as production of sufficient quantity of ASV. The government shall also make snakebite as notifiable disease and consider snakebites as an occupational disease and provide all the benefits of a laborer to snakebite victims also.

It is essential to initiate joint efforts through various ministries, departments/offices, and divisions/sections, such as education, industry, labor, forest, agriculture, animal husbandry, rural development, science and technology, finance, economics, internal and external security, revenue, health and family welfare, etc., in an interdisciplinary manner for dissemination of knowledge on snakes, snakebites, risks involved, first aid, early health-seeking behavior, treatment, and prevention aspects through audiovisual means among the community, school children, women self-help group, and all other risk-prone population; eliminate deterrents for early health-seeking behavior; and enhance social support system to the family members of the deceased victims of snakebite.

Different statutory bodies involved in health science education such as medical, dental, nursing, pharmacy, physiotherapy, and indigenous systems of medicine

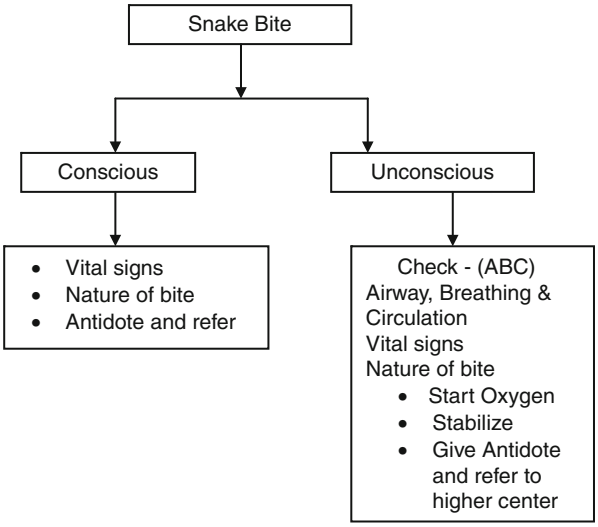
shall come out with a common module to teach and train students of various branches of health sciences and health-care professionals of all systems on snakebite management.

End users have to assess the applicability and limitations of syndromic approach to diagnose and treat snakebite in different parts of India, strengthen facilities to treat snakebites at all levels of health care, and ensure availability of facilities which shall be monitored by a local hospital committee. Research organizations shall be requested to allocate funds for undertaking health science research with reference to finding out biomarkers to diagnose envenoming, organ involvement, and outcome; conducting clinical trials on dose (low or high) of injection anti-snake venom; and carrying out works on social, cultural, economical, molecular, clinical, herpetological, phytochemical agents, and preventive aspects. Special efforts may be taken to find out the therapeutic use of snake venom in many other diseases.

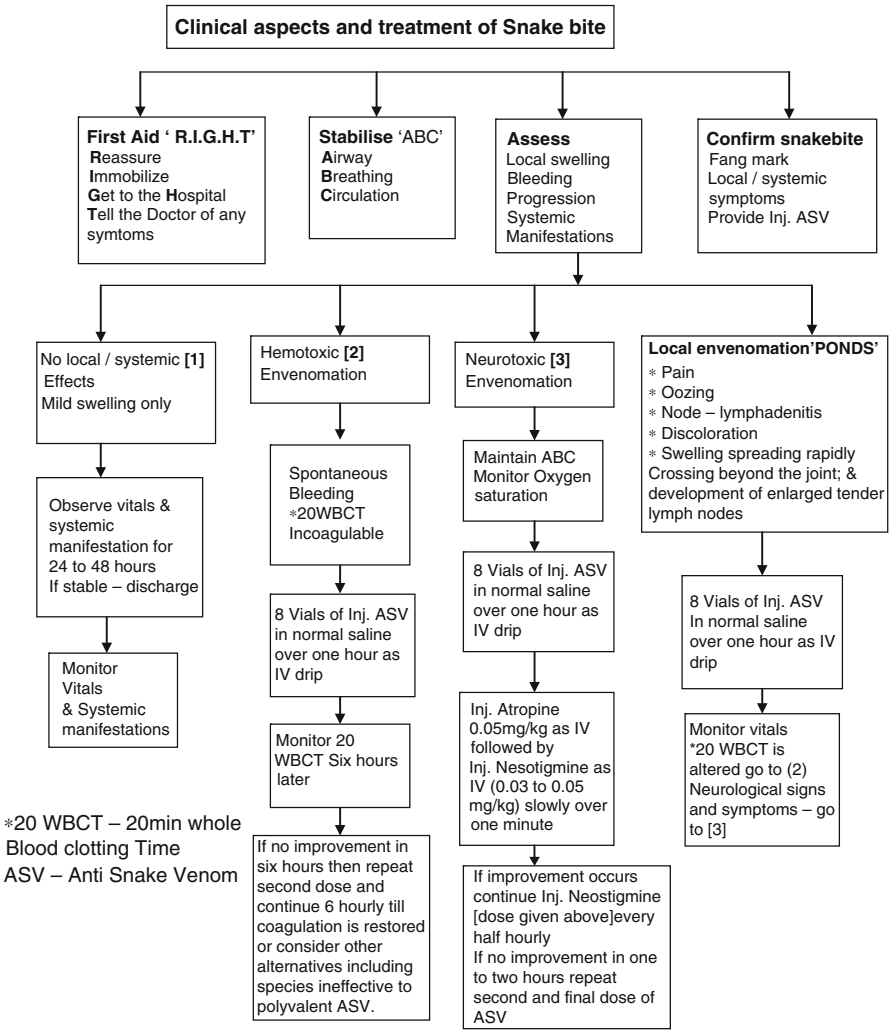
Cross-References

- ▶ Venomous Snakes and Snakebites in India
- ▶ Snake Venom and Hemostasis
- ▶ Venomous Snakes and Snakebites in India

Appendix I: Algorithmic Approach to Snake Bite at Primary Level



Appendix II: Algorithmic Approach to Snake Bite



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Viperidae Envenomation in India

9

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Contents

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Abstract

The estimated incidence of venomous snakebites in India is around 300,000 annually and the overall mortality is between 30,000 and 50,000. The majority of this is due to Viperidae envenomation. There is a lack of consensus regarding the management protocol. The symptoms, signs, diagnosis, and management of Viperidae envenomation are discussed. Well-known signs are due to hemorrhages and local reaction. Rare signs include bilateral parotid swellings, hypopituitarism, and capillary leak. A standard protocol for diagnosis and management is evolved in the light of treating a large number of Viperidae bites. Twenty minute whole blood clotting test (WBCT) is the gold standard for the diagnosis of Viperidae envenomation. When 20 min WBCT is positive, treatment is initiated with 10 vials of anti-snake venom [ASV], which is delivered in 100 ml of normal saline in 1 h. The repeat dose of ASV is given after 6 h if the 20 min WBCT is prolonged at 6 h after the first dose. Supportive treatment includes fresh frozen plasma and platelet transfusion. Antibiotics and tetanus immunization are given in all cases. The causes of mortality are bleeding into the lungs and brain, renal failure, intractable hypotension due to capillary leak syndrome, and hypotension due to unexplained etiology. Signs of recovery after ASV and bad prognostic signs are discussed.

Introduction

Venomous snakebite is a significant cause of human mortality and morbidity in the tropics. There is an estimated 300,000 victims of snakebite in India alone, every year. The mortality due to venomous snakebite annually in India is estimated at between 35,000 and 50,000. Of this the majority are by Viperidae envenomation.

In India, the venomous snakes belong to three broad families: Elapidae (cobras, kraits, and coral snakes); Hydrophidae (sea snakes); and Viperidae. The last has two subfamilies, Viperinae (Russell's viper, saw-scaled viper) and Crotalinae (pit viper).

The lethal dose of the venoms for a man has been reported to be as follows (Deoras 1965): Russell's viper 0.15 g and *Echis carinatus* 0.08 g.

About 80–90 % of Viperidae and 25–70 % of Elapidae venom consists of enzymes. The role of enzymes in envenomation is most clearly seen in the case

of venom procoagulants. For example, *Vipera russelli* venom contains glycoprotein which activates factor X and arginine ester hydrolase which activates factor V.

Echis carinatus venom (saw-scaled viper) contains a zinc metalloprotein – ecarin which activates prothrombin.

Many Crotalinae venoms (pit viper) contain proteases which cleaves fibrinogen molecule, for example, Arvin or ancrod (*C. rhodostoma*) and botorxin or reptilase (*B. atrain*). Phospholipase A2 is the most widespread and extensively studied of all venom enzymes. Up to 30 % of protein content is phospholipase A2, presenting in the form of seven isoenzymes. Under experimental condition, it damages mitochondria, red blood cells, leukocytes, platelets, peripheral nerve endings, skeletal muscle, vascular endothelium, and other membranes.

Hemorrhagins (HR 1 and HR 2)

Hemorrhagins, two immunologically distinct nonenzymatic hemorrhagic principles (HR 1 and HR 2) are typical components of crotalid (pit viper) and viperid (true viper) venoms. They cause acute, rapid hemorrhage. In many instances of severe envenomation, the hemorrhagins play a major lethal role by causing hemorrhage in the vital organs, e.g., the brain, lungs, kidneys, heart, and gastrointestinal tract. Pharmacologically, the hemorrhagins have been demonstrated to be separate entities from proteolytic enzymes in the venom. It has been observed that they cause severe vasoconstriction followed by vasodilation of microvessels and arterioles with hemorrhage in the capillary bed. The hemorrhagins act by directly disrupting the endothelial lining and by inhibiting platelet aggregation. Pharmacological studies have further shown that the hemorrhagic principles induce the release of certain autopharmacological mediators such as histamine and 5-HT from various tissues which open up endothelial cell junction and disrupt the isolated basement membrane, presumably in an enzymatic mode of action thus causing vascular damage and hemorrhage. Similarly, vasculotoxic changes have also been observed in the renal and cerebral vessels with crotalid venom.

Occupational Risks and Other Ecological Factors

The normal perception is that rural agricultural workers are most at risk and the bites occur first thing in the morning and last thing at night. However, this is of very little practical use to rural workers in preventing snakebite since it ignores the fact that:

- In rubber, coconut, and areca nut plantations, clearing the base of the tree to place manure causes significant number of bites.
- Harvesting high-growing crops like millet which requires attention focused away from the ground.
- Rubber tapping in the early hours – 03:00 a.m. to 06:00 a.m.

- Vegetable harvesting/fruit picking.
- Tea and coffee plantation workers face the risk of arboreal and terrestrial vipers when picking or tending bushes.
- Clearing weeds exposes workers to the same danger as their grass-cutting colleagues.
- Walking at night without a torch barefooted accounts for a significant number of bites.
- Bathing in ponds, streams, and rivers, in the evening. It should not be assumed that because the victim is bitten in water, the species is nonvenomous. Cobras and other venomous species are good swimmers and may enter the water to hunt.
- Walking along the edge of waterways.

Preventive Measures

- Walk at night with sturdy foot wear and use a torch, walk with a heavy step as snakes can detect vibration and will move away.
- Carry a stick when grass cutting or picking fruit or vegetables or clearing the base of trees. Use the stick to move the grass or leaves first. Give the snake chance to move away. If collecting grass that has previously been cut and placed in a pile, disturb the grass with the stick before picking the grass up.
- Keep checking the ground ahead when cutting crops like millet, which are often harvested at head height and concentration is fixed away from the ground.
- Pay close attention to the leaves and sticks on the ground when wood collecting.
- Keep animal feed and rubbish away from your house. They attract rats and snakes will follow.
- Try to avoid sleeping on the ground.
- Keep plants away from your door and windows. Snakes like to take refuge under the cover of plants, and plants help them climb up and into windows of the rooms and thus get into rooms.
- During trekking through forests or mountains, stay on clearly marked tracks. Do not step or reach into an area where you cannot see the ground. Wear boots, long-sleeved shirts, and long pants.

First Aid

When the snake bites its prey, it usually let it go allowing for the venom to take effect after which it follows the prey by means of its scent trail. Therefore, it is also very likely that after biting a human, the snake is found in the vicinity and it is no less dangerous after the first strike. So the victim should be moved away to a safe distance.

Increased activity would hasten the absorption of venom from the bite site. A bitten person should never run or indulge in any strenuous activity. This would

hasten the blood circulation and venom distribution. The bitten part should be kept below the level of the heart. If it is above the level of the heart, it would facilitate absorption.

Application of a compression bandage should be done in all extremity bites. A compression bandage (NOT A TOURNIQUET) should be firmly tied from the bite site upward. The idea is to compress the lymphatics and the venules but not retard arterial flow, if retarded could end up in gangrene or necrosis. The bandage should allow for the insinuation of one finger as is done for a fracture. A compressive bandage is a must in all Elapidae envenomation as this would retard the entry of venom into the central circulation.

The bandage should be released only 30 min after ASV has been infused. We had two cases of patient going into a respiratory paralysis on release of the compression bandage. However, there are lots of differences of opinion about the pressure immobilization.

Reassure the patient. Seventy percent of all snakebites are from nonvenomous species, and from the remaining 30 %, half of them are dry bites (i.e., 15 %).

Immobilize in the same way as a fracture limb. Use bandages or cloth to hold the splints.

Tell the doctor of any systemic symptoms such as ptosis that manifest on the way to the hospital.

The idea is to institute treatment as early as possible without wasting time in the hospital.

The snake, if killed should be carefully taken to the hospital for identification by the doctor. No time should be wasted in attempting to kill the snake or capture the snake. This solely wastes time and can lead to other victims.

Traditional Methods to Be Discarded

Tourniquets – The use of tight tourniquet made of rope, belt, string, and cloth have been traditionally used to stop venom flow into the body following snakebite. However, they have the following drawbacks and problem:

- Risk of ischemia and loss of the limb.
- Increased risk of necrosis with 4/5 of the medically significant snakes of India.
- Increased risk of massive neurotoxic blockade when the tourniquet is released.
- Risk of embolism if used in viper bites. Procoagulant enzymes will cause clotting in distal blood. In addition, the effect of the venom in causing vasodilation presents the danger of massive hypotension when the tourniquet is released.
- They do not work! Venom was not slowed by the tourniquet in several experimental studies as well as in field condition.
- They give patients a false sense of security, which encourages them to delay their journey to the hospital.

Cutting and Suction

Cutting a victim with incoagulable blood increases the risk of severe bleeding as the clotting mechanism is no longer effective and increases the risk of infection. No venom is removed by this method.

Washing the Wound

Victims and bystanders often want to wash the wound to remove any venom on the surface. This should not be done as the action of washing increases the flow of venom into the system by stimulating the lymphatic system.

Electrical Therapy and Cryotherapy

Electric shock therapy for snakebite received a significant amount of press in the 1980s. The theory behind it stated that applying an electric current to the wound denatures the venom. Research showed however that the venom is not denatured. In addition, it has been demonstrated that the electric shock has no beneficial effect and it has now been abandoned.

Cryotherapy involving the application of ice to the bite site was proposed in 1950s. It was subsequently shown that this method has no benefit and merely increased the necrotic effect of the venom.

Creating Awareness Among the Community About Dos and Don'ts

Awareness should be created among community about the dos and don'ts of the snakebite.

Dos

- Reassure the victim that death is not imminent and that medical care is available. Reassure that most of the bites are nonvenomous.
- Remain calm, make the victim comfortable. Control anxiety. Excitement may increase the heart rate and blood circulation. This will help to spread the venom through your body much faster.
- Lay down flat on the ground. Keep the bitten body part below heart level.
- Remove shoes, rings, watches, jewelry, and tight clothing from bitten areas. They may act as a tourniquet in the event swelling occurs.
- Immobilize the victim's bitten limb. Bandage it using a cotton bandage (or using any clean cloth material). Remember, venom travels through the lymphatic system, so the spread of the venom can be slowed by a bandage. Wrap the limb from the bite site to above the elbow or knee. Finally, apply a splint and do not allow the limb or the muscles in the area to be moved much.

- Be prepared to treat for shock and possibly administer CPR.
- Get the victim to the nearest hospital as soon as possible.

Don'ts

- Do not apply a tourniquet or constriction band. You could cut off blood flow to the limb causing more damage than the snakebite.
- Do not wash the bite site with water or any other solution to remove venom from the bite site. The action of washing increases the flow of venom into the system by stimulating the lymphatic system.
- Do not make cuts or incisions on or near the bite site. You could cut nerves and tendons or block vessels.
- Do not apply cold, electrocautery, heat to the bitten part.
- Do not apply any kind of potentially harmful herbal or folk remedies.
- Do not attempt to suck venom out with your mouth. You could have an ulcer or wound in your mouth, allowing venom to get into your bloodstream.
- Do not give the victim a drink, alcohol, or other drugs as it may confound clinical examination.
- Do not attempt to capture, handle, or kill a venomous snake. More people are bitten during these activities than in any other situation.
- Do not allow the victim to walk or run.

Diagnosis Phase: Symptoms

Hemostatic abnormalities are prima facie evidence of a viper bite. All the vipers can cause renal failure. Russell's viper can also manifest neurotoxic symptoms in a wide area of India, especially southern India. This can sometimes cause confusion, and further work is necessary to establish how wide this area might be. The neurotoxic symptoms of Russell's viper are believed to be presynaptic or krait-like in nature.

General Signs and Symptoms of Viperidae Envenomation

- Local pain and swelling and erythema over bitten part.
- Tender enlargement of local lymph nodes, as large molecular weight viper venom molecules enter the system via the lymphatic.
- Local necrosis and/or blistering.
- Vomiting, abdominal pain, and acute abdominal tenderness which may suggest gastrointestinal or retroperitoneal bleeding.
- Hypotension resulting from hypovolemia or direct vasodilation.
- Low back pain and loin pain indicative of an early renal failure or retroperitoneal bleeding.
- The passing of reddish or dark-brown urine or declining or no urine output.

- Lateralizing neurological symptoms and asymmetrical pupils may be indicative of intracranial bleeding.
- Muscle pain indicating rhabdomyolysis.
- Bilateral parotid swelling ("viper head"), conjunctival edema, and subconjunctival hemorrhage.
- Metallic taste.
- Confusional state and ptosis.
- Jaundice.
- The victim may bleed from any orifice or organ, hemoptysis, epistaxis, hematuria, hematemesis and melena, chemosis, macular bleed, excessive menstrual bleed, bleeding from the bite site or the cannula, bleeding into muscles, and bleeding from gingival sulci. Bleeding into the skin and mucous membrane may show evidence of petechiae, purpura, and epistaxis.

Late-Onset Envenomation

The patient should be kept under close observation for at least 24 h. Many species, particularly, the krait and the hump-nosed pit viper, are known for the length of time it can take for symptoms to manifest. Often this can take between 6 and 12 h. Late-onset envenomation is a well-documented occurrence.

This is also particularly pertinent at the start of the rainy season when snakes generally give birth to their young. Juvenile snakes, 8–10 in. long, tend to bite the victim lower down on the foot in the hard tissue area, and thus any signs of envenomation can take much longer to appear.

Diagnosis Phase: Investigation

Twenty minute whole blood clotting test (20 WBCT) is considered the most reliable test of coagulation in hematotoxic bite and can be carried out at the bedside without special training. It is significantly superior to the capillary tube method.

A few milliliters of fresh venous blood is placed in a new, clean, dry glass test tube and left at ambient temperature for 20 min. It is important that the tube is clean, glass, and dry as the mechanism under review is the contact clotting mechanism. The use of plastic bottles, tubes, or syringes will give false readings and should not be used.

The glass vessel should be left undisturbed for 20 min and then gently tilted, not shaken. If the blood is still liquid, then the patient has incoagulable blood. The test tube must not have been washed with detergent as this will inhibit the contact element of the clotting mechanism.

The test should be carried out every 30 min from admission for 3 h. If everything is normal, repeated at 1 h interval till 6 h after bite and twice more at 3 h intervals the next 6 h. If all reports are normal, no further triage would be needed. A normal 20 WBCT and clot lysis would exclude Viperidae species. But it occasionally

happens that the parameters become abnormal only 24 h after the bite especially in pit viper bites. Other investigations done in a case of Viperidae bites are:

Hematological: Hemoglobin, pack cell volume (PCV), total leukocyte count, differential leukocyte count, erythrocyte sedimentation rate (ESR), and peripheral smear. Platelet count – which is repeated 6 hourly the first 24 h in Viperidae bite.
Coagulation workup: clotting time (CT), bleeding time (BT), activated partial thromboplastin time (APTT), and prothrombin time (PT)
DIC workup: D-dimer, FDP (fibrin degradation product). Fibrinogen which is repeated on the third day.
Renal function: Blood urea and serum creatinine
Liver function: aspartate transaminase (AST), alanine transaminase (ALT), serum bilirubin, serum protein and albumin
Muscle enzymes: Creatinine phosphokinase (CPK)
Biochemistry: sodium (Na), potassium (K), and blood sugar
Urine: Checked for myoglobin, hemoglobin, and protein
Blood group: ABO and Rh (at the earliest as blood does not clot later)
Oxygen saturation/BP/postural blood pressure/PR/RR
Arterial blood gases (ABG): if facilities are available.

The same may have to be repeated depending on the clinical course of the patient.

- The tests repeated on a daily basis are hemoglobin (Hb), PCV, complete blood count (CBC), urea, creatinine, platelet, and urine protein.
- The coagulation workup usually normalizes within 24–48 h of treatment. Exceptions are in cases of certain pit viper species, where it may take up to 2–3 weeks to normalize.
- A peripheral smear is also sent for in which crenated red blood cells (RBC), schistocytes, or burr cells are looked for. These suggest systemic envenomation and along with thrombocytopenia are markers for MAHA (microangiopathic hemolytic anemia).

The signs specifically looked for are:

- Regional lymphadenopathy.
- Swelling of the bitten limb which is measured at 15 min intervals for the first hours and thereafter at 6 hourly intervals. An increase of 3 mm increases the circumference of the limb by 1 cm. As also the extent of spread of the swelling with time is kept. The upper limit of the spread of the swelling is marked and the time given, and the same is repeated at 3 hourly interval for the first 24 h.
- Discoloration of the urine.
- Look for gangrene or necrosis of the bitten part, especially, if a tight tourniquet has been tied.
- Urine output is measured and charted.

Snakebite Treatment Protocol: Treatment Phase

Managing Pain

Snakebite can often cause severe pain at the bite. This can be treated with pain killers such as paracetamol.

- Adult dose 500–1,000 mg 6 hourly orally
- Pediatric dose 10 mg/kg body weight every 6 hourly orally
- Mild opiates like Ketorol 50 mg can be used orally for relief of severe pain. In cases of severe pain at a tertiary center, Ketorol can be given IV

Handling Tourniquets

Though not recommended, the current practice is that many snakebite victims reaching the emergency with tightly tied tourniquets. Care must be taken when removing tight tourniquets. Sudden removal can lead to a massive surge of venom leading to neurological paralysis, hypotension due to vasodilation.

*Before removal of the tourniquet, check for the presence of pulse distal to the tourniquet. If the pulse distal to the tourniquet is absent ensure a doctor is present before removal.

- Be prepared to handle the complications such as sudden respiratory distress or hypotension. If the tourniquet has occluded the distal pulse, then a blood pressure cuff can be applied to reduce the pressure slowly.

Anti-snake Venom (ASV)

Anti-snake venom (ASV) is the mainstay of treatment. The ASV available in India is polyvalent, i.e., it is effective against all the four common species: Russell's viper (*Daboia russelii*), common cobra (*Naja naja*), common krait (*Bungarus caeruleus*), and saw-scaled viper (*Echis carinatus*). There are no current available monovalent ASVs primarily because there are no objective means of identifying the snake species, in the absence of the dead snake. It would be impossible for the physician to determine which type of monovalent ASV to employ in treating the patient.

There are known species such as the hump-nosed pit viper (*Hypnale hypnale*) where polyvalent ASV is known to be ineffective. In addition, there are regionally specific species such as sochureki saw-scaled viper (*Echis carinatus sochureki*) in Rajasthan, where the effectiveness of polyvalent ASV may be questionable. Further work is being carried out with ASV producers to address this issue.

ASV is produced in both liquid and lyophilized forms.

Liquid ASV requires a reliable cold chain and refrigeration and has a 2-year shelf life. Lyophilized ASV, in powder form, has a 5-year shelf life and requires only to be kept cool. This is a useful feature in remote areas where power supply is inconsistent.

ASV Administration Criteria

ASV is a scarce, costly commodity and should only be administered when there are definite signs of envenomation. Unbound, free-flowing venom can only be neutralized when it is in the bloodstream or tissue fluid. In addition, anti-snake venom carries risks of anaphylactic reactions and should not therefore be used unnecessarily. The doctor should be prepared for such reactions.

If a patient has evidence to suggest systemic envenomation or severe local envenomation, then only ASV will be administered.

Evidence of Systemic Envenomation

Evidence of coagulopathy: Primarily detected by 20 min WBCT or visible spontaneous systemic bleeding from gums. Further laboratory tests for thrombocytopenia, hemoglobin abnormalities, PCV, peripheral smear, provide confirmation, but 20 min WBCT is paramount.

Other determinants are:

- Cardiovascular abnormalities, hypotension, shock cardiac arrhythmia, abnormal ECG
- Persistent and severe vomiting or abdominal pain

Test Dose of ASV

Test dose have been shown to have no predictive value in detecting anaphylactoid or late serum reaction and should not be used. These reactions are not IgE mediated but complement activated. They may also pre-sensitize the patient and thereby create greater risk.

ASV Administration: Dosage

1 ml of ASV neutralizes 0.6 mg of Russell's viper venom
0.6 mg of cobra venom
0.45 mg of krait venom
0.45 mg of saw-scaled viper venom

Russell's Viper (Hematotoxic Bite)

- Russell's viper injects on an average 63 mg (5–147 mg $\pm$ 7) of venom.
- 1 ml of ASV neutralizes 0.6 mg of Russell's viper venom.
- So 1 vial, i.e., 10 ml of ASV, neutralizes 6 mg of Russell's viper venom.

- So the total required dose will be between 100 mg (10 vials) and 250 mg (25 vials).
- So starting with 10 vials ensures sufficient neutralizing power.
- Not all victims will require 10 vials as some may be injected with less than 63 mg. Not all victims will require 25 vials as very few are injected with a dose that is an outlier. However, starting with 10 vials ensures that there is sufficient neutralizing power to neutralize the average amount of venom injected and during the next 12 h to neutralize any remaining free-flowing venom.
- Start IV normal saline with wide-bore needle.
- Start 10 vials of ASV in 100 ml of normal saline over 1 h. *All ASV are to be administered over 1 h period at constant speed*. **Continue to monitor the vital signs at 5 min interval for first 30 min and then at 15 min interval for 2 h**.

Repeat Dose in Hematotoxic Envenomation

As already explained, initial blood test reveals coagulation abnormality and 10 vials of ASV given. No additional ASV until next 6 h (the liver is unable to replace clotting factors within 6 h). After initial 6 h, another 20 WBCT is done. If there is evidence of abnormality of 20 WBCT (continued coagulation disturbance), another 10 vials of ASV is administered in 1 h time. Repeat 20 WBCT and repeat ASV 6 hourly until coagulation is restored, unless a species is identified as one against which polyvalent ASV is not effective. (Usually in majority of cases, 20 vials ASV is enough).

ASV in Children

Children receive the same ASV dosage as adults. The ASV is targeted at neutralizing the venom. A snake injects the same amount of venom into adults and children.

Recovery Signs

If an adequate dose of appropriate antivenom has been administered, the following response may be seen:

- Spontaneous systemic bleeding such as gum bleeding, bleeding from venipuncture sites, usually stops within 15–30 min.
- Blood coagulability is usually restored in 6 h. Principal test is 20 WBCT.
- Active hemolysis and rhabdomyolysis may cease within few hours and urine may return to its normal color.
- In patient with shock, blood pressure may increase after 30 min.

Recurrent Envenomation

When coagulation has been restored, no further ASV should be administered, and unless proven, recurrence of a coagulation abnormality is established. Indian ASV a F(ab) 2 product and has a half-life of 90 h and therefore is not required in a prophylactic dose to prevent re-envenomation.

The Guidance to Give ASV to Achieve Homeostasis

The normal guidelines are to administer ASV every 6 h until coagulation has been restored. However, what should the clinician do if there is persistent incoagulation?

There are a number of questions that should be considered. Firstly, is the envenomation species one for which polyvalent ASV is effective? For example, it has been established that envenomation by the hump-nosed pit viper (*Hypnale hypnale*) does not respond to normal ASV. This may be a cause as in the case of *Hypnale*. Coagulopathy can continue up to 3 weeks.

The next point to consider is whether the coagulopathy is resulting from the action of the venom. Published evidence suggest that the maximum venom yield from, say, a Russell's viper is 147 mg which will reduce the moment the venom enters the system and starts binding to tissues. If 30 vials of ASV have been administered, that represents 180 mg of neutralizing capacity. This should certainly be enough to neutralize free-flowing venom. At this point, the clinician should consider whether the continued administration of ASV is serving any purpose, particularly in the absence of proven systemic bleeding. At this stage, the use of fresh frozen plasma (FFP) or factors can be considered if available.

ASV in Special Situation

Victims Requiring Lifesaving Surgery

In very rare cases, symptoms may develop which require that lifesaving surgery is to save the victim. An example would be a patient who presents with signs of an intracranial bleed. Before surgery, coagulation must be restored in the victim in order to avoid catastrophic bleeding. In such cases, a higher initial dose of ASV may be justified on the basis on guaranteeing a restoration of coagulation after 6 h.

Snakebite in Pregnancy

There is very little definitive data published on the effects of snakebite during pregnancy. There have been cases reported when spontaneous abortion of fetus has been reported, although this is not the outcome in majority of cases. It is not clear whether venom can pass the placental barrier.

Pregnant women are treated in exactly the same way as other victims. The same dosage of ASV is given.

Victims Who Arrive Late

A frequent problem witnessed in our country is victims who arrive late after the bite, often after several days, usually with acute renal failure. The key determining factor to decide on ASV treatment is to look for signs of current venom activity. Venom can only be neutralized if it is unbound in the circulation. Perform a 20 WBCT and determine if any coagulopathy is present. If coagulopathy is present, administer ASV. If no coagulopathy is evident, treat renal failure.

ASV Reactions

Anaphylaxis with ASV may be life-threatening. Anaphylaxis can be rapid onset and can deteriorate into a life-threatening emergency very rapidly. Adrenaline should always be immediately available.

The patient should be monitored closely for urticaria, itching, fever, shaking, chills, nausea, vomiting, diarrhea, abdominal cramps, tachycardia, hypotension, bronchospasm, and angioedema of the lips, mucous membrane, larynx (lump in throat hoarseness), and periorbital area.

If anaphylaxis is evident, then:

- ASV will be discontinued temporarily.
- 0.5 mg of 1:1,000 adrenaline will be given IM for adults. Children are given 0.01 mg/kg of body weight of adrenaline 1 M.
- In addition, to provide long-term protection against anaphylactoid reaction, 100 mg of hydrocortisone and an H2 antihistamine can be given.
- If after 10–15 min the patient condition has not improved or is worsening, a second dose of 0.5 mg of adrenaline 1:1,000 IM is given. This can be repeated for a third and final occasion, but in the vast majority of reaction, two doses of adrenaline will be sufficient. If there is hypotension or hemodynamic instability, IV fluids should be given.
- In persistent hypotension and life-threatening anaphylaxis, adrenaline 0.1 mg (1:10,000 dilution) IV bolus is given over 5 min. If hypotension refractory to bolus dose, start an adrenaline infusion.
- There is better patient outcome if adrenaline is used early.
- Pyrogen reactions (endotoxin)
  - 1–2 h after treatment
  - Chills and rigors

  - Fever
  - Vasodilation and hypotension
  - Febrile convulsion in children

  These are due to pyrogen contamination during manufacturing process.

- Adrenaline infusion:
  - Add 1 mg of adrenaline to 500 ml of 5 % dextrose (2 μg/ml). Infuse at 1 ml/min titrate upward to 4 ml/min 2–8 mg/min
- Can cause life-threatening arrhythmia. Cardiac monitoring is necessary.
- Late serum sickness reaction: 1–12 days after treatment, fever, arthralgia, myalgia, nausea, vomiting, diarrhea, lymphadenopathy, and proteinuria with immune complex nephropathy.
- Can be easily treated with oral steroids such as prednisolone, and antihistamine provides additional symptomatic relief.

When to restart the ASV after a reaction:

- Once the patient has stabilized.
- Once BP is under control.
- Once the manifestation of the reaction have subsided.
- In severe reactions, ASV can be restarted under cover of an adrenaline infusion.
- Rate of ASV infusion can be decreased initially.
- Patient should be under strict monitoring.

Poor Prognostic Indicators in Viper Bite

- Low platelets $< 20{,}000$
- Polymorphonuclear leukocytosis with the presence of band form
- Crenated RBC
- Hemoconcentration at presentation – indirectly denotes capillary leak.
- Raised D-dimer
- Low fibrinogen
- Low serum protein and albumin
- Hemoglobinuria
- Bilateral parotid swelling, “viper head” appearance
- Giddiness and syncope immediately following a snakebite
- Agitated behavior – cerebral anoxia, and profound thirst

Rare complications like bilateral parotid swelling due to capillary leak syndrome and hypopituitarism are also to be looked for.

Drugs Not to Be Used in Viper Bites

Heparin and Botropase

Heparin has been proposed as a means of reducing fibrin deposit in DIC. However, heparin use is controversial. Clinical trial evidence has shown that it has no beneficial effect. Venom-induced thrombin is resistant to heparin. The effect of heparin on antithrombin III are negated due to the elimination of antithrombin III by the time heparin is administered, and heparin can cause bleeding by its own action.

Botropase is a coagulant compound derived from one of the two South American pit vipers. It should not be used as a coagulant in viper bites as it simply prolongs the coagulation abnormality by causing consumption coagulopathy in the same way as the Indian viper venom currently affecting the victim.

Conclusion and Future Directions

A standard protocol for the treatment of Viperidae bites is now established. There is no doubt about the efficacy of ASV. The future is the shift to monovalent ASV for more effective, less costly, and significantly better side effect profile.

Before developing monovalent ASV, one should have the means to diagnose the species of snake for which ASV could then be developed. The most accurate means for the same would be an ELISA-based kit for the diagnosis of snakebite which would reliably tell us as to the species of the snake.

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Complications of Hemotoxic Snakebite in India

10

Jaideep C. Menon and Joseph K. Joseph

Contents

Abstract

Hemotoxic snakebite is the major cause of mortality and morbidity in India in victims of venomous snakebite. The hemotoxic species in India include the Russell's viper, saw-scaled viper, and various pit viper species.

The major complications related to hemotoxic snakebite, other than bleeding manifestations, which could occur anywhere from the bite site, mucosal

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membranes, serous cavities, and organs, include life-threatening ones like acute kidney injury (AKI), acute respiratory distress syndrome (ARDS) or its lesser variant acute lung injury (ALI), disseminated intravascular coagulation (DIC), and capillary leak syndrome (CLS). Mortality secondary to hemotoxic snakebite is high with complications like ARDS and CLS.

The prolonged hospital stay and mortality in hemotoxic snakebites as compared to Elapidae bites are mostly related to the multiorgan involvement seen in hemotoxic snakebites which is not seen in Elapidae bites.

The long-term complications related to hemotoxic snakebite include amputations and limb deformities, hypopituitarism, osteomyelitis, squamous cell carcinoma at sites of nonhealing ulcers, sequelae of acute coronary syndromes like left ventricular dysfunction, and sequelae of stroke like limb weakness or cognitive impairment.

Introduction

The one million death study estimated deaths due to venomous snakebites are in between 40,900 and 50,900 per year in India. Hemotoxic snakebites are the major cause of mortality and morbidity in most parts of India, other than certain pockets like North Bengal and parts of Orissa (Mohapatra et al. 2011).

Hemotoxic snakebites result from the bites of the *Daboia russelii* (Russell's viper), *Echis carinatus* (saw-scaled viper), and about 15 different species of pit vipers. Among the pit vipers, the *Hypnale hypnale* (hump-nosed pit viper), the *Ovophis monticola* (mountain pit viper), the *Trimeresurus malbaricus* (Malabar pit viper), and the *Trimeresurus gramineus* (bamboo pit viper) stand out as being medically significant in India (Government of India 2006, 2007, 2008, 2009; Alirol et al. 2010; Williams 2010).

Snake venom is a complex mixture of enzymes, nonenzymatic proteins, and glycoproteins. The primary function of venom is to both immobilize and digest the prey. Venom when injected into a live moving prey gets distributed to all parts of the body, and the process of digestion (autodigestion) actually starts even before the prey is swallowed (Bauchot 1994; Bottrall et al. 2010).

Venom Constituents and Its Function

The snakes have the most complex of all venoms. More than 90 % of the dry weight of venom is protein comprising a rich variety of enzymes, nonenzymatic polypeptide toxins, and nontoxic proteins such as nerve growth factor. Nonprotein ingredients of venom include carbohydrates and metals (often part of glycoproteins and metalloprotein enzymes), lipids, free amino acids, nucleosides, and biogenic amines such as serotonin and acetylcholine (Iyaniwura 1991; Marshall 2005).

About 80–90 % of Viperidae and 25–70 % of Elapidae venom consist of enzymes. The role of enzymes in envenoming is most clearly seen in the case of venom procoagulants. For example, *Daboia russelli* venom contains the following:

Glycoproteins which activate factor X
Glycoproteins which activate factor XI
Arginine ester hydrolases which activate factor V
Echis carinatus venom (saw-scaled viper) contains a zinc metalloprotein – ecarin which activates prothrombin

Many Crotalinae venoms (pit viper) contain proteases which cleave fibrinogen molecule, for example, Arvin or ancrod (*C. rhodostoma*) and Reptilase or Bothrops atrox (*B. atrain*).

The complex mixture of enzymes in snake venom has components which allow for better absorption from the bite site (hyaluronidases), better distribution through systemic vasodilatation (ablomin, arginine acetyl hydrolase, nucleotidases), breakdown of cell membranes and tissue (phospholipases), protein breakdown (proteases), and nucleic acid breakdown (nucleotidases).

The complex actions of venom allow for the breakdown and thereby digestion of most animal tissue. Most enzyme fractions have 37 °C as the ideal temperature for catalytic activity which is a reason for venom fractions having such devastating complications in humans (Marshall 2005; Kang et al. 2011).

Snake venom contains at least 26 different types of enzymes, 12 of these enzymes are common in all venoms, and the rest occur separately in certain species (Bailey 1998). Venoms of Viperidae and Crotalinae possess a very strong proteolytic activity, while those of Elapidae and Hydrophiidae have very weak proteolytic action (Farid et al. 1989; Braud et al. 2000).

Enzymes that are seen in snake venom are:

Fibrinogenolytic enzymes such as alpha-fibrinogenases, beta-fibrinogenases, and gamma-fibrinogenases
Plasminogen activator releasers such as ecarin
Prothrombin activators
Prothrombinase complex formation inhibitors such as phospholipases A_2, B, C and D
Factor X activator
Factor V activator
Factor XI activator
Protein C activator and fibrinogenolysin
Platelet aggregation inducers, either without coagulant activity or with coagulant activity
Platelet aggregation inhibitors, such as alpha-fibrinogenases or 5-nucleotidase, ADPase, fibrinogen receptor antagonists, and von Willebrand factor-dependent platelet aggregation inducers (Denson 1969; Djebari et al. 1995; Gutiérrez and Rucavado 2000; Bailey and Wilce 2001; Clemetson et al. 2007).

Zinc metalloproteases disrupt the endothelaial lining of blood vessels causing spontaneous bleeding, hyaluronidases (spreading factor), arginine esterase, and L-amino acid oxidase which is widely found in snake venoms and is responsible for the yellow coloration of snake venom due to the presence of riboflavin as a prosthetic group. Most of these enzymes are hydrolytic in nature except L-amino acid oxidase that causes oxidative deamination of amino acids. It is also reported to play a role in inhibiting platelet aggregation, induction of apoptosis, hemorrhagic effects, and cytotoxicity. Other enzymes present in snake venoms and considered as toxic components include phosphodiesterases, phosphatases (acid and alkaline), cholinesterases, transaminases, proteases, esterases, 5-nucleotidase, ATPase, and RNAase. However, none of these enzymes are responsible for the acute toxicity of snake venom inducers (Denson 1969; Djebari et al. 1995; Gutiérrez and Rucavado 2000; Bailey and Wilce 2001; Clemetson et al. 2007). Phospholipase A2 (PLA2) is one of the venom enzymes that catalyze the hydrolysis of fatty acid ester bonds and phospholipids. The specificity of the enzyme is directed toward the site of the fatty acid at the B-position rather than to the type of fatty acid. It is believed that phospholipase A2 is responsible for hemolysis produced by venoms. This may be due to its direct action on red blood cell membrane or indirect action by cleavage of lecithin, producing lysolecithin (a hemolytic factor). Phospholipase A2 is the most widespread and extensively studied of all venom enzymes. Up to 30 % of protein content is phospholipase A2, presenting in the form of seven isoenzymes. Under experimental condition, it damages the mitochondria, red blood cells, leukocytes, platelets, peripheral nerve endings, skeletal muscle, vascular endothelium, and other membranes. It also produces presynaptic neurotoxic activity, opiate-like sedative effects, and autopharmacological release of histamine. Phospholipase A2 activity contributes to many of the clinical manifestations of envenoming including hemolysis, rhabdomyolysis, presynaptic neurotoxicity, hepatic necrosis, platelet damage, edema formation, and vasodilatation causing shock and release of endogenous autacoids such as histamine serotonin and slow releasing substances, which may contribute to the local pain and permeability changes at the site of snakebite (Denson 1969; Djebari et al. 1995; Gutiérrez and Rucavado 2000; Bailey and Wilce 2001; Clemetson et al. 2007).

The complications of hemotoxic snakebite could be classified as:

- Local effects/envenoming at the site of the bite
- Systemic effects/envenoming due to organ involvement

Local Effects

The local effects at the site of the bite include swelling, tissue necrosis, and gangrene. Tissue necrosis is secondary to tissue destruction from snake venom enzymes in which the skin, subcutaneous tissue, and muscles get dissolved by enzyme action compounded by infections from anaerobic species in particular.

Fig. 10.1 Measuring intracompartmental pressure with a Stryker apparatus (Copyrighted to Dr Joseph K Joseph, Nephrologist LF Hospital)

This leads to an increase in intracompartmental pressure especially so in the limbs. An increase in intracompartmental pressure can lead to a decrease in limb perfusion, resulting in gangrene of the limb, digit, or toe. Intracompartmental pressure greater than 30 mm of Hg is an indication for fasciotomy. Intracompartmental pressure is measured with the Stryker device (Fig. 10.1). Tissue loss, scars, gangrene, and even amputations may be the end results of extensive local tissue damage. Another reason for extensive local tissue damage is the application of tight tourniquets which prevent arterial flow to the limb. Tourniquets prevent the release and therefore the dilution of venom into the larger central vascular space, leading to more extensive tissue loss at the site of bite more so if more than one tourniquet is applied (Dhananjaya et al. 2010; WHO Neglected Tropical Diseases 2010).

Nonhealing ulcers at the bite site have also been reported, one of the reasons for which is when a fang gets embedded at the bite site. Osteomyelitis can also result from venomous snakebite. Tenosynovitis has also been reported after hemotoxic snakebites of the foot (Hofer et al. 1993; Basavraj Nagoba et al. 2011). Squamous cell carcinoma has also been reported years after a venomous snakebite, having led to a nonhealing ulcer initially which in turn acts as a milieu for carcinomatous change (Mello et al. 2000). Amputation of a digit, toe, or limb is one of the dreaded complications of venomous snakebite. Amputations become necessary when the part becomes gangrenous. Extensive tissue necrosis secondary to the effect of venom enzymes and infection especially with anaerobes and gas-forming organisms compromise the arterial flow when the intracompartmental pressure in the limb rises. Fasciotomies help to relieve the built-up intracompartmental pressure and restore some degree of onward arterial blood flow. Signs of gangrene, pain, pallor, weakness of the limb, pulselessness, and paraesthesia are indications for an amputation (Abbas et al. 2009; Ajibade et al. 2011).

Systemic Effects

These result from the direct action of venom components or from the secondary venom action in the body. Hemotoxic snakebites are classified as such because of their effect on blood hemostasis. Bleeding manifestations are the "sine qua non" of hemotoxic snakebite. Snake venom components have both prothrombotic and anticoagulant effects. The effects of hemotoxic venom could be on coagulation factors by direct activation of, for example, factors X, V, and X1, increase of fibrinolytic activity by inducing tissue plasminogen activators, inhibition of plasminogen activator inhibitors, and actions on the platelets which include aggregation and platelet inhibition. Procoagulant effects – factor IX activation by cleavage of peptide bond *IX* by Russell's viper venom, factor *X* activation by Ca++ binding to gamma-glutamic residues in factor *X* with rapid change to the activated factor *X*a, direct prothrombin activation by cleavage of peptide bonds by venom, producing an intermediate which quickly converts to thrombin. Prolonged defibrination without thrombocytopenia occurs in *Echis carinatus* bites. Phospholipase A and a basic protein called direct lytic factor present in Russell's viper and *Echis carinatus* venom can lead to intravascular hemolysis. The viper venom activates the coagulation cascade at a number of sites, leading to rapid thrombin formation. Bleeding is due to VICC (venom-induced consumption coagulopathy) and due to the direct action of snake venom proteases:

Inhibition of platelet aggregation.
Inhibition of clotting factors or their activation.
Direct fibrinolysis or fibrinogenolysis.
Direct action on plasminogen or its proactivator is the mechanism by which snake venom enzymes exert their anticoagulant action.

The commonly observed bleeding manifestations include:

- Bleeding from the gums and oral cavity.
- Bleeding or ooze from the bite site.
- Gastrointestinal bleeds manifest as hematemesis and melena. Autopsy specimens of the stomach have shown gastric erosions and small punctate ulcers in the gastric mucosa as the causes of gastric bleeds.
- Epistaxis and hemoptysis.
- Subconjunctival bleeds.
- Erythema and purpuric patches.
- Increased menstrual bleed.
- Perinephric and renal bleeds.
- Intramuscular hematomas.
- Rarely intracerebral bleeds and hemopericardium and hemorrhagic pleural effusions

Bleeding from the gums and oral cavity is usually the first sign of a hemotoxic snake envenomation, especially so in individuals with poor oral hygiene. Bleeding is most common from the gingival sulcus (Devaraj 1979). Spontaneous systemic bleeding such as gum bleeding and bleeding from venipuncture sites usually stops within 15–30 min of instillation of ASV (anti-snake venom). Blood coagulability is usually restored in 6 h after ASV infusion, as tested by the 20 min WBCT (whole blood clotting test). Active hemolysis and rhabdomyolysis may cease within few hours, and urine returns to its normal color. In patient with shock, blood pressure may increase after 30 min of having been given ASV. Bites due to the hump-nosed pit viper (*Hypnale hypnale*) can lead to an abnormal coagulation profile, and the 20 min WBCT could take up to 2–3 weeks to normalize. This is usually not associated with spontaneous bleeding manifestations (Premawardena et al. 1998; Wijewantha and Sellahewa 2010).

Renal Failure

Renal failure is a common complication of Russell's viper, saw-scaled viper, and hump-nosed pit viper bites. Loin or lumbar pain in the first 12–24 h is a predictor of the likelihood of the victim developing renal failure. Acute kidney injury (AKI) is the most frequent and clinically important effect of envenomation on the kidneys. The *RIFLE criteria*, proposed by the Acute Dialysis Quality Initiative (ADQI) group, aid in the staging of patients with AKI.

According to the National Kidney Foundation, normal GFR ranges from 90 to 120 ml/min/1.73 m^2. GFR is calculated from the MDRD (modification of diet in renal disease) or *the* Cockcroft–Gault formulas:

- Risk: glomerular filtration rate (GFR) decreases >25 % and serum creatinine increases 1.5 times or urine production of <0.5 ml/kg/h for 6 h
- Injury: GFR decrease of >50 % and doubling of creatinine or urine production <0.5 ml/kg/h for 12 h
- Failure: GFR decrease of >75 %, tripling of creatinine or creatinine >355 μmol/l (with a rise of >44) (>4 mg/dl), or urine output below 0.3 ml/kg/h for 24 h
- Loss: persistent AKI or complete loss of kidney function for more than 4 weeks

The majority of cases are seen after viper bites, and the incidence varies with the distribution of Viperidae snakes in different geographic regions. About 13–32 % of those bitten by *Daboia russelli*, *Echis carinatus*, or the *Hypnale hypnale* develop AKI in India. The reported incidence from other countries varies between 1 % and 27 %. The symptoms depend on the dose of venom injected. Renal failure in a Viperidae bite is multifactorial – DIC (disseminated intravascular coagulation), acute tubular necrosis (ATN) resulting from shock, intravascular hemolysis, hemoglobinuria, and myoglobinuria from rhabdomyolysis along with direct nephrotoxicity of snake venom enzymes being the likely causes. Bleeding into the kidneys

and perinephric capsule could be another reason (Chugh et al. 1984; Chugh 1989). Apart from blood loss, hypotension and circulatory collapse can result from the release of kinins or depression of medullary vasomotor center or myocardium. Kininogenases are present in crotalid venom. There is also the possibility of immune complex deposits related to the venom–antivenom complex getting deposited in the glomerulus and causing injury. In view of the typical loin pain especially with Russell's viper preceding the development of acute kidney injury (AKI) could be a direct toxic effect of venom components (Chugh et al. 1984; Chugh 1989).

Renal Histopathology

Renal histology shows predominantly either acute tubular or cortical necrosis. A number of glomerular changes have been described, but their significance is not known. Acute tubular necrosis is the predominant lesion seen in 70–80 % of patients with ARF. On light microscopy, the tubules appear dilated and lined by flattened epithelium. Severe cases exhibit cell necrosis and desquamation of necrotic cells from the basement membrane. Hyaline, granular, or pigment casts are seen in tubular lumina. Varying degrees of interstitial edema, hemorrhage, and inflammatory cell infiltration are present. Later biopsies reveal regenerating tubular epithelium. Intrarenal blood vessels are usually unaffected.

On ultrastructural examination, proximal tubules show dense intracytoplasmic bodies representing degenerating organelles or protein resorption droplets. Small areas of basement membrane are denuded. Distal tubular cells have a dilated endoplasmic reticulum and many degenerating organelles. Apoptosis is a prominent feature in the distal tubules, indicating a high cell turnover. In the interstitium, fibroblasts appear active, with increased numbers of organelles and cytoplasmic processes. Mast cells and eosinophils show both granulated and partially degranulated forms.

Although the blood vessels appear normal under light microscopy, ultrastructural abnormalities are notable in both large- and small-caliber vessels. Medullary vessels are severely affected, with markedly swollen, focally necrotic, endothelial cells obliterating the lumen. Smooth-muscle cells show cytoplasmic vacuoles, which are empty or are filled with granular material. The severe vascular lesions, distal tubular apoptosis, and presence of mast cells, eosinophils, and active fibroblasts in the interstitium are features that have not been observed in acute tubular necrosis from other causes.

Acute Cortical Necrosis – Bilateral, diffuse, or patchy cortical necrosis has been observed following bites by *E. carinatus*. Cortical necrosis appears to be more common among Indian patients than among patients in Thailand, for unknown reasons. The presence of fibrin thrombi in the arterioles is a prominent feature in these patients. A narrow subcapsular rim of cortex often escapes necrosis. The area underlying this, however, shows necrosis of glomerular as well as tubular elements. The necrotic zone is often bordered by an area of hyperemia and leukocytic infiltration. Calcification of necrotic areas may occur at a later stage. Varying

numbers of glomeruli are spared in patients with patchy cortical necrosis. With healing, fibroblastic proliferation and organization of thrombi are seen following *Russell's viper bite*. Glomeruli with collapsed capillary basement membrane and denuded foot processes. No viable endothelial or mesangial cell could be identified, but swollen rounded cells, possibly of endothelial origin, were seen in some capillary lumina. Endothelial swelling of small arterioles and necrosis of peritubular capillaries were also seen. The tubular basement membrane was intact, but the epithelium showed degenerative changes. In the second patient, the biopsy was done 31 days after envenomation. In this patient, the urinary space contained unidentified cells with large cytoplasmic vacuoles. The tubular basement membrane was thickened, and the cortical tubules were lined by flattened epithelium, with large nuclei and a dilated endoplasmic reticulum. Fibroblastic proliferation was seen in the interstitium.

Glomerular Lesions – Whether or not specific glomerular lesions really occur is still controversial. Sant and Purandare reported a "proliferative glomerulonephritis" in patients bitten by E. *carinatus*. Later, Seedat et al reported two patients with crescentic glomerulonephritis, following puff adder bites, presenting as ARF. Because renal lesions of proliferative nephritis with crescents had developed within 24–48 h, these workers ascribed these lesions to an allergic reaction to snake venom. Sitprija and Boonpucknavig described two patients with crescentic glomerulonephritis after *Russell's viper* bites. In another study of 38 patients bitten by the green pit viper or *Russell's viper*, the authors observed thickening of the mesangial areas and mild mesangial proliferation in most of their patients and diffuse glomerular hypercellularity (ascribed to marked mesangial proliferation) in two patients. Other glomerular changes observed are ballooning of capillaries, endothelial swelling, mesangiolysis, and splitting of the glomerular basement membrane; however, the significance of these is difficult to ascertain. Immunofluorescence microscopy showed IgM, C3, and fibrin deposits. In occasional instances, a diffuse and intense mononuclear cell infiltrate has been noted in the interstitium, suggesting the occurrence of an acute interstitial nephritis.

The onset of renal failure is signaled by a decrease in urine output. This may occur within 1 h to as late as 4 days after the bite. Renal damage can develop very early in the case of Russell's viper bite, and even when the patient arrives at the hospital soon after bite, the damage may already have been done. Studies have shown that even when ASV is administered within 2 h of the bite, it is still incapable of preventing AKI.

About half of the patients give a history of passage of cola-colored urine, indicating intravascular hemolysis. Fibrin degradation products can be detected in the urine, indicating disseminated intravascular coagulation. Life-threatening hyperkalemia may develop in patients with hemolysis or myonecrosis. Renal failure is nonoliguric in less than 10 % of cases. Persistence of oliguria of more than 1 month indicates the possibility of acute cortical necrosis which may be confirmed by renal biopsy. Acute cortical necrosis due to snakebite is the second most common cause of acute cortical necrosis in India (Sitprija 2006; Patil and Bansod 2012).

Elevated urinary excretion of *N*-acetylglucosamine (NAG) is an early marker of renal damage in bitten patients. There is a strong correlation between AKI, platelet count, and albuminuria in envenomed victims of snakebite. Victims with a low platelet count <80,000/cmm and albuminuria are at a greater risk of progressing to AKI. Other causes of AKI include glomerulonephritis, direct nephrotoxicity of venom, tubulointerstitial nephritis, and rarely papillary necrosis. In patients with established acute kidney injury, the treatment of choice is dialysis. There is usually a period of anuria during which time the victim does not pass any urine at all. This usually lasts from a few days to a week. The patient then starts passing urine which improves progressively over a few days from a few ml to normal quantities. This can be followed by a period of polyuria which could last between 1 and 2 weeks. This is a period in which adequate hydration has to be maintained and care given to the maintenance of normal serum electrolytes, namely, serum sodium, potassium, chloride, bicarbonate, calcium, and phosphate.

Indications of Dialysis

Clinical

- Anuria of > 48 h
- Deterioration of general condition
- Severe metabolic acidosis
- Hyperkalemia
- Pulmonary edema

Biochemical

- Blood urea >120 mg/dl
- Serum creatinine > 5 mg/dl
- Serum potassium > 6 meq/l
- Daily rise of blood urea > 50 mg/dl
- Daily rise of serum creatinine > 1 mg/dl
- Daily fall of bicarbonates > 2 meq/dl

Hemodialysis is preferred in those who are hemodynamically stable. In those who are hemodynamically unstable, CAVHD (continuous arteriovenous hemodialysis), CAVH (continuous hemofiltration), CVVH (continuous venovenous hemofiltration), and CVVHD (continuous venovenous hemodiafiltration) can be employed. Hemodialysis is a method that is used to achieve the extracorporeal removal of waste products such as creatinine, urea, and free water from the blood when the kidneys have gone into failure. The principle of hemodialysis is the same as other methods of dialysis in that it involves diffusion of solutes across a semipermeable membrane. Hemodialysis utilizes countercurrent flow, where the dialysate is flowing in the opposite direction to blood flow in the extracorporeal circuit. Countercurrent flow maintains the concentration gradient across the membrane at a maximum and increases the efficiency of dialysis.

Fluid removal or ultrafiltration is achieved by altering the hydrostatic pressure of the dialysate compartment, causing free water and some dissolved solutes to move across the membrane along a created pressure gradient.

Hemofiltration is a renal replacement therapy similar to hemodialysis which is used almost exclusively in the intensive care setting. Thus, it is almost always used for acute kidney injury. It is a slow continuous therapy in which sessions usually last between 12 and 24 h and are usually performed daily. During hemofiltration, a patient's blood is passed through a set of tubing (a *filtration circuit*) via a machine to a semipermeable membrane (the *filter*) where waste products and water are removed. Replacement fluid is added and the blood is returned to the patient. As in dialysis, in hemofiltration, one achieves movement of solutes across a semipermeable membrane. However, solute movement with hemofiltration is governed by convection rather than by diffusion. With hemofiltration, dialysate is not used. Instead, a positive hydrostatic pressure drives water and solutes across the filter membrane from the blood compartment to the filtrate compartment, from which it is drained. Solutes, both small and large, get dragged through the membrane at a similar rate by the flow of water that has been engendered by the hydrostatic pressure. Thus, convection overcomes the reduced removal rate of larger solutes (due to their slow speed of diffusion) seen in hemodialysis. An isotonic replacement fluid is added to the blood to replace fluid volume and electrolytes. The replacement fluid must be of high purity, because it is infused directly into the blood line of the extracorporeal circuit. The replacement hemofiltration fluid usually contains lactate or acetate as a bicarbonate-generating base or bicarbonate itself. The use of lactate can occasionally be troublesome in patients with lactic acidosis or with severe liver disease, because in such cases the conversion of lactate to bicarbonate can be impaired. In such patients, the use of bicarbonate as a base is preferred.

Hemodiafiltration: Hemofiltration is sometimes used in combination with hemodialysis when it is termed hemodiafiltration. Blood is pumped through the blood compartment of a high-flux dialyser and a high rate of ultrafiltration is used, so there is a high rate of movement of water and solutes from the blood to the dialysate that must be replaced by substitution fluid that is infused directly into the blood line. However, dialysis solution is also run through the dialysate compartment of the dialyser. The combination is useful because it results in good removal of both large- and small-molecular-weight solutes (Brenner and Rectors 2001). **Peritoneal dialysis**, which is less effective, has a role in children and in hemodynamically unstable patients.

Acute Respiratory Distress Syndrome (ARDS)

Another major complication of hemotoxic snakebite is acute respiratory distress syndrome or acute lung syndrome. This too is multifactorial, the possible explanations being toxin related, DIC (disseminated intravascular coagulation) related, or aspiration related. The components of the venom are known to cause a capillary leak and could thereby directly cause ARDS. Another possibility is an immune

complex deposition resulting from venom–antivenom complexes getting deposited in the capillaries of the lung.

Acute respiratory distress syndrome (**ARDS**), also known as **adult respiratory distress syndrome**, is a life-threatening reaction to injuries or acute infection to the lung. ARDS is a severe lung syndrome with direct and indirect causes. Inflammation of the lung parenchyma leads to impaired gas exchange with systemic release of inflammatory mediators, causing inflammation, hypoxemia, and frequently multiple organ failure. This condition has a 90 % death rate in untreated patients. With treatment, usually mechanical ventilation in an intensive care unit, the death rate is ~50 % but advances in treatment using prone-positioning sessions while the patient is on mechanical ventilation have reduced mortality rates to ~25 %. A less severe form is called acute lung injury (ALI).

Signs and Symptoms

People usually present with shortness of breath and tachypnea leading to hypoxia to the brain, occasionally causing confusion. ARDS mostly occurs about 72 h after the trigger, such as an injury (trauma, burns, aspiration, massive blood transfusion, drug/alcohol abuse) or an acute illness (infectious pneumonia, sepsis, acute pancreatitis).

ARDS is characterized by the following four criteria:

1. Lung injury of acute onset, within 1 week of an apparent clinical insult and with progression of respiratory symptoms
2. Bilateral opacities on chest imaging not explained by other pulmonary pathologies (e.g., pleural effusions, lung collapse, or nodules)
3. Respiratory failure not explained by heart failure or volume overload
4. Decreased arterial PO_2/FiO_2 ratio

Mild ARDS: 201–300 mmHg ($\leq$39.9 kPa)
Moderate ARDS: 101–200 mmHg ($\leq$26.6 kPa)
Severe ARDS: $\leq$ 100 mmHg ($\leq$13.3 kPa)

A decreased PO_2/FiO_2 ratio indicates reduced arterial oxygen content relative to that of the inhaled gas, indicating a failure of the lung to transport oxygen into the blood. The above characteristics are the "Berlin criteria" of 2012 by the European Society of Intensive Care Medicine, endorsed by the American Thoracic Society and the Society of Critical Care Medicine. They are a modification of the previously used criteria:

- Acute onset:
- Bilateral infiltrates on chest radiograph sparing costophrenic angles.
- Pulmonary artery wedge pressure $<$ 18 mmHg (obtained by pulmonary artery catheterization), if this information is available; if unavailable, then there is lack of clinical evidence of left atrial hypertension.

- If $PaO_2/FiO_2 < 300$ mmHg (40 kPa), acute lung injury (ALI) is considered to be present.
- If $PaO_2/FiO_2 < 200$ mmHg (26.7 kPa), acute respiratory distress syndrome (ARDS) is considered to be present.

ARDS is an acute injury to the lungs that results in alveolar flooding, atelectasis, and a severe oxygenation defect but is not due to heart failure. Diffuse alveolar damage (DAD) is characterized by a diffuse inflammation of lung parenchyma. The triggering insult to the parenchyma usually results in an initial release of cytokines and other inflammatory mediators, secreted by local epithelial and endothelial cells. Neutrophils and some T lymphocytes quickly migrate into the inflamed lung parenchyma and contribute in the amplification of the phenomenon. Typical histological presentation involves diffuse alveolar damage and hyaline membrane formation in the alveolar walls.

Inflammation alone, as in sepsis, causes endothelial dysfunction, fluid extravasation from the capillaries, and impaired drainage of fluid from the lungs. Dysfunction of type II pulmonary epithelial cells may also be present, with a concomitant reduction in surfactant production. Elevated inspired oxygen concentration often becomes necessary at this stage and may facilitate a "respiratory burst" in immune cells. In a secondary phase, endothelial dysfunction causes cells and inflammatory exudate to enter the alveoli. This pulmonary edema increases the thickness of the alveolocapillary space, increasing the distance the oxygen must diffuse to reach the blood. This impairs gas exchange leading to hypoxia, increases the work of breathing, and eventually induces fibrosis of the air spaces. Moreover, edema and decreased surfactant production by type II pneumocytes may cause the whole alveoli to collapse or to completely flood. This *loss of aeration* contributes further to the right-to-left shunt in ARDS. As the alveoli contain progressively less gas, the blood flowing through the alveolar capillaries is progressively less oxygenated, resulting in massive intrapulmonary shunting. Collapsed alveoli (and small bronchi) do not allow gas exchange. It is not uncommon to see patients with a PaO_2 of 60 mmHg (8.0 kPa) despite mechanical ventilation with 100 % inspired oxygen. The loss of aeration may follow different patterns according to the nature of the underlying disease and other factors. In pneumonia-induced ARDS, for example, large, more commonly causes relatively compact areas of alveolar infiltrates. These are usually distributed to the lower lobes, in their posterior segments, and they roughly correspond to the initial infected area. In sepsis, trauma-induced ARDS, and also hemotoxic snakebite, infiltrates are usually more patchy and diffuse. The posterior and basal segments are always more affected, but the distribution is even less homogeneous. Loss of aeration also causes important changes in lung mechanical properties. These alterations are fundamental in the process of inflammation amplification and progression to ARDS in mechanically ventilated patients.

Acute respiratory distress syndrome is usually treated with mechanical ventilation in the intensive care unit. Ventilation is usually delivered through orotracheal intubation or tracheostomy whenever prolonged ventilation ($\geq$2 weeks) is deemed

inevitable. The possibilities of noninvasive ventilation are limited to the very early period of the disease or, better, to prevention in individuals at risk for the development of the disease (atypical pneumonias, pulmonary contusion, major surgery patients) (Cheung et al. 2006; Vincent and Zambon 2006; Malhotra 2007; Bakowitz et al. 2012; The ARDS Definition Task Force 2012; Guérin et al. 2013). In prone position, the distribution of lung infiltrates in acute respiratory distress syndrome is nonuniform. Repositioning the patient into prone position (face down) might improve oxygenation by relieving atelectasis and improving perfusion. The PROSEVA trial has shown apparent mortality benefit in patients with severe ARDS and a PaO_2/FIO_2 ratio of less than 150 mmHg (Guérin et al. 2013).

Nervous System Complications

This could range from a catastrophic **intracerebral bleed** in hemotoxic snakebites to **cerebral infarcts** from DIC. As mentioned above, hemorrhage could be due to venom-induced consumptive coagulopathy or the direct action of hemorrhagins in snake venom. The signs and symptoms are like any other hemorrhagic stroke including loss of consciousness, headache, lateralizing signs, vomiting, and signs of raised intracerebral pressure like papilledema, pupillary signs, and Cheynes–Stokes respiration. The treatment of a hemorrhagic stroke is like any other hemorrhagic stroke.

Ischemic strokes typically develop 2–3 days after the bite, the etiology being DIC (disseminated intravascular coagulation). The symptom complex corresponds to the cerebral artery involved. Treatment would be along the lines of an ischemic stroke (Malhotra 2007; Del Brutto and Del Brutto 2012). Rarely **pituitary infarcts** are known to occur, which present a few months after the bite with symptoms of fatigue, lassitude, somnolence, and loss of secondary sexual characteristics as a consequence of hypopituitarism. The symptoms are generally related to hypothyroidism, adrenal insufficiency, gonadotropin-releasing hormone deficiency, and growth hormone deficiency in that order, and the treatment is with hormone replacement (Uberoi et al. 1991; Antoypillai et al. 2011; Bhatt et al. 2013). **Ptosis** has been reported following bites by the *Daboia russelii* in Kerala, South India, and has been reported before by investigators in Sri Lanka and Burma. Ptosis following a Russell's viper bite is presynaptic and does not respond to treatment with neostigmine and atropine. Ptosis is usually short lasting and recovers spontaneously in 12–24 h of its onset. Muscle weakness following a Russell's viper bite is limited to the ocular muscles and is nonprogressive.

Cardiac Complications

The cardiac complications of Elapidae and Hydrophiidae bites include myocarditis which presents with symptoms of heart failure. Hemotoxic snakebites could lead to a hemorrhagic pericardial effusion.

Acute coronary syndromes (ACS) ranging from an ST-segment elevation acute myocardial infarction, non-ST-segment elevation myocardial infarction, to unstable angina have all been reported after a hemotoxic snakebite. This procoagulant phenomenon is secondary to DIC or could be due to preexisting disease. This being a life-threatening emergency is treated just as any other ACS with the standard measures of a primary intervention by way of thrombus aspiration and plain ballooning (POBA, plain old balloon angioplasty). The likelihood of stent thrombosis is high in DIC. Other treatment measures tried include intravenous thrombolysis in the standard doses, low-molecular-weight heparin, statins, and antiplatelet agents. Glycoprotein 11b/111a inhibitors like tirofiban, abciximab, and Integrilin and unfractionated heparin are to be avoided in cases with a low platelet count. Acute rhythm disturbances are common with bradycardia, first-degree heart block, or tachyarrhythmia in which paroxysmal supraventricular tachycardia (PSVT) and atrial fibrillation (AF) have all been reported following a hemotoxic snakebite.

Hepatobiliary System

A two to threefold increase in serum transaminases has been seen in most envenomed cases of hemotoxic snakebite. The rise in ASO (aspartate aminotransaminase) is more marked than in ALT (alanine aminotransaminase). The enzymes rise within 24 h of the bite. Jaundice has been noted with severe Viperidae bite, mostly due to hemolysis which carries a bad prognosis. The fall in serum albumin and total proteins in Viperidae bite is more of a consequence of capillary leak and proteinuria than due to liver failure.

Capillary Leak Syndrome (CLS)

The capillary leak syndrome was first described by Clarkson in 1960. It is characterized by hypotension with hemoconcentration, hypoalbuminemia without albuminuria, and generalized edema. This syndrome is due to a capillary hyperpermeability with massive extravasation of plasma containing macromolecules smaller than 200 kD and at times up to 900 kD. Hemorrhagins are nonenzymatic components of snake venom. These are zinc-containing metalloproteinases which degrade the compact proteins of basement membrane underlying the endothelial cells. They also act on the endothelial cells causing lysis or drifting apart of vascular endothelium leading to a shift of fluid from intravascular to interstitial space. Two immunologically distinct nonenzymatic hemorrhagic principles (HR 1 and HR 2) are typical components of crotalid (pit viper) and viperid (true viper) venoms. They cause acute, rapid hemorrhage. In many instances of severe envenoming, the hemorrhagins play a major lethal role by causing hemorrhage in the vital organs, e.g., the brain, lungs, kidneys, heart, and gastrointestinal tract. Pharmacologically, the hemorrhagins have been demonstrated to be separate entities from proteolytic enzymes in the venom. It has been observed that they cause severe vasoconstriction

followed by vasodilatation of microvessels and arterioles with hemorrhage in the capillary bed. The hemorrhagins act by directly disrupting the endothelial lining and by inhibiting platelet aggregation. Pharmacological studies have further shown that the hemorrhagic principles induce the release of certain autopharmacological mediators such as histamine and 5-HT from various tissues which open up endothelial cell junction and disrupt the isolated basement membrane, presumably in an enzymatic mode of action, thus causing vascular damage and hemorrhage. Similarly, vasculotoxic changes have also been observed in the renal and cerebral vessels with crotalid venom. The observed vasculotoxic changes resulting in severe cutaneous and systemic hemorrhage particularly in the kidneys, lungs, and brain bear a close resemblance to that of experimental Shwartzman phenomenon with bacterial toxins. Two vascular apoptosis-inducing proteins VAP1 and VAP2 that damage endothelial cells have also been identified. The apoptotic activity of VAP2 is specific for endothelial cells. Snake venom enzymes produce reactive oxygen species (ROS) which lead to apoptosis. Serine proteases are capillary permeability-increasing enzymes which have been isolated from hemotoxic snake venom.

The pathological change in the capillary brought about by BaH1, a hemorrhagic metalloproteinase isolated from bothrops asper, was studied in mouse gastrocnemius muscle after intravascular injection of the toxin. At the ultrastructural level, the earliest change was a decrease in the number of pericyte vesicles, cytoplasmic projections into vascular lumen, and detachment of the endothelial cell from the surrounding lamina with thinning of the endothelial cell. Capillaries showed gaps in the endothelium through which fluid leaks to the interstitial space.

The clinical features are:

Prodromal phase:
- Irritability, fatigue, and myalgia
- Nausea and abdominal pain
- Profound thirst and syncope

Initial phase (days 1–4):
- Anasarca
- Pleural and pericardial effusion
- Ascites
- Conjunctival chemosis
- Bilateral parotid swelling (viper head appearance)
- Hypotension

Recruitment phase:
- Pulmonary edema
- Polyuria

Initial capillary leak phase is characterized by hemoconcentration, leukocytosis, increase in IgM concentration, and decrease in serum albumin, IgG, C3, and C4 levels, and there may be an extravasation of up to 70 % of plasma. Pulmonary, cerebral, and renal circulations are involved infrequently in this phase. The second

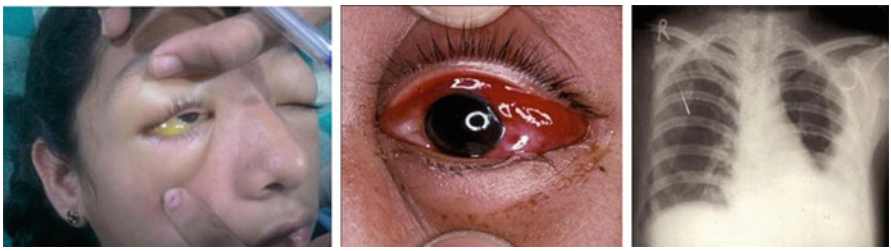

Fig. 10.2 Capillary leak syndrome manifested by periorbital edema and a left pleural effusion (Copyrighted to Dr Joseph K Joseph, Nephrologist LF Hospital)

phase is characterized by pulmonary edema, the most common cause of death in CLS (Assaly et al. 2001; Dutta 2007) (Fig. 10.2 – Capillary leak syndrome).

Treatment

Volume Replacement – Crystalloids and fresh frozen plasma are recommended in hemotoxic snakebite.

Vasopressors and Inotropic Support – When fluid resuscitation fails to maintain the mean arterial pressure, vasopressors like dopamine, norepinephrine, epinephrine, and phenylephrine are to be infused so as to maintain a systolic BP of 90 mm.

Disease-modifying agents tried include:

Theophylline
Terbutaline
Leukotriene inhibitors like montelukast
Plasmapheresis
Corticosteroids

Of these, corticosteroids are the agents that have shown the best response.

Prognosis in patients with hemotoxic snakebite developing CLS is uniformly dismal with a mortality as high as 90 %.

Disseminated Intravascular Coagulation (DIC)

The ISTH, International Society on Thrombosis and Haemostasis, has defined DIC as an acquired syndrome characterized by intravascular activation of coagulation with loss of localization arising from different causes.

The diagnosis of DIC requires the following:

1. An underlying disorder known to be associated with DIC
2. Clinical findings consistent with DIC
3. Laboratory findings

DIC is a clinicopathological syndrome characterized by systemic activation of pathways leading to and regulating coagulation, which can result in the generation of fibrin clots that may cause organ failure with concomitant consumption of platelets and coagulation factors that could result in bleeding.

ISTH diagnostic scoring system for DIC:

- Platelet count $> 100 \times 10^9$/l = 0
  $50–100 \times 10^9$/l = 1
  $< 50 \times 10^9$/l =2
- Elevated fibrin markers (D-dimer, fibrin degradation products)
  No increase =0
  Moderate increase =2
  Marked increase =3
- Prolonged PT
  < 3 s =0
  > 3 but <6 s =1
  > 6 s =2
- Fibrinogen level
  > 1 g/l =0
  < 1 g/l = 1

Interpretation of score: > _5 laboratory evidence suggestive of DIC

< 5 suggestive of non-overt or low-grade DIC

Using a cutoff of 5 gives the scoring system a 93 % sensitivity and a 98 % specificity for DIC.

The D-dimer is probably the single best test to rule out DIC, with a normal D-dimer effectively ruling out DIC. The suggested cutoff value for D-dimer is 0.6 μg/ml.

A moderate increase between 4 and 8 μg/ml scored as 2

Values >8 μg/ml scored as 3.

The laboratory abnormalities reported in decreasing order of frequency are low platelets, elevated fibrin degradation products, prolonged PT, prolonged APTT, and a low fibrinogen level.

Platelet Count – A low platelet count is seen in up to 98 % of cases of DIC.A low platelet count indicates thrombin generation as thrombin-induced platelet aggregation is primarily responsible for platelet consumption. A progressive drop in platelets suggests thrombin generation, and correspondingly, a stabilization of platelet values suggests that thrombin generation has stopped. It has to be pointed out that in hemotoxic snakebites the drop in platelets is also partly due to the direct destruction of platelets by venom enzymes.

Fibrin Degradation Products and **D-Dimer** – Fibrinolytic activity is measured as fibrin degradation products, FDP, or as one of the epitopes related to plasmin-degraded cross-linked polymer called the D-dimer. FDP are metabolized in the liver

and secreted in the kidneys; renal and hepatic damage could influence their levels. Soluble fibrin monomer (SFM) reflects thrombin action on fibrinogen. SFM is only generated intravascularly. Most clinical studies have shown a sensitivity of 90–100 % for the diagnosis of DIC with very low specificity (Horan and Francis 2001). However, its incorporation into the ISTH-DIC scoring system instead of D-dimer could help improve the specificity of the diagnosis of DIC.

Prothrombin Time (PT) and the **Activated Partial Thromboplastin Time** (APTT) – The PT and the APTT are abnormal in about 50–60 % of cases of DIC. In about half of patients with DIC, the PT and APTT are normal or shortened due to the presence of activated clotting factors like factor Xa and thrombin. It has to be pointed out that it is the PT and not the INR that is to be monitored as the INR is validated only for oral anticoagulant monitoring.

Fibrinogen – Fibrinogen levels could be normal in up to 57 % of cases of DIC. The sensitivity of low fibrinogen for the diagnosis of DIC was only 28 %, and hypofibrinogenemia was detected only in very severe cases of DIC. Fibrinogen is an acute phase reactant and remains in the normal range in spite of consumption coagulopathy. It has also to be borne in mind that measured fibrinogen levels may be influenced by the interference on the assay from high FDP levels.

Blood Film – In the presence of crenated RBCs, schistocytes provide confirmatory evidence of the process of DIC. Schistocytes rarely constitute more than 10 % of the red blood cells. Red blood cell membranes are disrupted directly by the action of venom enzymes especially phospholipases. In hemotoxic snakebite, the presence of crenated red blood cells could be due to either of the two mechanisms:

- Direct action of venom enzymes
- DIC as a complication of hemotoxic snakebite.

Newer Tests for Diagnosing DIC

An atypical light transmittance profile on the APTT has been associated with DIC. This transmittance profile seen as a biphasic waveform occurs independent of prolongation of the clotting times. It has been seen as a simple and rapid indicator of DIC. There is an increasing positive predictive value for DIC with increasing waveform abnormality, and this often precedes abnormalities in the coagulation parameters.

Treatment

The cornerstone of the treatment of DIC in hemotoxic snakebite is treatment of the snakebite itself.

Fresh Frozen Plasma and Platelets – Blood components are indicated in patients with active bleeding. In general, platelets are transfused in patients with bleeding who have a platelet count $<50 \times 10^9$/l. In nonbleeding patients, transfusions are indicated with a platelet count below $10–15 \times 10^9$/l.

For correction of the coagulation factors, fresh frozen plasma (FFP) is used. Initial doses of 15 ml/kg are suggested although there is evidence to suggest that a

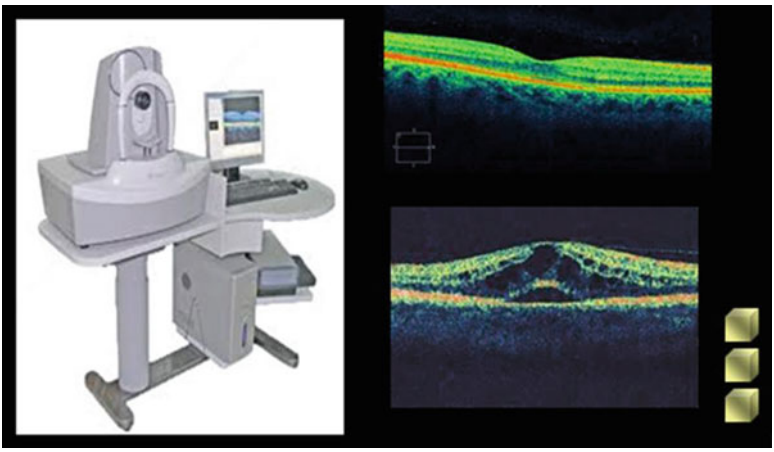

Fig. 10.3 Optical coherence tomography: retinal/macular edema (Copyrighted to Dr Joseph K Joseph, Nephrologist LF Hospital)

dose of 30 ml/kg produces a more complete correction of coagulation factors. Fibrinogen deficiency could be corrected by the administration of purified fibrinogen or cryoprecipitates. A dose of 3 g would raise the plasma fibrinogen levels by around 1 g/l (Carr et al. 1989; Nayak et al. 1990; Assaly et al. 2001; Cauchie et al. 2006; Collins et al. 2006; Dutta 2007; Levi et al. 2009).

Opthalmic complications in hemotoxic snakebite patients included conjunctival suffusion or hemorrhage, macular edema, vitreal hemorrhage, and retinal flame-shaped hemorrhages. Chemosis and macular edema were common in patients with capillary leak syndrome.

Optical coherence tomography (OCT) best demonstrates macular edema in a patient with CLS that generally appears before the onset of generalized anasarca (Fig. 10.3).

Orthopaedic Complications – Osteomyelitis of the bitten limb is one of the known complications following snakebite. They can develop draining fistulas which need excision. Tenosynovitis of the bitten joint is another complication of snakebite.

Long-Term Complications

The long-term complications of hemotoxic snakebite include:

- Sequelae secondary to local effects at the bite site
- As a consequence of vascular disease like stroke and myocardial infarction
- Hypopituitarism and its inherent hormone deficiencies
- Serum sickness
- Immune complex-related nephropathy and papillary necrosis
- Psychological stress related to snakebite and its treatment

Reaction to ASV

Reaction to ASV ranges from mild fever, skin rashes and urticaria, hypotension, vomiting, bronchospasm, to severe anaphylaxis. Allergic reactions are more common in patients with allergic bronchial asthma. Allergic reaction to the horse globulin is complement mediated. Medications to reduce allergic reactions include injections of chlorpheniramine maleate, hydrocortisone, and adrenaline. Allergic reactions in some form are seen in a third of patients. Severe anaphylaxis requiring stopping ASV is fortunately rare. Victims who have received ASV before are at an increased risk of developing allergic reactions in case they have to receive it again.

Conclusion and Future Directions

Hemotoxic snakebite is a major cause of mortality and morbidity especially in rural India. This is an eminently treatable disease, and treatment if initiated early leads to complete recovery other than in a small miniscule group from among those bitten who end up with long-term complications. Early diagnosis and prompt treatment are the only means of preventing the devastating complications listed above. A syndromic approach to the treatment of venomous bite is what is practiced in most centers that treat venomous snakebite. This has its inherent inadequacy and there is no uniformity in the presentation symptoms which most often are nonspecific. In a syndromic-based approach, the treatment is usually started after the signs and symptoms have set in, for example, muscular weakness in a case of Elapidae bite and bleeding manifestations in a case of a hemotoxic snakebite. The symptoms manifest after the venom has already affixed to the tissue and brought about changes. At this point, it is already a little too late to start treatment as the anti-snake venom neutralizes only the free form of the venom and not that that is already bound to the tissue. The symptom complex also tends to set in after a variable period of time after the bite depending on a number of factors like site of bite, size of the snake, whether a deflected bite in which the venom delivery is less, dry bite, season of bite, whether the snake had fed immediately before the bite in which case the delivery of venom tends to be less, age of the victim, amount of subcutaneous tissue at the bite site, and the presence of other comorbidities

The development of a kit for the diagnosis of snakebite would go a long way in the early diagnosis and the labeling of a bite as venomous, nonvenomous, or dry in case the snake was identified as being venomous. It would also help the unnecessary use of ASV in bites by species other than the Big 4 which are covered by the standard polyvalent ASV available in India. In the future, it could also result in the transition to monovalent ASV which could also cover the other Naja species and the pit viper species which are not presently covered by the available ASV. This would not only make the treatment more specific and thereby effective but would also help decrease adverse allergic reactions to ASV which are seen in up to a third of patients.

It has also been seen that the prothrombin time (PT) and the activated partial thromboplastin time (APTT) are seen to be abnormal in a significant time interval

before the 20 min whole blood clotting test (WBCT) which is the present standard for the diagnosis of hemotoxic snakebite. Facility being available, the PT and the APTT would help diagnose hemotoxic snakebite early after the bite.

Cross-References

- ▶ Clinical Uses of Snake Antivenoms
- ▶ Diversity and Distribution of Medically Important Snakes of India
- ▶ Kidney Injury and Animal Toxins
- ▶ Snake Venom and Hemostasis
- ▶ Venomous Snakes and Snakebites in India

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Snake Envenomation in Bangladesh

11

Aniruddha Ghose and Abul Faiz

Contents

Abstract

Snakebite is an important cause of morbidity and mortality in Bangladesh. Recent countrywide epidemiological survey estimated an annual incidence of 623/100,000 and 6,041 deaths annually. The prevalent groups of venomous snakes are cobras, kraits, and vipers. Bites are common during the monsoon and are mostly related to occupational activities. The identification of two previously unrecorded species (*Bungarus walli*, *B. niger*) among specimens brought to hospitals by victims has pointed out the deficiency of geo-epidemiological data and strongly argues for further research.

Most of the bites produce no features of envenoming. Among the venomous bites, main clinical features include neurotoxicity with local tissue necrosis in

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cobra bites, neurotoxicity in most krait (*Bungarus* sp.) bites, and neurotoxicity and myonecrosis in *B. niger* and local swelling with or without coagulopathy in green pit viper bites. In addition to the burden of acute morbidity and mortality, the injuries inflicted by the envenoming bites of snakes in Bangladesh frequently result in permanent disability and its associated long-term socioeconomic consequences.

In spite of recent scientific progress in management of the snakebite victim, treatment of this eminently treatable condition is complicated by social, cultural, geographical, economical, and ecological issues. The price of antivenom (AV) is still beyond reach of many people. Government supply is limited. The private sector has little interest in producing this only marginally profitable product because of limited demand. Polyvalent AV produced in India is being used in Bangladesh, exposing the victim to an unnecessarily wide spectrum of antibodies and to potentially fatal anaphylactic AV reactions. Yet the outcome is satisfactory enough to encourage its continuing use.

Introduction

Snakes and snakebites have been known to the people of Bangladesh and Indian subcontinent since ancient time. It has been cited in the ancient Indian literature like "Sushruta Samhita." Snakebite results most often from an unfortunate accidental interaction between a snake and a human victim. It is the single most important toxin-related injury, causing substantial mortality and morbidity in Bangladesh, especially in rural areas, as well as many parts of Africa, Asia, and the Americas.

The geographical position of Bangladesh in the junction of Indo-Malayan, Indo-Chinese, and Indo-Himalayan Regions provides excellent opportunities for having a great diversity of habitat for snakes and other wild animals. Due to its geographical location and climatic conditions, Bangladesh is a disaster-prone country also. Snakebite has a significant impact on human health and economy through treatment-related expenditure and loss of productivity.

Most often the victim of snakebite is a poor, young, and active individual. Biting occurs mostly when individuals are at work, engaged in activities such as cultivation, fishing, plantation, wood collection, or tending crops or gardens. Snakebite envenoming in Bangladesh is thus an occupational health hazard of the rural poor people who suffer bites while engaged in physical work, most often during cultivation (Faiz 2004; Faiz et al. 1995, 1997; Islam et al. 1999). However, bites are fairly common when the victims are walking on rural footpaths or while sleeping on the floor. Children have a particularly high risk of dying or suffering permanent disability from snakebite envenoming (Faiz et al. 1999). During the monsoon, snakebite occurrences increase as snakes leave their shelter due to rainfall. Most of the houses in the countryside of Bangladesh are not brick-built, and the snakes sometimes live in the holes of the muddy floors. Moreover, most of the houses have homestead bush, which offers ideal habitats for snakes. As a result, events of

snakebites are also common when people are at home. To go to the toilet and for other domestic purposes, people often come out of their houses and become victims. Village people store grains including paddy rice in their bedroom and keep the poultry in the same dwelling house, which also provides shelter to the snakes, therefore increasing the risk of snakebite.

Snakebite Epidemiology in Bangladesh

In the absence of any epidemiological survey data, there was a dearth of information about snakebite from Bangladesh. During 1988–1989, a small survey, conducted in 50 Upazilas (subdistricts) of Bangladesh, recorded 764 occurrences of snakebite of which 168 (22 %) died (Huq et al. 1995). A postal survey conducted in 21 of the 64 administrative districts in 1995–1996 estimated an annual incidence of 4.3 per 100,000 populations and a case fatality of 20 %. In this study, Chittagong Division and Barisal Division had the highest annual incidence of snakebites (7 per 100,000) (Sarker et al. 1999). These estimates were based on data from small studies, and due to methodological limitations, the estimates were unlikely to be representative of the whole country population. According to Faiz et al. 1995, 1,666 snakebite victims attended the Chittagong Medical College Hospital (CMCH) for treatment during 1993–2003. Among those victims, 28.5 % were bitten by venomous snakes and only eight (0.5 %) had died. Although the case fatality for CMCH is very low, it is usually very high in many rural areas. For example, 5 (25 %) out of 20 victims who attended Banshkhali Upazila Health Complex (subdistrict-level hospital) died.

It therefore seemed essential to conduct a countrywide study to determine the extent and magnitude of snakebites. Furthermore, it is imperative to assess epidemiological scenario of snakebites and its consequences in the context of rural Bangladesh, as this is mainly an issue for rural areas.

A nationwide community-based epidemiological study of snakebite and its socioeconomic consequences in Bangladesh was recently published (Rahman et al. 2010). The study reports an incidence density of snakebite in rural Bangladesh, which is substantially higher than previously estimated. The incidence density of snakebite is 623.4/100,000 person years (95 % CI 513.4–1595.3/100,000 person years) with an estimated 6,041 death annually. The majority of the snakebite victims are of young age. Bite occurred mostly when individuals were at work. The majority of the victims (71 %) received snakebites in their lower extremities. Eighty-six percent of the victims receive some form of management within 2 h of snakebite, although only 3 % of the victims went directly to either a medical doctor or a hospital. The study findings would be useful for planning and formulating strategies and specific interventions to combat snakebite-related health problems in Bangladesh.

Based on records of the surveillance system of the Directorate General of Health Services (DGHS), Bangladesh, snakebite envenoming was identified as a leading cause of mortality in the 2007 flood disaster, second only to drowning. DGHS data

suggested a similar situation in the 2004 flood disaster. Clinical observation suggests increased numbers of snakebite admissions in the Chittagong region after earthquakes and minor seismic activities (Faiz et al. unpublished data). This estimate assumes an annual occurrence of extreme weather events whose consequences for the affected parts of the population in Bangladesh qualify them as natural disasters, however, taking into account that the geographical area and the number of population affected as well as the severity and duration of the events are expected to be highly variable from year to year.

Snakes of Bangladesh

Historically the snake fauna of Bangladesh is undercollected and understudied and mostly restricted to species identification and distribution. A literature review from 1852 to 2013 identifies 100 species of which the venomous ones are 13 species of sea snakes, three cobras (including one king cobra), five kraits, two coral snakes, four green pit vipers, and one species of true viper (Prof. Farid Ahsan, personal communication).

One of the methods that proved beneficial is the prospective collection, labeling, preservation, and expert identification of snakes brought by bite victims to sentinel hospitals throughout the area. This method established the importance of *B. niger* and *B. walli* in Bangladesh.

Snakes of Medical Importance

Bites by green pit vipers (*Cryptelytrops erythrurus* (Fig. 11.1) and other species), cobras (*Naja* species), and kraits (*Bungarus*) are the most commonly identified ones in Bangladesh. Neurotoxic envenoming by kraits and cobras is the principal cause of snakebite mortality in Bangladesh.

Recent studies revealed that at least five different species of kraits contribute to snakebite mortality in Bangladesh (unpublished data). Based on their frequencies among proven krait bites in Bangladesh and their geographical distribution, we presently estimate that Wall's krait (*Bungarus walli*) causes about 40 % of all krait bites in the country, the greater black krait (*Bungarus niger*) and the common krait (*Bungarus caeruleus*) about 28 % each, and the banded krait (*Bungarus fasciatus*) and lesser black krait (*Bungarus lividus*) about 2 % each. In Bangladesh, *Bungarus lividus* is so far known only from the northwest. *Bungarus walli* and *B. caeruleus* are not known to occur in southeastern Bangladesh. *Bungarus fasciatus* and *B. niger* occur throughout the country.

Among the cobras, *Naja kaouthia* (Fig. 11.2) is expected to occur throughout the country and to cause the majority of cobra bites. It is the only species of *Naja* found in southeastern Bangladesh (here defined to include Chittagong District, Cox's Bazar District, and the three Chittagong Hill Tract Districts). *Naja naja* is known

Fig. 11.1 Green pit viper

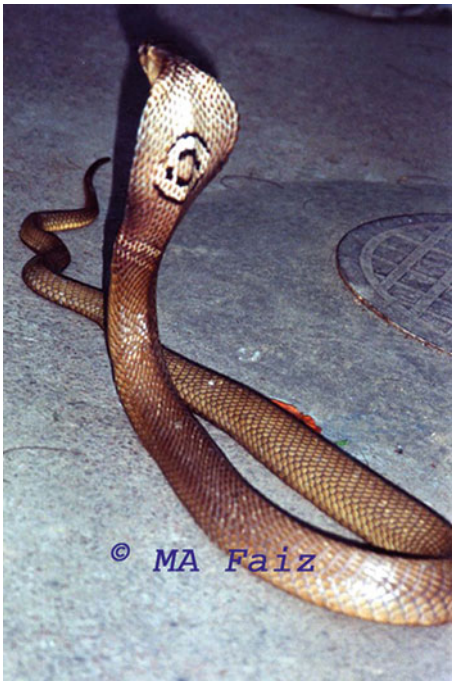

Fig. 11.2 Cobra (Naja kaouthia)

from the area around Dhaka and expected to be continuously distributed at least to the west and north of the capital. Its southern and eastern distributional limits are not known. King cobras (*Ophiophagus hannah*) occur wherever relatively undisturbed bamboo stands and forests remain in Bangladesh (northeastern and

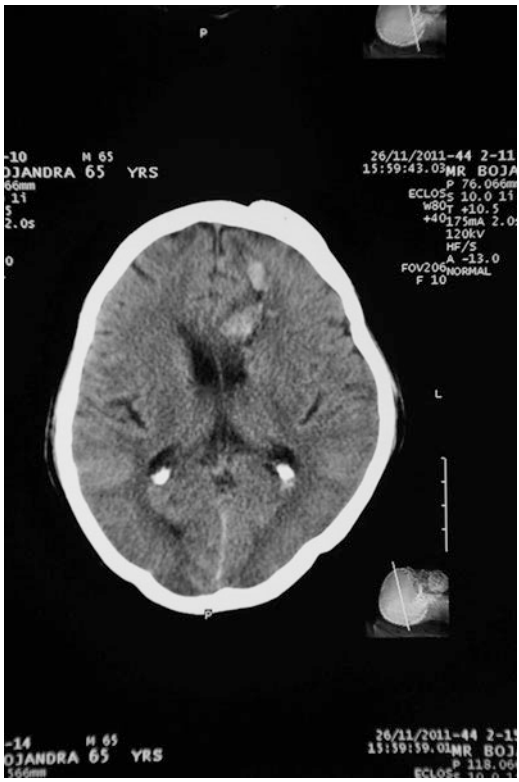

Fig. 11.3 Intracerebral haemorrhage after pit viper bite

southern part) but have not been documented to have caused envenoming bites in recent years.

Russell's viper (*Daboia russelii*) appears to be rare and its distribution patchy and/or restricted to western and northern parts of the country. There have been no recent reports of proven cases of Russell's viper envenoming in Bangladesh. Anecdotal notes (Banerji 1929) suggest that envenoming by Russell's viper used to occur in the southwest (Assasuni and Shyamnagar in Satkhira district; Koyra and Paikgachha in Khulna district) and possibly around Rajshahi and Dinajpur in the west and northwest of Bangladesh. There is no evidence to suggest that saw-scaled vipers (*Echis* species) occur anywhere in Bangladesh. Green pit vipers create a specific problem. It constitutes the largest proportion among the venomous bite cases, and most present with local swelling and pain only. Recently an increasing number of cases are being recognized to cause coagulopathy in the form of non-coagulation of blood in 20 WBCT (20 min whole blood clotting test) and prolong prothrombin time (PT) and activated partial thromboplastin time (APTT). This coagulopathy becomes evident after 24 h and persists for 5–6 days. Most of these do not cause any clinically significant features. But they are potentially dangerous as evidenced by reported cases of intracranial hemorrhage (Fig. 11.3) and torrential bleeding after fasciotomy (done to relieve the compartmental

syndrome caused by local swelling). There are documented cases of extensive ecchymosis, acute renal failure, and coagulopathy following bite by green pit viper.

Sea snakes also constitute an occupational hazard for fishermen in Bangladesh, but the incidence of their bites is unknown.

Snakebite

Clinical Features

Symptoms

Nonspecific symptoms: headache, nausea, vomiting, abdominal pain, swelling at bite site, loss of consciousness, difficulty in vision, and convulsions.

Neurological symptoms: muscle paralysis; difficulty in moving the jaw, tongue, and eye; heaviness of eyelids (ptosis); weakness of neck muscle ("broken neck sign"); difficulty in swallowing; dribbling of saliva; nasal regurgitation; nasal voice; difficulty in respiration; and extreme generalized weakness.

Hematological symptoms: spontaneous bleeding from gum, vomiting of blood, hemoptysis, hematuria, and persistent bleeding from bite site, venipuncture site, and inflicted wound if any.

Others: severe muscle pain, dark-colored urine, scanty urine volume, and collapse (cardiovascular).

Signs

Neurotoxic sign: ptosis (partial or complete) usually symmetrical and progressive, diplopia, external ophthalmoplegia (Fig. 11.4), loss of gag reflex/palatal palsy (bulbar palsy), dysphonia/nasal voice, facial paralysis, inability to open the mouth or protrude the tongue, paralysis of chest muscles and diaphragm (shallow breathing), "broken neck sign" (Fig 11.5), weak grip, and diminished tendon reflexes.

Signs of hematological abnormalities: persistent bleeding from bite site, venipuncture site and/or inflicted wound if any (Fig. 11.6), multiple bruise or large blood collection, hemorrhagic blisters (Fig. 11.7), bleeding from gingival sulci, hemoptysis, hematemesis, hematuria, and epistaxis.

Signs of renal failure: scanty or no urine output and dark-colored urine.

Clinical uremic syndrome: nausea, vomiting, hiccups, fetor, drowsiness, coma, flapping tremor, muscle twitching, convulsions, pericardial friction rub, and signs of fluid overload.

Signs of myotoxicity: muscle tenderness, weakness, respiratory failure, black urine (Fig. 11.8), and renal failure.

Signs of local envenomation: swelling (Fig. 11.9), tenderness, blisters, bleeding, ulceration, necrosis (Fig. 11.10), and local lymph node enlargement.

During the year 2012 in the snakebite clinic in Chittagong Medical College Hospital (in southeastern Bangladesh), a total of 769 patients were admitted with

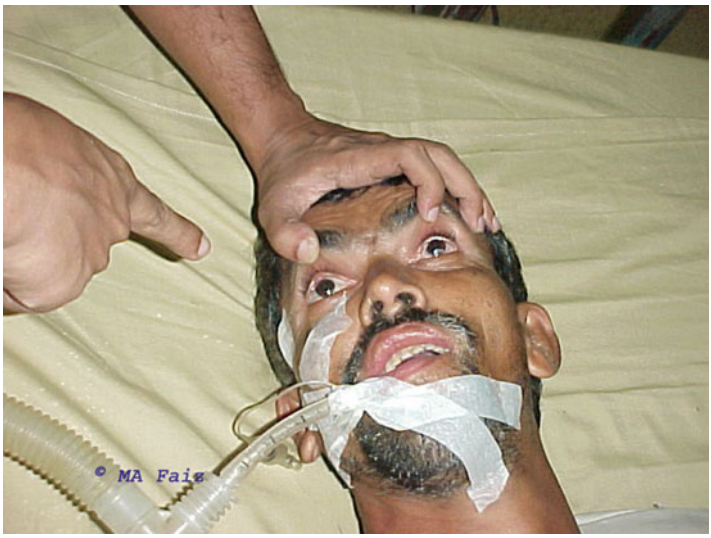

Fig. 11.4 Ophthalmoplegia and Respiratory Paralysis

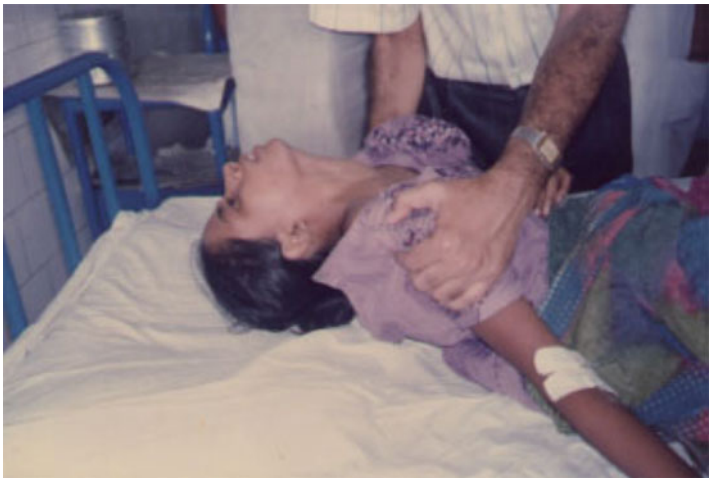

Fig. 11.5 Broken neck sign

snakebite. Among them 666 (86.6 %) patients had no features of envenoming, 61 (7.9 %) patients presented with features of local envenoming only, 15 (1.95 %) patients had features of local envenoming and coagulopathy (by WBCT and prolonged prothrombin time and APTT), 20 (2.6 %) patients presented with signs of neurotoxicity and local envenoming/tissue damage, and 07 (0.9 %) patients presented with only signs of neurotoxicity. Two patients (0.26 %) died (unpublished data, snakebite clinic, CMCH).

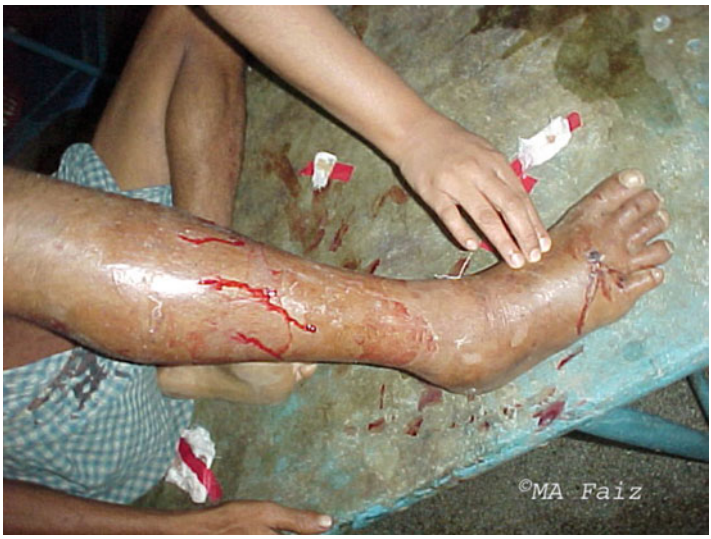

Fig. 11.6 Bleeding from incisions after pit viper bite

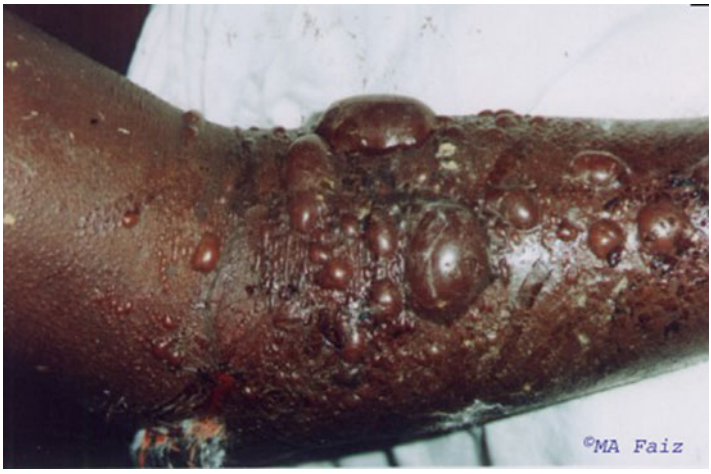

Fig. 11.7 Haemorrhagic Blister Following Bite by Cobra

Management

Diagnosis

From Bangladesh perspective, one of the most difficult aspects in management of snakebite is diagnosis. Due to absence of any serological test to detect presence of venom in victim's sample, diagnosis depends partly on identification of offending

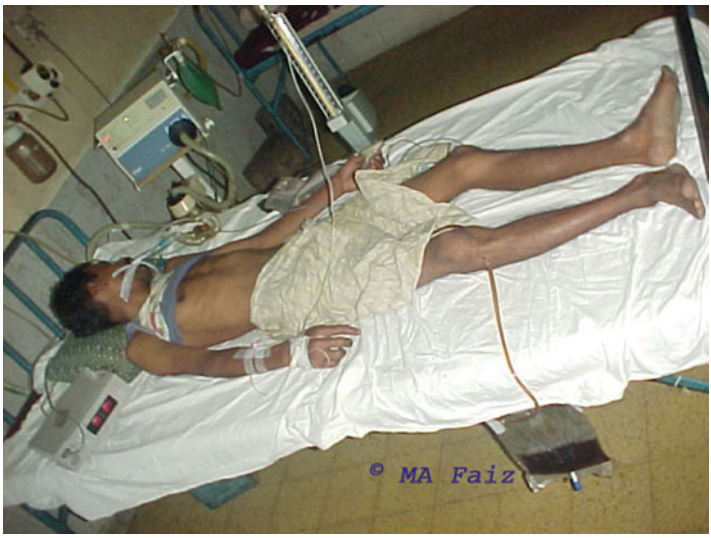

Fig. 11.8 Black urine/ myoglobulinuria following Bite by B Niger

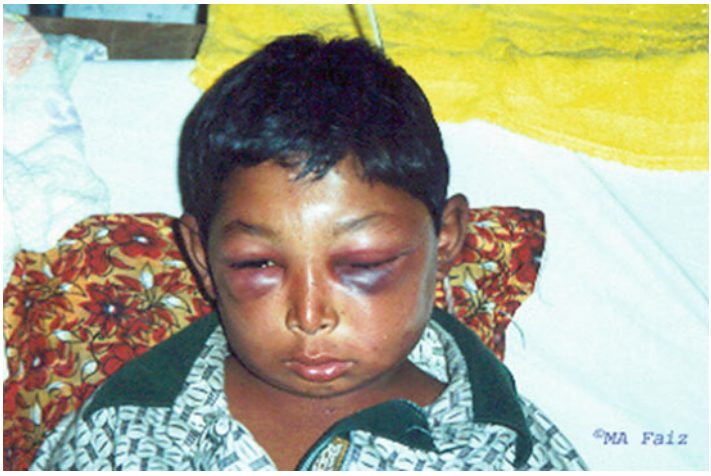

Fig. 11.9 Facial Swelling Following Green Pit Viper Bite on Head

snake by the victim or bystanders or by attending physician if the snake is brought. This is unreliable. The physicians use the presenting "syndrome" of features of envenomation as a way to predict the offender (below). It works reasonably well. But the established syndromes have been recently shattered by finding of some unusual features in some of the species. *Bungarus niger* has recently been reported from Bangladesh and found to cause neurotoxicity, myotoxicity resulting in myoglobulinemia, and renal failure (Faiz et al. 2010). So far kraits were known

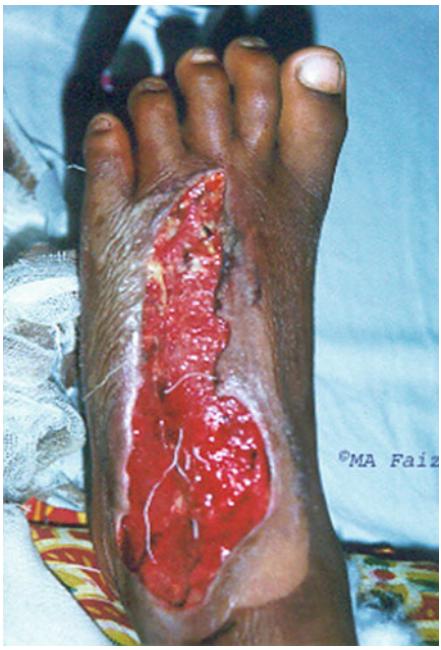

Fig. 11.10 Extensive Tissue Necrosis Following Cobra Bite

to cause only neurotoxicity. The syndromes now need to be rearranged. There is an ongoing scientific study which collects venom from local snake and will try to develop ELISA test using this. Another study is collecting bite site swab to detect the DNA of the offending snakes.

Clinical syndromes after snake envenoming

- Descending paralysis + severe local envenoming = *Naja* spp.
- Paralysis + negligible local envenoming + severe abdominal pain + bites at night while the victim is asleep = *Bungarus* spp. + rhabdomyolysis = *B. niger*.
- Local envenoming (swelling of extremities, etc.) with bleeding/clotting disturbance = Viperidae (all species) (*local swelling due to snakebite/due to bandage has to be determined).
- Local envenoming (swelling, etc.) with bleeding/clotting disturbances, shock, or renal failure with ptosis, external ophthalmoplegia, facial paralysis, etc., and dark brown urine = Russell's viper.
- Paralysis with dark brown urine and renal failure, severe muscle pain, no local envenoming, and no bleeding or clotting disturbances. Bite in the sea (no bleeding/clotting disturbances) = sea snake.

First Aid

Most of the first aid methods being practiced at field level are harmful to patients. These include application of tight tourniquets (Fig. 11.11); cutting or pricking at or around bite site; sucking of blood; applying seed, stone, mud, or different herbal

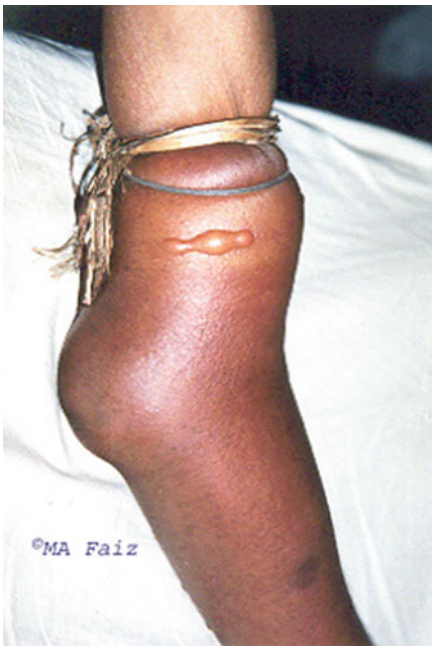

Fig. 11.11 Multiple Tight Tourniquets

remedy; induced vomiting and recitation of verses; and so on. Most of these are practiced by local healers (Ojha) (Fig. 11.12) who are consulted by the rural people for treatment of snakebite. These harm the patient physically and more importantly waste the precious time and cause delay to reach the health facility. There are many instances of loss of vital body parts or function (Figs. 11.13 and 11.14) due to these practices even in some of the patients who were bitten by nonvenomous snakes.

Treatment

Current Bangladesh National Guideline for Management of Snakebite recommends use of antivenom in case of bites fulfilling definite criteria. It includes neurotoxic signs, rapid extension of local swelling (more than half of bitten limb) not due to green snake bite or tight tourniquet, acute renal failure not due to sea snake bite, cardiovascular abnormalities, bleeding abnormalities, and hemoglobinuria/myoglobinuria not due to sea snakes.

The recommended dosage is 10 vials per dose irrespective of age. Additional dose is recommended if there are persistent signs of envenomation after 1 h. In 2012 only one patient received an additional dose at CMCH.

Bangladesh, yet to develop its own antivenom, is using antivenom from India. Antivenoms available in Bangladesh are polyvalent raised against *N. naja, B. caeruleus, D. russelii,* and *E. carinatus* and manufactured by different pharmaceuticals of India.

Clinical experience shows that it works reasonably well to neutralize the neurotoxic effects of cobras (*N. naja and N. kaouthia*) but very poorly against kraits of

Fig. 11.12 'Ojha' A traditional healer with Monocellate cobra

other species (*B. walli, B. candidus, B. niger*). *D russelii* (Russell's viper) and saw-scaled viper are not documented to cause any clinical cases so far.

In a prospective observational study carried out between 1999 and 2001, in the Snake Bite Study Clinic of Chittagong Medical College Hospital, among 35 neurotoxic-snake-bite patients who had received polyvalent anti-snake venom, the rate of anaphylaxis was 64.51 % and rate of pyrogenic reaction was 80.64 %. The common features of anaphylaxis were urticaria (80 %); vomiting and wheezing (40 %); and angioedema (10 %). The anti-snake venom reaction was treated mainly with adrenaline for anaphylaxis and paracetamol suppository in pyrogenic reactions. The average recovery time was 4.5 hours (Amin et al. 2008).

Ancillary treatment includes physostigmine. It also works well in cobra bite but not in krait bite, where a majority of neurotoxicity is due to deactivation of presynaptic receptors.

The coagulopathy in viper (pit viper) bites possesses an inherent problem. It is known to be due to consumption coagulopathy. It was used to be treated with fresh frozen plasma with the belief that it would improve the coagulopathy. Recently the concept has been questioned and clinicians now face a dilemma with these cases.

Supportive treatment includes resuscitation and respiratory support in cases with respiratory paralysis. In 2012 in Chittagong Medical College Hospital, out of 27 patients with neurotoxicity, only four required intubation and mechanical ventilation. Due to lack of facilities, many hospitals mainly the primary centers prefer not to treat and refer the cases to higher centers. Transfer without respiratory assistance may cause death en route to hospital. In most cases respiratory support

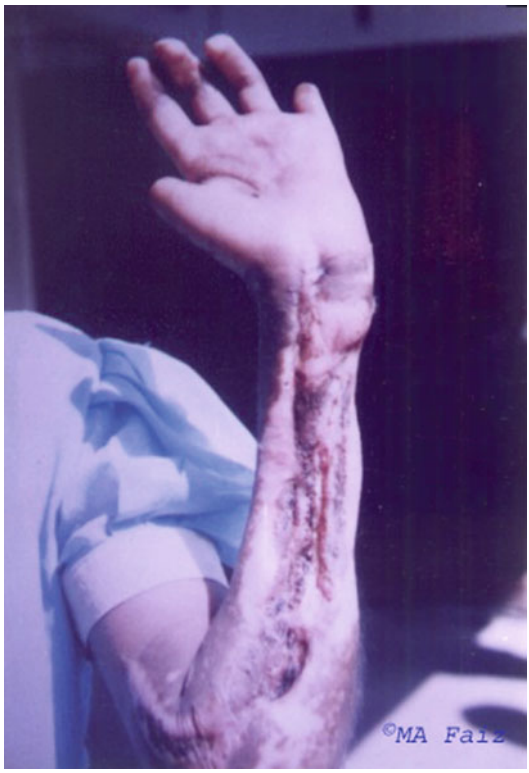

Fig. 11.13 Extensive disfigurement Following Prolonged Tourniquet

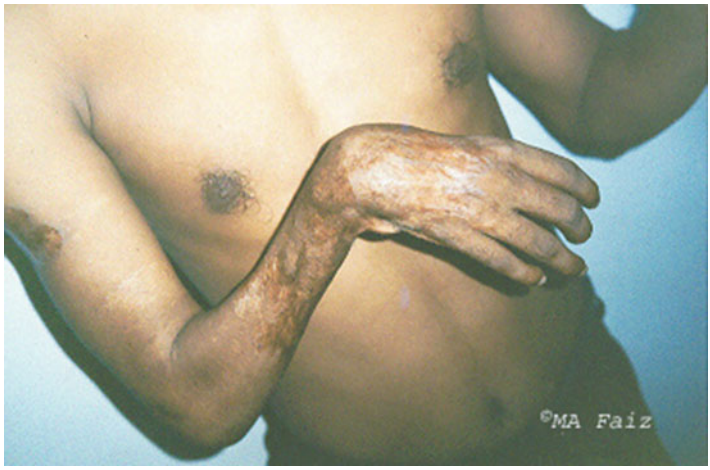

Fig. 11.14 Wrist Drop and muscle wasting as a Sequel of Prolong Tight Compression

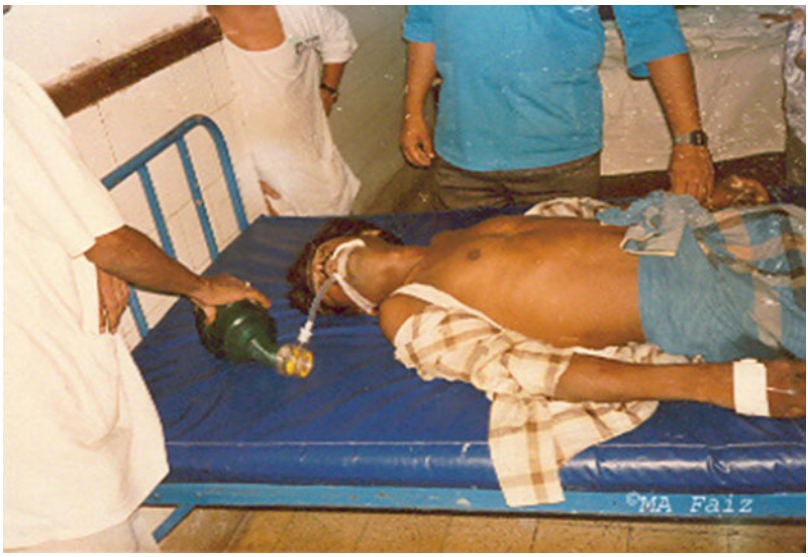

Fig. 11.15 Patient maitained on umboo bag ventilation after neurotoxic snake bite

is required for a short duration. This can also be achieved by intubation and manual ventilation with the help of Ambu bag (Fig. 11.15).

Disability and Sequelae

Some time effect of nerve compression like foot drop (Fig. 11.16) and wrist drop was found following effects of prolonged-tight compression. The surviving patients if followed through carefully are found to develop soft tissue necrosis in *N. kaouthia* bite toward the end of the first week. This requires careful debridement, surgical care and some time end up with disabling effects like contracture. Bites in tight compartment may result in compartmental syndrome requiring surgical treatment even amputation. If followed up on long term, victims of snakebite were found to have long-term disability in significant proportion which is yet to be quantified.

Conclusion and Future Directions

The field of "snakes and snakebite" has a mythological fragrance in the mind of people in this part of the world. Treatment of snakebite was largely dominated by traditional healer/snake charmers ("Ojha"). People used to be content with their traditional methods of tight tourniquet, multiple incisions at bite site, application of herbal products, and different rituals. The outcome was determined by chance.

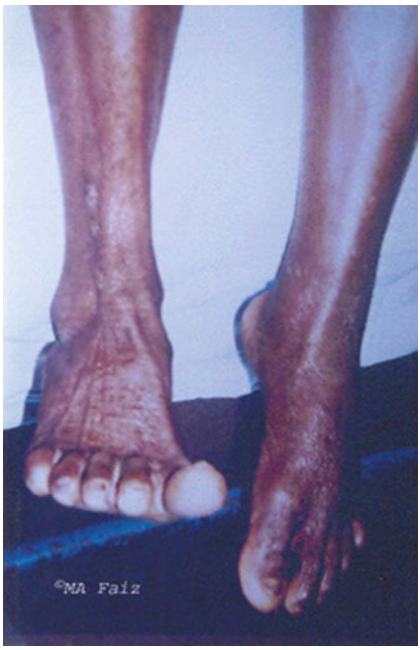

Fig. 11.16 Foot Drop as a Sequel of Prolong Tight Compression

Even the medical professionals were not well aware of the scientific methods of management. The first initiative was undertaken by the snakebite study clinic in a medicine ward in Chittagong Medical College Hospital, situated at the southeastern part of the country. It started to use antivenom (polyvalent) and conducted several public awareness meetings in rural areas involving the traditional snake charmers/healers. With some successes and "miraculous" recovery of nearly dead people, it soon became popular and well known in public. With repeated presentations at different scientific forums at home and abroad, the professionals and the policy makers were oriented. Bangladesh National Guidelines for Treatment of Snakebites had been developed. Then it was felt that the skill and knowledge of management has to be disseminated among the general physicians attending the victims at the primary level of health care. The training modules (learner's guide, teacher's guide) were then developed. So far more than 1,000 physicians have been trained through an ongoing program of DGHS. Several BCC materials have also been developed. It was mostly the effort of a group of interested clinicians that snakebite and its management had gained some success and a momentum. Now many of the trained physicians are doing research and providing service in this field. Following these successful examples and due to initiatives of DGHS, snakebite is now recognized as a rewardingly treatable medical condition in Bangladesh.

In spite of recent scientific progress in management of the snakebite victim, treatment of this eminently treatable condition is still complicated by social, cultural, geographical, economical, and ecological issues. The price of antivenom (AV) is still beyond reach of many people. Government supply is limited. The

private sector has little interest in producing this only marginally profitable product because of limited demand. Polyvalent AV (Indian) is being used in Bangladesh, exposing the victim to an unnecessarily wide spectrum of antibodies and to potentially fatal anaphylactic AV reactions. Yet the outcome is satisfactory enough to encourage its continuing use. Now the priority is to develop and produce detection kits and antivenoms which are developed against the venoms collected from local snakes.

Cross-References

- ▶ Clinical Uses of Snake Antivenoms
- ▶ Complications of Hemotoxic Snakebite in India
- ▶ Snake Venom and Hemostasis
- ▶ Venomous Snakes and Snakebites in India
- ▶ Viperidae Envenomation in India

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Venomous Snakes and Snakebites in Jordan

12

Zuhair S. Amr and Ahmad M. Disi

Contents

Abstract

Distributional data, diagnostic features, and habitat descriptions are given for seven species of venomous snakes found in Jordan. The epidemiology of snakebites in Jordan is discussed based on previous studies and recent data. Males were more vulnerable than females, and children less than 20 years old constituted the highest percentage of victims. Bites were more frequent in the spring and summer, peaking in April. Most bites occurred in Irbid Governorate. Symptoms associated with snakebites among hospitalized cases in Jordan are outlined. LD_{50} for venomous species known in Jordan and the Middle East are tabulated.

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Introduction

Jordan is a small country located in the heart of the Middle East with a total area of about 98,000 km^2 with a population of 6.5 million. Arid deserts constitute about 75 % of the total area and cover the southern and the eastern parts of the country. The climate of Jordan is characterized by a hot and dry summer, a cool and wet winter, and brief spring. Annual rain fall ranges from 700 to 100 mm annually in the northern mountains and the extreme eastern desert, respectively. Jordan is influenced by four major biogeographic zones (Mediterranean, Irano-Turanian, Saharo-Arabian, and Afrotropical). Vegetation cover, soil texture, altitude, and annual rain fall are among the major factors that shaped these biogeographic zones.

Thirty-seven species of snakes have been recorded from Jordan, whereas seven are considered to be venomous and life threatening to human. In this communication, venomous snakes of Jordan are presented with their current known distribution, and the epidemiology of snakebites is revised with additional new data.

Venomous Snakes of Jordan

Several papers addressed the taxonomic status of the snakes of Jordan (Disi 1983, 1985, 1987, 1990, 1993, 1996, 2002; Disi et al. 1988a, 1999, 2001, 2004; Al-Oran 2000; Al-Oran and Amr 1995; Al-Oran et al. 1997, 1998, Amr et al. 1994a, 1997; Abu Baker et al. 2002, 2004; El-Oran 2000; Modry et al. 2004; Joger et al. 2005; Venchi and Sindaco 2006; Amr 2008; Shwayat et al. 2009). Within the past 20 years, major changes in the systematics of the snakes of the Middle East took place. The most comprehensive study on the snakes of Jordan was published by Amr and Disi (2011) with updates of their taxonomic status and distribution. At present the snake fauna of Jordan consists of 37 species and subspecies belonging to seven families (Typhlopidae, Leptotyphlopidae, Boidae, Colubridae, Atractaspididae, Elapidae, and Viperidae). Venomous species belong to three families (Atractaspididae, Elapidae, and Viperidae) with a total of seven species on known toxicity.

	Key to families of snakes in Jordan	
1.	Ventral scales are not enlarged, eyes covered by scales, wormlike in appearance	2
	Ventral scales enlarged, eyes not covered by scales, not wormlike in appearance	3
2.	Midbody scales consist of 20–24 scales, tail length not exceeding its width	Typhlopidae
	Midbody scales consist of 14–16, tail length longer than its width	Leptotyphlopidae
3.	Head covered by small asymmetrical scales	4
	Head covered by symmetrical head shields	5
4.	Head not distinct from neck and ventral scales are narrow	Boidae
	Head distinct from neck and ventral scales are not narrow	Viperidae

(*continued*)

	Key to families of snakes in Jordan	
5.	First 2–9 caudal scales are entire, and the others are in pairs	Elapidae
	Caudal scales are single	6
6.	Loreal scale present	Colubridae
	Loreal scale absent	Atractaspididae

Family Atractaspididae Günther, 1858

Atractaspididae or the Mole Viper family is related to elapids. Externally, they are similar in appearance to colubrid snakes; however, species of this family are characterized by possessing powerful erectile hollow front fangs. The fangs are erected rather laterally. Species of this family are strictly fossorial and known as the burrowing asps or mole vipers. This family includes two genera known to occur in Jordan, *Atractaspis* and *Micrelaps* (Amr and Disi 2011). Species of the genus *Micrelaps* (*Micrelaps muelleri* and *Micrelaps tchernovi*) are not considered dangerous and will not be included in this entry.

Atractaspis engaddensis Haas, 1950

See Fig. 12.1.

Common name: En Gedi Mole Viper, Palestinian Mole Viper.

Diagnosis. Snout very short and moderately pointed. Frontal large and longer than broad; width of frontal equals length of parietal. One supraocular, one preocular, and one postocular. 2 + 3 scalelike temporal. Six upper labials, the third and fourth enter the eye. Ten lower labials; three or four in contact with the

Fig. 12.1 En Gedi Mole Viper, *Atractaspis engaddensis*

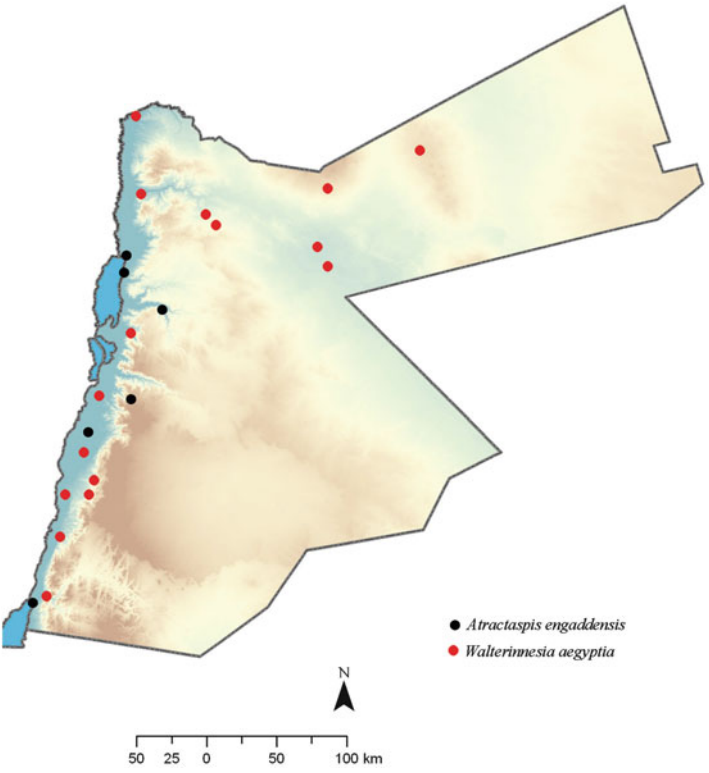

Fig. 12.2 Distribution of venomous snakes in Jordan. *Atractaspis engaddensis* (*black circles*), *Walterinnesia aegyptia* (*red circles*)

anterior chin shields. Lower jaw is retracted than the upper jaw and the mouth opening is located ventrally. Nasal divided in its lower half. Ten scales between chin shields and first ventral scales. 23–29 smooth middorsal scales, 263–282 ventrals; 31–39 entire subcaudals. Anal undivided. Snout-vent length reaches 700 mm or more, tail length reaches 60 mm. Maximum total length may reach up to 80 cm. Coloration**:** Dorsally it is usually shiny black. Rarely, the color is uniformly dark, shiny brown, or gray. Ventrals have an iridescent light color.

Habitats and ecology. The En Gedi Mole Viper is a strictly fossorial snake that seldom emerges above ground. However, it seems to move at night, this behavior was observed in the Wadi Al Mujib area, where an adult specimen was seen at night moving on the ground along small rocks. Subterraneous in vegetated places in hot and humid areas. Sometimes found in gardens while digging or under stones. It prefers loose soil and avoids loose sand or very arid situations. Also, it prefers oases or farms and may be encountered on arid hillsides (Al Mujib Wildlife Reserve). Two specimens were collected from At Tafilah Governorate (Al Oran and Amr 1995). Figure 12.2 shows the distribution of *A. engaddensis* in Jordan.

The sharp projections of the fangs may be related with the special striking behavior of *Atractaspis*. This projection helps ensure that the tiny amount of the venom is fully penetrated in to the prey (Kochva and Meier 1986). *Atractaspis* species exhibit an unmatched structure of venom glands with specific osteological and mycological modifications (Kochva et al. 1967).

Family Elapidae Boie, 1827

This family consists of about 100 venomous species, including the cobras. Members of this family are equipped with an anterior pair of grooved rigid fangs located on a fixed maxillary. Beyond the fangs none or a few teeth may present; fangs almost entirely tubular; proteroglyphous, with well-developed venom apparatus. Most species do not possess a loreal; oviparous. In Jordan, this family is represented by one genus (*Walterinnesia*) and one species (*Walterinnesia aegyptia*).

Walterinnesia aegyptia Lataste, 1887

See Fig. 12.3.

Common name: Black Desert Cobra, Walter Innes's Snake, Innes' Cobra.

Diagnosis. A stout snake with a small head slightly distinct from the neck, with large shields dorsally, and with a short tail. The fixed grooved fangs positioned near the front corner of the mouth under each nostril and in front of the eye. Rostral broader than deep. Internasals same length as prefrontals. Frontal length more than its width. Posterior nasal in contact with single preocular; two postoculars, one subocular. Temporals 2 + 3, posterior temporals 3/3. Seven upper labials, the third and fourth enter the eye. Nine lower labials, first four in contact with the anterior chin shield. Scale rows at midbody 21–23, 180–200 ventrals, 40–53 subcaudals, first 1–22 single, remainder paired. Anal divided. Sexual dimorphism is expressed in both males and females of *W. aegyptia*. Males have fewer ventrals, 178–190, than females, 191–210; males have more subcaudals (42–50) than females (39–48);

Fig. 12.3 The Black Desert Cobra, *Walterinnesia aegyptia*

also males have more undivided subcaudals (4–22) than females (1–5). The largest measured specimen from Jordan was 110 cm. Coloration: Dorsal body is uniformly shiny black and bluish black ventrally.

Habitats and ecology. The Black Desert Cobra was found in all types of habitats in Jordan. It was found in extreme desert habitats in the eastern desert and Wadi ‘Araba, as well as in mountain ranges near Al Karak and As Salt areas. In the last two decades a considerable increase in the number of the Black Desert Snakes were encountered. This increase is associated with the expansion of agricultural settlements in the Eastern Desert and Wadi ‘Araba resulting in an increase in number and range of distribution of the Green Toad (Amr and Disi 2011). The authors believes that this species is a follower of agriculture, toads inhabiting the newly established farms in the Eastern Desert may attract this snake since it constitutes one of its major food items in Jordan. This fossorial elapid was found frequently as roadkills in Wadi ‘Araba. Gaspertti (1988) stated that it is rare or rarely seen in Arabia. It is highly secretive spending most of its time in mammal burrows or those of the large spiny-tailed lizard (Leviton et al. 1992). Figure 12.2 shows the distribution of *Walterinnesia aegyptia* in Jordan.

Family Viperidae Oppel, 1811

Solenoglyphous. The maxilla has two sockets, where hollow and replacement fangs are fitted with highly modified, movable skull bones allowing for operative movement of the hollow long recurved anteriorly positioned fangs. Broad triangular heads covered by small scales, juxtaposed or imbricate, which are located high and oblique in the head. Nostrils point upwards. Elliptical pupils in the eye; 31–35 rows of keeled dorsal scales at midbody; those on flanks are slanted ventral and their keels are serrated. Ventrals broad (Gasperreti 1988). Tail is very short with either paired or entire subcaudal scales. Dorsal scales are keeled at various levels, and the ventral scales are wide and broad.

Recent molecular studies on this family in the Middle and Near East revealed new aspects of taxonomic treatment for many species (Herrmann et al. 1992; Lenk et al. 2001; Stümpel and Joger 2009). In Jordan, this family is represented by the subfamily Viperinae and includes five genera (*Cerastes*, *Daboia*, *Echis*, *Macrovipera*, and *Pseudocerastes*).

	Key to the family Viperidae	
1.	Horny projections that consist of small scales situated above the eyes	*Pseudocerastes fieldi*
	Horny projections that consist of one large scale situated above the eye are present or absent	2
2.	Horny projections that consist of one large scale situated above the eye are present	*Cerastes gasperettii gasperettii*
	Horny projections that consist of large scale situated above the eye are absent	3

(*continued*)

	Key to the family Viperidae	
3.	Subcaudals single, 3–4 layers of scales between upper labials and eye, lateral scales obliquely arranged	*Echis coloratus*
	Subcaudals divided, more than 4 layers of scales between the upper labials and eye, lateral scales not obliquely arranged	4
4.	Scale rows fewer than 30, 160–166 ventral scales	5
	Scale rows more than 30, 146–158 ventral scales	*Cerastes gasperettii mendelssohni*
5.	Supraoculars divided into 5 scales, ventral scales 155–181	*Macrovipera lebetina*
	Supraoculars intact and bordering eye, ventral scales l60–l66	*Daboia palaestinae*

Cerastes gasperettii gasperettii Leviton and Anderson, 1967

See Figs. 12.4 and 12.5.

Common name: Desert Sand Viper, Arabian Horned Viper.

Diagnosis. Head triangular and broad, wide flattened, and clearly distinct from the neck: head covered with small irregular tubercularly keeled scales. *C. g. gasperettii* is characterized by a thick body and short tail. Pupil elliptical. Eyes separated from labials by rows of small scale; absence of the cluster of enlarged scales at midoccipital region of the head between the eyes. A pair of supraocular hornlike spiny scales above the eye can be either present or absent. If horns are present they point externally. Four to five rows of supralabial scale, with the first supralabial scales relatively small. Upper labials, 12–15. Lower labials, 13–15. Number of scales in ocular ring, 12–14. More than four to five rows of scales between the eyes. Dorsal scales heavily keeled with apical pits. Lateral scales are smaller, laterally keeled, serrated, and arranged in an oblique series. Scale rows at midbody, 31–35; ventrals, 152164; subcaudals divided and vary from 33 to 37.

Fig. 12.4 The horned form of the Arabian Horned Viper, *Cerastes gasperettii gasperettii*

Fig. 12.5 The hornless form of the Arabian Horned Viper, *Cerastes gasperettii gasperettii*

Anal scale undivided. The Sand Horned Viper exhibits sexual dimorphism as follows: head of males larger including length, depth, and width; higher number of subcaudal and fewer dorsal scale rows; longer tail length and fewer ventrals (over 153 in males and more than 155 in females) and wider eye diameter. Maximum length 85 cm (most specimens are about half that size). Females grow larger than males. Coloration: The color varies regionally and can be reddish, yellowish, or gray, depending upon the actual color of the sand where a population lives. The pattern consists of indistinct brown spots in four to six longitudinal series, a dark streak on the tail, and a variable head pattern. The head pattern is accentuated in some populations of *C. g. gasperettii*, in which case the dark band between the eye and the angle of mouth is accompanied dorsally by a light band.

Habitats and ecology. The Arabian Horned Viper is a true psammophile species. It was seen and collected from sand dunes in Wadi Rum and Al Hazim. Also, they inhabit sandy soil where vegetation or rocky outcrops provide shelters. In Al Hazim, the area is dominated by *Haloxylon persicum* and *Nitraria retusa*. It is adapted to these habitats through its morphology, physiology, and behavior. During the daytime it hides in rodent borrows, and specimens have been seen buried in the sand with eyes protruding from the ground surface. It starts its activity after sunset and is active at night, moving across the sand searching for food, especially rodents. Side-winding trails are very characteristic for this viper. It was collected near roads in Wadi Ramm (Amr and Disi 2011). Figure 12.6 shows the distribution of *C. g. gasperettii* in Jordan.

Cerastes gasperettii mendelssohni Werner and Sivan, 1999

Common name. Not determined.

Diagnosis. Occipital tubercles and supraocular horns always absent; upper labials 12–13, lower labials 13–15, 12–14 scales surrounding the eye, 31–35 scale

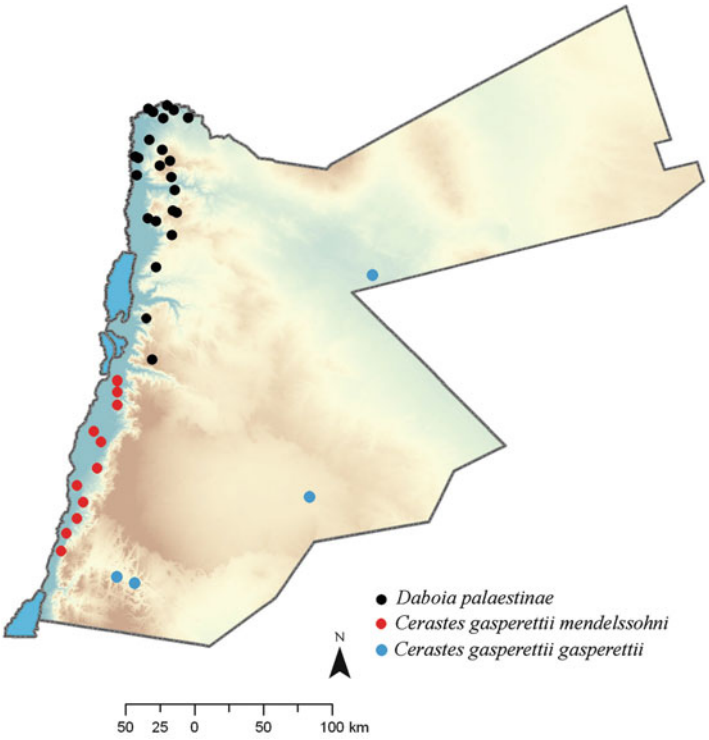

Fig. 12.6 Distribution of venomous snakes in Jordan. *Daboia palaestinae* (*black circles*), *Cerastes gasperettii mendelssohni* (*red circles*), *Cerastes gasperettii gasperettii* (*blue circles*)

rows at midbody, ventrals 146–158^{-}, anal divided: subcaudals divided and vary from 31 to 36. Extremely short tail. Sexual dimorphism appear clearly: Males in comparison with females have shorter snout-vent length, longer tail, dorsal scale length dorsals at midbody, less ventrals, and more caudals. Adult snout-vent length measures 443 mm; tail is 50–66 mm. Coloration: Irregular small dark dots with irregular shapes and sizes are scattered on head. A laterally dark band is accentuated by whitish upper margin extending between the eye corners of mouth. Dorsal background is sandy beige. Dorsal pattern differs within and among members of this subspecies. Middorsal blotches are represented by two dorsolateral series of smaller roundish blotches alternately positioned on left and right sides. These may form a zigzag pattern on same part of the body, oblique pairs of blotches or checkered throughout, but to a lesser degree regular discrete blotches have brown color. Ventrum is white.

Habitats and ecology. This viper is endemic to the sand dunes of Wadi ‘Araba between Ghawr as Safi in the north and ‘Aqaba in the south. It is nocturnal and encountered hiding into rodent burrows or at the base of shrubs buried in soft sand

except for the protruding nostrils and eyes. Figure 12.6 shows the distribution of *C. g. mendelssohni* in Jordan.

Daboia palaestinae (Werner, 1938)

See Fig. 12.7.

Common name: Palestine Viper.

Diagnosis. Head triangular, distinct from the neck, and covered by small scales. In contrast to other Jordanian viperids, there is a single large plate on top of each eye. Supraocular intact and bordering eye. Two scale rows between eye and upper labials. Upper labials, 9–11. Lower labials, 12–14. 24 or 25 midbody scale rows, l60–l66 ventral scales, 35–44 subcaudals. Anal undivided. Body stout and tail tapers abruptly behind cloaca. Largest *Daboia* species (both sexes up to 130 cm). Coloration: ground color gray to ocher, with a series of light brown, oval spots with lighter centers and pale edges; the spots may be fused to form a zigzag band. Top of the head has two V-shaped, brown occipital bands with dark edges in front of which is one large, round brown patch. Side of the head with yellow markings in adults (Amr and Disi 2011).

Habitats and ecology. The Palestine Viper is associated with oak and pine-forested areas. Some remnant populations are still existing in deforested mountains as in Al Karak Governorate. Nowadays it occurs in rocky hillsides, plantations, animal farms, and near human settlements. This viper is a nocturnal species. It climbs trees looking for fledging birds or to ambush arboreal reptiles and mammals. There is a considerable increase in the number of this viper in the Jordan Valley in correlation with the expansion of cultivated land and irrigated citrus and banana farms, which in turn has created an abundance of small rodents, especially mice and rats. Also, these places offer humid oviposition sites and the moisture needed by this viper to drink (Amr and Disi 2011). Figure 12.6 shows the distribution of *D. palaestinae* in Jordan.

Fig. 12.7 The Palestine Viper, *Daboia palaestinae*

Echis coloratus Günther, 1878

See Fig. 12.8.

Common name: Arabian Saw-Scaled Viper, Burton's Carpet Viper.

Diagnosis. Head very distinct from the neck. Three to four scale rows between the eyes and upper labials. 12–15 upper labials, 13–15 lower labials. Nostril is in a single or divided nasal and a series of scales separates the nasal from rostral. Scale rows 31–37, ventral scales 152–205, subcaudal scales 44–57. Anal scale entire, subcaudals single. *Echis* is different from other vipers by single (undivided) subcaudal scales. Males are larger than females and have longer tails. Maximum length 83 cm. Coloration: ground color quite variable, yellowish-gray or brownish-gray, but may be reddish-brown or pink in areas of red sandstone or granite. On the back, there is a row of grayish-white, elongate rhomboid blotches or crossbars with dark edges. Head without distinctive marks, except a brownish-gray band from the nostril to the edge of the mouth. The light dorsal blotches may have a narrow dark border. The pattern of orientation of the blotches and crossbars varies even on the same animal. On the side of the body, there is a row of brownish blotches.

Habitats and ecology. Carpet Vipers are abundant in the steep, dry rocky hillsides of the mountains which surround the Jordan Valley and Wadi 'Araba and also occurs in Petra and Wadi Ramm. It penetrates into the Mediterranean biotope through the wadi systems emerging from the Jordan Valley and Wadi 'Araba. It favors hard ground covered by rocks with widely scattered vegetation dominated by *Retama raetam*, *Salvia graveolens*, and *Urginea maritima* (Disi 1983). In El Quweira the Carpet Viper was captured on a tree (Amr and Disi 2011). It hides under medium-sized rocks. In early spring this viper is found close to the surface of the ground under rocks or logs. As the season becomes hotter in the summer, the vipers retract deeper into their burrows (Disi 1987). Vertical distribution in the southern part of its range reaches up to 2,600 m. Figure 12.9 shows the distribution of *E. coloratus* in Jordan.

Fig. 12.8 The Arabian Saw-Scaled Viper, *Echis coloratus*

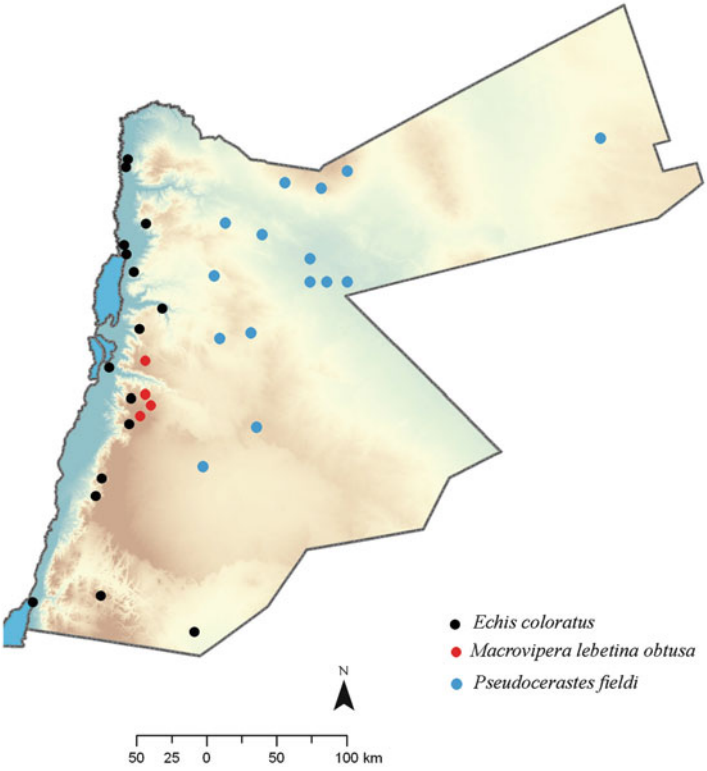

Fig. 12.9 Distribution of venomous snakes in Jordan. *Echis coloratus* (*black circles*), *Macrovipera lebetina obtusa* (*red circles*), *Pseudocerastes fieldi* (*blue circles*)

Macrovipera lebetina obtusa (Dwigubsky, 1832)

See Fig. 12.10.

Common name: Levantine Viper.

Diagnosis. The Levantine Viper has a fat body with a head triangular clearly separated from the neck, covered by small, imbricate, keeled, and smooth scales on tip of the snout. Solenoglyphous. Snout rounded obtusely. Supraoculars completely divided into five scales. Fourth supralabial enlarged, positioned under the eye. Nostril lateral, in large nasal shield. Eye surrounded by circle of 11–18 small circumorbital scales and separated from upper labials by two to three rows of scales; interocular scales 7–11. Two to three canthi. Two to three apicals. Lower labials 12–14. Dorsal scales keeled with the exception of lateral of most rows. Midbody dorsal scales, 23–25; ventral scales 155–181 and in females slightly higher; 35–44 divided subcaudals. Anal entire. Coloration: dorsal color yellowish to light gray, with about 35 Gy blotches in four longitudinal rows (two laterally and two dorsally), the latter meeting at the middorsal line, but in alternating positions. Head yellowish-gray. A gray stripe from the eye backwards, widening above the jaw angle. Ventrals

Fig. 12.10 The Levantine Viper, *Macrovipera lebetina obtusa* (Photo by D. Modry)

darkly pigmented, light posteriorly, powdered with fine dark spots (Amr and Disi 2011).

Habitats and ecology. Known from two locations; one specimen was taken from rocky terrain with scarce vegetation, while the other from an area covered by dense vegetation of *Artemisia herba-alba*. Both Jordanian localities are situated within the Irano-Turanian zone stretching south from Syria into Jordan to the 30th parallel. The Levantine Viper avoids deserts, high mountains, or densely forested areas; however, biogeographically, it is considered an Irano-Turanian species. At 'Ayn Lahza, it was encountered among thick bushes lying under stones (Al-Oran et al. 1998). In Dana Wildlife Reserve it is found at an altitude of 1,400 m. Also, it was observed during the hot summer at noon immersing its body in the water of a creek (Amr and Disi 2011). Figure 12.9 shows the distribution of *M. l. obtusa* in Jordan.

Pseudocerastes fieldi K. P. Schmidt, 1930

See Fig. 12.11.

Common name: False Horn Viper, Field's Horned Viper.

Diagnosis. Head triangular, wide, very distinct from the neck, covered with small, imbricate, keeled scales. Snout short and broadly rounded. One series of scales between the nasal and the nostril; nostrils are dorsolaterally positioned and valves present. Supranasal one or two. 14–18 (most common 15–17) scales in ocular ring. On both sides above the eye, there are erect hornlike projections formed of several small imbricate scales, its tip ending in two tiny scales. Three series of scales between the eye and labials. Upper labials 12–14. Lower labials 14–16, four of which are in contact with the chin shields. 21–22 strongly keeled scale rows at midbody, ventrals 127–142 (most common 131–135), subcaudals 34–46 (most common 33–38). Anal undivided. Tip of the tail black. Side-winding movements

Fig. 12.11 The False Horn Viper, *Pseudocerastes fieldi*

like *Echis* and *Cerastes*, but no differentiated noise-making lateral scales. Maximum length may reach up to 90 cm. Coloration: pale yellowish-gray or brown, with two rows of about 30 darker blotches on the back. In the eastern basalt desert, however, the ground color is dark gray. Opposite blotches sometimes fuse to form transverse crossbars. An additional row of smaller blotches laterally. Ends of ventrals and subcaudals, as well as many dorsal scales, with little black spots. Sides of the head with light brown band from the eye backwards and downwards. Maximum length 79 cm (females larger than males).

Habitats and ecology. In Jordan, it is common in the eastern deserts especially the black basalt desert. It is found in extremely arid regions with minimum vegetation. This viper avoids human habitations. The authors caught specimens inside rodent burrows and under large basalt rocks. According to Mendelssohn (1965), it inhabits semidesert with sandy soil and shrub vegetation and may be interspersed with rocks (but neither dune areas nor mountain slopes). Figure 12.9 shows the distribution of *P. fieldi* in Jordan.

Snakebites in Jordan

The problem of snakebites in Jordan is a noteworthy one which deserves more attention (Amr and Amr 1983), especially with the recent expansion of the cultivated landscape and human settlements in the Jordan valley, Wadi Araba, and eastern desert where most of the venomous snakes are present. It is indicated that the number and occurrence of venomous snakes have tremendously increased in association with the range of toads which forms mostly part of the diet of *W. aegyptia* and *E. coloratus*. It is also found that *D. palaestinae* expanded its range of distribution into the Jordan valley in correlation with the expansion of the irrigated farms (Mendelssohn 1965; Disi 1983). Moreover, a positive relationship exists between densities of *P. fieldi* and vegetation densities, where shrubs and bushes are found (Disi l988).

Most bites occurred in fields, near chicken coops, and around stone walls that are common in agricultural areas. In rural areas, wood and other dried bushy plants

used for heating and cooking purposes are piled near the dwellings. On many occasions, snakes seek refuge under these piles, and bites occurred when the unwary person picks up the wood. Few patients were bitten by snakes in house.

Most cases were reported from Irbid district, a highly populated area with abundant farming land. Additionally, health-care centers are situated in most villages. Cases of snakebites with severe manifestations are mostly attributed to the Palestine Viper, *D. palaestinae*. This is due to the overlapping of its geographical distribution with highly populated areas in Jordan. Bites caused by the saw-scaled viper, *E. coloratus*, are less common; however, renal failure and other complications that may result in amputation of the affected limb have been observed. Colubrid bites are more common; however, the symptoms are mainly psychological.

Epidemiology of Snakebites in Jordan

The earliest record of snakebites in Jordan was documented by Swaroop and Grab (1954). They reported a total of 84 recorded deaths from snakebite in the 5-year period (1948–1952) without mentioning the total number of cases. Almost half a decade after, Amr and Amr (1983) conducted a retrospective study on snakebites reported by the Ministry of Health during 1970–1972 and 1975–1980, with a total of 112 cases, including seven fatalities. Another study by Disi et al. (1988b) documented 65 cases of snakebites during 1982–1986. Jaghbir and Khoury (1989) examined records of snakebites in Balqa Governorate with a total of 20 confirmed cases. Amr et al. (1994b) presented an epidemiological study on snakebite cases reported during 1982–1992, with a total of 99 cases (Table 12.1). The previous authors believe that the actual number of snakebites is much higher than the above figures, since snakebites were treated with folklore medications and snakebites in remote areas and military personnel were not included in the official records.

Al Shamari (2002) presented a retrospective study on snakebites in the Ghor es-Safi, southern Jordan Valley. He reported a total of 60 cases admitted to the emergency department at Ghor Safi Hospital. Age of victims ranged between 46 and 50 years, with 9:1 male to female ratio. Severe cases constituted 12 %.

Table 12.1 Summary for snakebite cases in Jordan

Years	No. of cases	No. of deaths	References
1948–1952	?	84	Swaroop and Grab (1954)
1970–1972 and 1975–1980	112	7	Amr and Amr (1983)
1982–1986	65	0	Disi et al. (1988b)
1982–1992	99	5	Amr et al. (1994b)
1986	20	0	Jaghbir and Khoury (1989)
2001	60	0	Al Shamari (2002)

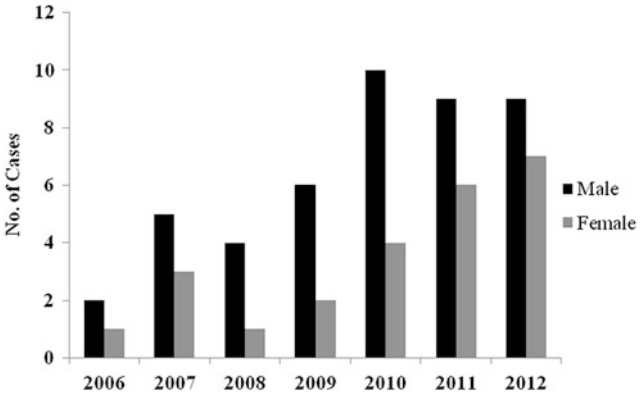

Fig. 12.12 Snakebites according to age reported to DEH, NDPIC, and PHH (Source: DEH, NDPIC, and PHH)

Snakebite data were obtained from Princess Haya Hospital (PHH) in Aqaba, southern Jordan, from 2004 to 2012 (21 cases), the National Drug and Poison Information Center (NDPIC), Jordan University Hospital covering the years 2009–2012 (15 cases), and records of the Ministry of Health, Directorate of Environmental Health (DEH) from 2006 to 2012 (34 cases).

Of the total snakebites (70 cases), only one case of death occurred in Ajlune (55-year-old male). Also, we witnessed two cases of snakebites caused by *D. palaestinae* that resulted in death in As Salt Hospital. Both cases were not among the documented cases by the MOH, as data obtained from the NDPIC showed that 13 out of 15 snakebite cases occurred outdoors, while only two cases occurred at patients' residence. Two, four, and nine patients showed minor, moderate, and severe symptoms, respectively.

Snakebites are more common among males compared to females all through the study period (Fig. 12.12). Males are more exposed to snakes due to their activity outdoors, especially among farmers. According to the records (1982–1986), snakebites in males and females constituted 72.3 % and 27.7 %, respectively (Disi et al. 1988b), and almost twice among males in Balqa Governorate (Jaghbir and Khoury 1989).

Data concerning age were obtained for only 51 patients. Children and young adults were at higher risk and constituted about 55 % of the total number of cases (Table 12.2). Patients over 20 years old were the most affected group (Jaghbir and Khoury 1989). The foot and ankle were the most affected sites for snakebites, followed by finger and hand (Jaghbir and Khoury 1989).

Seasonality

The highest number of snakebites reported to DEH, NDPIC, and PHH occurred during April and the summer months (Fig. 12.13). In the past, Disi et al. (1988b) found that the highest incidence of snakebites happened in July (35.4 %) followed

Table 12.2 Snakebites according to age groups (Source: DEH, NDPIC, and PHH)

Age group	M	F	Total
1–10	6	5	11
11–20	13	4	17
21–30	9	0	9
31–40	6	2	8
41–50	1	4	5
51–60	1	0	1

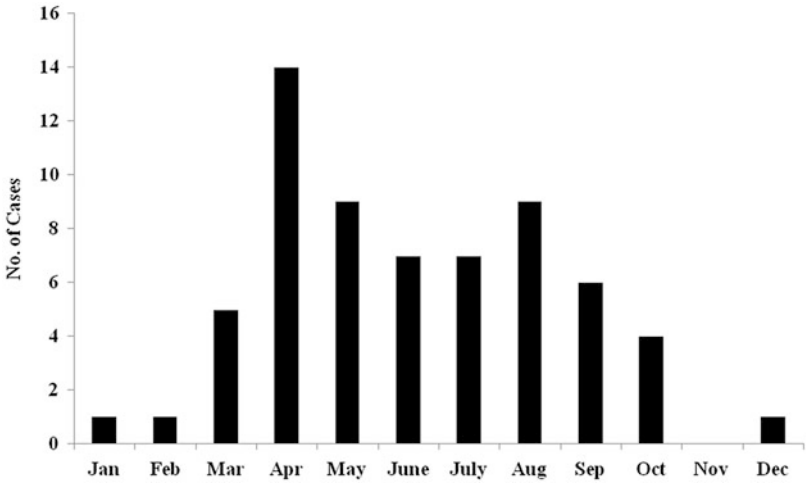

Fig. 12.13 Seasonal distribution of the snakebites based on data from DEH, NDPIC, and PHH (Source: DEH, NDPIC, and PHH)

by June (21.5 %) while the lowest incidence occurred in October (1.5 %), while Jaghbir and Khoury (1989) found that most snakebites occurred in April. Amr et al. (1994a) reported similar findings with lowest incidence in November. The present results may suggest the effect of climate change, where snakes are becoming more active during the spring as they emerge in March after hibernation.

According to geographic areas, data were obtained for 56 cases. The highest incidence of snakebites occurred in Irbid Governorate with 34 cases (60.7 %), followed by Balqa Governorate with 8 cases (14.3 %). Bites were mostly from the Middle and northern Jordan Valley (Table 12.3). The Palestine Viper, *D. palaestinae*, is the most dominant viper in the northern part and the Jordan Valley. Also, both areas are agricultural areas were farmers are more exposed to snakes.

Clinical Manifestation and Treatment

The following are summaries extracted from medical records of snakebite patients from three hospitals. Symptoms associated with snakebites varied from flushing in

Table 12.3 Snakebites according to Governorates (Source: DEH, NDPIC, and PHH)

Governorate	No. of cases	%
Amman	3	5.4
Ajlune	1	1.8
Aqaba	4	7.1
Balqa	8	14.3
Irbid	34	60.7
Mafraq	1	1.8
Tafilah	1	1.8
Zarqa	4	7.1

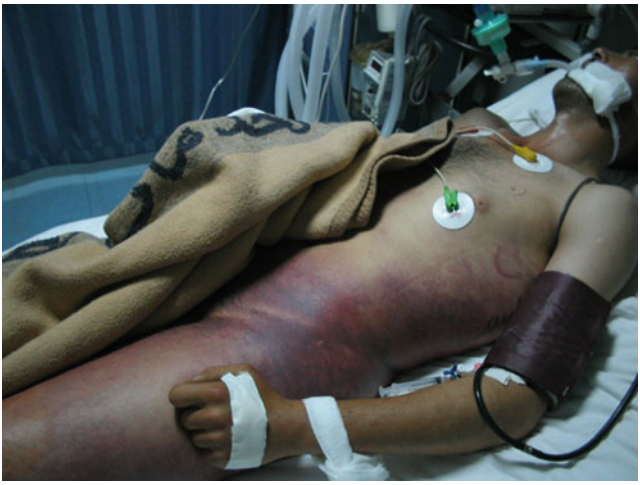

Fig. 12.14 A patient bitten by *D. palaestinae*, showing extensive hemorrhage (Source: Dr. Malik Dabbas, Royal Medical Services, Jordan)

respiration, headache, tenderness, and slight swelling at the site of the bite which may develop later into skin eruption. Anaphylactic shock, vomiting, shivering, and abdominal pain were also reported. Some patients required hospitalization for 2–14 days.

Others include cases that exhibited fear, pain, impairment of consciousness and irritability, severe pallor and sweating, and swelling of the affected limb (Fig. 12.14). Upon arrival, vomiting, lower abdominal pain, drowsiness, bloody diarrhea, and progressive swelling of the affected limb with local hemorrhage developed within 24 h of the bite. Blisters of variable size started to appear on the third day.

A 30-year-old farmer that had been bitten in his right hand by *D. palaestinae* developed melena and hematuria with marked swelling and bleeding at the site of the bite. His clotting time was 2 h and 40 min (normal 9–15 min), and his

prothrombin time (PT) was 300 s (control 12 s). He developed hypofibrinogenemia of 90 mg/dl (normal 150–300 mg/dl). He required blood transfusions and the patient had to be hospitalized for 12 days (Amr and Amr 1983).

In most cases, treatment consisted of administering corticosteroids, vitamin K, Avil, and anti-snake venom if available. Fresh plasma, one million units of crystalline penicillin, anti-tetanus injection, intravenous streptokinase (250,000 units), and blood transfusion were also used for treatment of severe cases of *D. palaestinae* bite.

Saadeh (2001) presented a case of envenoming by *D. palaestinae*. Although several antivenom infusions were given to the patient, complications including swelling that spread to the groin and large well-defined ecchymoses developed over at the medial aspect of the left leg and thigh. Four hours after admission, the patient experienced severe retrosternal chest pain associated with nausea and vomiting, and the electrocardiogram showed a pattern of acute inferior myocardial infarction several hours after the bite.

Lethal Dose for Snakes of Medical Importance in Jordan

Seven out of the 37 snake species in Jordan are considered venomous. Deadly species in Jordan are members of families Atractaspididae, Elapidae, and Viperidae. Lethal doses 50 (LD_{50}) vary among venomous species (Table 12.4). The LD_{50} for four local venomous snakes in Jordan was determined. The LD_{50} for *C. gasperettii* was found to be 1.285 mg/ kg IP (Nofal 1993) and 1.75 mg/ kg IP (Shakhanbeh 1985), 1.9 mg/ kg IP for *D. palaestinae* (Disi et al. 1988a), 0.675 mg/ kg IP for *P. fieldi*, and 0.45 mg/ kg IP for *W. aegyptia*.

Antivenins

Two polyvalent antivenins have been used in Jordan during the last 9 years. The imported antivenins cost Jordan about 60,000 US$ in the period 1982–1986 (Ministry of Health Statistics). One of these antivenins is prepared in Pasteur Institute for *Bitis*, *Echis*, and *Naja* (Isper Africa). None of the snakes which were used to develop this antivenin are present in Jordan. The other antivenin is Behringwerke (Near and Middle East), with only *Cerastes gasperettii* present in Jordan (Disi et al. 1988b). Further research on scorpion's antivenom is developed in the Middle East ("► Chap. 16, Snake Venoms and Scorpion Venom Research in the Middle East: A Review").

Behringwerke antivenin has a higher titer of neutralization antibodies against the crude venom of *Cerastes cerastes* and *Daboia palaestinae* than Pasteur antivenin. However, Pasteur antivenin provided no protection against *Pseudocerastes fieldi*, while Behringwerke antivenin provided a partial protection against this viper. Both antivenins provided full protection against 10 LD_{50} of *Walterinnesia aegyptia* venoms, but no protection was provided at higher doses (Disi et al. 1988b).

Table 12.4 Reported toxicities of snakes of medical importance in Jordan

Species	Method	LD_{50}	References
Atractaspis engaddensis	IV	0.06–0.075 μg/g	Weiser et al. (1984)
	IV	15 μg/kg	Wollberg et al. (1988)
Walterinnesia aegyptia	IV	0.30 mg/kg	Ovadia and Kochva (1977)
	IP	0.285–0.45 mg/kg	Gitter et al. (1962), Disi et al. (1988a)
Cerastes gasperettii	SC	15 mg/kg	Minton (1974)
	IP	1.285–1.75 mg/kg	Mohamed et al. (1980), Shakhanbeh (1985)
	IV	0.45 mg/kg	Hassan and Hawary (1977)
Daboia palaestinae	SC	9.4 mg/kg	Minton (1974)
	IV	0.18 mg/kg	Minton (1974)
	IP	1.9 mg/kg	Krupinck et al. (1968), Disi et al. (1988a)
Echis coloratus	IV	0.575 mg/kg	Gitter et al. (1960)
	IP	1.55–1.75 mg/kg	Gitter et al. (1960), Rechnic et al. (1962)
	SC	3.875 mg/kg	Gitter et al. (1960)
Macrovipera lebetina	IP	7.58 mg/kg	Nalbantsoy et al. (2012)
Pseudocerastes fieldi	IP	0.675–1.00 mg/kg	Gitter et al. (1962), Disi et al. (1988a)
	IV	0.25–0.30 mg/kg	Ovadia and Kochva (1977), Batzri-Izraeli and Bdolah (1982)

All doses are expressed in mg or μg of venom per g or kg of mouse
IP intraperitoneal injection, *IV* intravenous injection, *SC* subcutaneous injection

Disi et al. (1988b) indicated that both imported antivenins were inefficient to protect against most of the snakebites in Jordan, because of venom variations in different species of vipers and even within the same species over its wide range of distribution.

Various concentrations of date extracts were found to inhibit the hemolytic activity of the venom of *Cerastes cerastes*. 60 % of mice injected with LD_{100} of C. *cerastes* venom and fed with dates survived (Sallal et al. 1997).

Conclusion and Future Directions

There is an urgent need for proper documentation of snakebites countrywide through the different health providers in the country. Furthermore, the production of antivenom for the local venomous species is among the high priority research. Further studies should focus on the determination of the LD_{50} for the Jordanian venomous snakes. Educating the public health sector on handling snakebite accident should be considered. Protocol for snakebite treatment should be formulated by the Ministry of Health and circulated over all health centers in the country.

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Venomous Snakes and Snake Envenomation in Nigeria

13

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Abstract

Three snake species carpet viper (*Echis ocellatus*), black-necked spitting cobra (*Naja nigricollis*), and puff adder (*Bitis arietans*) belonging to the Viperidae and Elapidae families are the most important snakes associated with envenoming in Nigeria. The incidence of bites has been reported as 497 per 100,000 population per year with a 12 % natural mortality, with *Echis ocellatus* accounting for at least 66 % in certain foci. Bites occur more often while victims are farming, herding, or walking mostly among agricultural workers, the economically productive members of society. Carpet viper venom contains a prothrombin-activating procoagulant, hemorrhagin, and cytolytic fractions which cause hemorrhage, incoagulable blood, shock, and local reactions/necrosis. The spitting cobra bite manifests with local tissue reaction and occasionally with bleeding from the site of bite. Classic neurotoxic feature has been observed following Egyptian cobra (*N. haje*) bites. Cardiotoxicity and renal failure may occasionally occur following bites by the carpet viper and the puff adder. In the laboratory, hematological features are noted, while immunodiagnosis has a role in species identification and monitoring of therapy. Immobilization of the bitten limb is the single most important first aid measure. Effective antivenom remains the only proven remedy for snakebite envenomation, but they are prohibitive, scarce, and often inappropriate and ineffective. Antivenom should be used cautiously when indicated, and use of boots among workers should be the main preventive measures. However, availability, distribution, and utilization of antivenom remain challenging although two new antivenoms (monospecific EchiTAb G and tri-specific EchiTAb-Plus-ICP) raised from Nigerian snake venoms proved very effective and safe in clinical trials. Strategies for broadening antivenom access to endemic rural areas together with instituting quality assurance, standardization, community, and healthcare workers' education should be implemented. With the advent of antivenomics, health authorities must be helped to select and purchase antivenoms appropriate to their national needs, while manufacturers should be helped in practical ways to improve the safety, efficacy, and potential coverage of their products.

Introduction

Snakebite envenoming is a major public health problem among communities of the savanna region of West Africa, notably in Benin, Burkina-Faso, Cameroon, Ghana, Nigeria, and Togo (Chippaux 2011; Visser et al. 2004; Warrell and Arnett 1976; WHO 2007). The precise incidence of snakebite is difficult to determine and is often grossly underestimated, but a recent global reappraisal estimated 10,001–100,000 snakebite envenoming with an incidence of 8.9–93.3/100,000 persons per year with an estimated 1,001–10,000 deaths and a mortality rate of 0.5–5.9/100,000 persons per year occur in the West African region (Kasturiratne et al. 2008). A more recent study using meta-analytic approach estimated over 314, 000 bites, 7,300 deaths, and nearly 6,000 amputations occur annually in sub-Saharan Africa (Chippaux 2011). An earlier estimate in northeastern Nigeria

reported a bite incidence of 497 per 100,000 population per year with a 12 % natural mortality, with *Echis ocellatus* accounting for at least 66 % (Pugh and Theakston 1980). It is probable that Nigeria, the most populous nation in Africa with over 160 million people, has the highest burden of snakebite morbidity and mortality in sub-Saharan Africa (Fig. 13.1).

Venomous Snakes and Geographic Distribution in Nigeria

There are more than 3,000 species of snakes in the world, but in Africa, only a few of these are known to cause mortality, and these mainly belong to four families, Viperidae (*vipers and adders*), Elapidae (*cobras and mambas*), Colubridae (*boomslang*), and Hydrophiidae (*sea snakes found in Coastal East and South Africa)*. The Hydrophiidae, now grouped by some taxonomists with Elapidae, have not been reported from Nigeria. The subfamily Crotalinae is not found in Africa. The vipers are relatively shorter, thicker snakes with long hinged front fangs. The main example in Nigeria is the saw-scaled or carpet viper (*Echis* spp.) of which there are two main species in West Africa – *Echis ocellatus* and *Echis leucogaster*. *Echis ocellatus* has an average length of 35–40 cm and resides in the semiarid rocky terrain. It is the most prevalent cause of bites in the middle belt and the northern states of Nigeria (Warrell and Arnett 1976; Warrell et al. 1977) (Figs. 13.1 and 13.2). Roman's carpet viper *(E. leucogaster*) lives in the Sahel belt of Nigeria and is probably underrecognized as cause of human bites (Warrell and Arnett 1976; WHO 2010). In the country, *Echis ocellatus* has also been seen as far south as the Shaki and Kishi in Oyo state and in Enugu and Udi hills (Fig. 13.1). The snake is mainly nocturnal entering human dwellings only rarely unlike the spitting cobra. *Echis ocellatus* may be confused with at least four other snakes in West Africa – *Dasypeltis scabra*, *Telescopus variegatus*, *Bitis arietans* (juvenile type), and *Causus maculatus*; the former two lack fangs, while the latter two are also venomous. The puff adder, *B. arietans*, is another viper which is bigger and thicker but less common than *E. ocellatus* in some areas. Additionally, it has the characteristic V or U pattern along its dorsal aspect that helps in differentiation (Fig. 13.2). The night adder *Causus maculatus* is very similar to the carpet viper but has smooth scales and therefore does not produce a rasping sound and has large symmetrical scales on top of the head (Warrell and Arnett 1976), but differentiation between the two could be difficult. The *Atractaspis* spp. (borrowing asps or stiletto snakes) together with *C. maculatus* are small vipers which are only mildly venomous. The former are now regarded as a separate family, the *Atractaspididae*, and are present in Nigeria (WHO 2010).

The Elapid snakes are longer with short hinged front fangs. The spitting cobra, *Naja nigricollis*, is the commonest and most widely distributed African cobra and is indeed a familiar snake in Nigerian states within the savannah terrain, where it is usually colored black, dark brown, or steel gray with pink or reddish throat bars. The average length is 117 cm (Warrell et al. 1976a) (Fig. 13.2). It is the most

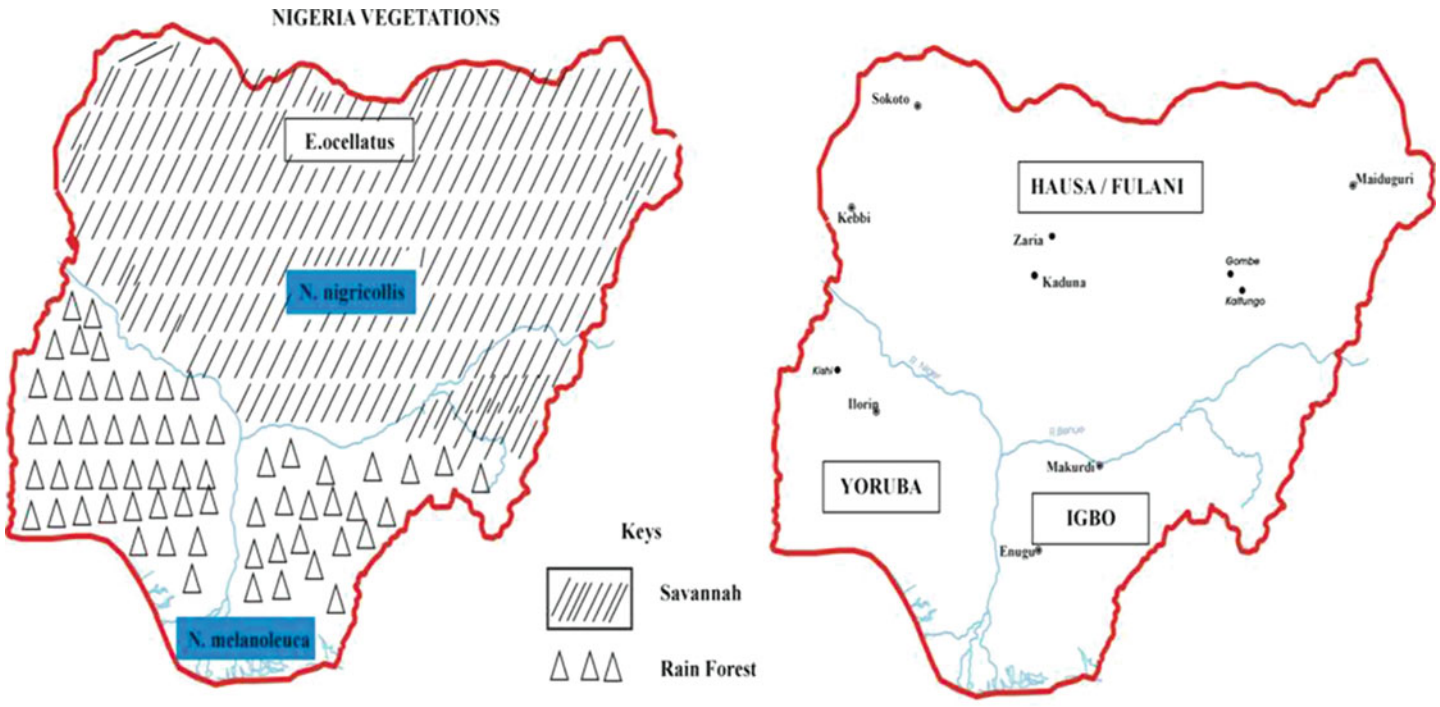

Fig. 13.1 Maps of Nigeria showing major vegetation zones with distributions of two major snakes (*left*) and depicting towns where carpet viperbites have been reported and distributions of main tribal groups (*right*)

Fig. 13.2 *Left* to *right*: carpet viper with "white-black eyes" markings on its sides; cobra with hooded neck; puff adder with dorsal U-shaped markings

important snake at Malumfashi district of Katsina state, though another cobra *Naja katiensis* which has a shorter length and brown color along its back is also found (Pugh et al. 1980). *Naja nigricollis*, unlike *N. Katiensis*, may inhabit the savannah bordering the forest zones. The forest cobra *N. melanoleuca* inhabits the southern forests of Nigeria and is usually black in color (WHO 2010) (Fig. 13.1). In forested rainy areas of southern Nigeria, these two species, the black forest cobra (*Naja melanoleuca*) (Fig. 13.1) and following relatively recent introductions the spitting cobra (*Naja nigricollis*), are known to inhabit the region. The two species may be contiguously parapatric or even sympatric especially in the recently deforested areas. It has been suggested that *Naja nigricollis* has been extending its range, especially by exploiting recently deforested "derived savannah" in southeastern Nigeria. The overlap in their geographic range is thought to result from catastrophic deforestation that has characterized Nigeria over the last four decades mainly following the oil industry boom in the 1970s (Luiselli 2001). The Egyptian cobra, *N. haje*, is seen mostly in the northern states with snake charmers (Pugh et al. 1980; Warrell et al. 1976a) (Fig. 13.2). The *Naja* spp. are also nocturnal. The mambas (*Dendroaspis* spp.) occasionally cause bites in northern and to a lesser extent other parts of the country. The boomslang, *Dispholidus typus*, is a member of Colubridae which possess short non-hinged fangs situated far back in the mouth. At least 18 venomous species have been reported in Nigeria [*Echis ocellatus*, *Echis leucogaster*, *Bitis arietans*, *Bitis gabonica*, *Naja nigricollis*, *Naja haje*, *Naja katiensis*, *Naja melanoleuca*, *Dendroaspis jamesoni*, *Dispholidus typus*, *Causus maculatus*, *Causus rhombeatus*, *Causus lichtensteini*, *Atractaspis aterrima*, *Atractaspis corpulenta*, *Atractaspis microlepidota*, *Thelotornis kirtlandii*, *Elapsoidea semiannulata*] (see Table 13.1 for names of common snakes in local vernacular and English and Fig. 13.2 for their pictures) (Habib et al. 2001; WHO 2010). Studies of *Bitis* spp. and *Dendroaspis* spp. populations in southern Nigeria suggested snake populations have been declining from several causes such as global climate change, habitat quality deterioration, and prey availability (Reading et al. 2010).

Table 13.1 English and vernacular names of snakes of medical importance in Nigeria (Habib et al. 2001; Warrell and Arnett 1976; see also Fig. 13.1 distribution of major vernacular/tribal groups and main snakes' distribution)

Scientific name	*Echis ocellatus*	*Naja nigricollis*	*Bitis arietans*
English	Carpet viper or Saw-scaled viper	Black spitting cobra	Puff adder
Hausa	Kububuwa	Baki	Kasa
	Gobe da nisa	Kumurci	
Fulani	Buneri	Sharkori	Kasari
Yoruba	Paramole	Oka	Olufa
		Isebe	
Igbo	Echi-e-teka	Agwoegwu	Abuana

Epidemiology

The incidence of snakebite and its related morbidity and mortality have been difficult to determine precisely in tropical and subtropical regions including Nigeria. Both underreporting and errors in epidemiological methods which include difficulties in snake identification by medical workers and patients have obscured the true incidence of bites and their effects.

Snakebite is mainly a problem of rural dwellers, the occupational groups most at risk being farmers, herdsmen, hunters (including adolescents hunting rabbits and rodents by putting their hands in holes), and firewood collectors. Accordingly, most bites occur in farms, bushes, and rural paths, but a substantial percentage (17.3 %) occurs in the house. Bites are most often in the limbs but have been reported to occur on the genitalia (scrotum) and the tongue.

A national snakebite survey was conducted in 1993 to document cases from randomly selected households and health facilities for the preceding 5 years (i.e., from 1989 to 1993) from four high-prevalence states (Bauchi, Enugu, Kogi, Taraba) and from four low-prevalence states (Jigawa, Katsina, Osun, and Rivers). The result of the health facilities survey showed the highest number of cases was reported in Taraba state with 684 cases representing 10.3 per 1,000 hospital admissions during the period. Enugu and Osun states had the least with 128 cases (3.7 per 1,000 hospital admissions) and 152 cases (2.7 per 1,000 hospital admissions), respectively. Jigawa state had the highest case per hospital admissions and case fatality rates. However, it had been noted that hospital snakebite admissions have been declining progressively at ABUTH, Zaria, from 1980 to 1989 due to lack or high cost of antivenoms. This implies health institution data should be interpreted cautiously. In the Benue valley and the eastern parts of northern Nigeria, incidence of 497 bites per 100,000 populations per year with a 12.2 % mortality (mostly from *E. ocellatus* bites) was found (Pugh and Theakston 1980). The estimated incidence of *E. ocellatus* bites at Bambur in Taraba state was about 120 per 100,000 populations per year with the mortality following bites at

8 per 100,000 populations per year. Left untreated, mortality may be more than 16 %, and in these parts of the Nigerian savannah, its victims may occupy 10 % of hospital beds (Warrell and Arnett 1976). Carpet viper was found to be responsible for 66 % of all snakebites in that area. In 2007, 1803 victims of snakebite with 26 deaths were seen at Kaltungo General Hospital, Kaltungo, Gombe state, with over 90 % of the bites due to *E. ocellatus* and 21 due to *Naja* spp., 4 due to *B. arietans*, and the rest due to *Atractaspis* spp., *Telescopus variegatus*, or unidentified or unknown snakes (Habib et al. 2008). This makes carpet viper the commonest and medically the most important snake in the area (Warrell et al. 1977). The victims of *E. ocellatus* bites were found to be mostly males bitten during the months of February to May on their lower limbs in about 81 % of cases, and this occurred while walking or farming (53 %) and herding (8 %) but none while asleep (Warrell and Arnett 1976; Warrell et al. 1977). In Nigeria, *E. ocellatus* or its bite has been reported from Bambur, Birnin Kebbi, Enugu and Udi hills, Gombe, Ilorin, Jos, Nassarawa, Kaduna, Kaltungo, Karim Lamido, Makurdi, Shaki/Kishi (Oyo state), Shendam, Sokoto, Takum, Wukari, Yelwa, Zaria, Zungeru, and Rivers Benue and Niger valley (Fig. 13.1) (Njoku et al. 2008; Onuaguluchi 1960; Pugh et al. 1979; Pugh and Theakston 1980; Warrell and Arnett 1976; Warrell et al. 1977). It is probable that it is medically the most important snake in the country causing an estimated mortality of many hundreds in the savannah of Nigeria (Pugh and Theakston 1980). Indeed *Echis* spp. has been described as the most dangerous species complex of snakes in the world causing more bites and deaths than any other snake largely due to its wide geographic distribution, irritability, venom toxicity, and occurrence in farming communities. Bites by the puff adder have been reported; ten (10) patients were bitten by *Bitis arietans* over a period of 4 years in Zaria, and, in two, the bites were fatal (Warrell et al. 1975). The small African adder *C. maculatus* and *Atractaspis* spp. were found to have caused 19 bites over a 4-year period mainly when they were trodden or inadvertently touched in the dark, thus reflecting the snakes' nocturnal habit, but none of the bites was fatal (Warrell et al. 1976b). The spitting cobra *N. nigricollis* is responsible for most cases of snakebite in Malumfashi area of Katsina state with an incidence of 15–20 bites per 100,000 populations per year and an estimated natural mortality of 5 % (Pugh et al. 1980). Of the 253 previous snakebites in Malumfashi, *N. nigricollis* accounted for 106 with a further 40 having been spat at in the eyes. Of these146 spitting cobra victims, 79 % were males and 21 % females most being Hausa youths (Pugh et al. 1980). It is noteworthy that majority of encounters had occurred near human dwellings at night as has been noted with Krait bites in Asia (Warrell et al. 1976a). The colubrid *Dispholidus typus* (boomslang) is less common, and it bites rarely except when handled (WHO 2010). In Niger Delta region of southern Nigeria, correlation was observed between annual activity patterns of venomous snakes and rural people. In particular, snake activity peaked in the wet season between April and August when human activity was also at its peak. Paradoxically potential risks of encounters were found to be highest between snakes and women and children, despite they are less often in the field compared to men (Akani et al. 2013).

Economic Impact of Snakebite

The burden of human suffering caused by snakebites has been greatly underestimated, ignored, and neglected for far too long. Snakebites, common in rural areas of many tropical developing countries including Nigeria, mainly affect the youth or agricultural workers who lack the political voice adequately to protest their needs. From the foregoing, it is evident that several thousands of Nigerians fall victims to snakebite annually. These are mostly farmers, herdsmen, and their rural-dwelling families. Each bite, whether accompanied by envenoming or not, leads to loss of work days. The high-risk groups are also the groups responsible for cash and food crops production and their prolonged or short-term incapacitation at periods which coincide with the most intense farming activities can only lead to reduced agricultural production and low economic performance (Akani et al. 2013). Furthermore, a recent study of 109 snakebite victims showed in multivariate analysis that delayed presentation of over 24 h after bite had adjusted (OR: 5.8; 95 % CI, 2.0–17.0) and hospital stay >2days had adjusted (OR: 19.5; 95 % CI, 2.0–192.3) as independent risk factors of high cost of care. Apart from loss of work days, the victims and their families have to bear the cost of treatment which could be quite high. It is not unusual for families to sell one or more cattle or a substantial portion of their harvest to pay for such treatment. Added to this, a variable but unacceptably high percentage of victims die or are left with permanent mutilation each year. This constitutes a major loss to the families concerned as the victims are usually the bread winners, and it is a permanent loss to the manpower resources available for the agricultural sector. Even the fear of snakebite keeps many people away from such economic activities as peri-domestic poultry farming, a favorite haunt of *Naja nigricollis*, or from working late in the farm for fear of being bitten on the way home in the darkening hours of the evening.

Recently, snakebite-induced mortality was shown to be inversely associated to Human Development Index, the Per Capita Government Expenditure on Health, and Gross Domestic Product Per Capita and directly associated with the Percentage Labour Force in Agriculture. It was further shown that snake envenoming is negatively associated with the government's expenditure on health, reiterating it as a disease of the poor. Clearly therefore, poverty predisposes to snakebite, and it is not only a major health problem but it is also a major impediment to economic prosperity.

Future health economic studies should not only factor the burden of mortality but also incorporate the consequences resulting from amputation, blindness, disability, disfigurement, maternofetal loss, mutilation, and tissue destruction (Habib et al. 2008; Pugh et al. 1980; Warrell et al. 1976c). In particular studies should derive quantitative estimates of economic and productivity loss with computation of disability-adjusted life years (DALYs) and quality-adjusted life years (QALYs).

Composition and Effect of Nigerian Snake Venoms on Physiologic Cascades

The snakes' venom apparatus superbly evolved to deliver a lethal dose of venom to its natural prey but may occasionally inject significant doses into humans. It must be remembered that snake venom is not a single toxin but a complex mixture of perhaps 20 or more components including enzymes, nonenzymatic polypeptides, toxic and nontoxic proteins, carbohydrates, metals, lipids, free amino acids, nucleotides, and biogenic amines. The venom of some snakes may indeed contain exogenous pathogenic bacterial flora (Theakston et al. 1990). Snake venoms may cause various adverse effects on body function as detailed in the section below.

The clinical biochemistry, pathophysiologic effects, and laboratory uses of Nigerian snakes' venoms have been reviewed in details elsewhere (Habib et al. 2001). Their main components have been described individually as detailed (Habib et al. 2001). Certain components and effects are summarized below:

(a) Ecarin, a prothrombin procoagulant fraction from *Echis ocellatus* venom (Stoker et al. 1986)
(b) Echistatin, a platelet inhibitor from *Echis ocellatus* venom
(c) Hemorrhagin, a vasculotoxic factor from *Echis ocellatus* venom
(d) A prothrombin procoagulant from *Dispholidus typus* venom
(e) Inhibitor of fibrinolytic inhibitors from *Bitis arietans* venom
(f) Botrocetin, a platelet stimulator from *Bothrops atrox* (from South America) and *Bitis arietans* venoms
(g) Complement activator in *Naja nigricollis* venom that also affects the kinin system (Warrell et al. 1976a)
(h) Complement activator in *Dispholidus typus* venom
(i) A fibrogenase stimulator of fibrinolytic system from *Naja nigricollis* venom
(j) Burrowing asp *Atractaspis* spp. venom is rich in sarafotoxins an endothelin homologue

Clinical Manifestations

Bites by most venomous snakes produce local swelling and tissue damage, and these are the first signs of envenoming. Local swelling and pains occurred in nearly 100 % of *E. ocellatus* bites. Its bites were associated with local blistering, tissue necrosis, and enlarged tender regional lymph nodes (Warrell et al. 1977). Associated constitutional symptoms such as fever, vomiting, headache, dizziness, drowsiness, and abdominal pains may occur. Local and systemic bleeding results from incoagulable blood and hemorrhagins in the viper venom particularly the venoms of *E. ocellatus* (Warrell et al. 1977) and *D. typus*. Bleeding may manifest at the site of bite or from any affected organ/system (Fig. 13.3), e.g., as bleeding from the

Fig. 13.3 Images (**a**–**d**) of snakebite victims with different manifestations and complications (unconsciousness in **d**) and killed snakes in panels **a** and **b** (Source: SB Abubakar and AG Habib)

urinary tract in urine (hematuria), bleeding from the lungs in sputum (hemoptysis), bleeding from the gastrointestinal tract in vomiting (hematemesis) or stools (hematochezia), bleeding in skin or tissues (hematomas), bleeding from the nostrils (epistaxis), and bleeding in the brain as intracranial hemorrhage; this is an important cause of death (Warrell et al. 1977). Rarely bleeding may be into the retroperitoneal tissue, retina of the eye with subsequent loss of vision (as in three cases, one each reported from Kaduna, Maiduguri, and Makurdi (Mustapha et al. 2010)) (Figs. 13.1 and 13.3), retroperitoneal nerve roots, and other organs. In summary, *E. ocellatus* envenoming in Nigeria manifests with hemorrhage, incoagulable blood, shock, and local reaction (necrosis). Venoms of some elapid snakes including *N. nigricollis* may also cause severe local effects (Warrell et al. 1976a). The local effects of venom may be aggravated by secondary phenomena such as ischemia caused by thrombosis, external compression caused by tight tourniquets, raised tissue pressure within a tight fascial compartment, and tissue infarction.

Spontaneous hemorrhage may occur following *N. nigricollis* bites (Warrell et al. 1976a) and occasionally following bites of puff adder (*B. arietans)* due to the damaging effect of the venom on the vascular endothelium aggravated by thrombocytopenia (Warrell et al. 1975).

Envenoming by *B. arietans* bite is usually characterized by severe local reactions (Warrell et al. 1975). Its venom may have a direct depressive effect on the myocardium as seen in one of the ten victims reported from Nigeria (Warrell et al. 1975). This may lead to bradycardia and occasionally cardiac arrhythmias. Similar cardiac complications occasionally follow *E. ocellatus* bites. In a recent study of 108 patients predominantly bitten by carpet viper, cardiac and hemodynamic abnormalities were widespread, and electrocardiographic (ECG) abnormalities were observed in 60 % although only 1 patient had elevated cardiac troponin T. The three victims of burrowing asp had nonspecific cardiac ECG abnormalities: ischemia, conduction defects, and heart blocks (Karaye et al. 2012). Renal failure may also follow puff adder or carpet viper bites due to hypovolemia and consumptive coagulopathy (Warrell et al. 1975; Warrell et al. 1977).

Traditionally elapid bites have been associated with neurotoxic features although in this country it has only been reported following *Dendroaspis* spp., *N. haje*, and *N. melanoleuca* but not *N. nigricollis* bites despite having alpha presynaptic toxin containing venom (Warrell et al. 1974a, 1976a; WHO 2010). In Nigeria and elsewhere, *N. nigricollis* may be associated with ophthalmologic features resulting from venom spitting into the eyes by cobra (Warrell and Ormerod 1976). The features range from acute photophobia, pains, blepharospasm, and lachrymation to chronic keratitis conjunctivitis, corneal opacity, and blindness (Warrell and Ormerod 1976). Additionally most snakebite victims tend to be anxious, exhausted, and occasionally drowsy and sleepy. This may be related to endogenous opiates (or related substances) in the venom rather than neurotoxicity as was seen in Russell's viper venom. Psoas bleeding following *Echis ocellatus* bite may lead to iliofemoral nerve compression.

Clinical Course and Prognosis

Most cases of snakebite recover completely, but mortality may be as high as 16–20 % in untreated carpet viper bites which with the advent of appropriate and effective antivenom has been reduced to 78 deaths (1.27 %) among 6,187 victims in northeastern Nigeria (Habib and Abubakar 2011; Warrell et al. 1977). In untreated cobra bites, the mortality is about 5 % (Pugh et al. 1980; Warrell et al. 1976a). Some of the factors contributing to deaths among snakebite victims include intracranial hemorrhage (Habib and Abubakar 2011; Warrell et al. 1977) (in viper bites), complications of local wound necrosis including tetanus (Habib 2003; Theakston et al. 1990), renal failure (Warrell et al. 1975; Warrell et al. 1977), and logistic problems regarding cost, availability, or efficacy of antivenoms and delays between bites and presentation to equipped hospitals (Habib and Abubakar 2011; Habib and Warrell 2013). The interval between *E. ocellatus* bites and deaths ranges from 25 h

to 41 days with a median of 5 days (Warrell and Arnett 1976). Maternal and fetal loss has been reported; fetal loss was observed in 1 of 10 pregnant women bitten by *E. ocellatus* (Habib et al. 2008). Only a small fraction of treated survivors may have lifelong deformities like scars, amputations, and blindness. The bad prognostic factors which may be related to dose of venom and the rapidity of its circulation include small young victims; bites on trunk, face, or vessels; bite by large snakes; presence of bacteria in venom or mouth of snake (Theakston et al. 1990); and mobilization or exertion following bite.

Investigations and Laboratory Findings

Anemia, thrombocytopenia, and leukocytosis may result, but in case of envenoming by *E. ocellatus*, the platelet count may remain normal despite severe consumptive coagulopathy (Warrell et al. 1977). Polymorphic blood film may be seen with schizocytes resulting from microangiopathic hemolytic anemia and poikilocytosis especially in *E. ocellatus* envenoming. Depressed clotting factors and raised fibrin degradation products (FDPs) together with other features of disseminated intravascular coagulation (DIC) have been noted (Warrell et al. 1977). Bleeding, clotting, prothrombin, and partial thromboplastin times may be prolonged following *E. ocellatus bites*. In *N. nigricollis* bites, depressed complement levels like C3, C4, and total hemolytic component may be noted (Warrell et al. 1976a). Nonclotting blood when the 20 min whole blood clotting test is performed is diagnostic of systemic envenoming by *E. ocellatus* in Nigeria (Meyer et al. 1997; Warrell et al. 1977; Warrell and Arnett 1976), and failure of clot retraction may suggest *N. nigricollis* (Warrell et al. 1976a). The estimation of blood urea, electrolytes, and creatinine would be helpful if renal failure complicates the presentation as in *E. ocellatus* and *B. arietans* (Warrell et al. 1975; Warrell et al. 1977) bites. Serum concentrations of creatine phosphokinase or aspartate aminotransferase may be raised in severe envenomation caused by *Naja* spp. Features of intravascular hemolysis should be sought. Urine should be examined for red blood cells, hemoglobin, and proteins. Electrocardiographic (ECG) studies are important where cardiotoxicity is considered (Karaye et al. 2012; Warrell et al. 1975, 1976b, 1977).

Immunological and Molecular Tests

Immunological methods such as immunodiffusion using antisera against the specific snake venoms have been used in Zaria, Nigeria, where it was particularly successful in diagnosing patients bitten by *B. arietans* and *N. nigricollis* (Greenwood et al. 1974). Enzyme-linked immunosorbent assay (ELISA) has found use in epidemiology, diagnosis, and management of snakebite. It was also used to study kinetics of venom antigen and antibodies in victims of bites 40 years previously at Malumfashi in Katsina state (Pugh et al. 1980) and in monitoring venom-antivenom levels in clinical trials of new antivenoms (Meyer et al. 1997).

Thus, immunological methods are important in venom detection, snake species identification, antivenom titration, and general assessment of management and elucidation of pathophysiology. A polymerase chain reaction (PCR)-based swab test with samples collected from the area surrounding the site of bite has shown promise for identification of responsible snakes causing bites in Asia and is currently being evaluated in northeastern Nigeria.

Management

First Aid, Prehospital Care, and Clinical Management

First aid measures include reassurance of patients and immobilization of the bitten limb with a splint or sling. First aid administrators should hasten transfer of patients to hospital taking the dead snake along if found. Avoid harmful and time-wasting procedures, such as incisions application of native herbs, ice packs, or electric shock, as they have not been confirmed to be effective in controlled studies. Avoid the use of tourniquet or constricting band as it has minimal or no beneficial effect unless the snake was identified as dangerous neurotoxic (Tun-pe et al. 1987) like *N. haje* and *Dendroaspis* spp. as they may worsen ischemia and necrosis. Such unproven traditional first aid measures lead to delay and further worsen outcome (Habib and Abubakar 2011; Ogunfowokan et al. 2011; Michael et al. 2011). Recently, prehospital practices of 72 consecutive snakebite victims at a hospital in north central Nigeria were reported. The primary outcome assessed was death or disability at hospital discharge. Victims were predominantly male farmers, and in 54 cases (75 %), the snake was identified as a carpet viper (*Echis ocellatus*), with the remainder unidentified. Most subjects (58, 81 %) attempted at least one first aid measure after the bite, including tourniquet application (53, 74 %), application (15, 21 %) or ingestion (10, 14 %) of traditional concoctions, bite site incision (8, 11 %), black stone application (4, 5.6 %), and suction (3, 4.2 %). The majority (44, 61 %) presented late (after 4 h). Most (53, 74 %) had full recovery at hospital discharge. Three deaths (4.2 %) and 13 (18 %) disabilities (mainly tissue necrosis) occurred. The use of any first aid was associated with a longer hospital stay than no use (4.6 ± 2.0 days vs 3.6 ± 2.7 days, respectively, $P = 0.02$). The antivenom requirement was greater in subjects who had used a tourniquet ($P = 0.03$) and in those who presented late ($P = 0.02$). Topical application (odds ratio 15, 95 % CI 1.4–708) or ingestion of traditional concoctions (OR 20, 95 % CI 1.4–963) was associated with increased risk of death or disability. The authors concluded that ingestion and application of concoctions were associated with a longer time interval before presentation, a higher cost of hospitalization, and an increased risk of wound infection (Michael et al. 2011).

All victims should be admitted to hospitals for at least 24 h except in clear nonvenomous snakebites where the snake has been reliably identified. Pains may be managed with either oral paracetamol or narcotics. Persistent vomiting may be

treated with intravenous chlorpromazine or other antiemetics. Intramuscular injections should be avoided because of susceptibility to hematoma formation in carpet viper bites.

Shock usually responds to replenishment of the circulating volume or infusion of dopamine. Fresh whole blood may be required. Renal failure may require peritoneal or hemodialysis if volume replenishment, diuretics, and dopamine fail to increase urine flow. Tetanus toxoid and antimicrobial agents particularly ceftriaxone and metronidazole (or amoxicillin-clavulanic acid or penicillin) and aminoglycosides are indicated to prevent or treat secondary wound infection. A rise in intracompartmental pressure (commonly in the anterior tibial compartment) above 45 mmHg probably justifies fasciotomy provided that adequate antivenom has been given and blood coagulability restored (Habib et al. 2001; Warrell et al. 1977). However, the results of fasciotomy are usually disappointing as muscle swelling and necrosis are directly attributable to envenoming. Surgical debridement with skin grafting may be required for severe local necrosis. For persistent acute snake venom, ophthalmia treatment with local antibiotics like tetracycline or chloramphenicol is recommended. Irrigation with bland fluid is imperative, but the value of antivenom for ophthalmia appears unproven (Pugh et al. 1980; Warrell et al. 1976c). Recently, it has been found that those presenting with local blisters following envenoming have a poorer outcome with longer hospital stay, wound complications necessitating debridement/amputation, and use of more antivenoms. Although secondary wound infection may complicate "de-blistering," it is probable such intervention may be needed in managing bite injuries.

Use of Antivenoms

Antivenom remains the hallmark of therapy, and it should be used only if there is evidence of envenoming. Indeed, distance and delay from bite to hospitalization or antivenom administration predicted poor prognosis and mortality (Habib and Abubakar 2011; Ogunfowokan et al. 2011; Michael et al. 2011). As most antivenoms are of equine or ovine origin, anaphylactic reactions may follow their use. Anaphylactic reactions often are not predicted by cutaneous or conjunctival hypersensitivity testing (Malasit et al. 1986). The features and signs of envenoming which constitute indications for treatment with antivenom are:

Systemic Envenoming

1. Hypotension, shock, or signs of cardiotoxicity
2. Neurotoxicity (ptosis, ophthalmoplegia, dysphagia, respiratory paralysis)
3. Impaired consciousness often due to subarachnoid hemorrhage manifesting as neck rigidity and subhyaloid hemorrhage on fundoscopy or secondary to respiratory failure or shock
4. Spontaneous/systemic bleeding
5. Nonclotting blood using the simple 20 min whole blood clotting test in glass tube

6. Leukocytosis with white blood cell counts of 20,000/ml or elevated serum enzymes, e.g., aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatine kinase
7. Acidosis – more with ensuring respiratory paralysis

Local Envenoming

1. Known necrotic venom
2. Swelling involving more than half limb
3. Rapid progression of swelling
4. Bites on digits and into tight fascial compartments

It has been shown that either 15.2 ml of South African Institute for Medical Research (SAIMR) or 37.9 ml of Behringwerke antivenom is needed to restore normal coagulability among carpet viper victims (Warrell et al. 1974b), while at least 80 ml of specific polyvalent antivenom should be given to puff adder victims as reported from Zaria (Warrell et al. 1975) and at least 4–5 ampoules (40–50 ml) should be given regardless of the age of victim. Administration of antivenom is either by slow intravenous drip infusion or bolus injection over 10 min. Antihistamines such as chlorpheniramine, hydrocortisone, and adrenaline should be available to treat early antivenom reaction. Effect is judged by the clinical response or clotting time in the case of massive procoagulant envenomation by *E. ocellatus*. The initial dose is repeated 6 hourly until the blood clots within 20 min. Anticoagulants have no place in the management. Fresh whole blood transfusion may be useful to replace lost blood and provide coagulant fractions preferably after administration of effective antivenom. Without achieving venom neutralization, such transfusions may "fuel the fire" and worsen envenoming. The most dramatic and effective treatment for hemostatic disorders is specific antivenom. After envenoming by most species including *E. ocellatus*, blood coagulability will be restored within 6 h of antivenom treatment (Habib and Warrell 2013; Meyer et al. 1997; Warrell et al. 1977). The very rapid recovery from virtually total defibrinogenation must indicate extreme stimulation of hepatic synthesis perhaps by fibrin degradation products (FDP) and acute phase proteins.

Are Antivenoms Effective for Echis Ocellatus Envenoming?

Studies in Nigeria reported rapid restoration of blood coagulability and resolution of spontaneous hemorrhage following administration of effective antivenoms after carpet viper envenoming (Habib and Warrell 2013; Meyer et al. 1997; Warrell et al. 1974b, 1976d, 1977; Warrell and Arnett 1976). However, observations on envenoming by the brown snake (*Pseudonaja textilis*) and taipan (*Oxyuranus scutellatus*) in Australasia suggest that antivenom may be ineffective for correcting hemostatic abnormalities if not given almost immediately after the bite (Isbister et al. 2009). This is of concern as *E. ocellatus* venom, like the venoms of the two

Australasian species, causes consumptive coagulopathy by activating prothrombin. But *Echis* venoms studied so far have groups A and B prothrombin activators with substantially different molecular mechanisms of action from the prothrombinase-mimicking groups C and D prothrombin activators in Australian snake venoms (Kini 2005). As there had never been a double-blind placebo randomized controlled trial confirming antivenom effectiveness, this has threatened its use as a therapeutic agent especially given known potential life-threatening adverse reactions and cost considerations. However, a recent critical reanalyses and meta-analysis of published studies found appropriate antivenoms confer 75 % (95 % confidence interval, 55–86 %) protection against mortality from carpet viper (Habib and Warrell 2013). Furthermore, in a short period during a 3-year period, lack of effective antivenom resulted in doubling of mortality to 16/550 (2.91 %) compared to 78/6137 (1.27 %) ($p < 0.002$) when it was available (Habib and Abubakar 2011). It is unclear whether differences in molecular mechanisms of prothrombin activation by venoms of Australasian snakes and carpet viper (Kini 2005) might explain the potential disparity in responses to antivenom therapy (Isbister et al. 2009). Further experimental studies are needed to elucidate the interaction dynamics between these venoms, antivenoms, and human tissues.

Crisis in Antivenom Supply

Antivenoms are the only proven remedies for snakebite, but in sub-Saharan Africa, deficiencies in the quality, quantity, specificity, access, and distribution of antivenoms are responsible for the huge morbidity and mortality burden. Although many previously available antivenoms in Nigeria were effective, few currently marketed antivenoms are neither specific to West African snake venoms nor effective. Indeed, dangerous inappropriate foreign products are commonly being marketed by unscrupulous manufacturers in rural West Africa (Bregani et al. 2006; Habib and Abubakar 2011; Habib and Warrell 2013; Visser et al. 2008; Warrell 2008).

Antivenoms Raised Specifically Against Nigerian Snakes

Given the protracted problem of inappropriate and ineffective antivenoms in the country, the Nigerian Government since 1990 has supported development of antivenoms that are appropriate for Nigerian needs through collaborative efforts with scientists in the United Kingdom. In early 1990s, a new antivenom EchiTAb was made specifically against the venom of Nigerian *Echis ocellatus*. It is unusual in being papain-digested Fab fragment of gammaglobulin (IgG) rather than conventional pepsin-refined Fab. Fab has the theoretical advantage of rapid tissue penetration, large apparent volume of distribution, and less risk of complement activation (and early adverse reactions) compared to Fab. EchiTAb is lyophilized and has a longer half-life and has proved excellent in preclinical studies. A clinical

trial was undertaken in Kaltungo, Gombe state, Nigeria, comparing EchiTAb with another antivenom Pasteur Ipser Afrique – which had earlier been found effective against *E. ocellatus* in Nigeria (Daudu and Theakston 1988; Meyer et al. 1997). Intramuscular route for mildly envenomed patients was also investigated as that may allow antivenoms to be given at primary health centers by junior health workers if proven safe and effective. Although EchiTAb proved effective at the initial dose of 20 mls, unfortunately the batch used in the trial contained impurities resulting in higher anaphylactic reactions. Presumably owing to its small molecular weight, restoration of clotting was not permanently sustained in few patients at the lower dose of 10 ml (0.5 g) used in the main trial (Meyer et al. 1997).

Subsequently, through international collaborations involving a number of different partners (Abubakar et al. 2010a), two new antivenoms have been developed, tri-specific Costa Rican EchiTAb Plus (raised against the venoms of Nigerian *E. ocellatus*, *B. arietans*, and *N. nigricollis*) [ET-Plus] and monospecific EchiTAb G (raised against the venom of Nigerian *E. ocellatus*) [ET]. Both consist of caprylic acid purified whole IgG rather than Fab fragment in the initial EchiTAb. Both antivenoms passed preclinical assessments and were effective and safe in preliminary clinical dose-finding/safety studies (Abubakar et al. 2010a). The efficacy and safety of the initial doses of the two antivenoms were then compared in a randomized double-blind comparative trial in Kaltungo Hospital from 2006 to 2008 (Abubakar et al. 2010b). The main outcome measure in the patients all of whom presented with incoagulable blood was permanent restoration of blood coagulability after 6 and 24 h, assessed by the 20 min whole blood clotting test. Permanent restoration of coagulability at 6 h by the initial doses was achieved in 83 % of ET-Plus and 76 % of ET patients ($p = 0.074$) with no fatalities, and the incidence of early adverse reactions (nonsevere) was similar (26 % ET-Plus and 19 % ET, $p = 0.100$), respectively. Thus, both antivenoms proved effective and acceptably safe, and both were recommended for the treatment of *E. ocellatus* envenoming in Nigeria and probably elsewhere in the savannah West Africa (Abubakar et al. 2010b).

Nigerian Antivenoms in Randomized Controlled Trials (RCT) and Preclinical Studies

Several observational studies were conducted in Nigeria with the antivenoms currently or previously available in the country as described above and in the table (Meyer et al. 1997; Warrell et al. 1974b, 1977, 1980, 2010c) (Table 13.2). Some of the studies were non-placebo randomized controlled trials (RCTs) (Meyer et al. 1997; Warrell et al. 1974b, Warrell et al. 1980, c) or preclinical studies conducted outside the country (Abubakar et al. 2010a; Calvete et al. 2010; Casewell et al. 2010; Segura et al. 2010; Petras et al. 2011) (Tables 13.2 and 13.3). These five (5) preclinical studies of antivenoms raised against venoms from Nigerian snakes (including the two mentioned above in the second RCT (Abubakar et al. 2010b)) used experimental approaches utilizing immunological assays (venom-antivenom

Table 13.2 Non-placebo randomized controlled trials (RCTs) of antivenoms following carpet viper envenoming in Nigeria (Habib and Warrell 2013)

Author year	Design	Trial antivenoms (intravenous dose)	Successful outcome (RC: restoration of clotting)	Early adverse reactions (EAR)
Warrell et al. 1974b	Open	SAIMR antivenom (10[a]–20 mls) vs Behringwerke antivenom (20[a]–80 mls)	23/23 vs 18/23 (after 1 or more doses)	4/23 vs 6/23
Warrell et al. 1980	Open	Pasteur Paris monospecific (20[a]–40 mls) vs Behringwerke antivenom (60[a]–180 mls)	7/7 vs 4/7 (after 1 or more doses)	1/7 vs 2/7
Meyer et al. 1997	Open	EchiTAb Fab (10 mls) vs Pasteur Ipser Afrique (40 mls)	8/22 vs 6/17 (after 1 dose)	5/22 vs 2/17
Abubakar et al. 2010b	Double blind	EchiTAb G (10 mls) vs EchiTAb-Plus-ICP (30 mls)	156/206 vs 161/194 (after 1 dose)	39/206 vs 50/194

[a]Lower volume equals one dose; *SAIMR* South African Institute for Medical Research

neutralization tests), venom lethality assessments in mice, and proteomics (venomics, antivenomics) analyses in various combinations (Abubakar et al. 2010a; Calvete et al. 2010; Casewell et al. 2010; Segura et al. 2010; Petras et al. 2011). The two antivenoms cited in Table 13.3 were raised against venoms of *Echis ocellatus* and were confirmed to be efficacious in neutralizing its venom; one of the 2 EchiTAb Plus is trivalent with additional activity against *Bitis arietans* and *Naja nigricollis*. The studies further showed that neutralization may extend to other *Echis* species, notably *E. coloratus*, *E. jogeri*, *E. leucogaster*, and *E. pyramidum* in larger areas of SSA (Table 13.3). However, all these antivenoms were ineffective against Asian *Echis carinatus sochureki*. In fact, the antivenoms (imported from India or Iran) that had been raised against venoms of *E. c. carinatus* and *E. c. sochureki* proved ineffective in clinical studies (Bregani et al. 2006; Habib and Warrell 2013; Visser et al. 2008; Warrell and Arnett 1976; Warrell et al. 1977). Utilizing proteomics science (venomics, antivenomics), the activity of the trivalent antivenom was also shown to extend to other *Bitis* spp. and *Naja* spp. in SSA (Calvete et al. 2010; Petras et al. 2011) (Table 13.3).

Prevention

As a preventive measure, protective clothing, boots, and long trousers should be worn while working in snake-infested areas. Newer and improved techniques of animal immunization, effective and safer antivenom production (e.g., whole immunoglobulin vs digested fragments), and best formulation with extended shelf life (freeze-dried vs liquid formulation) in African setting should be critically explored. The field of proteomics (venomics, antivenomics) should complement preclinical studies and clinical trials in evaluating and selecting antivenoms

Table 13.3 Preclinical studies of antivenoms against venoms of Nigerian and African snakes (Habib and Warrell 2013)

Author and antivenom	Experimental method	Objective	Findings	Comment/ conclusion
Casewell et al. 2010 on EchiTAb IgG: it was raised against venom of *E. ocellatus*	Immunological assays (ELIZA); venom lethality and AV neutralization	Determine utility across *Echis* spp. in Africa	Neutralized venoms *E. pyramidum leakeyi* and *E. coloratus*	Not active against *E. carinatus sochureki* venoms
Abubakar et al. 2010b on EchiTAb G and EchiTAb- Plus-ICP. The latter was raised against venoms of 3 Nigerian snakes – *E. ocellatus*, *B. arietans*, and *N. nigricollis*	Venom lethality and AV neutralization in mice Open dose finding	Comparison of several (8) AVs in mice neutralization; efficacy and tolerance in 24 human patients	Compared AVs; doses were determined (in 24 patients)	Confirmed viability to test the 2 AVs in RCT
Segura et al. 2010 EchiTAb-Plus-ICP	Venom lethality and AV neutralization	Determine cross neutralization	Showed cross neutralization to *E. leakeyi* and *E. pyramidum* venoms. Neutralized hemorrhagic, anti-hemostatic, and necrotic activities of *E. ocellatus* venom	Likelihood for its use across broad areas of SSA as it was active against other genera
Calvete et al. 2010 on EchiTAb-Plus-ICP	Proteomics: antivenomics and immune-depletion studies	Determine cross-reactivity to other species	Showed immunological cross-reactivity to *E. leucogaster* and *E. pyramidum leakeyi* venoms in SSA	Likelihood for its use across broad areas of SSA as it was active against other genera
Petras et al. 2011 on EchiTAb- Plus-ICP	Antivenomics and immune-depletion studies; lethality and dermonecrotic neutralization tests	Determine cross-reactivity against other African *Naja* spp.	Neutralized lethality against venoms of *N. nigricollis*, *N. mossambica*, and *N. pallida*. Neutralized dermonecrotic activity of all African *Naja* spp	Likelihood of effectiveness against other African cobras in SSA

for use in the region (Calvete et al. 2010; Petras et al. 2011). The proteomics approach brings with it the potential to design new immunizing mixtures from which to raise potent antivenoms with wider therapeutic ranges (Habib and Warrell 2013). As only about 8.5 % of snakebite victims attend hospitals in Nigeria (Pugh et al. 1980), communities should be educated about the benefits of orthodox medicine (antivenom therapy) so as to reduce time-wasting unproven traditional first aid care, improve hospital attendance, and eventually reduce the resulting morbidity and mortality. Healthcare workers managing snakebite should also be educated on the appropriate and standardized ways for managing snakebite as such intervention has been shown to improve outcome (Visser et al. 2004). As delay, distance, and remoteness from accessing care and use of time-wasting unproven traditional first aid measures worsen outcome (Habib and Abubakar 2011; Ogunfowokan et al. 2011; Michael et al. 2011), strategies for providing antivenoms in rural inaccessible areas that will reduce distance traveled and delay to care should be made available as reviewed elsewhere (Habib and Warrell 2013). Certain herbs in Nigeria (*Aristolochia albida*, *Guiera senegalensis*, *Schumaniophyton magnificum*, etc.) were found to have activities on snake venoms in experimental animals but studies are ongoing and remain inconclusive (Habib et al. 2001).

Policy Recommendations and Future Directions

Expert advice and encouragement must be offered to Nigerian health authorities, regulatory agencies, antivenom producers, and medical personnel to improve every aspect of the management and control of snakebite. In particular:

1. Antivenom manufacturers must be helped in practical ways to improve the safety and efficacy of their products. This demands the design and implementation of long-term technology transfer programs involving both North–south (i.e., collaboration between Nigeria and industrialized countries) and South-South partnerships.
2. National health authorities must be helped to select and purchase antivenoms appropriate to their national needs.
3. Adequate policies for antivenom distribution must be developed in the country and in each health zone (or state) in order to provide antivenoms where they are most needed.
4. Medical personnel must be trained in the modern management of snakebites and, most crucially, in the selective use of antivenoms through use of educational pamphlets and didactic and practical lectures.
5. Communities must be informed and educated about snakebite risks through the use of posters and leaflets. They should be offered realistic solutions that ameliorate the hazards and empower the people themselves to help manage the problem in practical and sustainable ways.

6. Research directed at improving the available methods of first aid, primary clinical care, and patient rehabilitation must be accorded priority and funded at national and state levels.
7. Surveillance and reporting systems that enable collation of reliable epidemiological and clinical data need to be developed, tested, and implemented and the data used to support rational resource allocation and distribution and appropriate prioritization of snakebite as a neglected tropical disease at all levels.

Conclusion

Venomous snakebites are common in Nigeria. Carpet viper, cobra, and puff adder are medically the most important snakes causing bites, morbidity, and mortality mainly among agricultural workers and rural dwellers. Carpet viper causes most bites, and envenoming manifests with hemorrhage, incoagulable blood, shock, and local reaction (necrosis). Bites and spits may be complicated with amputations, venom ophthalmia, wound infection, scarring, and malignant transformation. Use of inappropriate antivenoms has increased morbidity and mortality, but two antivenoms have recently been developed against Nigerian snakes' venoms, and they have proved effective and safe in clinical trials. However, availability, distribution, and utilization of antivenoms remain challenging, and strategies for broadening antivenom access to endemic rural areas together with instituting quality assurance, standardization, community, and healthcare workers' education should be implemented. With the advent of antivenomics, health authorities must be helped to select and purchase antivenoms appropriate to their national needs, while manufacturers should be helped in practical ways to improve the safety, efficacy, and potential coverage of their products.

Cross-References

► Disability and Impairment Following Snakebite in Africa
► Socioeconomic Aspects of Snakebite in Africa and the Tropics

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Socioeconomic Aspects of Snakebite in Africa and the Tropics

14

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Contents

Abstract

Snakebite remains predominantly a problem of the poor and neglected. It is an occupational disease of young agrarian farmers that occasionally results in significant morbidity, mortality, and economic loss. It kills more people than many of the earlier recognized neglected tropical diseases (NTDs). Unfortunately, countries affected with snakebite are incapable of appropriately

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responding to the challenges it poses. Accessibility and affordability of snake antivenoms where it is most needed remains a major challenge in these countries. The intricate relationship between snakebite and poverty has been recognized. Families often spend significant part of their time and income as a result of snakebite incidents. There is need for an all-encompassing approach to address the disparities in access to effective health-care interventions for snakebite victims. Furthermore, the long-term disabilities and economic consequences should be properly addressed.

Introduction

Snakebite remains an important, though neglected, global public health problem. Despite potential underreporting, an estimated global incidence of 2.5 million bites and 85,000 deaths occur yearly (Gutierrez et al. 2010). The work of Kasturiratne, considering unreported cases, projected an estimated 5.5 million bites/year (Kasturiratne et al. 2008). In the West African subregion, it is a major medical problem among rural communities of the savannah. The region arguably bears the heaviest burden of snakebite as reported from earlier studies. An estimated incidence of 497 per 100,000 population per year, with a mortality of 12.2 %, was reported in the Benue Valley of northeastern Nigeria (Pugh and Theakston 1980). This high burden is mainly the result of frequent accidental snake-human contact necessitated by agricultural activities. High incidence of bites and stings by snakes and other animals among rubber tappers and Amazonian Indians of the Jurua valley, Acre State, Brazil, has been reported. In such settings, snakebite is an important cause of morbidity and death. Overall, 13 % of a surveyed population had been bitten during their lifetime (Pierini et al. 1996).

The local tissue damage is characterized by swelling, blistering, hemorrhage, and necrosis of skeletal muscle. Any delay in the access to health facilities could lead to immediate (bleeding, necrosis, death) and long-term complications or permanent disability. Large numbers of victims survive with permanent physical and consequently psychological sequelae, mostly due to the tissue-damaging effects of snake venoms and the psychological trauma of an encounter. Young agricultural workers, especially males, are the most frequently affected, making snakebite envenoming a truly occupational disease. This is frequently overlooked by national authorities. The non-mechanized nature of farming in much of the tropics, the fact that rainy season forces the snakes from their holes to the surface, the failure of the farmers to use protective boots and gloves, and the attraction of snakes by poultry or rodents that are in turn attracted to grains usually stored close to human habitation are some of the factors predisposing agrarian farmers in Africa to snakebites.

Children and pregnant women are also common victims of snakebites. Snakebites have been reported to contribute to poor pregnancy outcomes with resultant psychological trauma.

The fact that snakebite and its resultant morbidity and mortality mainly affect the poor has been established by local observations and studies that compared socioeconomic indices of nations, spending on health and mortality from snakebite (Harrison et al. 2009). An important, though indirect, relationship also exists between snakebites and poor rural dwellers and nomadic populations (Habib 2013). In such settings, poverty from snakebite could be a cause or effect. This is because snakebite predisposes to poverty through immobility and its resultant disabilities notably amputation, disfigurement, and blindness among others. On the other hand, poverty compels agrarian farmers, their wives, and children to circumstances that put them at risk of snakebite.

Snakebite victims tend to be in the economically productive age group. The economic impact of any disability could be very high. There is paucity of data regarding the socioeconomic impact of snakebite on victims. However, there are enough case reports and isolated studies to suggest that the impact is high.

At a global level, Harrison and colleagues demonstrated that snake envenoming is a disease of the poor. They established a negative association between snakebite deaths and government expenditure on health. Furthermore, they confirmed that the burden of snakebite mortality is highest in countries least able to deal with its financial cost (Harrison et al. 2009).

The WHO lamenting the challenges of having reliable data on snakebites estimated about five million snakebites/year, 2.5 million envenomings, and 100,000 deaths with approximately thrice as many amputations and other permanent disabilities. Furthermore, snakebite kills more people in the tropics than some of the world's recognized neglected tropical diseases (NTDs). This argument among others led to its recent categorization as an NTD.

Despite several attempts to establish the global and regional burden of snakebite, there is a dearth of well-designed, well-conducted studies to determine the exact socioeconomic burden of snakebite on affected populations in the tropics (Mohapatra et al. 2011; Snow et al. 1994; Pugh and Theakston 1980). The WHO age-standardized estimated DALYs for all poisoning in Nigeria are as high as 182/100,000 population (WHO 2009). The need for getting more accurate estimates specific for snakebite cannot be overemphasized.

The Poverty-Snakebite Cycle

There appears to be a vicious circle of cause-effect between snakebite and poverty. Poverty is an important risk factor for snakebite. On the other hand, snakebite could lead to disability and thereby predispose to poverty. In addition, snakebite could stretch the very scarce resources available to the health system in most of the areas endemic for snakebites. An estimated 10 % of hospital beds could be occupied by victims in the Niger Valley of Nigeria (Pugh et al. 1979; Fig. 14.1).

It is not uncommon for families to spend more than their monthly incomes on transport and treatment of snakebites. In Kaltungo, Nigeria, the median cost of a

Fig. 14.1 Snakebite victims overflowing the available bed spaces in Kaltungo, Nigeria

snakebite incident on families (excluding antivenoms) was US$47. Furthermore, households could spend more than 100 % of their monthly income on hospital care for snakebite (Dalhat et al. 2011). A similar strain on family income was noted with other NTDs. For instance, the median total cost of one episode of visceral leishmaniasis in Nepal was US$ 165 or 11 % of annual household income. Direct and indirect costs (income losses) represented 47 % and 53 % of total costs, respectively. It was also reported that households could go as far as taking loans to cope with the cost of illness.

Snakebite-Disability Socioeconomic Cycle

The long-standing disability from snakebite often incapacitates the breadwinners, thereby compromising the overall well-being of families. Complications like blindness, amputations, chronic osteomyelitis, stroke, and hypopituitarism could result in long-term disabilities. Given that most cases of snakebites occur in young productive farmers that are frequently the source of support for their families, the victims become incapable of providing sustenance to their families. An illustrative case is a young man bitten over 40 years by puff adder (Warrell 1975) with subsequent lingering recurrent chronic osteomyelitis of the tibia. He spent the better part of his life depending on handouts from others to treat his ailment, generate resources to get married, and take care of his family. In such cases, the

health workers are frequently tasked with the responsibility of providing support or ensuring income-generating activities for him.

The relationship between poverty and NTDs could be complex. The global health community cannot afford to write off the poor. Casting destitution as intractable, or epidemics that afflict the poor as accidental, erroneously exonerates us from responsibility for caring for those most in need (Alsan et al. 2011). There is the need for clear understanding of the social and psychological effects of diseases of the poor by public health physicians if they are to be properly addressed. High-tech cost-effectiveness analyses are not enough to properly capture the sufferings of the poor. Economic analyses shouldn't be from institutional perspective alone; individual and societal perspectives are also important. They should also focus on immediate as well as remote socioeconomic implications of diseases. Above all, the inequalities regarding access to health care should be the central focus of all efforts.

Snakebite-Related Socioeconomic Challenges

Depending on the type of snake and resulting complication, situations could arise with grievous economic implications. They range from loss of jobs due to inability to carry out routine tasks, reduced productivity and difficult to measure, loss of quality of life. Below are examples of disabilities and scenarios that could result in significant economic loss:

Central Nervous System (CNS) Complications

Long-term morbidity could follow snakebite complications like strokes (Mosquera et al. 2003; Machado et al. 2010; Santos-Soares et al. 2007). This may result in longer hospital stay, need for long-term rehabilitation, and permanent disability. These have serious economic/financial implications on the immediate need for spending on hospital care, high cost of rehabilitation, and loss of productivity from permanent disabilities. Even though this has not been well documented for snakebite, the high cost of hospital care, rehabilitation, and permanent disability has been established in other settings. The need for expensive investigations like CT scan and MRI and the challenge of stroke care on the health system that is mostly poorly trained and grossly underfunded are a serious cause for concern. Furthermore, areas with the highest burden of snakebites don't usually have access for these investigations. In the places that have, they are frequently unaffordable to the victims. Other CNS complications like cerebellar ataxia and Guillain-Barre syndrome have similar implications (Chuang et al. 1996). The fact that antivenoms have been implicated in causing some of the complications worsened an already bad situation.

Musculoskeletal System

Chronic ulcers and chronic osteomyelitis could complicate snakebite (Garg et al. 2009). The immediate and long-term medical care for victims is a source of stress on families and the overall health system. Immediate burden of care includes the cost of antivenins and the cost of wound care (debridement, antibiotics, wound dressing, analgesia, and direct cost of amputation). Long-term challenges include management of chronic ulcers, skin graft, chronic osteomyelitis which may necessitate bone resection, and malignant transformation. Remote indirect economic implications include loss of productivity due to frequent hospital visits, unemployment, and destitution. The above complications have been linked to huge economic loss and poverty in other settings.

Renal Impairment

Acute or chronic kidney disease could result from snakebite. The poor access to health care in most communities affected by snakebite implies that they are unlikely to get optimum care for acute kidney injury (AKI), and this could result in chronic kidney disease. The huge financial burden of renal replacement therapy – dialysis or renal transplant – could be enormous. The extent to which frequent dialysis sessions affect quality of life cannot be quantified. Furthermore, quality time spent on efforts to ensure renal replacement therapy is enormous. It denies the victims and their families the opportunity to engage in economically beneficial activities. The need for constant central venous access, dialysate, and dialysis fee in SSA is enormous. Postrenal transplant care could be very expensive. The need for long-term immunosuppressant drugs, prophylactic anti-infective drugs, recurrent breakthrough infections, and restrictions that significantly reduce quality of life.

Psychosocial Stress

The relationship between snakebite and psychological disorders is well established (Williams et al. 2011). Several case reports and studies showed the extent of psychological disturbances related to snakebite. Anxiety, depression, and posttraumatic stress disorders are known sequelae of snakebite. Even though there is no physically identifiable disability seen, the degree to which psychological trauma could affect victims' ability to perform simple tasks is well established in other settings (Williams et al. 2011). Insecurity and hopelessness, rapid social change, and risks of violence and physical ill-health are additional reasons why the poor are vulnerable to common mental common mental disorders. Consequently, a vicious cycle of poverty and mental health could result from the direct and indirect costs of mental ill-health (Patel and Kleinman 2003). There is the need to conduct more studies to evaluate and address the immediate and long-term psychological effects of snakebite as it affects productivity.

Pregnancy Lost

The relationship between snakebite and abortion has been established (Habib et al. 2008; Adam and Gerais 2005). Considering the poor availability and access to basic maternal health care in areas where snakebite is common, the obstetric challenges posed by snake envenomation need special attention. The fact that even pregnant women have to work in the farms and walk long distances to the markets or rivers (to fetch drinking water) shows the level of poverty among these communities predisposing them to snake envenomation.

Visual Loss

The African spitting cobra is known to cause blindness. This results from either the direct effect of the venom in the eyes (venom ophthalmia) or the application of harmful, unhygienic, and traditional herbs. Blindness could lead to severe disabilities and handicap that prevent the victims from carrying out their normal activities. In sub-Saharan Africa (SSA), without social welfare support it is common for the blind to resort to destitution. Furthermore, the blind often needs someone to depend on for directions in order to move around. This responsibility frequently rests on a child who is supposed to be in school.

Delayed Presentation

There is an established relationship between delay in seeking appropriate care and high cost of care. The use of harmful traditional interventions is also associated with delayed presentation, prolonged hospital stay, and consequently high cost of care (Dalhat et al. 2012). This frequently results from the activities of traditional healers. Economic loss could be from direct payment for their services, delay in seeking care in established snakebite centers, or poor outcomes in terms of complication, disability, and death. Studies in Kaltungo, Gombe State, Nigeria, showed the relationship between distance from standard snakebite centers, use of time wasting and ineffective first aid, and local traditional treatment with poor outcomes from snakebite (Habib et al. 2013).

Unscrupulous Marketing of Ineffective Antivenoms

The lack of an established, regular supply of effective antivenoms has been associated with huge financial loss from the marketing of expensive, ineffective antivenoms. This results in high rate of the complications and consequent financial loss as highlighted above.

Mortality from Snakebite

This confers the largest imaginable socioeconomic challenge on families of snakebite victims. Snakebite could result in the loss of breadwinners that are responsible for the overall care of the family. In traditional African setting, the husband is responsible for providing shelter, feeding, security, and psychological support to all members of the household. In some instances, one could be responsible for the welfare of the whole extended family whose size could be as high as 30–50 members. The same individual might be responsible for taking care of all the children, nephews, nieces, cousins, uncles, and grandparents. The young ones could be under his care up till their marriage or beyond and the elderly until death. The loss of such a strong pillar from snakebite could leave so many devastated. In cases where such a person happened to be in charge of a business enterprise, it could collapse following his death. Mortality from snakebite could also affect the economy of the state. The fact that in SSA most victims of snakebite are young productive farmers, death from snakebite could decimate this productive age group, thereby impacting on food production.

The Need to Reverse the "Inverse Care Law"

There is probably no better demonstration of the inverse care law in any disease than snakebite. It states thus: "the availability of good medical care tends to vary inversely with the need for it in the population served." This is due to the fact that even though snakebite mainly affects poor rural agrarian farmers, the trained manpower and antivenoms are mainly found in the sophisticated tertiary institutions in the big cities. These disparities are as true 40 years ago as they are now and should be corrected (Hart 1971). In areas endemic for snakebites, care should be demystified. Local and national authorities should prioritize training of available manpower and provision of antivenoms and hospital beds for care of victims. For instance, in areas like Kaltungo, Gombe State, Nigeria, victims should not be seen lying on the floor when there are unoccupied beds in the adjoining surgical or pediatric wards (see Fig. 14.1). In such settings, snakebite should have as many bed spaces or more as there are beds for other major specialities. Health service equity here should mean that access to care should be demand driven. Snakebite victims should have as much access to expertise and treatment as other endemic diseases. Antivenoms should be part of the essential drug list just as commonly used antibiotics. Efforts to update health workers through, for instance, continuing medical education (CME) should include topics related to snakebite care. On a global level, researchers should develop innovative approaches to develop a body of evidence that will enable advocacy for addressing the health inequities as has been done with other diseases (Schellenberg et al. 2003; Gwatkin 2003). Thus far,

there remains a lot of criticism of how well we are moving towards addressing health inequities not just for snakebite but for all diseases (Gwatkin et al. 2004).

People Should Set the Agenda!

Even though advocacy by public health practitioners for increased spending on neglected diseases has not changed the thinking of policy makers, direct pressure from the affected populations could force the hands of decision makers. The populace should be guided on innovative ways to advocate for redistribution of services in favor of areas of need. For instance, in areas with high incidence of snakebite, there might be more justification to develop infrastructure and capacity for snakebite care rather than routine expansion of frequently ineffective primary and/or secondary health-care facilities. The inhabitants of such communities should be able to make such a case to their policy makers. Optimizing care for snakebite should be part of campaign debates and promises in such areas. Above all, public health practitioners and professional bodies with special interest in neglected tropical diseases should be in the forefront of providing leadership and advocacy based on sound evidence. They should start and sustain viable debates on health-care disparities as it affects populations in snakebite endemic regions.

The Need for an All-Encompassing Approach

An integrated multifocal approach as proposed by the Global Snake Bite Initiative of the International Society on Toxinology and the World Health Organization will help to alleviate the enormous burden of human suffering inflicted by snakebite. This includes among others implementation of programs to support those people whose snakebites resulted in chronic disabilities as well as preventive and educational programs at the community level, with the active involvement of local organizations and employing modern methods of health promotion (Gutierrez et al. 2010; Williams et al. 2010). Another innovative way is the use of geographic information systems to identify areas with high incidence of snakebite that are remote from formal snakebite treatment centers. The maldistribution of antivenoms to "specialized" snakebite centers and tertiary institutions should be abolished. Mechanisms should be evolved to ensure basic training to enable primary health-care workers at all levels have the capacity to identify patients requiring antivenom treatment or otherwise. Such training may include some hands-on, on-the-job training in the "specialized" centers mentioned above. This will guarantee that those in need of antivenoms are given promptly, thereby reducing high cost of care as well as prevent wastage of antivenoms from panic administration of antivenoms to patients with no clinical evidence of envenomation.

Any approach should be pro-poor, multi-sectoral, and multidimensional. It should involve governmental and nongovernmental organizations.

Conclusions and Future Directions

1. The true burden of snakebite and its attendant socioeconomic consequences should be determined using well-designed, well-conducted studies. This will serve as an advocacy tool to influence global health policy in favor of snakebite prevention and treatment. Studies that assess the cost-effectiveness of proven interventions could help in convincing decision makers that monies voted for snakebite are money well spent. These studies should be comprehensive enough to factor individual, societal, and institutional perspectives.
2. Given that snakebite may have a multisystemic presentation, advocacy across the various clinical and public subspecialties such as cardiology, hematology, nephrology, neurology, psychiatry, infectious diseases, health economics, environmental health, and occupational health, among others, could help in resolving the poor political willpower to support snakebite care.
3. Rehabilitation should be an integral part of snakebite care. Victims should have counseling and support to enable them return to their normal lives. This should be done with all the physical and psychological disabilities in mind. Simple provision of hand gloves and boots to farmers could give them the required confidence.
4. The need for psychological assessment for snakebite patients should be determined while they are on admission and during follow-up visits. Considering the high incidence of delayed psychological morbidity reported by previous studies, snakebite centers may organize periodic visits by counselors/psychologists to offer additional care to patients during follow-up.
5. Patients with disabilities and handicap precluding them from going back to their jobs should be referred to appropriate skills acquisition centers to learn new trades and preventive and educational programs at the community level, with the active involvement of local organizations and employing modern methods of health promotion.

Cross-References

► Disability and Impairment Following Snakebite in Africa
► Venomous Snakes and Snake Envenomation in Nigeria

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Disability and Impairment Following Snakebite in Africa

15

Ahmad Maifada Yakasai

Contents

Abstract

Snakebite is a neglected disease that constitutes major health crisis in poor resource rural areas of Sub-Saharan Africa (SSA). Young people in the productive age group that are involved in farming activities are commonly affected. The complex physiological effects of snake venom either systemically or locally leads to organ dysfunction with resultant disabilities and impairments if not properly managed. About 6000 amputations occur annually in SSA due to

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snakebite. Other disabilities and impairments are chronic non healing leg ulcers, visual loss, chronic kidney disease, myocardial ischemia, foetal loss, stroke and depression. Several factors that are associated with increased risk of developing these disabilities include poor health care delivery services, shortage of medical personnel and lack of appropriate potent antivenom. Other environmental triggers are poor housing facilities, lack of protective clothing during farming, specie of snake, site of snakebite, severity of envenomation and involvement in high risk activities like snake charmers. The risk of morbidity and disability exponentially increases with increasing bite-to-hospital time which ranges from 0.5 to 216 hours. Difficult transportation terrain, harmful ineffective traditional practices and visiting local traditional/spiritual healers immensely contributes to delay in presentation to health care facilities. Ultimately the delay in commencing antivenom and other supportive therapy accelerates the development of disabilities and impairments that are preventable. Provision of antivenom, educating patients and health workers on first aid measures and providing easier means of transporting patients to health care facilities could reduce the burden of disability and impairment from snakebite in Africa.

Introduction

Snakebite is a major health crisis in poor resource rural areas and has recently been recognized by the World Health Organization (WHO) as a neglected disease (WHO 2009). The highest burden has been reported from sub-Saharan Africa (SSA), South Asia, and Southeast Asia. Victims of snakebite envenomation could occupy $\geq$10 % of hospital beds in West Africa (Warrell and Ormerod 1976). Snakebites commonly occur in poor resource settings and mostly affect males in the productive age group. Disabilities following snakebite envenomations are important but often unrecognized complications. The complex physiological effects of snake venom either systemically or locally lead to organ dysfunction with resultant permanent disability in some cases. Delay in hospital presentation, lack of appropriate antivenom, shortage of medical personnel, poor health-care services, and harmful ineffective traditional beliefs and practices contribute to the development of disabilities following snakebite envenomations. Use of traditional medications prescribed by snake charmers, application of tourniquet, inappropriate incisions, sucking at site of bite, and use of black stone are specific harmful practices performed.

Epidemiology

In West Africa snakebite envenomation incidence has been estimated at 8.9–93.3/100,000 population per year (ppy) (Kasturiratne et al. 2008). These figures are underestimation due to the heterogenous nature of health-care services and

Table 15.1 Disabilities following snakebite envenomation

System involved	Disabilities
Musculoskeletal	Amputation – limb, finger, toe
	Volkmann's contractures
	Joint stiffness with loss of sensibility
	Arthritis
	Disseminated osteomyelitis
	Chronic ulceration/nonhealing ulcers
	Malignant transformation/squamous cell carcinoma
	Raynaud's phenomenon
	Loss of mass muscle
Eye	Visual loss
Endocrine	Anterior pituitary insufficiency
	Chronic panhypopituitarism
	Diabetes insipidus
	Hypoadrenalism
Kidneys	Acute kidney injury (AKI)
	Chronic kidney disease (CKD)
Central nervous system	Stroke
	Cerebellar ataxia
	Complex regional pain syndrome type 1
	Acute disseminated encephalomyelitis (ADEM)
	Guillain-Barre syndrome (GBS)
	Parkinsonism
	Anterograde memory loss
Psychological	Depression
	Posttraumatic stress disorder
	Anxiety disorder
Cardiovascular	Myocardial ischemia
	Left ventricular hypertrophy
	Heart block
	Hemodynamic changes
Others	Intestinal ischemia, bowel obstruction, fetal loss, hearing loss

reporting systems worldwide. Moreover, hospital data do not reflect the true incidence and burden because up to 80 % of victims of snakebite get to hospital or primary health facilities after visiting local traditional and spiritual healers (Chippaux 1988). The process of seeking treatment from traditional healers coupled with difficult terrain in transporting victims to health facilities leads to unnecessary delay that immensely contributes to morbidity and mortality in Africa. Thus, the true incidence and burden of disabilities following snakebite envenomations are unknown, though there have been reports of various types of disabilities worldwide. A hospital-based retrospective study in northeastern Nigeria found 0.4 % incidence of amputation among victims of snakebite envenomations (Abubakar and Habib 2010) (Table 15.1).

Specific Disabilities

Amputation

In sub-Saharan Africa, a meta-analysis found that up to 5,908–14,614 amputations occur annually from snakebite and mostly affect young productive men (Chippaux 2011). These amputations could affect the limbs, fingers, or toes. Other musculoskeletal disabilities following snakebite envenomations included arthritis, Volkmann's contractures, joint stiffness, disseminated osteomyelitis, chronic ulcers, malignant transformation/squamous cell carcinoma, Raynaud's phenomenon, foot drop, fibular palsy, and loss of mass muscle. Several studies in SSA highlighted the contribution of snakebite envenomation to development of disabilities in rural communities (Abubakar and Habib 2010; Kidmas et al. 2004; Wood et al. 2009). Victims are usually bitten on the limb with subsequent application of traditional herbs and tourniquet. Self-amputation of a bitten limb following snakebite has been reported from Africa (Newman and Moran 1997). Victims of snakebite envenomation from poor resource settings usually present to the hospital 2–3 days after the bite. Bite-to-hospital presentation time ranges from 0.5 to 216 h, and the risk of morbidity and disability exponentially increases with increasing bite-to-hospital time (Ogunfowokan et al. 2011). Factors that accelerate development of disabilities include local effect of snake venom, development of compartmental syndrome, and harmful first aid practices such as use of tourniquet. Extremities are very common sites of snakebite and are prone to compartmental syndrome and necrotizing fasciitis. As pressure within the fascial compartment builds up, blood supply is compromised and the risk of developing complications is high. If compartmental pressure is not addressed within 12 h, the incidence of permanent functional loss is high (Matsen and Krugmire 1998). Based on clinical presentation, compartmental syndrome could be divided in to two forms, namely, imminent and manifest compartmental syndromes. Imminent type is characterized by severe pain at site of bite that does not correlate with degree of trauma, moderately raised intracompartmental pressure (30–40 mmHg), moderate impairment in muscle perfusion, and lack of neurological manifestations. The second type of compartmental syndrome is more severe presenting with functional loss, neurological manifestations, and severely elevated intracompartmental pressure >40–45 mmHg (Bucaretchi et al. 2010). An emergency exist when the difference between diastolic blood pressure and the compartmental pressure is less than 30 mmHg. Measurement of intracompartmental pressure is therefore vital in differentiating true compartmental syndrome from the local effects of snake venom. However, for bites affecting hand and fingers, measurement of intracompartmental pressure is difficult as no definite method is currently available. These deserve special attention as fingers and hands are particularly prone to tissue necrosis and severe functional disability. High index of suspicion with good clinical acumen is required to identify compartmental syndrome involving hand and the digits. A decrease in vibratory sensation has been found to be the earliest sensory impairment that occur in upper extremity compartmental syndrome and could be used as a

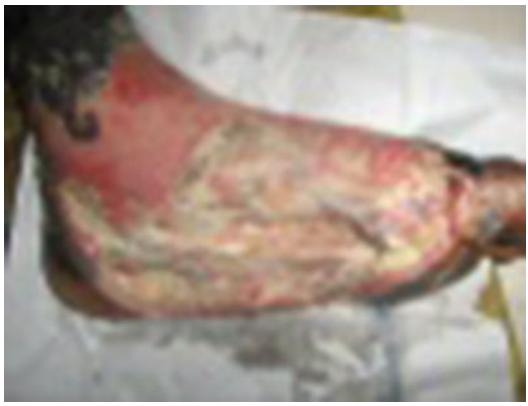

Fig. 15.1 Extensive tissue destruction and loss of muscles following snakebite on the foot

guide (Gelberman et al. 1983). If compartmental syndrome is not properly managed, subsequently patients could develop Volkmann's contracture. Magnetic resonance imaging (MRI) is important in order to delineate the affected compartment for ease of surgery. Management of compartmental syndrome entails early commencement of antivenom, restoration of blood coagulability, limb elevation, mannitol to reduce compartmental edema, and 100 % hyperbaric oxygen therapy. Debridement of necrotic tissue should be done at least 3 days after snakebite when patient is hemodynamically stable and had received adequate anti-snake venom (ASV). Many surgical procedures are available, but not all have clear-cut benefits to the patients. Incision and excision have been discouraged as no conclusive evidence to show their benefit and were associated with high risk of complications like osteomyelitis, wound infection, and damage to nerves and tendons. Fasciotomy should be performed when intracompartmental pressure remained elevated (>45 mmHg) despite administration of ASV and local debridement. In order to save digits, dermotomy with decompression of the digits should be done urgently when indicated. Hand reconstruction is indicated to minimize functional loss. Psychosocial complications are common following amputation. These include phantom limb, depression, anxiety, posttraumatic stress disorder (PTSD), unemployment, and stigma. Long-term management of patients should include social rehabilitation, job rehabilitation, and referral to psychiatrist (Fig. 15.1).

Visual Loss

Snakebite envenomations could cause loss of vision either by direct spit in to the eyes or indirectly via hemostatic or neurological manifestations. From Nigeria, it has been reported that the eyes were involved in up to 38 % of snakebites by cobra (Abubakar and Habib 2010). Ocular disturbances leading to loss of vision included ophthalmoplegia, keratomalacia, vitreous hemorrhage, uveitis, glaucoma, central retinal artery occlusion, macular infarction, optic neuritis, penetrating eye injury, globe necrosis, ptosis, and accommodation paralysis. Cortical infarction could also

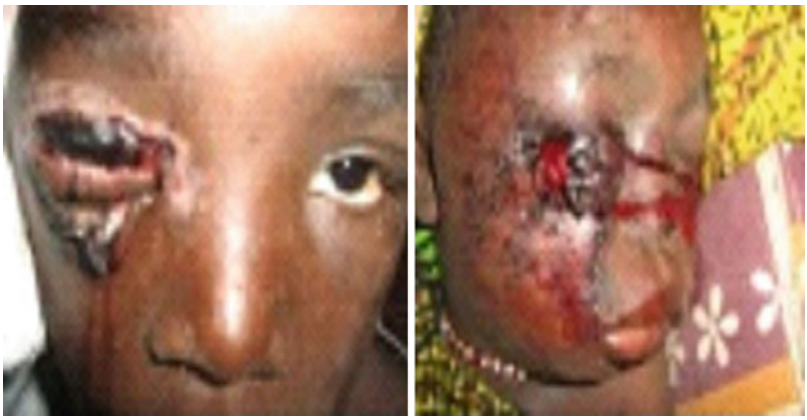

Fig. 15.2 Facial swelling, exophthalmos, periorbital ecchymoses, and subconjunctival hemorrhage following snakebite envenomation

lead to loss of vision. Patients may present with periorbital edema, subconjunctival hemorrhage, exophthalmos, and corneal edema. Local saline irrigation of the eye with subsequent application of topical ASV should be done immediately. Pain relief has been shown to be faster with topical ASV as compared to local anesthetic agents (Fung et al. 2010). Patients that presented early and received immediate topical ASV may recover within 1–15 days (Warrell and Ormerod 1976). Severely ill patients may present with facial swelling and breathing difficulty from progressive airway edema and complete blindness. Management involves immediate maintenance of breathing and prevention of infection. Patient should be intubated in the intensive care unit (ICU) and systemic antibiotics commenced in addition to ASV. Early evisceration is recommended in penetrating ocular injury as it helps in removing snake venom (Chen et al. 2005). Anti-snake venom rarely causes uveitis and retinal necrosis. Bilateral corneal blindness following the use of traditional topical eye medicines has been reported (Kandar et al. 2010). Poor quality of life, impaired social functioning, phobic anxiety, and low self-esteem are common sequelae of visual loss (Fig. 15.2).

Hypopituitarism

Both acute and chronic panhypopituitarism with diabetes insipidus have occurred with Russell's viper bite envenomations. The clinical features and the biochemical derangements usually resemble Sheehan's syndrome. The functional consequences were as a result of fibrin deposition and bleeding into the pituitary gland arising from the action of hemorrhagins and procoagulant enzymes contained in the snake venom. In chronic hypopituitarism, features of pituitary insufficiency are not seen immediately following envenomations but appeared much later. It has been observed that acute pituitary insufficiency could persist for several weeks thereby

becoming chronic hypopituitarism. Patients with acute pituitary insufficiency present with sudden collapse, hypotension, and hypoglycemia in addition to other features of envenomations. Chronic pituitary insufficiency on the other hand presents with body weakness, hoarseness of voice, body swelling, reduced body hair, loss of libido/erectile dysfunction, and menstrual disturbances in women (Antonypillai et al. 2011; Tun-Pe et al. 1987). High index of suspicion is required to make diagnosis. The biochemical derangement in acute hypopituitarism included low serum cortisol, growth hormone (GH), prolactin, thyroid-stimulating hormone (TSH), thyroxine, and estrogen concentrations. Gonadototropin levels are usually low or inappropriately normal (Antonypillai et al. 2011; Tun-Pe et al. 1987). Thyroid function tests should be interpreted cautiously as the picture may resemble sick euthyroid syndrome especially in the acute state (Antonypillai et al. 2011). Hormonal assay is expensive and not readily available in areas most affected by snakebite. Initial investigation should include serum-free thyroxine, cortisol, and testosterone or estradiol. If these hormones are deranged, diagnosis should be confirmed with pituitary function tests (TSH, FSH, and LH assay) and insulin tolerance test (ITT) (Antonypillai et al. 2011). Acute pituitary insufficiency is a medical emergency and should be managed aggressively. Hypoglycemia should be corrected with glucose infusion, while hypotension should be managed with saline infusion, inotropes, and hydrocortisone. Due to risk of hematoma formation, hydrocortisone should be given intravenously rather than intramuscularly. Because of the difficulty in making diagnosis in poor resource settings, empirical therapy could reduce morbidity and mortality from hypopituitarism resulting from snakebite envenomations. Hormonal replacement should start with steroids followed by thyroid hormones in order to avoid precipitating an adrenal crisis. Subsequently steroids should be tapered off and later withdrawn after the acute phase which usually last for 3 weeks or more.

Stroke

Cerebral vascular compromise in form of hemorrhage or infarction has been reported following snakebite envenomations (Mosquera et al. 2003) and is associated with high morbidity and mortality. Victims that survived usually had disabling neurological sequelae commonly as a result of intracerebral hemorrhage (ICH) or subarachnoid hemorrhage (SAH) and rarely cerebral infarction (Mugundhan et al. 2008). The anterior cerebral artery, middle cerebral artery, posterior cerebral artery, and other arterial territories could be affected. Venoms of snakes of the Viperidae and Crotalidae families are mainly hemotoxic. However, some species of vipers additionally have a neurotoxin which could block neuromuscular transmission resulting into eye muscle paralysis (Re et al. 1999). Envenomations following bite by vipers and Crotalidae (*Bothrops lanceolatus*) snakes lead to thrombocytopenia, disseminated intravascular coagulation (DIC), and consumption of clotting factors. These present clinically as internal and external bleeding from multiple sites including the brain, skin, gum, and mouth. Rarely victims of hemotoxic

snakebite present with thrombotic complications in form of cerebral infarction and acute myocardial infarction (Merle et al. 2005). The mechanism of this prothrombotic state is unclear but possibly related to vascular wall changes with subsequent adhesion and platelets aggregation (Bogarin et al. 1999), hyperviscosity from hemoconcentration, and cardiotoxicity of snake venom leading to dysrrythmias with cardiac thromboembolism. Other mechanisms included antiphospholipid antibodies, mutation in factor V, and deficiencies in protein C, S, or antithrombin III which are procoagulant states (Hoskote et al. 2009). Moreover vessel-occlusive thrombi from DIC and variations in venom composition in favor of occlusive thrombosis in contrast to hemorrhage (Hoskote et al. 2009) have been proposed as possible explanations for the unexpected cerebral infarction in the setting of snake venom-induced hemostatic failure.

Clinical features of stroke from snake envenomations include loss of consciousness, seizures, dehydration, symptoms of raised intracranial pressure, and other features of envenomation. Brain computed tomography (CT) scan or magnetic resonance imaging (MRI) should be done to confirm and localize cerebral hemorrhage or infarction. Other routine investigations such as 20 minutes whole blood clotting test (WBCT20), full blood count, ECG, and cardiac enzymes should be carried out immediately. Patients should be managed in the intensive care unit (ICU) where available. Assisted ventilation and adequate hydration should be provided in addition to anti-snake venom, tetanus toxoid, antibiotics, and anticonvulsants like phenytoin. Generally clinical outcome for stroke following snakebite envenomation is poor. All patients presenting with neurological deterioration following snakebite should be evaluated for possible stroke.

Cerebellar Ataxia

Cerebellar ataxia is one of the delayed neurological manifestations seen after neurotoxic snakebite envenomation. Victims rapidly develop neurological signs such as diplopia, ptosis, weakness of limbs, ophthalmoplegia, confusion, restlessness, and deteriorating level of sensorium (Awasthi et al. 2010). Cardiac and respiratory paralysis sets in, and urgent ventilatory and circulatory support in addition to early anti-snake venom (ASV) administration is lifesaving. At least 1 week after successful management, some patients may experience involuntary movement of limbs with features of cerebellar disease such as ataxia, dysdiadokinesia, positive finger-nose test, dysmetria, inability to perform tandem walking, and broad-based gait (Awasthi et al. 2010). Prolonged respiratory paralysis following snakebite results in widespread cerebral hypoxia which on recovery usually manifests as focal neurological deficit which could be confirmed with neuroimaging. Other proposed mechanisms of delayed neurotoxicity include neurotoxin-induced ultrastructural damage to nerve endings and nerve fibers or demyelination and ASV hypersensitivity. Delayed (serum sickness type) reactions could occur several days following treatment with ASV (Awasthi et al. 2010). Possible reasons for this included immune complex-mediated reactions and

possible compliment activation. Polyvalent ASV is only effective on free venom in circulation and does not affect the venom already bound in neuromuscular junction. Hence, it has no significant benefit in reversing respiratory paralysis and preventing development of delayed neurological complications following snakebite envenomation (Kularatne 2003).

Parkinsonism

Parkinsonism usually follows asymmetric leukoencephalopathy secondary to snakebite envenomation (Chaudhary et al. 2013). Clinical presentation is with loss of consciousness, diminished tendon reflexes, and bilateral flexor plantar response. Direct toxic effect of snake venom has been suggested as the likely pathogenesis of leukoencephalopathy. High index of suspicion is required to make diagnosis. Cerebrospinal fluid analysis may be normal and also negative for viral markers such as herpes simplex, Japanese encephalitis, and dengue. Magnetic resonance imaging may reveal signal intensity alteration in the caudate nuclei, lenticular nuclei, and the thalami with involvement of the cortical rim suggestive of an asymmetrical leukoencephalopathy. Diffusion-weighted imaging and the apparent diffusion coefficient should not be suggestive of stroke. Management involves use of specific ASV, mannitol infusion, and other supportive therapy. With above measures, sensorium may improve and subsequently patient may regain full consciousness. However, this may be accompanied by an akinetic-mute state that could improve to a lesser disabling bradykinetic state. Motor abilities and verbal output may improve over weeks. Parkinsonism features should be managed with levodopa and carbidopa.

Chronic Kidney Disease (CKD)

A substantial portion of cardiac output goes to the kidney, thus making it a well-vascularized organ that is predisposed to circulatory toxins such as snake venom. There is a wide spectrum of clinical manifestations following renal injury from snakebite envenomation. These include proteinuria, hematuria, hemoglobinuria, myoglobinuria, acute kidney injury, and chronic kidney disease (CKD). CKD in snakebite victims commonly result from acute kidney injury (AKI) that persisted. The incidence and outcome of renal impairment following snakebite envenomation could be influenced by species of the snake, severity of envenomation, availability of appropriate antivenom, and time of commencement of renal replacement therapy. Early commencement of hemodialysis could help abort progression of AKI to CKD. Moreover, alkalinization of urine in myotoxic snakebite envenomation could help prevent development of AKI if started early. It has been reported in a prospective study that 37 % of patients who had AKI following snakebite envenomation develop CKD after 1 year of follow-up (Herath et al. 2012). Several mechanisms have been postulated to explain the pathogenesis of AKI in snakebite

envenomation. These include DIC, microangiopathic hemolytic anemia, rhabdomyolysis, intravascular hemolysis, and intravascular contraction from hypovolemic shock. Also kidney injury from nephrotoxic effects of snake venom has been implicated (Sitprija 2006). Enzymatic action of metalloproteases and phospholipase A2 is responsible for the direct nephrotoxic effect of snake venom. All tissues of kidney are susceptible to injury from snake venom. Cellular injury is mainly due to hemodynamic and inflammatory changes from mediators of inflammation such as cytokines and other vasoactive substances (Thamaree et al. 2001). Renal pathologies reported in patients with CKD following snakebite include bilateral cortical necrosis and calcification (De Silva et al. 1979), chronic interstitial inflammation, and glomerular sclerosis (Thamaree et al. 2001).

AKI should be managed aggressively in order to halt progression to CKD. Serum creatinine at presentation, persistently elevated creatinine, female sex, duration of oliguria, advancing age, duration of renal replacement therapy (RRT), late initiation of RRT, and presence of comorbid illnesses have been reported to predict development of CKD (Herath et al. 2012; Oeyen et al. 2007). Nephrotoxins should be avoided in patients with AKI. Sepsis, hypovolemia, and other potential sources of kidney injury should be addressed immediately to prevent acceleration of renal damage. Hyperkalemia and hyperuricemia in AKI from myotoxic snake envenomation should be managed immediately. Anti-snake venom (ASV) should be given to patients immediately. Plasmapheresis when ASV is not available could be given immediately before venom is attached to tissues, otherwise not beneficial. Snakebite victims may not be suitable kidney donors even after full recovery of renal function because the kidney concentrates venom toxins in the circulation. Traces of venom stored in the donor kidney have been proposed to be responsible for massive hemorrhage and rupture of graft in the recipient. Hence, patients who died of snakebite should not donate kidneys for transplantation (Herath et al. 2012).

Intestinal Ischemia

Intestinal ischemia following snakebite envenomation could lead to bowel obstruction secondary to massive bowel necrosis. Following development of DIC in snakebite victims, ischemia with obstruction of blood vessels could occur. Victims may present with abdominal pain few days after successful management of the DIC. Initial abdominal ultrasound scan and plain abdominal x-ray may be normal likewise platelets and coagulation parameters (Rosenthal et al. 2002). There could be raised C-reactive protein (CRP) and mild leukocytosis. Over the next few days, signs and symptoms of bowel obstruction may start appearing with classical clinical and radiological features. A double-contrast barium enema examination could reveal multiple stenoses of the colon, regional edema, and longitudinal ulcer (Iwakiri et al. 1995). Abdominal CT scan is important in identifying obstruction of superior mesenteric artery and vein. At this stage, fibrin degradation products (FDP) may be elevated. Recurrent coagulopathy after treatment of crotalid snakebite envenomation with polyvalent ASV has been reported (Bogdan et al. 2000).

Hence, sufficient doses of ASV should always be given immediately after snakebite envenomation. For patients with bowel obstruction, surgical intervention is recommended, and during laparotomy the necrotic portion of the colon should be resected. It is advisable to do histological examination of the stenotic lesion which may reveal hemorrhagic necrosis of the bowel and thrombosed peripheral submucosal arteries. Short bowel syndrome has been reported after extended bowel resection in a victim of snakebite envenomation (Iwakiri et al. 1995). Therefore, patients who had bowel resection should have nutritional supplement in addition to oral anticoagulation.

Myocardial Infarction

Snakebite envenomation could present with cardiac and hemodynamic alterations. A prospective study in northeastern Nigeria reported palpitations, tachycardia, bradycardia, hypotension, hypertension, heart block, chamber enlargement, and myocardial ischemia among snakebite victims (Karaye et al. 2011). Rhythm disorders such as torsade de pointes (Gaballa et al. 2005) have been reported and contribute to mortality. Mechanisms of myocardial infarction from snake venom include vasoconstriction and vasospasm of coronary arteries, DIC, anaphylactic shock, hyperviscosity, and direct cardiotoxicity (myocarditis and myonecrosis) (Gaballa et al. 2005; Saadeh 2001; Maheshwari and Mittal 2004; Blondheim et al. 1996; Menon). Vasoconstriction is mainly due to the effects of safaratoxins (an endothelin homologue), whereas occlusion of coronary arteries is due to thrombus formation in setting of DIC (Copley et al. 1973). Onset of myocardial infarction may be rapid (<1 h) (Dissanayake and Sellahewa 1996) or delayed for several hours (Saadeh 2001) after envenomation. Clinically patients usually present with excessive sweating, nausea, vomiting, severe retrosternal chest pain, chest tightness, and loss of consciousness which may be immediate or delayed. Blood pressure may be low, normal, or even high. Local manifestation of envenomation may be present at site of bite: fang marks, edema, redness, bleeding, and pain. On arrival at health facility, cardiopulmonary resuscitation should commence immediately. If tachypneic, assisted ventilation should be provided in ICU where available. Anti-snake venom should be administered in addition to antiplatelets (aspirin/clopidogrel), β-blockers, and angiotensin converting enzyme inhibitors (ACEI). Other supportive medications include antihistamines (chlorpheniramine), antiemetics (promethazine), hydrocortisone, prophylactic antibiotic, nonsteroidal anti-inflammatory drugs (NSAIDs), tetanus toxoid, intravenous fluid, and antihypertensives where indicated. ECG changes may include sinus tachycardia, ST elevation/depression, T wave inversion, and prolonged QT interval. Cardiac enzymes may be normal or elevated. Twenty minutes whole blood clotting time (WBCT) is usually abnormal (>20 min). Leukocytosis with predominant neutrophilia may be seen in full blood count. Echocardiography may not show much. Cardiac catheterization may show normal coronary arteries with or without thrombus (Gaballa et al. 2005; Saadeh 2001). Clotting profile may show prolonged

prothrombin time (PT) and activated partial thrombin time (APTT). Serum fibrinogen level may be elevated. Low oxygen saturation could occur with development of acute pulmonary edema. For those with hypotension adrenaline, inotropes and boluses of intravenous fluid could raise the blood pressure. For those patients with rhythm disorders, defibrillation may be required where facilities are available. Renal function should be monitored with fluid input/output chart, serum electrolytes, and creatinine. Serial ECGs should be done to monitor extent and progression of myocardial injury. It is important to note that normal ECG in the early hours following snakebite envenomation does not rule out myocardial injury. Typical ECG changes in acute myocardial infarction may not be evident in the hyperacute phase. Hence, to facilitate early detection of myocardial infarction, high index of suspicion is needed with repeated ECG monitoring in the hyperacute phase. During follow-up visit, stress ECG should be done.

Delayed Psychological Morbidity

Up to 4 years after snakebite , envenomation victims were reported to experience an ongoing psychological morbidity in the form of anxiety, posttraumatic stress disorder, somatic symptoms, functional disability, and poor quality of life (Williams et al. 2011). The burden of these is likely to be substantial given that the majority of people commonly affected resides in resource-poor countries of SSA and other less developed countries of the world. Psychological morbidity could be explored using Beck Depression Inventory (BDI), Hopkins Somatic Symptoms Checklist, and Posttraumatic Stress Symptom Scale. Disability following snakebite envenomation could be assessed using Sheehan Disability Inventory. The prevalence of depression among previous snakebite victims was reported to be higher than the WHO estimated baseline community prevalence (54 % and 15 %, respectively) from one study (Williams et al. 2011; Patel and Kleinman 2003). The prevalence of moderate to severe depression was found to be higher among victims of snakebite than controls (16 % and 1 %, respectively). Posttraumatic stress disorder (PTSD) prevalence was 21.6 %, negative change in employment occurred in 27 % of victims, and up to 10.2 % already had stopped working. Residual physical disability was reported in 17 % of previous snakebite victims. Common somatic symptoms reported were blindness, tooth decay, body aches, tiredness, and weakness (Williams et al. 2011). Snakebite is commoner in rural areas, and the high prevalence of psychological distress among snakebite victims could be partly related to social disadvantages in those areas. In such communities, the level of poverty is high with lack of infrastructures and prevalent social stressors. Majority of people in rural areas are low-income earners and mostly engaged in small-scale non-mechanized farming under difficult conditions with potential health risk. People in poor resource settings adjust to these harsh living conditions to live a normal life. A negative life event like snakebite may easily distort this balance in favor of neuropsychological distress. High incidence of psychological morbidity following trauma from other causes has been reported (Ameratunga et al. 2009).

There are different cultural perceptions of snakebite across the world. These perceptions are usually complex and integrated into the belief of people. Intense fear of snakes is common among people, and ophidiophobia (abnormal fear of snakes) persists despite most species of snakes being nonvenomous.

Fetal Loss

In tropical areas of Africa, snakebite envenomation contributes to maternal morbidity and mortality. The fetus is not exempted from the toxic effects of snake venom. It has been reported from South Africa that pregnant women constituted 0.4 % of patients admitted into the hospital with snakebite envenomation (McNally and Reitz 1987). Rate of fetal loss could be influenced by severity of envenomation and availability of anti-snake venom. It has been found that the rate of fetal loss could be up to 100 % if there is no anti-snake venom (Nasu et al. 2004). With use of anti-snake venom, rate of fetal loss ranges from 0 % to 57 % depending on the type of snake involved (Seneviratne et al. 2002; Chang et al. 2005). Snakebite-induced fetal loss could occur at any trimester during pregnancy (Habib et al. 2008). Fetus could have systemic envenomation in the absence of florid systemic manifestation of envenomation in the mother. This is related to the ability of the snake venom to cross the placenta in doses enough to cause systemic envenomation in fetus (McNally and Reitz 1987). Several factors are involved in the pathogenesis of fetal loss after venomous snakebite. Maternal hemodynamic changes lead to hypovolemia and subsequent fetal hypoxia. Excessive uterine contractions generated by snake venom actions and direct toxic effects on fetus lead to fetal wastage (Kumar et al. 2011). Furthermore, DIC presenting with placental bleeding also adversely affect the fetus (Habib et al. 2008). Apart from fetal loss, other obstetric complications arising from envenomation include threatened abortion, vaginal bleeding, intrauterine fetal death (IUFD), abruption placenta, and premature labor (Kumar et al. 2011). Congenital malformations associated with snake envenomation include hydrocephalus, polydactyly, cerebral ventricular dilatation, and intracranial hemorrhage (Langley 2010; Seneviratne et al. 2002; Malz 1967; Entman and Moise 1984). In Africa rate of fetal loss following venomous snakebite could be reduced with adequate supply of appropriate and potent ASV. If patients develop hypersensitivity reactions to ASV, phenylephrine and ephedrine should be given. Epinephrine should be avoided because it could impair placental blood flow (Entman and Moise 1984).

Conclusion and Future Directions

The interaction between man and his environment in Africa predisposed him to snakebite. Most people affected were within productive age group and occupations mostly at risk include farmers, hunters, and the herdsmen. Poor housing facilities, lack of protective clothing for people at risk of snakebite, difficult transportation terrain, poor health facilities, and involvement in high risk activities (like snake

charmers) are important factors related to snakebite in Africa. The implication of these factors is that the incidence of snakebite envenomation in Africa is poverty-driven, and snakebite-related disabilities that are preventable could cause a huge socioeconomic loss. No organ of the body is spared following snakebite and disabilities and impairments are common following envenomations in Africa. Delay in hospital presentation, lack of antivenom, shortage of medical personnel, poor health-care services, and harmful traditional practices contribute to development of disabilities and impairments. Provision of antivenom, educating patients and health workers on first aid measures, and providing easier means of transporting patients to health facilities could reduce the burden of disabilities and impairment from snakebite in Africa.

Cross-References

- ▶ Kidney Injury and Animal Toxins
- ▶ Socioeconomic Aspects of Snakebite in Africa and the Tropics
- ▶ Venomous Snakes and Snake Envenomation in Nigeria

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Snake Venoms and Scorpion Venom Research in the Middle East: A Review

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Contents

Abstract

Modern research during the last decades has shown that both snakebites and scorpion stings are among the most important causes of envenoming and are responsible for significant morbidity and mortality, and hence they are a major

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public health issue in tropical and desert areas in many regions including the Middle East. This has generated a plethora of research in several regions, not only the classification and determination of the fauna but also of the intricate mechanisms of actions and resultant effects on humans. This chapter highlighted the major tracks of research undertaken to gain a better understanding of the intricacies of scorpions and snakes that share our ecosystem. The distribution of snakes and scorpions, classification, and mechanism of action of their venoms will be summarized. Management modalities, whether with serotherapy or supportive, will be discussed to enlighten on researches undertaken to protect humans from these ancient foes. Moreover, research undertaken to study and fully characterize venom toxins will be outlined with emphasis on new trends of utilizing venom toxins as beneficial therapeutic biological interventions in several disease states such as hypertension, thromboembolic disease, and cancer.

Introduction

Snakes and scorpions are ancient culprits that have been in close contact with human beings and various fauna for centuries, in the "Old World" including the Middle East, with myth intermingled with facts in ancient scrolls and literature. In ancient Egypt the cobra was used to decorate crowns of emperors, and in ancient Greece it was associated with medicine. A glass entwined with a snake is the symbol still used to represent the guilds of medicine and pharmacy (Balozet 1971; Deghani and Fathi 2012; Koh and Kini 2012).

Modern research during the last decades has shown that both snakebites and scorpion stings are among the most important causes of arachnid envenoming and are responsible for significant morbidity and mortality and hence a major public health issue in tropical and desert areas in many countries including the Middle East, Asia, Northern and Southern Africa, Central and South America, and Australia (Balozet 1971; Freire-Maia et al. 1994; Ismail 1995; Abroug et al. 1999; Jahan et al. 2007).

This has generated a plethora of research in several regions, not only the classification and/or determination of the fauna but also of intricate mechanisms of actions and resultant effects on humans and other creatures. Efforts were undertaken to both protect and treat from the cascade of ensuing events, especially in regions of high interaction between the human inhabitants and the desert and mountainous terrains known to harbor different species of scorpions and snakes. Another recent research theme that emerged concentrated on utilization of the venom toxins to better understand different biological systems and to extract possible novel treatment modalities (Vachon 1979; Warrell 1993; Ibrahim et al. 2013; Al-Hajjaj 2005; Al-Lawati et al. 2009).

This chapter will attempt to highlight the major research themes undertaken in the Middle East to gain a better grasp of the intricacies of scorpions and snakes that share our ecosystem and historical foes to be protected from yet future sources of beneficial biomarkers and therapeutic biological interventions.

Distribution of Snakes and Scorpions in the Middle East

With regard to snakes, approximately 3,000 species have been reported to be present worldwide, about 600 are venomous. These are generally found in four main families: *Atractaspididae*, *Colubridae*, *Elapidae*, and *Viperidae*. Geographically the Middle East includes Saudi Arabia, the Arabian Peninsula, Asian Turkey, Syria, Lebanon, Jordan, Israel, Palestine, Iran, Iraq, Yemen, Oman, Qatar, and Emirates. The snake fauna contains species in common with Northern Africa, Europe, and Central Asia, and toward the east there is infiltration of species characteristic of tropical Asia (Ismail and Memish 2003). At least 51 species of snakes are present in the Arabian Peninsula and surrounding regions in the Middle East, as well as Northern and Southeastern Africa, nine of which are venomous including *Naja haje arabicus* and *Walterinnesia aegyptia* from the family *Elapidae*; *Echis carinatus*, *Echis coloratus*, *Cerastes cerastes*, *and Bitis arietans* of the family *Viperidae*; *and Atractaspis microlepidota of the family Atractaspididae*. Indeed, the latter alone, for example, contains 64 species in 12 genera. In the Middle East, vipers cause most of the snakebites; cobras and other elapids occur but appear to be restricted in range, inflicting fewer bites (Warrell 1993; Ismail and Memish 2003; Al-Lawati et al. 2009; Sajevic et al. 2011; Abd-Elsalam 2011; Fahmi et al. 2012) (Table 16.1).

With regard to scorpions, approximately 1,500 species are known, with 25 of them considered dangerous and might cause human death without medical treatment. According to the review undertaken by Al-Asmari et al. (2012), regular updates of scorpion taxonomy, particularly in higher-level systematics and in relation to other arthropods, have been undertaken. Nine families were identified during the last previous decades, and at least 16 families have been added in the current decade. Detailed recent information about scorpions is available on websites of scorpiology (Al-Asmari et al. 2009, 2012).

Dehghani and Fathi (2012) reported that the earliest study of the scorpion fauna began in 1807, when Olivier described *Androctonus crassicauda* from Kashan City, central of Iran. The study was continued by other researchers in several countries to classify scorpion families. According to Abdel-Rahman et al. (2010), within the phylum of *Arthropoda*, scorpions (*Chelicerata*, *Scorpionida*) are the oldest known terrestrial species and are common in the Mediterranean, the Middle East, Saudi Arabia, and Jordan regions.

In the Arabian Peninsula including the Kingdom of Saudi Arabia with its abundance of desert areas, several species of scorpions abound. Fourteen species or subspecies belonging to two families, the *Buthidae* and the *Scorpionidae*, have been identified (Vachon 1979). For example, from the *Buthidae* family eight genera were documented: *Leiurus*, *Androctonus*, *Compsobuthus*, *Parabuthus*, *Buthacus*, *Orthochirus*, *Apistobuthus*, *and Vachonioulus*. Moreover, several species that belong to the families *Scorpionidae* and *Hemiscorpiidae* have also been documented (Vachon 1979; Ismail 1995; Al-Asmari and Jahan et al. 2007; AL-Saif 2008; Al Asmari et al. 2012).

Table 16.1 Species of medicinally important snakes (Adapted from Vyas et al. 2013)

Species no.	Family	Scientific names	Common names
1	Elapids	*Naja haje*	Egyptian or brown cobra
2	Elapids	*Naja oxiana*	Central Asian cobra
3	Elapids	*Ophiophagus*	Hannah-king cobra
4	Viperids	*Echis carinatus*	Russell's viper
5	Viperids	*Vipera russelli*	Saw scaled viper
6	Crotalids	*Agkistrodon bilineatus*	Canti
7	Crotalids	*Agkistrodon contortrix*	Copperhead
8	Crotalids	*Agkistrodon halys*	Mamushi
9	Crotalids	*Agkistrodon piscivorus*	Eastern cottonmouth
10	Crotalids	*Calloselasma rhodostoma*	Malayan pit viper
11	Crotalids	*Bothrops asper and/or atrox*	Fer-de-lance
12	Crotalids	*Bothrops jararaca*	Jararaca
13	Crotalids	*Bothrops jararacussu*	Jararacussu
14	Crotalids	*Bothrops neuwiedi*	Jararaca pintada
15	Crotalids	*Crotalus adamanteus*	Eastern diamondback rattlesnake
16	Crotalids	*Crotalus atrox*	Western diamondback rattlesnake
17	Crotalids	*Crotalus scutulatus*	Mojave rattlesnake

In Iran and the surrounding regions, a similar trend prevailed, whereas scorpion families were reported to include 22 genera, about 52 species and 25 subspecies, with less than a dozen species, mainly from *Buthidae* family appearing to be responsible for the reported envenoming. *Buthidae* appears to be the largest scorpion family, distributed throughout numerous regions of the globe and widespread in the Old World, especially in the tropical areas of Africa, with *A. crassicauda*, *Leiurus quinquestriatus*, and *H. lepturus* usually cited as the most dangerous species (Balozet 1971; Shahbazzadeh et al. 2009; Jalali et al. 2010; Dehghani and Fathi 2012). Occupied Palestine and the surrounding regions are inhabited by three out of the 16 recognized scorpion families: *Buthidae*, *Scorpionidae*, and *Diplocentridae*, comprising nine genera, 19 species and subspecies, out of about 1,200 known species, which belong to three faunal elements, Saharo-Sindian (most species), Mediterranean, and Central Asian (Raz et al. 2009).

The Importance of Researches on Snake and Scorpion Envenomation in the Middle East

Epidemiological studies have shown that snakebites and scorpion stings have caused morbidity and mortality, especially in rural areas in various countries, thus encouraging a cascade of research especially in the regions with the highest encounter incidences such as the Middle East, South and Southeast Asia, Africa, South America, and Australia. These researchers studied the basic concepts underlying envenomation by snake and scorpion venoms. Results attained in different

studies pertaining to biochemical composition and biological activities of these creatures' venoms were extrapolated with studies concentrating on their mode of action on their targets: molecular, pharmacological, toxicological, immunological, and clinical aspects. The following sections will attempt to highlight the most pertinent findings.

Snake and Scorpion Venom Components

It is known that animal venoms constitute a diverse and synergistic cocktail of enzymes, bioactive peptides, and proteins, as well as other small molecules (e.g., neurotransmitters, nucleotides, and inorganic salts), selected for the survival of the particular species in its natural habitat. Venomous animals (including snakes and scorpions) employ their toxins in both offensive and defensive scenarios (Warrell 1993; Abdel Rahman et al. 2009).

Snake venoms are complex mixtures that according to snake type may contain specific species-dependent mixtures of pharmacologically active proteins, peptides, biogenic amines, and nonprotein components including inorganic cations. Some of these proteins exhibit lethal and debilitating effects as a consequence of neurotoxic, cardiotoxic, and tissue necrotizing effects. Kini (2002) in his review described the structure-function relationships of what he characterized as three-finger toxins, since they shared a common structure of three-stranded loops extending from a central core. Moreover, they bind to different receptors/acceptors and exhibit a wide variety of biological effects. These toxins affect the human body according to their potency, type, species, geographic location, habitat, climate, gender, and age. This will be expanded upon in a subsequent section. Hemorrhagin (a protein that possesses hemorrhagic activities), coagulopathic and defibrination factors, and enzymes can also be found in different snake venoms including proteolytic enzymes and those that affect hemostasis: phospholipases A2, L-amino acid oxidases and 50-nucleotidases, proteinases, hydrolases, collagenases, hyaluronidases, acetylcholinesterases, and/or phosphodiesterases (see reviews by Kini 2002, 2005, 2006, 2011; Sajevic et al. 2011; Vyas et al. 2013).

Scorpion venoms are complex mixtures composed of a wide array of substances. They contain mucopolysaccharides, hyaluronidase, phospholipase, low-molecular-mass molecules like serotonin and histamine, protease inhibitors, histamine releasers, and polypeptidyl compounds (Balozet 1971; Freire-Maia et al. 1994; Abdel-Rahman et al. 2009, 2010).

Scorpion venom peptides likewise exhibit a vast variety of biochemical activities and pharmacological functions. The composition and venom potency of these scorpion toxins can vary from species to species. Differences have been described within the same species concerning protein content and toxicity of the venoms. These differences can be observed in different individual venoms collected from different specimens at the same time and in the venom of the same specimen following multiple extractions (Abdel-Rahman et al. 2009, 2010).

Classification and Actions of Snake and Scorpion Venoms

Venomous snakes belong to four families *Atractaspididae*, *Elapidae, Viperidae*, and *Hydrophiidae*. Snake venoms, particularly crotalid and viperid venoms common in the Middle East, are rich sources of serine proteases and metalloproteases. These proteases affect various physiological functions such as platelet aggregation, blood coagulation, fibrinolysis, complement system, and blood pressure and the nervous system, with several authors classifying them according to their content of proteases: procoagulant, anticoagulant, and fibrinolytic proteases (see reviews by Kini 2002, 2005, 2006; Ismail and Memish 2003; Duncan et al. 2008; Sajevic et al. 2011; Vyas et al. 2013) (Table 16.2).

More specifically, Isbister et al. (2010) classified snake venom according to their ability to affect coagulation and cause venom-induced consumptive coagulopathy (VICC) which according to the authors are common manifestations of snake

Table 16.2 Snake venom proteins acting on the hemostatic system at different points resulting in inhibition or augmentation of blood coagulation (Sajevic et al. 2011)

Protein family	Hemostatic system target	Effect
Serine proteinases	Platelets	Aggregation
	FX, FVII, FV, FII; PC	Activation
	Plasminogen	Activation
	Fibrinogen	Clotting
	Fibrin(ogen)	Degradation
	Serpins	Inactivation
Metalloproteinases	Endothelial cells, basement membrane	Hemorrhage
	Platelets	Inhibition of aggregation
	FX, FII	Activation
	Fibrin(ogen)	Degradation
	Serpins	Inactivation
Phospholipases A_2	FXa, FIIa; TF/FVII	Inhibition
	Platelets	Aggregation
L-amino acid oxidases	Endothelial cells	Hemorrhage
	Platelets	Aggregation
		Inhibition of aggregation
5′-Nucleotidases	Platelets	Inhibition of aggregation
Disintegrins	Platelets	Inhibition of aggregation
C-type lectin-like	FIX, FX, FIIa	Inhibition
Proteins	FX, FII	Activation
	Platelets	Aggregation
		Inhibition of aggregation
Three-finger toxins	FVIIa,	Inhibition
	Platelets	Inhibition of aggregation

Abbreviations: *F* coagulation factor, *PC* protein C

envenoming worldwide. The authors compared venoms from Australian elapids with African and Middle Eastern vipers, including species from the genus *Echis* spp. (carpet vipers), which contain prothrombinase-like toxins. The prothrombin activators in elapid venoms were classified as group C and D prothrombin activators by their ability to cleave the two peptide bonds necessary to convert prothrombin to functional thrombin. Venoms with group C prothrombin activators [*Oxyuranus scutellatus* (coastal taipan) and *Pseudonaja textilis* (common brown snake)] could catalyze this reaction without human factor V, whereas venoms with group D prothrombin activators [*Notechis scutatus* (common tiger snake), *T. carinatus* (rough-scale snake), and *Hoplocephalus stephensii* (Stephen's-banded snake)] depend on the presence of plasma factor V (Isbister et al. 2010).

In his review Vyas et al. (2013) simplified snake venoms dividing them into three types according to their effects: hemotoxic venoms affecting the cardiovascular system and blood functions, cytotoxic venoms targeting specific cellular sites, or muscle and neurotoxic venoms that harm the nervous system and the body. As its natural destiny the predator snake needs to paralyze and digest its prey; therefore, the venom as mentioned earlier contains a variety of requirements as well such as enzymes that can hydrolyze proteins plus membrane components and toxins capable of paralyzing the prey (Vyas et al. 2013).

With regard to scorpion venoms, their main components are the neurotoxins that act on sodium channels and have been divided into two major classes, α- and β-toxins (Catterall 1980; Couraud and Jover 1984). The α-toxins bind to site "3" in voltage-gated sodium channels (VGSC) inhibiting their inactivation and are mainly found in Africa and Asia (Old World scorpion venoms such as *Androctonus*, *Leiurus*, *Compsobuthus*, *Buthus*). On the other hand, scorpion venom β-toxins bind to site "4" in VGSC causing prolongation of the activated sodium channels to more negative potentials. The β-class may be further subdivided into three main groups: classical, depressant, and excitatory β-scorpion toxins (Couraud and Jover 1984). The classical β-toxins have been assigned to scorpions of the New World (such as *Centruroides* and *Tityus*). Moreover, scorpion toxins have also been classified into three types: acting on mammals, insects, or both (Zlotkin et al. 1985; Abdel-Rahman et al. 2010).

Toxic manifestations of α- and β-scorpion toxins are in general due to their ability to act mainly on Na^+ channels and to a lesser degree *on voltage-gated or calcium-activated potassium channels*, ryanodine-sensitive large-conductance Ca^{2+}-selective channels in skeletal and cardiac endoplasmic reticulum, and small-conductance chloride channels (Possani 1984; Harvey et al. 1994; El-Hayek et al. 1995; Abdel-Rahman et al. 2009, 2010). This will ultimately lead to prolonging depolarization, causing massive release of neurotransmitters such as adrenaline, noradrenaline, dopamine, and acetylcholine from both sympathetic and parasympathetic nerve terminals – "autonomic storm" (Gueron et al. 1992; Ismail et al. 1992; Fatani et al. 2010). Several mediators and modulators have also been reported to be released following scorpion envenomation in both the peripheral and nervous systems such as histamine, glutamate, GABA, kinins, prostaglandin,

platelet-activating factor (PAF), cytokines, and nitric oxide (NO) (Ismail 1995; Meki and Mohey El-Deen 1998; Fukuhara et al. 2003).

Subsequently, especially in children and elderly, this will lead to multiple organ dysfunction (MOD) and ultimately failure (MOF), with death due mainly to cardiovascular and respiratory manifestations (Mekki and Mohey El-Deen 1998; Fukuhara et al. 2003; D'Suze et al. 2007).

Researches unraveling intricacies of snake and scorpion envenomation have been undertaken over the last decades, and the following sections will attempt to summarize the most relevant.

Manifestations of Snake and Scorpion Envenomation

Snakebite accidents are common around areas of human habitation, and symptoms may vary according to the type of snake and condition of victims. These may include local symptoms at site of bite such as pain, swelling, bruising, and extensive edema. General signs and symptoms include nausea, vomiting, lactic academia lipoproteinemia, urinary retention, tachycardia, and hypertension followed by hypotension. Snake venoms also result in hematological abnormality and coagulopathy manifesting with necrosis, systemic bleeding, increased fibrinolysis, thrombocytopenia, microangiopathic hemolytic anemia, and/or disseminated intravascular coagulopathy. When combined with other actions such as respiratory distress, labored breathing, pulmonary edema, shock, and oliguria, this may lead to acute renal failure and multiple organ damage. Biochemical findings of metabolic disturbances in envenomed humans and animals have included elevation of AST and ALT, enhanced glucose serum levels as a glycolytic response, hepatocyte glycogen depletion and damage as indicated by nuclear alterations mainly pyknosis and karyorrhexis, amyloidosis indicative of hepatocytes necrosis, and cell destruction due to proteinases and phospholipases; the latter hydrolyze phospholipids in the cell membrane. Death may occur within less than an hour to several days according to the snake type, condition of the victim, and severity of signs and symptoms (Sajevic et al. 2011; Fahmi et al. 2012; Vyas et al. 2013).

Over the past decades, several researchers from different countries in the Middle East have studied clinical signs and symptoms following scorpion envenomation with a variety of scorpion types, with relatively similar patterns emerging. These ranged from immediate or delayed local and systemic manifestations with the former including pain, erythema, edema, and necrosis. Systemic manifestations include neurological alterations such as hyperthermia/hypothermia, restlessness, anxiety, sweating, myoclonia, agitation, priapism, miosis, mydriasis, anisocoria, nystagmus, squint, erratic eye movements generalized, localized convulsions, and in severe cases coma and/or convulsions (Mesquita et al. 2002; Fatani et al. 2010). Gastrointestinal manifestations, enhanced secretions, and neuronal simulation are usually exhibited such as hypersalivation, sweating, rhinorrhea and tearing, nausea, vomiting, diarrhea, defecation, urination, gastric distension, abdominal cramps, muscle spasms, and convulsions. In more advanced cases, especially in children

and the elderly, tachypnea, shortness of breath with wheezing, Cheyne-Stokes respiratory pattern, hypo- and/or hypertension, cardiac rhythm disturbances, myocardial infarction, and cardiogenic plus noncardiogenic pulmonary edema have been reported. Eventually the neuromuscular, respiratory, and cardiac abnormalities lead to systemic multiple organ dysfunction (MOD)/failure (MOF) and death. These symptoms are usually accompanied by biochemical changes including abnormal sodium and potassium serum levels, and blood gases were prominent biochemical features accompanied by enhanced levels of white blood cells, blood urea nitrogen (BUN), creatinine, CK, LDH, AST, and ALT, plus markers indicative of apoptosis, lipid peroxidation, and cellular damage (Freire-Maia et al. 1994; Ismail 1995; Tarasiuk et al. 2003; Shahbazzadeh et al. 2007; Fatani et al. 2010; Jalali et al. 2010; Dehghani and Fathi 2012).

It is widely believed that the majority of the signs and symptoms are due to the massive release of neurotransmitters and modulators as a consequence of venom toxin action mainly on sodium channels and to a lesser degree potassium and calcium channels, resulting in systemic alterations, in addition to possible direct actions on different organs. For example, venom-evoked cardiac injury has been postulated to be caused by Ca^{2+} influx in cardiomyocytes, producing increased inotropic activity and structural alterations. The initial positive inotropic effect, associated with the high oxygen demand due to the massive release of catecholamines, might play a role in the cardiac function deterioration that ultimately leads to death (Freire-Maia et al. 1994; Ismail 1995; Fatani et al. 2000, 2010; Al-Sadoon and Al-Farraj 2008; Al-Asmari et al. 2008, 2012).

Proteomics: Understanding Snake Venom Toxins

As is apparent from the previous sections, for decades researchers have attempted to unravel the intricacies of envenomation by two deadly species, snakes and scorpions. This was undertaken to better understand the deep linkages between their composition and biochemistry, molecular biology, pharmacology, and toxicology, for a better understanding of their interactions with other organisms. Research regarding toxins has become a very exciting field to study because of the recent advances in genomic and proteomic technologies, such as the venomous systems genome project and the development of methods to screen venoms and toxins (Heinan 2011), allowing better alternatives and means to study the pharmacologically active substances found so far. The next paragraphs will shed some light on newer researches pertaining to genomics and proteomics of snake and scorpion venom toxins for a more in-depth understanding of creatures that share our ecosystem and affect our health and lives.

Proteomics (venomics), the new approach for detection and characterization of venom proteins, plus their molecular masses, whereas isolated fractions are subjected to amino acid sequence analysis and compared against available viperid protein sequence data banks (Fahmi et al. 2012). Researchers have begun to explore newer techniques that would give a better understanding of snake and scorpion venom components while relating results with venom effects in similar species. For

example, Fahmi et al. (2012) performed proteomic analysis on a common snake *C. cerastes* and showed that despite its broad distributional range in Morocco, Tunisia, and Egypt, it has a low-complexity proteome composed of 25–30 toxins belonging to six protein families. They were able to ascertain that Zn^{2+}-metalloproteinases (SVMPs) comprised the most abundant toxin family (61 %) followed by phospholipases (PLA2s) (19 %), dimeric disintegrins (8.5 %), and serine proteinases (7 %). Cysteine-rich secretory proteins (CRISP) and C-type lectin-like molecules (CTL) each accounted for less than 4 % of the total venom toxins. The authors concluded that the venom toxin profile was consistent with the pathophysiology of *C. cerastes* envenoming since these components cause local hemorrhage as a primary consequence of the degradation of extracellular matrix proteins of the vascular subendothelium and are responsible of the hemorrhagic manifestations of viper envenoming in different regions (Fahmi et al. 2012).

The overview by the authors explained that PI-SVMPs are nonhemorrhagic, fibrino(geno)lytic enzymes, which significantly decrease the plasma fibrinogen levels resulting in consumptive coagulopathy. Disintegrins are small cysteine-rich polypeptides released into viper venoms by proteolytic processing of PII snake venom metalloprotease precursors or synthesized from short-coding mRNAs. Disintegrins selectively block the function of β1 and β3 integrin receptors. Fahmi et al. (2012) also explained that the dimeric disintegrins corresponded to others previously characterized in the venom of *C. cerastes* from other regions such as Tunisia. It is known that blocking fibrinogen binding to its receptor, integrin αIIbβ3, on the surface of agonist-activated platelets; additionally disintegrins prevent platelet aggregation and thereby promote bleeding. Snake venom serine proteinases exhibit selective activities at various steps of the hemostasis cascade, i.e., activating prothrombin, FV, FVII, FX, protein C, and plasminogen and inactivating the fibrinolysis inhibitors, PA inhibitor-1 (PAI-1) and α2-antiplasmin. Snake venom serine proteinases often display non-species-specific thrombin-like activity. Snake venom thrombin-like proteinases are thus regarded as defibrinogenating enzymes that cause consumptive coagulopathy. The family of C-type lectin-like venom proteins comprises inhibitors and activators of coagulation factors IX/X and von Willebrand factor, proteins that bind to the platelet membrane GPIb/IX complex and either block or promote platelet aggregation, potent activators of the platelet collagen receptor GPVI, and selective inhibitors of the platelet collagen receptor, integrin α2β1 (Fahmi et al. 2012) (Fig. 16.1).

Fahmi et al. (2012) also utilized proteomics to ascertain geographic variability comparing venom composition of the same species in Morocco and Tunisia. The authors concluded that the venom of *C. cerastes* from the former exhibited remarkable compositional variation from that of the latter. It contained lower amounts of C-type lectin-like molecules and serine proteinases and was devoid of L-amino acid oxidase, an enzyme that catalyzes the oxidative deamination of a number of L-amino acids which affect platelets, have antibacterial and hemorrhagic effects, and have an ability to induce apoptosis. On the other hand, the venom from Morocco expressed higher amounts of dimeric disintegrins and contained a PI-SVMP and a CRISP molecule not found in the venom from Tunisia. Reverse-phase HPLC separations

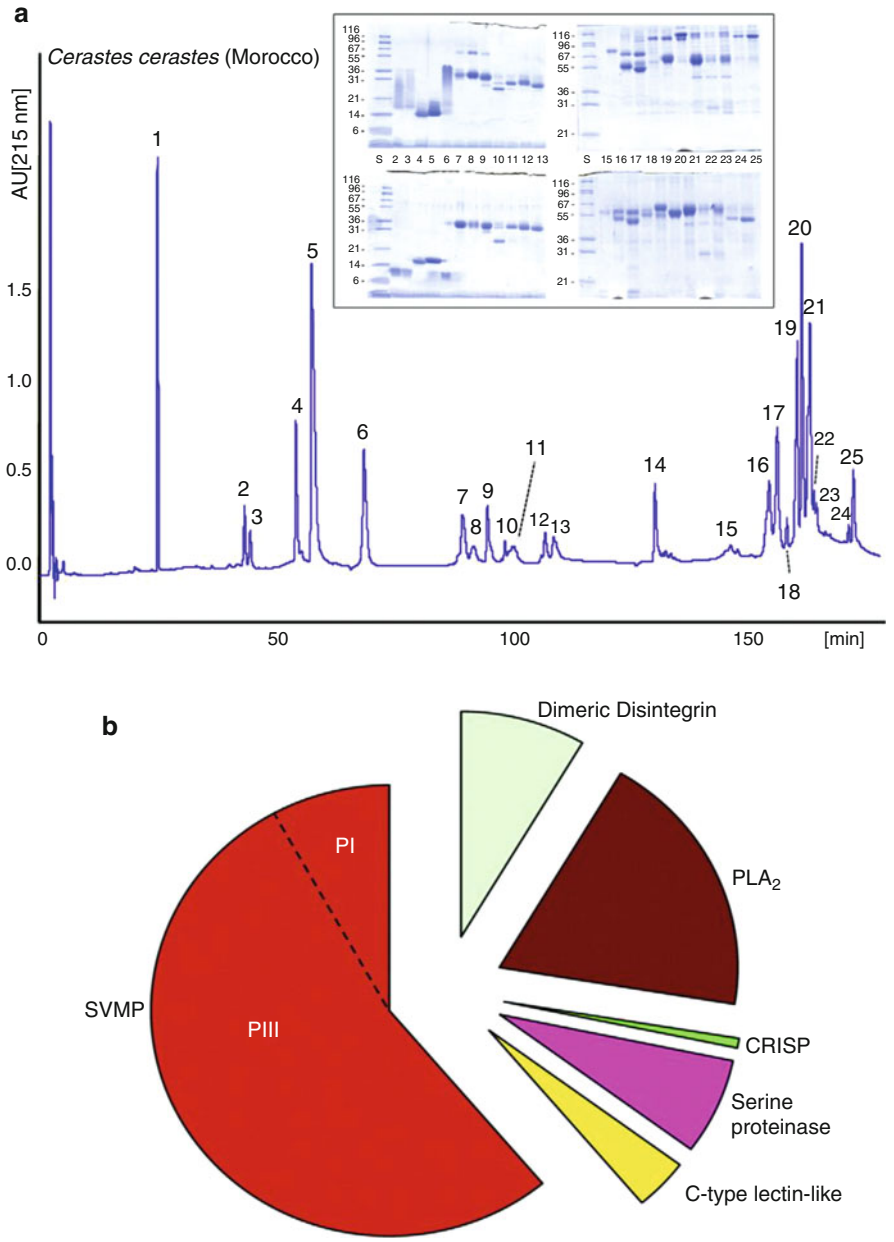

Fig. 16.1 *Upper-left panel* – Characterization of venom proteome of *Cerastes cerastes*, Morocco. (**a**) Reverse-phase HPLC separation of venom proteins (0.25 mg) of *C. cerastes*. Isolated proteins initially characterized by *N*-terminal sequencing and SDS-PAGE (*insert*), run under nonreduced (*upper panel*) and reduced (*lower panel*) conditions. Molecular mass markers (in kDa) indicated at side of each gel. Protein bands were excised and characterized by mass fingerprinting and CID-MS/MS of selected doubly or triply charged peptide ions. (**b**) Relative occurrence of proteins

suggested a high degree of phenotypic similarity between the venoms of *C. cerastes* from Tunisia and Egypt. The authors concluded that venoms represent trophic adaptations, and different venom formulations have evolved in different taxa for the same purpose: rapid immobilization of prey. They affirmed that intraspecific venom variation represented a well-documented phenomenon particularly evident among species that have a wide distribution range. They supported the concept that these species should be considered as a group of metapopulations, in which, from a medical standpoint, intraspecific geographic variability may have an impact in the clinical picture of envenomation treatment (Fahmi et al. 2012) (Fig. 16.2).

It is hoped that pharmacological activities of snake venoms including hemolytic, cardiotoxic, myotoxic, anticoagulant, convulsant, hypotensive, edema-inducing, and local necrotic effects can be better characterized by utilizing proteomics.

Researches on Proteomics and Genomics of Scorpion Venom Toxins

Several researchers have performed proteomic analyses and shown that single scorpion venoms might contain more than 100 peptidic components (Calvete et al. 2007; Abdel-Rahman et al. 2009, 2010). According to Abdel-Rahman et al. (2009), from more than 1,500 different species of scorpions known to exist in the world, many of their species have been characterized by either proteins or individual peptides; theoretically, estimates approach nearly 100,000 for distinct components in scorpion venom peptides. The concept of the "venome": the taxonomic protein profile of a particular venom is the analysis of venom components of utilization of peptides and proteins to produce a valuable fingerprint that can be used as a useful reference tool in taxonomic analysis, as a complementary method to morphology and behavioral characterization for species identification and classification of related specimens. Abdel-Rahman et al. (2009) affirmed that intraspecific venom variation is not a novel concept and reviewed what had been previously studied in various venomous animal species such as bees, wasps, ants, fish-hunting *Conus* snails, spiders, scorpions, and snakes. Its greatest asset, according to Abdel-Rahman et al. (2009), in addition to the study of the ecology and evolution of venomous animals, is relevance to snakebite and scorpion sting therapy. Additionally, scorpion and snake venoms also represent a valuable natural source of biochemicals for basic research and biomedicine, and the abundance of particular components in selected individuals would be of value.

Fig. 16.1 (continued) from different toxin families in venom pooled from adult *C. cerastes*. PI- and PIII-SVMP, snake venom Zn2+-metalloproteinase (SVMPs) of classes I and III; *PLA*$_2$ phospholipase A$_2$, *CRISP* cysteine-rich secretory protein. *Upper-right panel* – Comparison of RP-HPLC profiles of *C. cerastes* venom from Tunisia (**a**) and Egypt (**b**). Peaks numbered as in the *left panel*. Proteins identified by *N*-terminal sequencing and MS/MS (**a**). *DD* dimeric disintegrin, *LAO* L-amino-acid oxidase (Adapted from Fahmi et al. 2012)

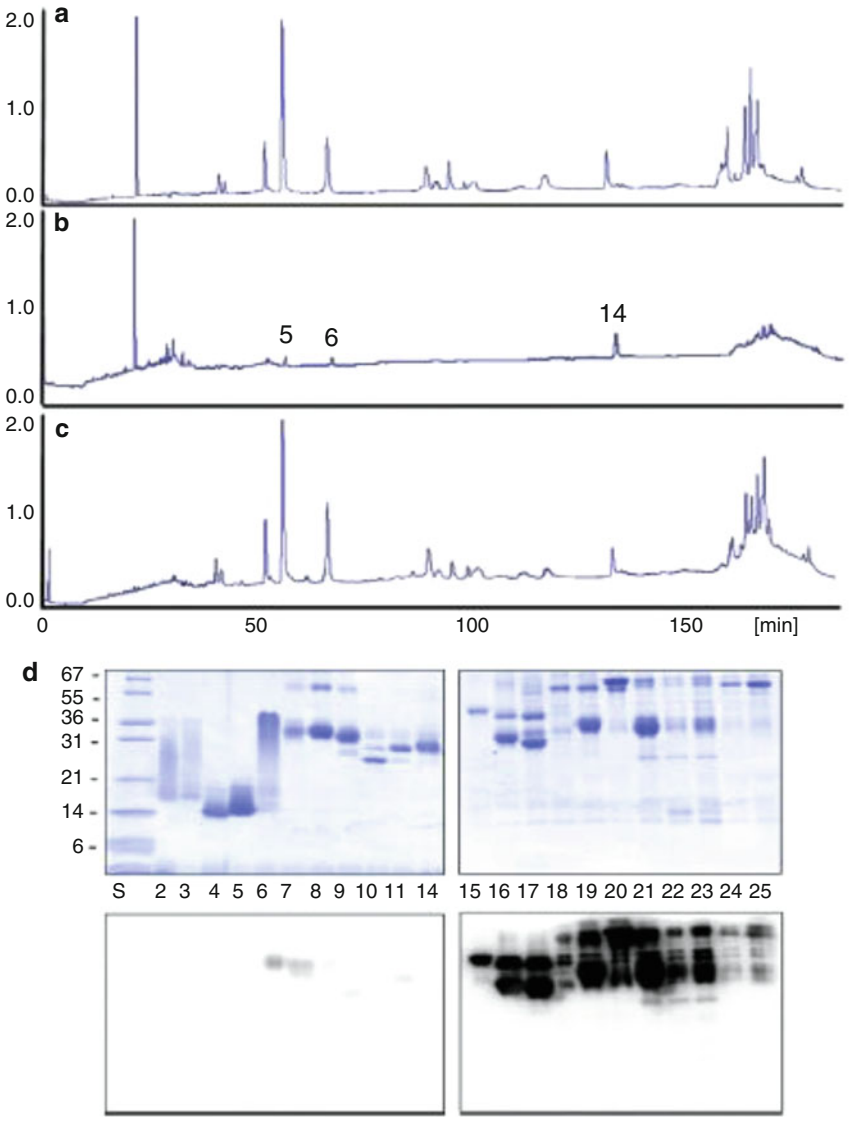

Fig. 16.2 Characterization of the venom proteome of *Cerastes cerastes* from Morocco. (**a**) Reverse-phase HPLC separation of the venom proteins (0.25 mg) of *C. cerastes*. Isolated proteins were initially characterized by *N*-terminal sequencing and SDS-PAGE (*insert*), run under nonreduced (*upper panel*) and reduced (*lower panel*) conditions. Molecular mass markers (in kDa) are indicated at the side of each gel. Protein bands were excised and characterized by mass fingerprinting and CID-MS/MS of selected doubly or triply charged peptide ions. (**b**) Relative occurrence of proteins from different toxin families in the venom pooled from adult *C. cerastes*. PI- and PIII-SVMP, snake venom Zn2+-metalloproteinase (SVMPs) of classes I and III, respectively; *PLA*$_2$, phospholipase A$_2$, *CRISP* cysteine-rich secretory protein; *svVEGF* snake venom vascular endothelial growth factor (From Fahmi et al. 2012)

Abdel-Rahman et al. (2009) reviewed methodologies to assess venom variability such as matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOFMS) analyses used to detect intraspecific protein diversity (using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)) and genetic variation in venom production among the scorpion strains from different regions. Abdel-Rahman analyzed researches done on intraspecific variation for various scorpions collected from different areas in Egypt wherein differences were found (Abdel-Rahman et al. 2009).

Moreover, Abdel-Rahman et al. (2009) discussed another utilized technique: discriminant functions analysis (Figs. 16.3 and 16.4) showing that scorpion venom of the Egyptian *S. m. palmatus* collected from different biotopes exhibited an intraspecific diversity in neurotoxic and cytotoxic effects, with results consistent with molecular data on the level of genes and expressed protein of this scorpion species (Abdel-Rahman et al. 2009). The authors examined the RAPD banding and genetic distance (FST) profile produced for four scorpions (Fig. 16.5). Fifteen primers were tested for their ability to amplify polymorphic RAPD fragments from gDNA samples obtained from four different populations. Utilizing RAPD, SDS-PAGE techniques, and dendrograms, the authors reported the presence of selection pressure plus intraspecific variation of scorpion venoms, probably due to a combination of local environmental conditions, geographic separation, ecogenetic diversity, and migratory trends, with various populations reflecting clear differences in patterns of venom composition and their potency (Abdel-Rahman et al. 2009, 2010).

According to Abdel-Rahman et al. (2009), individual venom variation can be viewed as either population markers (intraspecific variability related to geographic and/or gender status) or individual markers (variability within the same specimen related to temporary influences such as age, seasonal changes, feeding behavior, or dynamics in gland production and peptide maturation). They advocated the utilization of a well-supported RAPD-based tree of the scorpion populations that would reveal correlation between genetic polymorphism and geographic origin. Moreover, the qualitative and quantitative variations in the venom composition of scorpions of the same species could partially explain the disparity of symptoms in the victims of scorpion envenomation and may play a role in future tailored therapeutic regimens.

Treatment Modules for Snakes and Scorpions: Serotherapy

In general treatment of envenomation whether snakes or scorpions, although each has its differences according to characteristics and prevailing signs and symptoms, is usually based on symptomatic interventions and the use of neutralizing antivenoms. The latter has supporters and skeptics to its benefit. The upcoming sections will attempt to summarize the latest research findings in this regard.

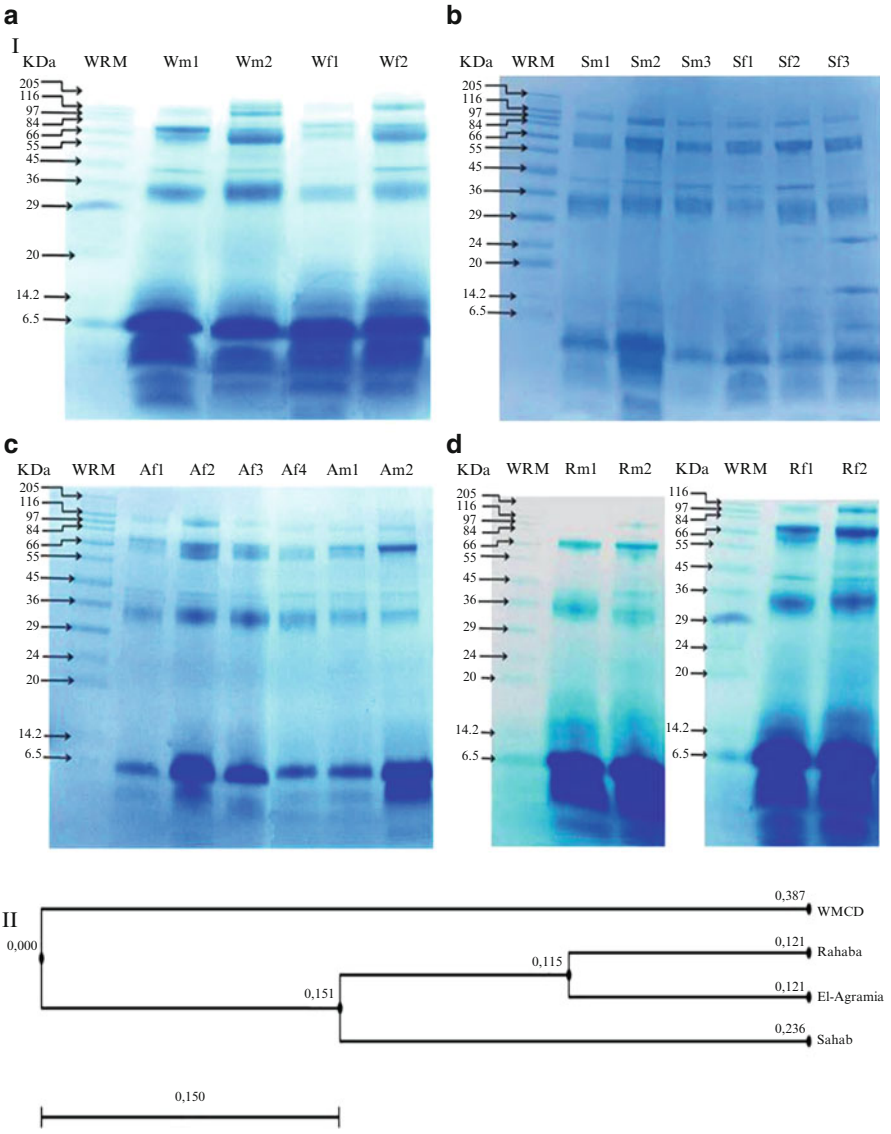

Fig. 16.3 (**I**) Electrophoretic protein pattern of the scorpion venom samples of WMCD (*a*), Sahab (*b*), El-Agramia (*c*), and Rahaba (*d*). (**II**) Hierarchical cluster analysis based on the electrophoretic protein pattern of the scorpion venom collected from the four different locations. The cluster was derived using SPSS software. *WRM* wide range marker, *f* female, *m* male, *W* WMCD, *S* Sahab, *A* El-Agramia, *R* Rahaba (From Abdel-Rahman et al. 2009)

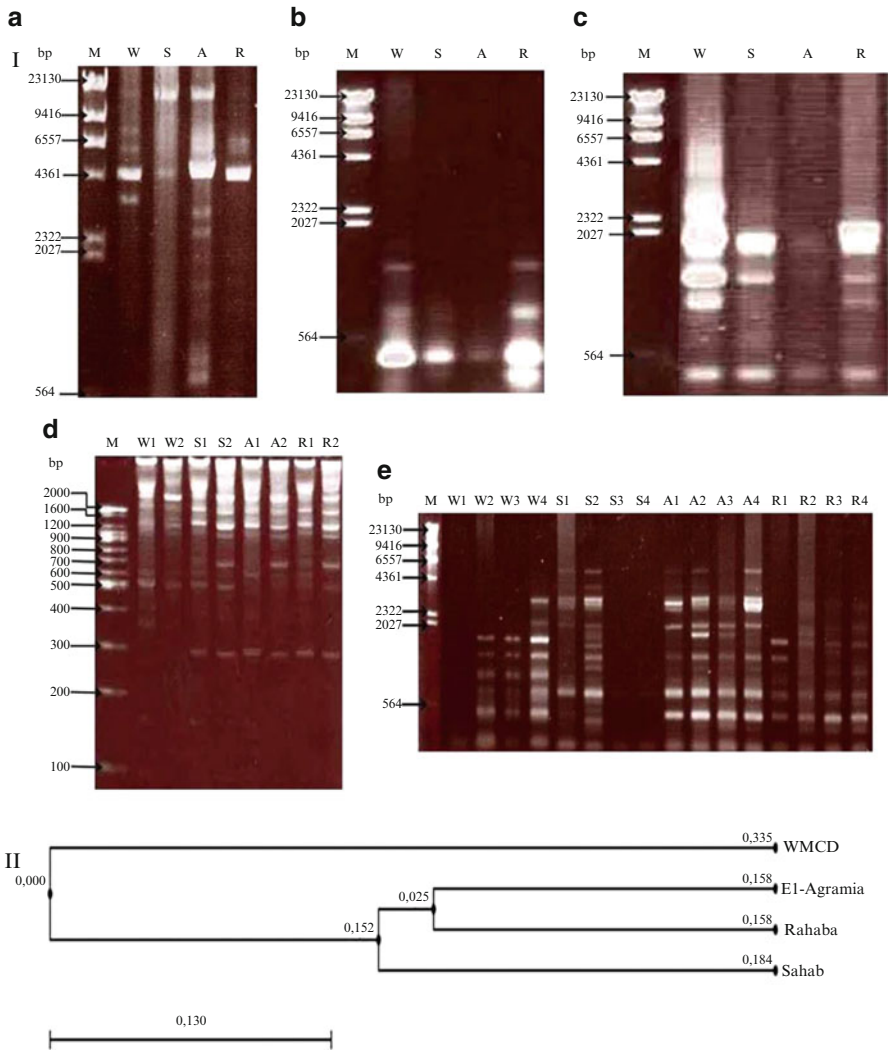

Fig. 16.4 (**I**) Electrophoretic protein pattern of the scorpion venom samples of WMCD (*a*), Sahab (*b*), El-Agramia (*c*), and Rahaba (*d*). (**II**) Hierarchical cluster analysis based on the electrophoretic protein pattern of the scorpion venom collected from the four different locations. The cluster was derived using SPSS software. *WRM* wide range marker, *f* female, *m* male, *W* WMCD, *S* Sahab, *A* El-Agramia, *R* Rahaba (Abdel-Rahman et al. 2009)

Antivenoms Against Snake Venom Toxins

Serotherapy against envenomation using highly specific effective antivenoms, in the appropriate dose and route of administration, is reported to be the main determinants capable of neutralizing venoms of local snakes or scorpions, hence

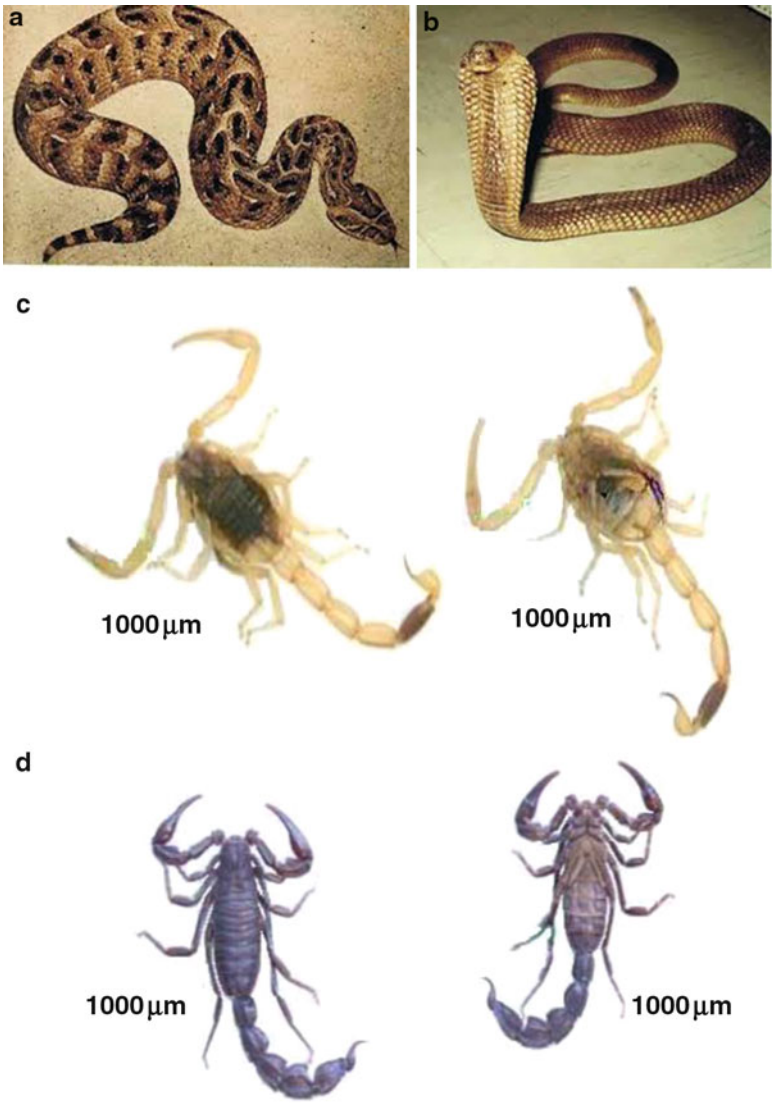

Fig. 16.5 Examples of snakes and scorpions common in the Middle East. (**a**) *Bitis arietans* (puff adder), a Saudi viper (Ismail and Memish 2003). (**b**) *Naja haje arabicus* (Arabian cobra), a Saudi elapid (Ismail and Memish 2003). (**c**) *Leiurus quinquestriatus* (Al-Asmari et al. 2009). (**d**) *Androctonus crassicauda* (Al-Asmari et al. 2009)

alleviating the suffering of the victims, reducing both morbidity and mortality (Ismail et al. 2007; Al-Lawati et al. 2009; Fatani et al. 2010; Abd-Elsalam 2011).

With regard to snakes, several types are considered to be extremely dangerous to victims (Fig. 16.5) requiring immediate attention, rapid judgment, and special medical care (Ismail et al. 2007; Isbister et al. 2010; Abdel ElSalam 2011; Ibrahim

et al. 2013). Antivenom therapy is the mainstay for management of snake envenomation; however, deciding on the most appropriate protocol and method for preparation of specific effective economical antivenom has posed several challenges (Ismail et al. 2007; Abd-Elsalam 2011).

The initial step must be highly efficient venom detection kits that act as a means to specify certain monovalent antivenom, assure positive signs of envenomation, avoid confusion with less severe bites, and eventually aid in the design of the proper individualized treatment protocol for each envenomed victim (Ibrahim et al. 2013). Wahby and Ibrahim (2008) summarized the development of sensitive practical immunoassays, adapted for use by clinicians for detection of snake venoms in human victims, which kept in mind that venoms of the various species are different in composition and demonstrated high immunological cross-reactivity, which would be significantly reduced using monoclonal Abs (Hutton and Warrell 1993; Wahby and Ibrahim 2007; Ibrahim et al. 2013).

Moreover, immuno-affinity purification of species-specific Abs (SS-Abs) has become an indispensable successful immunodiagnostic venom detection kit using either their specific reactivity or avidity, defining groups of venoms in specific geographic areas for more tailored antivenom therapy (Wahby and Ibrahim 2008). Ibrahim et al. (2013) examined the advantages offered by avidity, which can be used to discriminate the immunologically high cross-reactive venoms. The power of the venom-specific antibodies (VS-Abs) obtained after one-step purification has been reported to outweigh the specificity of the species-specific antibodies (SS-Abs) obtained after further purification (Wahbi and Ibrahim 2007; Ibrahim et al. 2013).

Fahmi et al. (2012) reviewed how researchers incorporated proteomics and antivenomics in the analysis of venoms of the medically relevant common snakes. The authors showed how antivenomics was conceived as a proteomic tool for the qualitative and quantitative analysis of the immunoreactivity of antivenoms. They mentioned that the original antivenomics protocol was based on the immunodepletion of toxins upon incubation of whole venom with purified antivenom IgGs, followed by the addition of a secondary antibody or immobilized IgG-binding moiety, such as protein-A or protein-G. The authors stated that usually antigen antibody complexes were immune-depleted from the reaction mixture containing the toxins against which antibodies in the antivenom were directed. By contrast, venom components that remain in the supernatant are those which failed to raise antibodies in the antivenom, or which triggered the production of low-affinity antibodies (Fahmi et al. 2012). These components can be easily identified by comparison of reverse-phase HPLC separation of the non-precipitated fraction with the HPLC pattern of the whole venom previously characterized by a venomic approach. For example, Fahmi et al. (2012) compared immunoreactivity profiles of *Cerastes cerastes* and *Cerastes vipera* snake venoms of various geographic regions against monospecific Fab (ab')2 and gamma-VIP divalent antivenoms. They showed similar immunocapturing capability toward snakes from several regions such as Moroccan, Tunisian, and Egyptian *C. cerastes* venom proteins but not necessarily toward *C. vipera*

venom toxins not accounted in the antivenom preparation. Generation of a pan-*Cerastes* antivenom was recommended to ascertain more complete therapeutic cover in the formulation of venom immunization mixtures (Fahmi et al. 2012).

The importance of administration of appropriate antivenom via the effective route must be remembered as well as using the appropriate assay to test antivenom efficacy and dose that would take into account "physiological" concentrations of venom closer to those seen in patients with snake envenoming (Ismail et al. 2007; Isbister et al. 2010; Abd-Elsalam 2011). Another aspect to keep in mind is that in several parts of the world, there are continuing crises in the production, deployment, and accessibility of antivenom (Al-Lawati et al. 2009), whereas alternative therapeutic modules must be implemented and these will be discussed in upcoming sections. Moreover, the antigenic nature of antivenoms in general has led several authors to advocate a trial dose of antivenom to be given subcutaneously to the victim especially one who has a history of allergic reactions. General signs of a reaction have been reported previously resulting in recommendations that patients are monitored over 30 min, and in the absence of reactions, the whole dose may be given slowly and resuscitation facilities ensured (Malasit et al. 1986; Ismail et al. 2007; Fahmi et al. 2012).

Antivenoms Against Scorpion Venom Toxins

Serotherapy and symptomatic treatment have been the major therapeutic measures used in the treatment of scorpion envenoming during the last decade in several countries in the Middle East, Asia, Africa, and South America. Researchers and clinicians have demonstrated that the correct polyvalent scorpion antivenom, in the right dose and route, administered at an appropriate time after the sting, appears to be effective in ameliorating various manifestations of scorpionism, especially if the exact species specimen is correctly identified and the correct antivenin is available (Ismail et al. 2007; Jahan et al. 2007; Shahbazzadeh et al. 2009; Fatani et al. 2010; Jalali et al. 2010).

As is the case with treatment of snake envenomation with serotherapy, several authors have debated extensively the appropriate treatment protocol for antivenoms to protect patients from the deleterious effects of venom toxins, reduce the severity of symptoms, and protect from morbid outcomes (Freire-Maia et al. 1994; Ismail 1995; Dehghani and Fathi 2012). The authors concluded that the amount of antivenom required for treatment varied from case to case and depended on the species, victim age, body weight, previous health conditions, severity of envenomation, or delayed time of treatment; severely envenomed victims need regularly repeated larger doses of the appropriate antivenom. The administration route in severe cases is intravenous (given slowly, either as a single bolus or preferably by a slow drip infusion of antivenin diluted in physiological solutions). Intramuscular injection was the preferred route in mild to moderate cases (Ismail et al. 1998, 2007; Fatani et al. 2010).

Despite the ability of antivenoms to neutralize the venom, their utilization is argued by some authors, especially in mild to moderate cases. Certain clinicians concentrated on symptomatic treatment due to inavailability, while others are skeptic of their need or benefit (Bawaskar and Bawaskar 1992, 2007; Gueron et al. 1992; Al Asmari et al. 2012; Deghani and Fathi 2012). One of the issues regarding antivenoms in general is the possibility of anaphylaxis or serum sickness. With snake antivenom therapy, there is an agreement that the benefit outweighs the risk; this, however, is not agreed upon in scorpion envenomation with discussions centering around potency, standardization methods, effective route, and dose of antivenoms (Freire-Maia et al. 1994; Ismail 1995; Ismail et al. 2007; Jahan et al. 2007; Shahbazzadeh et al. 2009; Fatani et al. 2010; Jalali et al. 2010).

Due to the presence and involvement of more than one scorpion species in the Middle East (Fig. 16.5), the development of specific immunoassay methods for the determination of venom type and quantification of its antigens in body fluids of stung patients is crucial to evaluate the severity and appropriate therapeutic regimens. Additionally, investigators have mentioned differences in the amount and frequency of administration of antivenom recommended by guidelines produced by different countries and organizations and advocate evidence-based unified strategies for the treatment of comparable signs and symptoms resulting from similar species (Shahbazzadeh et al. 2009; Dehghani and Fathi 2012). The quantification of venom antigens in body fluids of stung patients, by the development of immunoassay methods, is a crucial matter to evaluate the severity of envenomation by scorpions. It is possible to identify the type of scorpion venom in the sera of stung patients in each area via specific immunoenzyme assay kit. Past and future collaborative studies on scorpions of similar geographic and zoological zones such as the Middle East have been useful in improving understanding of the intricacies of envenomation and hence producing more effective antivenoms (Ismail et al. 2007; Jahan et al. 2007; Shahbazzadeh et al. 2009; Fatani et al. 2010; Jalali et al. 2010).

General Prophylactic and Therapeutic Interventions Following Envenomation

Over the past decades, health professionals, experts, and scientific researchers have shown that envenomation, by snake or scorpion venom toxins, is a health problem and medical emergency in several countries, and part of the issue must be to reduce the rate of encounters between humans and these venomous creatures. The use of long-term and precise programs for control and prevention of scorpion stings was reported to effectively reduce consequences. The necessity of developing safer environments was stressed, by improving housing construction, fencing, and population control in areas with high encounter rates (Balozet 1971; Freire-Maia et al. 1994; Ismail 1995; Deghani and Fathi 2012).

Deghani and Fathi (2012) recommended the need to increase the knowledge of urban and rural people, especially those who are living in the areas with high rates

of incidents, by public media, training workshops, and publication of educational leaflets. These should cover general information about these creatures, their lifestyle, seasonal patterns, mode of action, venom characteristics, and prophylactic and first aid procedures, plus general concepts of management of envenomation.

Likewise Deghani and Fathi (2012) advocated that due to variability of scorpion venoms and the fact that severity of envenoming is species dependent, determination of the species responsible for sting is critical and can affect the clinical procedures of patient's treatment. They strongly recommended that it was necessary to acquaint health professionals with full details about native medically important snake and scorpion species in their local area. Researches pertaining to the prevention and control of scorpion stings abound especially in regions where it is a long-standing public health problem (Warrell 1993; Ismail et al. 2007; Al-Lawati et al. 2009; Abd-Elsalam 2011; Al kaabi et al. 2011; Dehghani and Fathi 2012).

Several authors have reported that in cases where snake was the culprit, patients were given various medications including serotherapy and symptomatic treatments, which included epinephrine, antihistamines, and steroids, the latter to protect from the possible hypersensitivity reactions that may occur with serotherapy. Some authors have mentioned the utilization of antibiotics for swelling or necrosis at bite site and leukocytosis or prophylactically to avert future complications. Laboratory investigations are often essential, especially coagulation profile in cases of envenomation from snakes known to possess venoms that affect clotting. This would ensure tailored therapeutic interventions and subsequent monitoring. Arrival time at health facilities remains an essential factor in the treatment of envenomation, whereas early presentation was closely linked to increased efficacy of therapeutic regimens and more favorable outcomes (Warrell 1993; Ismail et al. 2007; Wahby and Ibrahim 2008; Al-Lawati et al. 2009; Sajevic et al. 2011).

A similar pattern was observed in researches dealing with treatment of scorpion envenomation. Several authors showed that therapeutic interventions following scorpion envenomation are usually based on zoogeographic regions and their distinctive fauna where the incident occurred, plus their emergency room protocols. Other than therapy with antivenoms, treatment generally included paramedical care, monitoring, and supportive plus symptomatic conventional protocols. This has included lidocaine for local pain, acetaminophen for fever, chlorpromazine or promethazine for vomiting, hydralazine or nifedipine for hypertension, oxygen or furosemide for pulmonary edema, diazepam for convulsion, and antihistamines and corticosteroids, for allergic reactions (due to venom or antivenom) (Balozet 1971; Ismail et al. 1992; Freire-Maia et al. 1994; Ismail 1995; Shahbazzadeh et al. 2007; Al-Asmari et al. 2012; Dehghani and Fathi 2012).

As an alternative to antivenom therapy, due to either lack or doubt of effectiveness, various treatment modalities have been utilized in a variety of regions to treat scorpion envenomation, not necessarily based on detailed clinical, pharmacological, and toxicological studies, but in some cases based on personal experiences and expertise. These include, for example, blockers of transmitters or modulators

ultimately released by the venom such as atropine, propranolol, prazosin, dobutamine, sodium nitroprusside, as well as insulin to offset venom-elicited metabolic changes or chloroquine, a multifaceted drug (Bawaskar and Bawaskar 1992, 2007; Gueron et al. 1992; Warrell 1993; Murthy and Hase 1994; Al-Asmari et al. 2008; Patil 2009).

Several other probable therapeutic interventions have been investigated experimentally in different animals, based on their ability to counteract pathological mechanisms of actions of scorpion venom toxins. These included the use of sodium and calcium channel blockers, in doses capable of blocking their initial actions on these channels which ultimately leads to neurotransmitters and modulators release (Couraud and Jover 1984; Ismail 1995; Fatani et al. 2010). A thorough investigation of these treatment modules is necessary to compare outcomes observed following blockade of channels compared with that following blockade of neurotransmitter or modulator release. Other interventions being investigated include the use of phenobarbital, phenytoin, or carbamazepine to counteract the central effects of scorpion venom toxins, leukocyte inhibitors such as benzydamine and cyclophosphamide, and aprotinin, a kallikrein-kinin synthesis inhibitor (Ismail et al. 1992; Mesquita et al. 2002).

Venom Toxins and the Potential for Novel Drug Discoveries

The plethora of data generated from decades of research encompassing a multitude of areas and angles related to snake and scorpion envenomation in various regions including the Middle East has enabled the scientific community to expand its knowledge exponentially. Researchers attempting to learn how to protect themselves from harrowing encounters with these venomous creatures found en route great potential for novel drug discoveries. Researchers vied to explore the pharmacological potential of venoms and their toxins and whether they could generate therapeutic drugs capable of impacting certain disease states. Several studied the vast array of components and active secretions originating from animals of different species and their ability to affect various disease states such as cancer, hypertension, and thrombosis.

Clinical Applications of Venom Components as Antitumor Agents

Many active principles produced by animals, plants, and microorganisms have been employed in the development of new drugs to treat diseases such as cancer. Among the animals that produce pharmacologically active molecules capable of interfering in human cellular physiology, the highlights are venomous creatures, such as snakes, scorpions, bees, wasps, spiders, ants, and caterpillars. The substances found in the venom of these animals present great potential as antitumor agents. Heinen and Gorini da Veiga (2011) reviewed the main results of years of research

involving in particular the active compounds of arachnid/arthropod venoms that have anticancer activity.

Vyas et al. (2013) reviewed several researches showing remission of tumor cells after treatment with proteins, peptides, and/or enzymes derived from snake venoms. For example, certain components bind specifically to cancer cell membranes, affecting cell adhesion, migration, tissue organization, growth, and proliferation, thus presenting a great potential as wide-range antitumor agents (De Carvalho et al. 2001). Vyas et al. (2013) reported that venom components worked by either induction of apoptosis in cancer cells to control tumor size and cell number, generation of intracellular reactive oxygen species, increasing Ca^{2+} influx, inducing cytochrome C release, containing lethal cardiotoxic-cytotoxic protein components, increasing or decreasing expression of proteins controlling the cell cycle, or causing damage to cell membranes.

Researchers are currently examining delivery systems for maximum efficacy and safety. Currently, much attention has been given to the advances of nanotechnology in the fight against cancer, as chemotherapy-delivery agents to induce apoptosis or DNA/siRNA to regulate oncogene expression. However, its application has been limited by the low specificity against therapeutic targets. Badr et al. (2013) have shown enhanced antitumor activity of venoms from the snake (*Walterinnesia aegyptia*) in vitro in breast carcinoma, prostate cancer, and multiple myeloma cell lines, by combining with silica nanoparticles. Moreover, they reported greater in vivo therapeutic efficacy exhibited as reduction in tumor volume in xenograft breast and prostate cancer-bearing experimental mouse models. Enhanced induction of apoptosis, altered mitochondrial membrane potential, tumor cells to growth arrest, marked elevations in levels of reactive oxygen species, and hydroperoxide and nitric oxide were moreover documented by the authors. Robust reductions in the levels of several chemokines and decreased surface expression of their cognate chemokine receptors were moreover reported, with subsequent reductions in the chemokine-dependent migration of both breast and prostate cancer cells. The authors also documented inhibition of insulin-like growth factor 1 (IGF-1) and epidermal growth factor (EGF)-mediated proliferation of breast and prostate cancer cells. Hence, the authors confirmed the multifaceted actions of the venoms of this snake may have therapeutic potential against different cancer cell types, especially when combined with a nanoparticle-sustained delivery system. The latter was said to enhance bioavailability and help direct these drugs to specific sites in vivo (Badr et al. 2013).

With regard to scorpions, Heinen and Gorini da Veiga (2011) in their review of arthropods and cancer therapy stated that although over 1,500 scorpion species have been identified each producing a different type of venom composed of 50–100 different toxic polypeptides, not many have been well studied. According to the authors, more than 250 bioactive proteins and peptides have been characterized, and their actions on selective ion channels and subsequent sequelae examined, revealing promise in several therapeutic interventions including cancer therapy.

Clinical Applications of Venom Proteins in Cardiovascular Manifestations

In their review, Koh and Kini (2012) summarized the utilization of snake venom toxins in the treatment of cardiovascular manifestations starting with captopril, the first drug based on snake venom protein, more than 30 years ago. Since then snake venom toxins, valuable natural pharmacopeia of bioactive molecules, have continued to provide lead compounds for the development of new drugs for the treatment of cardiovascular conditions such as hypertension and thromboembolic diseases. The authors highlight in their review the molecular basis of developing therapeutic agents, such as captopril, tirofiban, and eptifibatide, from snake venom proteins, highlighting successes and challenges. It has long been noted that some snake venoms drastically lower the blood pressure in human victims and experimental animals. This can either be through direct hypotensive agents, which are useful as future antihypertensive agents. Koh and Kini (2012) summarized the variety of proteins with diverse molecular structures and mechanism of actions to bradykinin-potentiating peptides, natriuretic peptides L-type Ca^{2+}-channel blockers. The authors also mentioned that hypotensive actions of snake venom toxins can be via indirect actions on hemostasis which will be summarized in the next section.

Clinical Applications of Venom Proteins Affecting Hemostasis

Coronary heart disease, stroke, and other cerebrovascular diseases are the major cause of mortality resulting in a higher percentage of deaths than other medical conditions. The precise control of blood coagulation is essential to the life of humans and animals, with atherosclerosis playing a central role in the pathophysiology of coagulopathic diseases (World Health Organization (WHO) http://www.who.int/mediacentre/factsheets/fs310_2008.pdf). Since arterial thrombi are composed of platelets and fibrin, treatment strategies for thromboembolic disorders have been developed that target coagulation, fibrinolysis, and platelet functions (White 2005). These strategies may include inhibiting thrombus formation or modulation of platelet adhesion and function. Venomous snakes contain components that target a variety of processes in hemostasis as a natural response to disable their prey or enemy and alternatively have proven useful in the treatment of a variety of coagulopathies. Both Koh and Kini (2012) and Sajevic et al. (2011) summarized components of snake venoms that have been approved for use as drugs to treat thromboembolic disorders and these included different structural classes of snake venom toxins that act through various mechanisms. These included (1) disintegrins, (2) C-type lectin-like proteins, (3) three-finger toxins, (4) phospholipases A2, (5) serine proteinases, (6) metalloproteinases, (7) nucleotidases, and (8) L-amino oxidases.

Specific research tools and diagnostic agents as well as new therapeutic agents have been discovered from snake venom toxins. These included diagnostic agents and research tools commonly used in hematology laboratories such as batroxobin,

botrocetin, convulxin, ecarin, echicetin, echistatin, factor X (FX), and factor V (FV) activators from Russell's viper venom (RVV-X and RVV-V), protein C activator (Protac), Reptilase, and thrombocytin (see reviews [1, 2]). Some life-saving drugs developed based on exogenous proteins include captopril (Capoten) and enalapril (Vasotec) that inhibit angiotensin-converting enzyme, eptifibatide (Integrilin) and tirofiban (Aggrastat) that block platelet receptor aIIbb3, and bivalirudin (Angiomax) (Kini 2011).

Collaborative efforts, multidisciplinary knowledge of the advancements in all the basic investigational techniques, and better understanding of toxin structures, functions, and possible applications ensure an exciting future in the development of toxins into therapeutics.

Venom Therapy in Multiple Sclerosis

Mirshafiey (2007) reviewed treatment methodologies for multiple sclerosis (MS), concentrating on adjuncts to conventional therapies: complementary and alternative medicines, utilized to treat their symptoms. The authors noted that despite extensive research efforts, specific effective treatments are still being developed for this is an autoimmune disease associated with chronic inflammatory demyelination of the central nervous system, probably due to disease complexity and heterogeneity, and vague pathogenesis. Among the common therapies, the authors concentrated on the therapeutic potential of venoms in MS and stated that therapeutic value required further studies. Venom-based therapy using bee, snake, and scorpion venom and/or sea anemone toxins is currently under investigations, with various components and molecular mechanism of the effects of venoms being examined under in vitro and in vivo conditions. The authors reviewed relevant researches and findings regarding the role of venoms and their components in the treatment of MS. They summarized the actions of several toxins including those obtained from various snake and scorpion venoms known to block different types of ionic channels including a variety of potassium channels (Harvey 1994). The authors summarized researches pertaining to their modes of action and possible benefits in MS and concluded that although basic research is providing new insights into the efficacy of venoms for approaching to the treatment of multiple sclerosis, it is strongly recommended a deep and long research on clinical trials of this traditional procedure in order to reach a logical decision for choosing this therapeutic method in MS disease.

Applications of Venom Toxins as Insecticides

Abdel-Rahman et al. (2010) summarized researches related to identification and classification of scorpion-derived insecticidal toxins, concluding the availability of an immense combinatorial peptide library for the potential development of insecticides and future pharmaceuticals. The authors mentioned the classifications of

insecticidal toxins including the contracture-inducing insect toxins or excitatory toxins, and the depressant toxins are potent both on mammals and on insects (Zlotkin et al. 1985; Abdel-Rahman et al. 2010). The authors mentioned studies demonstrating the use of insect-specific natural toxins in construction recombinant viral insecticides and the research trends of their utilization for control of agricultural pest insects.

Conclusion

For thousands of years, humanity has been blessed with an abundance of fauna and flora containing natural medicinal products. Among them snake and scorpion venoms have an important niche, for their richness in bioactive molecules, such as peptides, proteins, and enzymes with important pharmacological activities. With the advent of new technologies unraveling protein fold structures, proteomic and genomic information, a better understanding of these toxins and their implications on the development of effective drugs targeted to particular protein functions has ensured their status and future role in therapeutics for decades to come.

Cross-References

- ▶ Clinical Uses of Snake Antivenoms
- ▶ Complications of Hemotoxic Snakebite in India
- ▶ Developing Snake Antivenom Sera by Genetic Immunization: A Review
- ▶ Scorpion Sting and Envenomation
- ▶ Snake Venom and Hemostasis

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Snake Envenomation in Children

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Abstract

The highest burden of snake envenomation exists in South Asia, Southeast Asia, and sub-Saharan Africa. India has emerged as the country with the highest mortality among the Southeast Asian countries. Important species causing envenomation in India are spectacled cobra (*Naja naja*) and common krait (*Bungarus caeruleus*), which are neurotoxic, and the saw-scaled viper (*Echis carinatus*) and Russell's viper (*Daboia russelii*), which are hemotoxic. Snake venom is the most complex of all natural venom and poisons. Of which, 90 % are pharmacologically active peptides and proteins, which is responsible for almost all of its biological effects. Clinically relevant components of the venom have cytotoxic, hypotensive, neurotoxic, or anticoagulant effects.

Systemic manifestation usually due to vasodilatation and capillary leakage, alone or together with the hypovolemia resulting from acute bleeding, may cause arterial hypotension and shock. Neurotoxic features vary from early morning neuroparalytic syndrome to several cranial nerve palsies and locked-in syndrome (LIS) in snakebite (central /peripheral). The time lag between the bite and onset of paralysis is usually 4–12 h. Physicians should recognize the "locked-in" syndrome (LIS) to prevent the dangerous error of diagnosing brain death. The most common coagulopathy associated with snake envenoming is a procoagulant or consumption coagulopathy. There is no obvious fibrin deposition, microvascular thrombotic obstruction, and resultant end-organ damage or organ failure in contrast to disseminated intravascular coagulopathy. Treatment with heparin, warfarin, Fresh Frozen Plasma (FFP), and cryoprecipitate is ineffective. The treatment of thrombotic microangiopathy associated with snake envenoming is controversial. Renal involvement following snakebite envenomation is seen predominantly with the bite of the vipers. Compartment syndrome is a rare phenomenon particularly in children and it usually affects the upper limb. Early treatment with anti-snake venom (ASV) remains the best way of preventing mortality and morbidity.

Introduction

Globally, 421,000 envenoming and 20,000 deaths occur each year due to snakebite (Kasturiratne et al. 2008). The highest burden existed in South Asia, Southeast Asia, and sub-Saharan Africa where >100,000 envenoming occur annually. Among the Southeast Asian countries, India has emerged as the country with the highest mortality (Table 17.1, Fig. 17.1) (Sankar et al. 2013). More than 330 species of snakes are found in India, of which more than 60 species are venomous snakes – some of which are abundant and can cause severe envenomation (Mohapatra et al. 2011). Approximately 70 % of snakebites are "dry" bites and do not result in envenomation. Recent snakebite mortality survey in India (Mohapatra et al. 2011) found that snakebite-related deaths occurred more in rural areas (97 %), were more common in males (59 %) than females (41 %), and peaked at ages 15–29 years (25 %) and during the monsoon months of June to September.

Table 17.1 Global estimates of deaths due to snakebites in 2007 by region (Kasturiratne et al. 2008)

Global burden region	Number of deaths per year			
	Low estimate	Rate/ 100,000	High estimate	Rate/ 100,000
Asia Pacific, high income	12	0.007	18	0.010
Asia, Central	9	0.011	29	0.037
Asia, East	462	0.033	4,829	0.347
Asia, South	**14,112**	**0.912**	**33,666**	**2.175**
Asia, Southeast	**790**	**0.134**	**19,094**	**3.245**
Australasia	2	0.008	4	0.016
Caribbean	107	0.275	1,161	2.983
Europe region[a]	16	0.008	43	0.057
Latin America[b]	135	0.156	575	0.468
North Africa/Middle East	43	0.010	78	0.018
North America, high income	5	0.001	7	0.002
Oceania	227	2.434	516	5.533
Sub-Saharan Africa[c]	882	0.406	8,029	3.226
Total	**19,886**	**0.297**	**93,945**	**1.403**

[a]Including Central, Eastern, and Western Europe
[b]Including Andean, central, southern, and tropical region of Latin America
[c]Including central, east, southern and west region

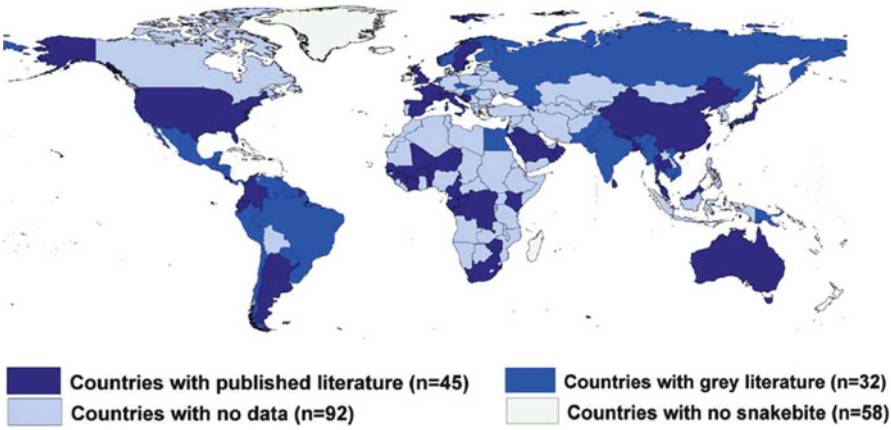

Fig. 17.1 Countries with data on snakebite envenoming (Kasturiratne et al. 2008)

This proportion represents about 45,900 annual snakebite deaths nationally or an annual age-standardized rate of 4.1/100,000, with higher rates in rural areas (5.4/100,000) (Mohapatra et al. 2011).

Most important species causing envenomation in India are spectacled cobra (*Naja naja*) and common krait (*Bungarus caeruleus*), which are neurotoxic, and

the saw-scaled viper (*Echis carinatus*) and Russell's viper (*Daboia russelii*), which are hemotoxic (Mohapatra et al. 2011), but other species may cause fatal snakebite envenomation in particular areas, such as the Central Asian cobra (*Naja oxiana*) in the far northwest, monocellate cobra (*N. kaouthia*) in the northeast, greater black krait (*B. niger*) in the far northeast, Wall's and Sind krait (*B. walli* and *B. sindanus*) in the east and west, and hump-nosed pit viper (*Hypnale hypnale*) in the southwest coast (Mohapatra et al. 2011).

This chapter discusses the pathophysiology of snake venom and critical care issues of systemic manifestation, neurological complications (locked-in syndrome), venom-induced consumption coagulopathy (VICC), acute kidney injury (AKI), compartmental syndrome, and management of snake envenomation.

Pathophysiology of Snake Venom

Snake venom is the most complex of all natural venom and poisons (Warrell 2010; Daltry et al. 1996; Fry 2005). The venom of any species might contain more than 100 different toxic and nontoxic proteins and peptides, and also nonprotein toxins, carbohydrates, lipids, amines, and other small molecules (Fry 2005). Of which, 90 % are pharmacologically active peptides and proteins, which is responsible for almost all of its biological effects (Warrell 2010; Daltry et al. 1996). Clinically relevant components of the venom have cytotoxic, hypotensive, neurotoxic, or anticoagulant effects. The composition of the venom is species specific, i.e., neurotoxins most often predominate in the venom of elapids, while cytotoxic and anticoagulant/procoagulant substances are most often found in the venom of vipers and colubrids (Doley and Kini 2009). The amount of venom injected to the prey is not related to the size of the snake or the fangs, or the number of strikes. Some venomous snakes fail to inoculate their victims with venom ("dry bites").

Cytotoxic enzymes (phospholipases A2, metalloproteinases) activate proinflammatory mechanisms that cause edema, blister formation, and local tissue necrosis at the site of a venomous snakebite. These enzymes also favor the release of bradykinin, prostaglandin, cytokines, and sympathomimetic amines, which are responsible for the pain experienced by the victims (Del Brutto and Del Brutto 2012; Vaiyapuri et al. 2010). Aminopeptidases modify the physiological function of the victims, and peptides of the venom act as angiotensin-converting enzyme inhibitors, causing a drop in arterial blood pressure (Vaiyapuri et al. 2010; Joseph et al. 2004). Safarotoxins and endothelins are potent vasoconstrictors of the coronary arteries and may cause myocardial ischemia or cardiac arrhythmias (Vaiyapuri et al. 2010; Joseph et al. 2004).

Neurotoxins are the major components of elapids. These toxins do not cross the blood–brain barrier, but cause paralysis by affecting the neuromuscular transmission at either presynaptic or postsynaptic levels. Presynaptic neurotoxins are phospholipase A2 complexes (β-neurotoxins such as taipoxin, paradoxyn,

trimucrotoxin, viperotoxin, *Pseudocerastes* neurotoxin, textilotoxin, and crotoxin) that inhibit the release of acetylcholine from the presynaptic terminal. Such inhibition may be irreversible as these toxins interfere with the formation of new acetylcholine vesicles (Tedesco et al. 2009). On the other hand, postsynaptic neurotoxins are three-finger protein complexes (α-neurotoxins) that have a curare-like mechanism of action, causing a reversible blockage of acetylcholine receptors (Karsani and Othman 2009; Roy et al. 2010). The best characterized α-neurotoxins are irditoxin (Doley and Kini 2009). Some of the venoms contain both α- and β-neurotoxins, producing complex blockages of neuromuscular transmission.

Metalloproteinases activate factor X and serine proteases are potent prothrombin activators. In addition, a number of nonenzymatic proteins (snake venom C-type lectins) and some of the three-finger toxins have anticoagulant or procoagulant activity and may be either agonists or antagonists of platelet aggregation (Doley and Kini 2009). Paradoxically, components of the snake venom may also have immunomodulatory, anti-inflammatory, and antitumor effects and are currently under investigation as potential therapeutic agents for human diseases (Koh et al. 2006; Reid 2007). Toxin "ancrod" a serine protease derived from the venom of the Malayan pit viper has been used for years for therapy of patients with acute ischemic stroke because of its defibrinogenating properties (Levy et al. 2009).

Therefore, every patient envenomed by snakebite becomes a natural experiment, providing new insights into the pathophysiological actions of venom toxins, while presenting a therapeutic challenge to the treating physician (Warrell 2010). This experiment is, however, biologically inappropriate since venoms have been evolutionarily selected to subdue prey animals that are much smaller than human beings (Warrell 2010).

Systemic Manifestations

Venom components cause vasodilatation and capillary leakage, which, alone or together with the hypovolemia resulting from acute bleeding, may cause arterial hypotension and shock (Del Brutto and Del Brutto 2012; Otero-Patino 2009). Acute renal failure may occur in severe envenomation and may be related to hypovolemic shock, consumption coagulopathy, rhabdomyolysis, and direct nephrotoxicity causing tubular necrosis (Del Brutto and Del Brutto 2012; Sitprija 2008). Pituitary hemorrhages, causing acute hypopituitarism, may occur after a Russell's vipers bite, because of the presence of hemorrhagins in their venom (Del Brutto and Del Brutto 2012). Clinical manifestations associated to thrombotic and hemorrhagic complications are common in those bitten by vipers and colubrids. The toxins alter the coagulation system and the function of platelets in different ways, representing the basis for the occurrence of thrombosis and hemorrhages in every site of the body (Del Brutto and Del Brutto 2012; White 2005).

Neurological Complications

Pathophysiology

Neurotoxic features of snakebite vary from early morning neuroparalytic syndrome to several cranial nerve palsies (Azad et al. 2013). In literature it is described under different names like brain stem suppression reflex, locked-in-syndrome (LIS) in snakebite, early morning snakebite syndrome, and peripheral LIS (Smith and Delargy 2005). Most often it is related to the toxic effects of the venom, i.e., anticoagulant/procoagulant activity or neurotoxicity. Both anticoagulant/procoagulant and neurotoxic effects may be seen in the same venom, and it can lead to a more complex and severe neurological damage (Doley and Kini 2009). Most venom neurotoxins bind to receptors with high affinity, making reversal of paralysis by antivenom implausible (Warrell 2010). However, rapid improvement in neurotoxicity has been noted when postsynaptic toxins were implicated, e.g., after envenoming by Asian cobras (Sitprija 2008). Binding of toxin α, a three-finger-fold polypeptide (venom of black-necked spitting cobra), to the acetylcholine receptor was reversible by antibodies in vitro and rodents, although this venom is not neurotoxic in man (Sitprija 2008).

Clinical Manifestation

The time lag between the bite and onset of paralysis is usually 4–12 h (Agarwal et al. 2006). The earliest manifestation is ptosis followed by external ophthalmoplegia. Paralysis then progresses to involve muscles of palate, jaw, tongue, larynx, and neck and muscles of deglutition – usually but not strictly in that order (Warrell 2010; Agarwal et al. 2006). The proximal muscles of the limbs are involved earlier than distal, and there can be complete quadriplegia and "locked-in" state (Warrell 2010; Agarwal et al. 2006). Recovery starts in the reverse order, and the median time of onset for recovery of respiratory failure is 2 days (Agarwal et al. 2006). The initial involvement of levator palpebrae superioris, as in botulism, myasthenia gravis, and Graves' disease, might be attributable to the small size, unusual anatomy and physiology, and the low safety part of the neuromuscular junctions of this muscle, features shared by all the extraocular muscles (Warrell 2010). The subsequent pattern of descending paralysis is difficult to explain neurophysiologically (Warrell 2010; Del Brutto and Del Brutto 2012).

Locked-in Syndrome (LIS) in Snakebite

In LIS, patient is conscious yet unable to communicate and has absent pupillary reflex (internal ophthalmoplegia due to autonomic dysfunction) uncommon in snakebite (Azad et al. 2013; Smith and Delargy 2005; Agarwal et al. 2006). It can be of three types (Azad et al. 2013; Smith and Delargy 2005): *classic* LIS, in which patient has quadriplegia and anarthria with preservation of consciousness and vertical eye movements; *incomplete* LIS is similar to classic except remnants of voluntary movement other than vertical eye movement are present; and in *total* LIS, there is total immobility and inability to communicate, with preserved consciousness. Usual causes of LIS are stroke, trauma, or encephalitis of ventral pontine area,

but it can also be caused by extensive bilateral destruction of corticobulbar and corticospinal tracts in the cerebral peduncles (Smith and Delargy 2005). LIS can also be caused by peripheral causes such as severe Guillain–Barre syndrome, neuromuscular junction blockade (myasthenia gravis, toxins, and snakebite), etc. (Smith and Delargy 2005). Duration of LIS varied from 30 h to 6 days (Azad et al. 2013). The common krait is a nocturnally active snake with painless bite; so many patients with neurological manifestations present to the emergency without history of snakebite (Azad et al. 2013).

LIS in snakebite occurs due to neuromuscular paralysis of voluntary muscles which in turn is caused by neuromuscular transmission blockade (krait venom acts presynaptically, while cobra venom acts postsynaptically) (Warrell 2010; Azad et al. 2013). Irreversible binding of the toxin to presynaptic portion makes clinical recovery slow in krait envenomation as recovery occurs only with the formation of new neuromuscular junctions (Warrell 2010; Azad et al. 2013). Physicians should recognize the **LIS**, so as to prevent the dangerous error of diagnosing brain death. Both internal and external ophthalmoplegia can occur in snake envenomation, which would mimic brain death in many ways, thus prompting an intensivist to consider withdrawing ventilator support, which would be disastrous to the patients (Sodhi et al. 2012). Supportive care needs to be continued until the effects of the venom wear off with excellent outcomes. The diagnosis of brain death includes documentation of coma, lack of brain stem reflexes, and apnea in the absence of conditions that mimic brain death like severe electrolyte and acid–base disturbances, drug intoxication, neuromuscular blocking agents, etc. (Nakagawa et al. 2011). In fact, confirmatory tests like cerebral angiography, electroencephalography, etc., are considered in situations like LIS, where a misdiagnosis of brain death is possible (Agarwal et al. 2006).

Snakebite-Induced Coagulopathy: Disseminated Intravascular Coagulation (DIC) or Venom-Induced Consumption Coagulopathy (VICC)

Pathophysiology

The most common coagulopathy associated with snake envenoming is a procoagulant or consumption coagulopathy (Isbister 2010). The diagnostic features of DIC are problematic for snakebite because an elevated D-dimer, prolonged prothrombin time, and low fibrinogen are features of VICC that are always present, and thrombocytopenia is often associated with VICC, as well. But, VICC is not characterized by the other significant features of DIC, such as evidence of systemic microthrombi and end-organ failure. Patients with VICC may have no other systemic manifestations of illness and appear asymptomatic (Isbister 2010; Isbister 2009). The course of VICC differs from DIC with the rapid onset of the coagulopathy within hours of the snakebite and the resolution over 24–48 h. VICC can either resolve spontaneously or after antivenom therapy over 24–48 h (Isbister 2009, 2010).

The pathogenesis of initiation of coagulation activation in VICC differs from DIC (Isbister 2010). In DIC, the activation of the coagulation system leading to thrombin generation is mediated by the tissue factor/factor VIIa pathway, which is not balanced by anticoagulant system due to impairment in the major anticoagulant pathways. In addition, there may be impairment of the fibrin removal due to depression of the fibrinolytic system. This series of events does not occur in VICC. In contrast, the initiation of coagulation activation in VICC is usually due to the action of a snake procoagulant toxin at one point in the coagulation pathway and not via the tissue factor/factor VIIa pathway (Isbister 2009). Depending on which part of the pathway the toxin acts, the resultant coagulopathy can range from mild, with thrombin-like enzymes (vipers) that cause a partial or complete consumption of fibrinogen alone, to more severe coagulopathy, seen with prothrombin activators (elapids), factor X activators (Russell's viper) that cause severe deficiencies of fibrinogen, factor V, factor VIII, and factor X activation, respectively (Swenson and Markland 2005).

Clinical Manifestation and Differentiation

A significant difference between VICC and DIC is that, in VICC, there is no obvious fibrin deposition, microvascular thrombotic obstruction, and resultant end-organ damage or organ failure. VICC is usually only complicated by bleeding, whereas DIC is characterized by both end-organ failures resulting from microvascular thrombi as well as bleeding complications. Metalloproteinase prothrombin activators activate the coagulopathy pathway and simultaneously cause injury to the blood vessel integrity, increasing the risk of bleeding. This differs from DIC where there is no such injury to vessel walls (Isbister 2010).

Current consensus diagnostic criteria for DIC when applied to VICC often produce a score of 5 and therefore suggest overt DIC (Kumar and Gupta 2008; Levi 2004). VICC presents possibly a unique clinical syndrome that will often meet the currently accepted diagnostic criteria for DIC, but is clearly different based on the current understanding of the pathophysiology of conditions, the time course, and the prognosis. This is the reason that it has been believed for so long that snakebite can cause DIC. However, it is also the reason why it is important that the term VICC is used to clarify the clinical syndrome (Isbister 2010).

Treatment Issues of VICC

Coagulopathy is usually a direct effect of toxins in the venom. It follows that the removal of those toxins, using antivenom, should allow the return to normal homeostasis. Of course, antivenom cannot repair injuries caused by the coagulopathy, such as critical organ damage, nor can it "switch off" secondary phenomena activated during the coagulopathy, such as hyperfibrinolysis (White 2005). Once coagulopathy is detected, it is therefore necessary to give the correct antivenom as early as possible, in sufficient amounts, and be prepared to give supplementary doses if required. Equally, it is important to avoid giving other therapies that may exacerbate the coagulopathic process like heparin, warfarin, FFP, and cryoprecipitate (White 2005). In snakebite coagulopathy,

such treatments are generally ineffective and may be potentially dangerous. Heparin will not "switch off" the pathologic VICC, so will not help, yet may induce its own degree of pathologic changes to clotting, thus making things worse. The addition of FFP or cryo may only add fuel to the venom-stoked fire, especially with procoagulant unless all venom has been removed (White 2005; Isbister 2010).

Snakebite-Induced Thrombotic Microangiopathy (TMA)

In a proportion of patients with VICC, a clinical syndrome consistent with thrombotic microangiopathy has been reported and is characterized by acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia (Isbister 2009, 2010; Morling et al. 1989). The etiology of TMA associated with snake envenoming remains unclear, and even a good definition is currently unavailable. This thrombotic microangiopathy appears to only occur in conjunction with VICC but several different snakes worldwide including vipers and elapids. Consistent with thrombotic microangiopathy, it progresses despite the resolution of the coagulopathy, suggesting a different but related process (Isbister 2009, 2010). The existence of the overlapping clinical syndromes of VICC and thrombotic microangiopathy in snake envenoming is the likely cause for the mistaken idea that snakebite causes DIC (Isbister 2010).

Treatment Issues of Snakebite-Induced TMA

The treatment of TMA associated with snake envenoming is controversial, and it is unclear that approaches based on most forms of TMA are appropriate (Isbister 2010). Recommendations for the administration of FFP and plasmapheresis are problematic. In most patients, TMA associated with snake envenoming is resolved with supportive care, and in many cases it is not recognized as such (Isbister 2010).

Acute Kidney Injury

Pathophysiology

Renal involvement following snakebite envenomation has been reported with many snake species, but the most severe of these, i.e., acute renal failure, is seen predominantly with the bite of the vipers. In India, this is usually seen with Russell's viper and the saw-scaled viper that are the most widespread viper species in India (Athappan et al. 2008). The frequency of snakebite as a cause of acute renal failure has been variably reported as 1.2 % in Thailand, 3–32 % in India, and as high as 40 % in Myanmar (Athappan et al. 2008; Waikhom et al. 2013; Golay et al. 2012). Snake venom can cause cellular injury through enzymes, polypeptide toxins, cytokines, and mediators. Snakes that cause renal failure are either myotoxic or hemotoxic snakes causing rhabdomyolysis, intravascular hemolysis, disseminated intravascular coagulation (DIC), or hemorrhage (Golay et al. 2012).

Clinical Manifestation

Renal failure occurs a few hours to several hours after the bite, with a rapid rise of blood urea nitrogen and serum creatinine. Nonoliguric renal failure is not uncommon and averages 2–3 weeks in duration. The renal histology mainly consists of acute tubular necrosis (ATN) in 73 % and acute interstitial nephritis (AIN) in 5–15 %, while glomerular changes are rare (Athappan et al. 2008; Golay et al. 2012). Degeneration, necrosis, and regeneration of tubular epithelial cells have been seen in bite by either hematotoxic or myotoxic snakes (metalloproteases and phospholipase A2), in addition to interstitial edema and cellular infiltration, which consist of lymphocytes, plasma cells, and mononuclear phagocytic cells (Athappan et al. 2008; Golay et al. 2012; Sitprija 2006). Immunologic mechanism plays a minor role in the pathogenesis of AKI (Sitprija 2006). However, diffuse AIN out of proportion to tubular degeneration has been rarely seen in Russell's viper bite (Waikhom et al. 2013; Golay et al. 2012). Although many factors can contribute to the development of AIN, snake venom has been postulated to directly result in the development of the interstitial inflammation via various cytokines, mediators, and adhesion molecules (Athappan et al. 2008; Golay et al. 2012).

Nevertheless, the role of direct nephrotoxicity of snake venom is still not clear, but hypersensitivity to venomous or antivenom protein has been occasionally found to cause acute renal failure (Golay et al. 2012). The contribution of the antivenom to the development of the AIN needs to be determined as there is no significant data to implicate this relationship. The histological finding of AIN correlated with poor prognosis for chronic kidney disease (CKD) (Athappan et al. 2008).

Risk Factors and Treatment Issues of Snakebite-Induced AKI

Independent risk factors associated with renal failure and dialysis requirement are, delayed administration of an adequate dose of ASV (hazard ratio of two times), presence of cellulitis, and bite during the winter months, low platelet count, bleeding, intravascular hemolysis and hypotension at presentation (Athappan et al. 2008; Waikhom et al. 2013). Management of snakebite induced renal failure is primarily of supportive care along with timely administration of ASV. Rather than being concentrated in referral hospitals, ASV should be made available in all emergency and primary health centers near local communities.

Compartmental Syndrome

Envenomation of a limb can lead to cutaneous necrosis, compartment syndrome, and even necrotizing fasciitis (Hamdi et al. 2010). Compartment syndrome in snakebite is a rare phenomenon particularly in children and it usually affects the upper limb (Hamdi et al. 2010; Pietrangiolillo et al. 2012). In fact, the same amount of venom affects children more severely than adults because of the reduced total dilution volume in children (Pietrangiolillo et al. 2012). The principal local effect of venom is edema which occurs within 2 h after a bite and intensifies during the

following 3 days (Hamdi et al. 2010). Swelling and vasoconstriction lead to ischemia and compromise the vitality of the limb.

Consider compartment syndrome if any of the following are noted (**6 Ps**): pain on passive stretching, pain out of proportion, pulselessness, pallor, paresthesia, and paralysis. However, detection of arterial pulses by palpation or Doppler ultrasound probes does not exclude intracompartmental ischemia. The most reliable test is to measure intracompartmental pressure directly through a cannula introduced into the compartment and connected to a pressure transducer or manometer. McQueen and Court-Brown consider that a difference of 30 mmHg between diastolic and compartment pressure is the threshold of fasciotomy (McQueen and Court-Brown 1996). Early treatment with antivenom remains the best way of preventing irreversible muscle damage.

Management of Snake Envenomation

The following steps or stages are often involved in the management of snake envenomation: (i) first-aid treatment, (ii) transport to hospital, (iii) rapid clinical assessment and resuscitation, (iv) detailed clinical assessment and species diagnosis, (v) diagnosis and investigations/laboratory tests, (vi) antivenom treatment, (vii) observing the response to antivenom, (viii) decision about further dose(s) of antivenom, (ix) supportive care, (x) treatment of the bitten part, (xi) rehabilitation, and (xii) treatment of chronic complications. Treatment in the field consists of safe identification of the species of snake whenever possible and rapid transport of the patient to the nearest health-care facility (Simpson 2007).

First-Aid Treatment

Aims of first-aid treatment include (i) attempt to retard systemic absorption of venom, (ii) preserve life and prevent complications before the patient can receive medical care, (iii) control distressing or dangerous early symptoms of envenoming, (iv) arrange the transport of the patient to a place where they can receive medical care, and (v) to do *NO HARM* (Fig. 17.2) (Simpson 2007). The priorities for the treatment of people bitten by snakes are the transport to medical care as quickly as possible irrespective of the nature of bite and symptoms and the delay of life-threatening shock and respiratory paralysis until professional care is available (Warrell 2010; Simpson 2007). First-aid treatment is carried out immediately or very soon after the bite, before the patient reaches a dispensary or hospital. It can be performed by the snakebite victim or by anyone else who is present and able.

In most of the tropical developing countries, traditional healers undertake the immediate treatment of snakebite; unfortunately these traditional, popular, available, and affordable first-aid methods have proved to be useless or even frankly dangerous (Fig. 17.2). These methods include: tying tight bands (tourniquets)

Fig. 17.2 "DO NO HARM" during first-aid treatment

around the limb, making local incisions or pricks/punctures ("tattooing") at the site of the bite or in the bitten limb, attempts to suck the venom out of the wound, use of (black) snake stones, electric shock, and topical instillation or application of chemicals, herbs, or ice packs. Local people may have great confidence in traditional (herbal) treatments, but they must not be allowed to delay medical treatment or to do harm. Traditional treatment delays presentation, distorts the clinical picture, and can cause bleeding, infection, gangrene, and other complications (Simpson 2007).

Tying tight bands (tourniquets) around the limb remains as the main first-aid method adopted by victims. Research shows that tourniquets expose the victim to the risk of ischemic damage, potentially increase the necrotic action of the venom, present dangers of neurotoxic blockage and clotting when the tourniquet is released, and are ineffective in retarding venom flow. Hence, early transport to hospital or dispensary should be encouraged and ineffective and harmful traditional treatments should be discouraged. In light of these problems, the pressure immobilization method (PIM) was developed in Australia in the late 1970s and was advocated as a reliable technique to inhibit venom flow into the system (Warrell 2010; Simpson 2007).

Pressure Immobilization Method (*PIM*) (Simpson 2007): Unless a bite by a neurotoxic elapid can be excluded, the bitten limb should be bandaged at a pressure of about 50–70 mmHg and immobilized with a splint (pressure immobilization), or a pressure pad should be applied at the site of the bite in the same way as for a sprain. Obstruction of lymphatic and venous drainage delays systemic absorption of

large molecular weight neurotoxins without the use of tight tourniquets, which are dangerous. However, the clinical efficacy of these methods has not been adequately investigated. Further research demonstrated that the required bandage pressure varied between the upper and lower limbs, that lay people and emergency room physicians were unable to apply the technique correctly in a simulated environment, and that the requirement for complete immobilization was the key. Walking for more than 10 min, even if the bandage was applied to the correct range of pressure, invalidated the effect of the bandage.

Do It RIGHT

Both techniques and PIM require the use of equipment, diminishing their practicability in developing countries, and pressure immobilization has been difficult to teach and apply effectively. In view of these limitations both tourniquets and PIM are rejected for use in India by "Pediatric Management of snakebite: The National Protocol" (Simpson 2007). The first-aid treatment is based around the mnemonic: "*Do it R.I.G.H.T.*" as recommended by The National Protocol. It consists of: *R* = Reassure the patient. Seventy percent of all snakebites are from nonvenomous species. Only 50 % of bites by venomous species actually envenomate the patient. *I* = Immobilize in the same way as a fractured limb. Children can be carried. Use bandages or cloth to hold the splints, not to block the blood supply or apply pressure. Do not apply any compression in the form of tight ligatures, they do not work and can be dangerous! *G.H* = Get to the Hospital immediately. Traditional remedies have *NO PROVEN* benefit in treating snakebite. *T* = Tell the doctor of any systemic symptoms such as ptosis that manifest on the way to hospital. Ideally, tight bands, bandages, and ligatures if applied should not be released until the patient is under medical care in the hospital, resuscitation facilities are available, and antivenom treatment has been started.

Identification of Envenoming Snake Species

The enormous interspecies diversity of snake venom actions is ignored in reports of unidentified snakebites. Such descriptions are as futile as those of cases of undiagnosed fever. Attempts to capture or kill the snake that has bitten someone are dangerous and ecologically destructive and should be discouraged (Warrell 2010). However, even if the snake is available for examination, it might be misidentified, leading to inappropriate treatment. Expert herpetologists have made fatal errors of species recognition. Identification of the species on the basis of descriptions provided by the victims or their companions or recognition from pictures is often unreliable. A useful method is to distinguish *clinical syndromes* of envenoming by analysis of a series of reliably identified bites (Fig. 17.3). When the identification of the snake species cannot be confirmed by examination by an

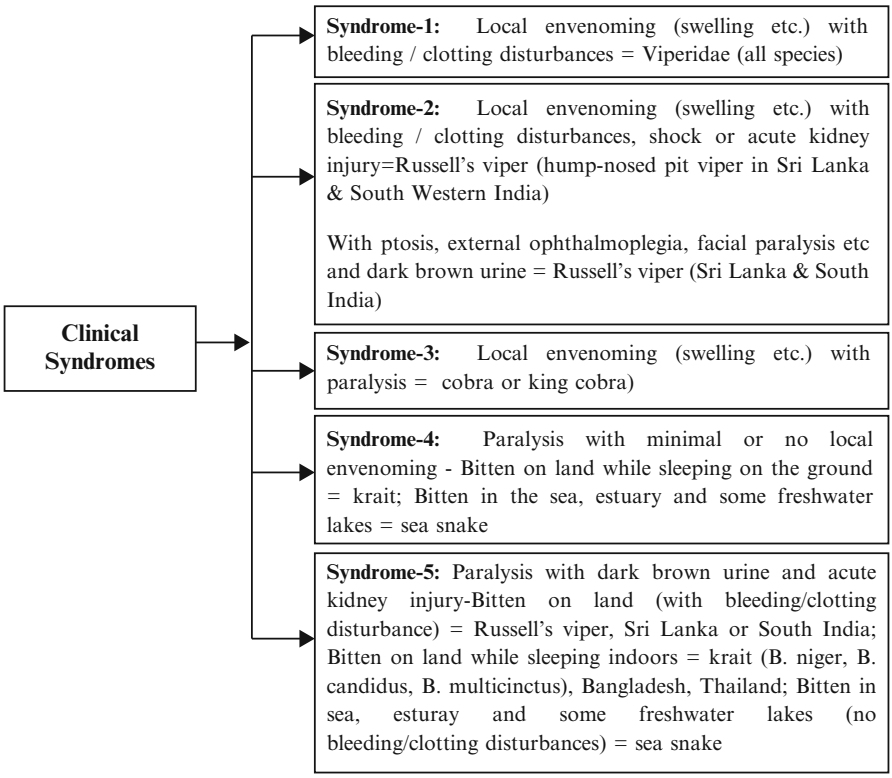

Fig. 17.3 Approach based on clinical syndrome

expert, indirect confirmation is possible by immunological detection of toxin antigens in the victim's blood or tissue fluids. A limitation of the use of immunoassays is that venom antigens differ in their immunogenicity (Warrell 2010).

Diagnosis and Testing

Bite marks to determine whether the biting species was venomous or nonvenomous are of *no* use (Simpson 2007). Many venomous species are in possession of more than one set of fangs, and nonvenomous species can leave just two punctures from enlarged teeth, which can appear to be fang-like. The diagnosis of snake envenomation was based on one or more of the following features: history of snakebite; presence of fang marks; presence of local manifestations, such as pain and swelling at the site of the bite, or systemic manifestations, such as spontaneous bleeding or features of neurotoxicity; and/or if the dead snake was brought in for identification.

Twenty-Minute Whole Blood Clotting Test (20WBCT)

It is a very useful and informative bedside standard test for coagulopathy in the management of snake envenomation (Warrell 2010; Simpson 2007). It is simple to carry out but crucially requires a clean, new, and dry test tube. A 2 ml of fresh venous blood is left undisturbed for 20 min and then gently tilted once. If the blood is still liquid (*unclotted*) and runs out, the patient has hypofibrinogenemia (*incoagulable blood*) as a result of venom-induced consumption coagulopathy. In India, incoagulable blood is diagnostic of a viper bite and rules out an elapid bite. If the vessel used for the test is not made of ordinary glass, or if it has been cleaned with detergent, its wall may not stimulate clotting of the blood sample (surface activation of factor XI – Hageman factor) and test will be invalid. If there is any doubt, repeat the test in duplicate, including a *control* (blood from a healthy person such as a relative).

Other Tests

Hematological Abnormalities: A transient increase of hemoglobin (Hb) indicates hemoconcentration resulting from a generalized increase in capillary permeability (e.g., in Russell's viper bite). More often, there is a decrease in Hb reflecting blood loss or, in the case of Indian and Sri Lankan Russell's viper bite, intravascular hemolysis. Decreased platelet count may be seen in the victims of envenoming by vipers and Australasian elapids. An early neutrophil leukocytosis is evidence of systemic envenoming from any species. On blood film, fragmented red cells ("helmet cell," schistocytes) are seen when there is microangiopathic hemolysis. Plasma/serum may be pinkish or brownish if there is gross hemoglobinemia or myoglobinemia.

Biochemical Abnormalities: Aminotransferases and muscle enzymes (creatine kinase, aldolase, etc.) will be elevated if there is severe local muscle damage or, particularly, if there is generalized muscle damage (sea snake and Sri Lankan and South Indian Russell's viper bites). Mild hepatic dysfunction is reflected in slight increases in other serum enzymes. Bilirubin is elevated following massive extravasation of blood. Potassium, creatinine, urea, or blood urea nitrogen levels are raised in the renal failure of Russell's viper and hump-nosed viper bites and sea snakebites. Early hyperkalemia may be seen following extensive rhabdomyolysis in sea snakebites. Bicarbonate will be low in metabolic acidosis (e.g., renal failure).

Anti-Snake Venom (ASV)

ASV is the only specific antidote to snake venom. A most important decision in the management of a snakebite victim is whether or not to administer antivenom. First introduced by Albert Calmette at the Institute Pasteur in Saigon in the 1890s for the

treatment of envenoming, it was quickly accepted without formal clinical trials (Warrell 2010). ASV is immunoglobulin [usually pepsin-refined F(ab')2 fragment of whole IgG] purified from the plasma of a horse, mule or donkey (equine), or sheep (ovine) that has been immunized with the venoms of one or more species of snake (Warrell 2010). "Specific" antivenom implies that the antivenom has been raised against the venom of the snake that has bitten the patient and that it can therefore be expected to contain specific antibody that will neutralize that particular venom and perhaps the venoms of closely related species (paraspecific neutralization). *Monovalent* (*nonspecific*) antivenom neutralizes the venom of only one species of snake. *Polyvalent* (*polyspecific*) antivenom neutralizes the venoms of several different species of snakes, usually the most important species, from a medical point of view, in a particular geographical area. In India, ASV (Serum Institute of India, Pune) is polyvalent venom which contains antisera against *Naja naja*, *Bungarus caeruleus*, *Vipera russelli*, and *Echis carinatus*. It is available in both liquid and lyophilized form (Simpson 2007).

ASV Administration Criteria

ASV treatment should be given as soon as it is indicated. ASV should be given only to patients in whom its benefits are considered likely to exceed its risks. Since ASV is relatively costly and often in limited supply, it should not be used indiscriminately. The prophylactic use of ASV should be avoided due to inherent risk of hypersensitivity reaction (Simpson 2007). Symptomology is no help as a means of determining severity of envenomation as it is too dynamic and constantly evolving.

Essentially systemic envenomation will be evident from the 20WBCT, signs of spontaneous bleeding, or by visual recognition of neurological impairment such as ptosis. Severe local symptoms are defined as swelling rapidly crossing a joint or involving half the bitten limb, in the absence of a tourniquet. Once the tourniquet has been removed for more than 1 h, if the swelling rapidly continues, this should be viewed as venom generated and not due to the continuing effect of the tourniquet. Isolated local swelling is not grounds for administering ASV. It may reverse systemic envenoming even when this has persisted for several days or, in the case of hemostatic abnormalities, for two or more weeks. Therefore, it is appropriate to give ASV for as long as evidence of the coagulopathy persists. Whether ASV can prevent local necrosis remains controversial, but there is some clinical evidence that, to be effective in this situation, it must be administered within the first few hours after the bite.

ASV Doses and Administration

The initial dose of ASV to be given to a patient has been the subject of much debate. Published research has indicated that Russell's viper injects on average 63 mg (SD 7 mg) of venom in the first bite to both adults and children (Warrell 2010; Simpson 2007). Hence, the initial dose should be calculated to neutralize the average dose of venom injected. This ensures that the majority of victims should be covered by the initial dose and keeps the cost of ASV to acceptable levels. As

each vial of polyvalent ASV neutralizes 6 mg of Russell's viper venom, the initial dose is 8–10 vials for both adults and children. A maximum ASV dose is of around 25 vials because range of venom injected was shown to be 5–147 mg. There is no good evidence to suggest children should receive either more ASV because of body mass or less in order to avoid adverse reactions (Simpson 2007).

Reconstituted ASV is diluted in 5–10 ml/kg of body weight of normal saline or 5 % dextrose or lactated Ringer's solution and should be administered over one hour at constant rate with hemodynamic monitoring because slow (over 120 min) or rapid (over 20 min) infusion would not reduce the rate of severe systemic hypersensitivity reactions. There is no benefit in administering each dose over longer periods, and indeed lengthening the period before the ASV is able to neutralize the venom is counterintuitive (Simpson 2007).

Adverse Reactions of ASV

Adverse reactions, either anaphylactic or pyrogenic, have often been identified as reasons not to administer ASV in smaller local hospitals/dispensary. The fear of these potentially life-threatening reactions has caused reluctance among some doctors to treat snakebite (Simpson 2007). However, if handled early and with the primary drug of choice, these reactions are easily surmountable and should not restrict doctors from treating snakebite. Early intervention against these kinds of reactions has been shown to have more positive outcomes. Patients should be monitored closely as there is evidence that many anaphylactic reactions go unnoticed.

ASV should be discontinued, and administration of adrenaline at a dose of 0.01 mg/kg of body weight IM should be given if any of following *first signs* appeared: urticaria, itching, fever, shaking chills, nausea, vomiting, diarrhea, abdominal cramps, tachycardia, hypotension, bronchospasm, and angioedema. In addition, 2 mg/kg of hydrocortisone IV and 0.2 mg/kg of antihistamine IV should be administered to provide long-term protection against anaphylactic reactions.

A proportion of patients, usually more than 10 %, develop a reaction either early (within a few hours) or late (5 days or more) after being given ASV. Recent systematic review and meta-analysis found substantial beneficial effect of adrenaline premedication, but a marginal benefit with the combination of antihistamines and corticosteroids premedications used against early adverse reaction (Isbister et al. 2012; Habib 2011). Once the patient has recovered, the ASV can be restarted slowly for 10–15 min, keeping the patient under close observation. Then the normal drip rate should be resumed. *ASV test doses have been abandoned*. They have no predictive value in anaphylactic or late serum reactions and may pre-sensitize the patient to the protein. Inappropriate use of ASV should be strongly discouraged as they expose patients who may not need treatment to the risks of ASV reactions, and they also waste valuable and scarce stocks of ASV. To retain their full potency within the limits of stated expiry dates, lyophilized ASV (shelf life about 5 years) should be stored at below 25 °C and liquid ASV (shelf life 2–3 years) should be stored at 2–8 °C and not frozen.

Trial of Anticholinesterase

Anticholinesterase drugs have a variable, but potentially very useful effect in patients with neurotoxic envenoming, especially those bitten by cobras (Watt et al. 1989; WHO/SEARO 1999). A trial of anticholinesterase (e.g., "Tensilon test") should be performed in every patient with neurotoxic envenoming, as it would be in any patient with suspected myasthenia gravis. However, this should not delay ASV treatment or endotracheal intubation. Patients must be observed closely as they may deteriorate while the trial of anticholinesterase is being carried out.

Atropine sulfate (50 μg/kg) or glycopyrronium is given by intravenous injection followed by neostigmine bromide by intramuscular injection 0.04 mg/kg. Short-acting edrophonium chloride (Tensilon) is ideal for this test but is rarely available in the region. It is given by slow intravenous injection in dose of 0.25 mg/kg. The patient is observed over the next 30–60 min (neostigmine) or 10–20 min (edrophonium) for signs of improved neuromuscular transmission. Ptosis may disappear and ventilator capacity (peak flow, FEV-1 or maximum expiratory pressure) may improve. Patients who respond convincingly can be maintained on neostigmine methylsulfate, 0.01–0.04 mg/kg every 2–4 h for 24 h by intramuscular, intravenous, or subcutaneous injection together with atropine to block muscarinic side effects.

Repeat Doses of ASV

In anti-hemostatic bites, once the initial dose has been administered over 1 h, no further dose of ASV is given for 6 h (Simpson 2007). This reflects the period the liver requires restoring clotting factors (WHO/SEARO 1999). Consider a repeat dose of ASV if there is persistence or recurrence of blood incoagulability after 6 h or of bleeding after 1–2 h and if there are deteriorating neurotoxic or cardiovascular signs after 1–2 h. In the case of neurotoxic bites, once the first dose has been administered, and a neostigmine test given, the victim is closely monitored. If after 1–2 h the victim has not improved or has worsened, then a second and final dose should be given. At this point the victim will have received sufficient neutralizing capacity from the ASV and will either recover or require mechanical ventilation; in either event further ASV will achieve nothing (Simpson 2007; WHO/SEARO 1999).

Supportive Care

ASV treatment can be expected to neutralize free circulating venom, prevent progression of envenoming, and allow recovery. However, these processes take time and the severely envenomed patient may require life support systems such as treatment of shock, assisted ventilation, and renal dialysis until the severely damaged organs and tissues have had time to recover.

Follow-Up

Close follow-up is mandatory in those patients discharged early (24–48 h) from hospital. Patient should be advised to return to hospital immediately if there is any worsening of symptoms such as bleeding, pain or swelling at the site of bite, difficulty in breathing, altered sensorium, etc. The patients should also be explained about late manifestation of ASV reaction like serum sickness which may manifest after 5–10 days of administration of ASV.

Conclusion and Future Direction

Snake envenomation is a neglected problem in tropical countries. The first aid treatment should avoid compression and emphasize immobilization and ensure speedy transport to hospital. Early administration of ASV is the only beat strategy to prevent mortality and morbidity due to snake envenomation. There is more for governments to do in ensuring that all doctors are aware of the best methods of treating snakebites in local settings. Allocating resources in a sustainable manner and ensuring availability of ASV in primary health-care setting and enhancing the confidence levels of doctors to administer ASV at a facility nearest to the occurrence of bite, management of adverse reactions, and timely transport with appropriate airway support are indeed important. It deserves further investigation in relation to adjunctive therapy for snakebite-induced acute renal failure and mechanisms of snake venom causing various complications like coagulopathy and thrombotic microangiopathy (Levi 2004; Sitprija 2006).

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Snake Venom Detection Kit (SVDK): Update on Current Aspects and Challenges

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Abstract

Snakebite is a medical emergency causing considerable morbidity and mortality worldwide, particularly in the tropics. Snake venom components are known to vary greatly leading to varied clinical manifestations following snakebite. The success of antivenom therapy, which is the mainstay of therapy, usually depends on the snake species involved, and uncertainties concerning the species involved remain a major hurdle in effective management of snakebite. Therefore, proper identification of snake species is of prime importance, consequently leading to the development of the Snake Venom Diagnostic Kit (SVDK). Over the years, various detections tests have been developed, with the immunological-reaction-based **enzyme-linked immunosorbent assay** (ELISA) method being the most widely used. However, in recent times various other techniques, such as optical immunoassays (OIA), venom/antibody microarray assay, PCR based assays, etc., are also being developed with much more promise in real-time applications. Furthermore, the tests tend to be highly species-specific, reliable, sensitive, rapid, inexpensive, stable, simple, and portable for field use. It is desirable that each country develops and optimizes its own regional species-specific diagnosis kits for effective management of snakebite. Considering doubts in the commercial viability of developing SVDK, more public or private partnerships have to be developed and nurtured. This work attempts to summarize existing techniques of snake venom detection in current use, especially their advantages and disadvantages. It also focuses on recent developments and discusses the present challenges to the development and application of SVDK for successful clinical usage in the future.

Introduction

Snake Envenomation

Snakebite is a significant public health problem causing considerable morbidity and mortality worldwide, particularly in the tropics. Snakebite is now recognized as a Neglected Tropical Disease (NTD) by the World Health Organization (WHO) (Warrell et al. 2013). An accurate measure of the global burden of snakebite envenoming remains elusive (Alirol et al. 2010; Kasturiratne et al. 2008;

Mohapatra et al. 2011; Warrell et al. 2013) and the vast majority of snakebite-induced deaths occur in Asia (15,400–57,600 deaths per annum) and Sub-Saharan Africa (3,500–32,100 deaths per annum) (Kasturiratne et al. 2008). According to World Health Organization (WHO) direct estimates, the highest rate of mortality due to venomous snakebite in the world is between 35,000–50,000 per annum in India (Kasturiratne et al. 2008; Alirol et al. 2010; Mohapatra et al. 2011; Warrell et al. 2013). Most estimates of incidence, morbidity, and mortality associated with venomous snakebite are extrapolations from a few regional studies (Kasturiratne et al. 2008). The actual incidence and burden would only be known from community-based studies, as most of the snakebites are not reported or treated by traditional healers (Kasturiratne et al. 2008; Warrell et al. 2013).

Snakes are found in various part of the world, except in Artic and Antarctic regions. About 2,000 species of snakes are distributed around the world, of which 256 are venomous. Snakes are classified under various families and the venomous snakes are broadly classified into (i) Elapidae – e.g., *Naja naja* (Common cobra), *Ophiophagus hannah* (King cobra), etc.; (ii) Viperidae – e.g., *Daboia russelii* (Russell's viper), etc.; (iii) Crotalidae – e.g., *Trimeresurus malabaricus* (Malabar pit viper), etc.; and (iv) Hydrophidae – e.g., *Aipysurus eydouxii* (Marbled sea snake), etc. Snakes are also classified as hemotoxic (primarily affecting hemostatic components) and neurotoxic (primarily affecting neuronal components) snakes, based on the clinical manifestations they exhibit upon bite. Therefore, it can be generalized broadly that the principle effects of envenomation are on the nervous system, kidneys, heart, liver, blood coagulation system, vascular endothelium, and local effects at the site of bite (Alirol et al. 2010). However other complications also may be observed (Alirol et al. 2010). The snake venom, which is known to be responsible for bringing about the clinical manifestations of a snake envenomation, is known to consist of a diverse and synergistically acting cocktail of biologically active molecules that evolved with precise function to intervene in biological systems, thus helping in prey acquisition and its subsequent digestion (Aird 2002; Dhananjaya and D'Souza 2010). Snakes are known to vary in their composition of venoms based on their sex, diet, and geographical locations, as well as seasonal factors (Alape-Giron et al. 2008; Chippaux et al. 1991; Daltry et al. 1996). This variation is known to influence the observed clinical symptoms of envenomation, such as neurotoxicity, myotoxicity, hemotoxicity, anticoagulant, procoagulant, hemorraghic, necrosis, renal damage, and muscular paralysis in prey/victims (Alirol et al. 2010). This variation in composition influences the presentation, morbidity and mortality of snakebite victims, thus posing certain challenges in the management of snakebite.

Management of Snakebite

In the treatment of snake envenomation, specific antivenom therapy is the mainstay of therapy, and its selection usually depends on the species diagnosed. The species diagnosis, in the absence of the culprit snake being brought in or identified, is

usually based on configuration of the bite, differentiating the clinical manifestations, knowledge of species commonly recognized at a given geographical area, and from information gathered from the victim or witness. In many parts of the world, differentiating the culprit species is based on the clinical manifestations. However, this is difficult due to the fact that toxins contained in venoms of different species are often physiochemically and pharmacologically similar and therefore may elicit similar clinical effects. For example, venoms of *Naja naja siamensis, Ophiophagus hannah*, and *Bungarus fasciatus* all contain postsynaptic neurotoxins inhibiting neuromuscular transmission leading to similar predominant clinical features, namely paralysis, respiratory failure, and eventually death (Chang 1979). Even when the culprit species of snakes is brought, there is a chance of misidentification and, thus, misadministration of antivenom leading to complications as observed in many cases in the past (Joseph et al. 2007). Further, it is observed that the same species (morphological similar) exhibits different clinical effects due to the variation of venom components based on variations in geographic locations (Boldrini-França et al. 2010). Due to the limitations for accurate identification of snake species, clinicians may find it difficult to administer appropriate antivenom upon presentation. In the absence of rapid, sensitive, and dependable diagnostic tests for the identification of species responsible for envenomation; the polyvalent antiserum raised against a mixture of venoms is commonly used for treatment. Although it might be effective, recovery is slow and large volumes are needed for treatment of any particular snakebite. Additionally, it is associated with severe allergic reactions, serum sickness, and other side effects (Thachil et al. 1992; Warrell et al. 2013). Furthermore, production of polyvalent serum is a long, complex, and expensive process (Warrell et al. 2013). Considering these limitations and drawbacks of the snake venom antiserum (SVA) treatment, establishing identity of the species of snake inflicting the bite would facilitate administration of monovalent (species-specific) antiserum for rapid and effective recovery with reduced side effects.

The fact that clinical manifestations alone are not reliable factors because of the overlap in symptoms due to variations in venom components detection of snake venom and venom antibodies in body fluids plays an important role in management of snake envenomation. It is believed that the lack of a rapid, sensitive, and reliable diagnostic test for identification of snake species has limited the potential use of antivenoms, which has caused delay in treatment and resulted in severe disability or death in many cases (Alirol et al. 2010; Ratanabanangkoon et al. 1987; Selvanayagam and Gopalakrishnakone 1999; Theakston 1983; Warrell et al. 2013). Therefore, it is to be noted that whatever might be the choice of therapy, establishing for assisting in the correct administration of poly/monovalent antivenom for rapid and/or more effective prognosis and recovery of the victim with minimal side effects. Some cheap and reliable diagnostic assays exist for snake venom, i.e. 20WBCT used in West Africa to diagnose *Echis ocellatus*, *Bothrops sp*. in South America, *Echis carinatus* in South Asia, and Malayan pit viper in South-east Asia envenoming. However when it comes to various snake species, applying diagnostic assays is limited only to species that cause hemotoxic bites and,

therefore, is restricted in its application to a few isolated places due to the great diversity of snakes and its varied clinical manifesto that are known to exhibited due to bite at other regions. Therefore, because of the inability to achieve a prompt, unequivocal, and accurate identification of the species that caused the envenomation in most instances, it is highly desirable that an optimal rapid, sensitive, reliable, inexpensive, and portable Snake Venom Diagnostic Kit (SVDK) be available in the field to help properly identify the snake species. Such an SVDK would help in the prognosis, treatment, and improved management of snakebite.

Tests for Detection of Snake Envenomation: Snake Venom Diagnostic Kit (SVDK)

Venoms of many snakes share similar physicochemical and pharmacological properties and exhibit similar clinical manifestations. Detection of specific snake species becomes of paramount importance in effective treatment of snakebite victims by the administration of specific antivenom. It is accepted that, for the success of antivenom therapy in snake envenomation, detection and measurement of snake venom in body fluids is extremely important to assess antivenom effectiveness. Coulter et al. (1974) demonstrated the measurement of snake venom in human, animal tissue and serum with the use of solid-phase radioimmunoassay. Subsequently, over the years various assays and diagnostic tests based on different techniques and principles have been developed (viz., bioassays, immunodiffusion, immunoelectrophoresis, immunofluorescence, haemagglutination, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), optical immunoassay, etc.) with varied degrees of success (Ratanabanangkoon et al. 1987; Selvanayagam and Gopalakrishnakone 1999; Selvanayagam et al. 1999; Theakston 1983). Due to the specific nature of the antigen–antibody interactions, immunological reactions offer better methods for snake venom detection; the ELISA method is the most widely used test (Minton 1987; Ratanabanangkoon et al. 1987; Selvanayagam and Gopalakrishnakone 1999; Theakston 1983). Recently, important contributions have been made to improve the specificity, sensitivity, rapidity, and simplicity of the ELISA methods (Selvanayagam and Gopalakrishnakone 1999). Monoclonal antibodies and affinity-purified venom-specific antibodies are being used to improve ELIZA specificity, and this seems to be ideal for venom detection (Dong le et al. 2003a, 2004; Kulawickrama et al. 2010; Selvanayagam and Gopalakrishnakone 1999). However, various other techniques, such as optical immunoassays, PCR based assays, etc., which are in the developmental stage, are also coming up with a much more promise in real-time application for snake venom detection.

There have been several reports on the detection of snake venoms from various parts of the world, and the techniques that have been used have been extensively reviewed (Minton 1987; Ratanabanangkoon et al. 1987; Selvanayagam and Gopalakrishnakone 1999; Theakston 1983). Therefore, this work attempts to summarize the various techniques available for detection of snake venom, focusing on

their advantages and disadvantages. It also focuses on updating recent developments and discussing the present challenges to developing a SVDK, both within the present context and for its future prospects.

Snake Venom Detection Kit (SVDK)

The snake venom detection kit (SVDK) is primarily a diagnostic tool that is designed and/or developed to help clinicians and health care workers make the right decisions about the most appropriate type of antivenom (Poly/monovalent) to administer to a person who has been bitten and envenomed by a venomous snake. This also helps in prognosis during treatment. Specifically, it is developed to qualitatively and quantitatively identify the presence and type of snake venom (species-specific kit). The main aim of SVDK development is to reduce the time it takes to reach a decision on the appropriate antivenom to administer as this potentially may improve the prognosis and outcome for bitten patients. The principle around which it works is that venom antigen in the body fluids (blood, urine and/or swabs around the bitten region) reacts, which can be measured quantitatively and also identify the specific snake species. This provides an informed choice of appropriate antivenom to be given to the envenomed victim and also helps monitor its effect during therapy. Over the years various assays and diagnostic tests based on various principles have been carried out in an attempt to develop an effective, reliable, sensitive, cost-effective, and portable SVDK. Although there have been several reports on venom detection and assay protocol development from various parts of the world (Bhatti et al. 1993; Chavez-Olortegui et al. 1993; Coulter et al. 1980; Dong le et al. 2002, 2003, 2004; Dong le 2004; Gao et al. 2008; Kittigul and Ratanabanangkoon 1993; Theakston et al. 1977), so far only a few have been effectively developed for its application as field kits. The Commonwealth Serum Laboratories (CSL) SVDK, which has been developed and successfully applied to detect envenomation by common venomous snakes of Australia, helps in administration of the appropriate monovalent antivenom to the affected patients. Based on the specific nature of the antigen–antibody interaction, immunological-reactions-based techniques are known to offer better results for snake venom detection. Hence, our focus is on some of the various immunological techniques that have been developed over the years and on updating recent developments in snake envenomation detection. Table 18.1 shows the various immunoassays developed and sensitivities achieved.

Immunodiffusion

Invented by Oudin and Ouchterlong in the late 1940, this technique is commonly used for its simplicity. The endpoint is dependent on the combination of antigen and antibody forming a visible precipitate. Muelling et al. (1957) first used this technique for the detection of snake venom in tissue at the site of the bite. Over the

Table 18.1 Comparison of sensitivity of various immunoassays for snake venom detection

Assay type	Sensitivity (per ml)	Source	Snake species	Reference
Radioimmunoassay	0.4 ng	Rabbit serum & Urine	*N. scutatus*	Coulter et al. 1974
Reverse latex agglutation	0.16–12 ug	Human serum & wound swab	*B. fasciatus, C. rhodostoma N. hannah, N. kaouthia, T. albolabris, V. russelii*	Chinonavanig et al. 1991
Reverse passive agglutination	2.0 ng	Human serum & wound swab	*B. fasciatus, C. rhodostoma N. hannah, N. kaouthia, T. albolabris, V. russelii*	Kittigul and Ratanabanangkoon 1993
Fluorescence immunoassay	1.5 ng	Tissue homogenates	*N. naja, C. adamentus*	Tiru-Chelvam 1972
Fluorescence immunoassay	0.1 pg	Biological fluids	*V. russelii*	Bhatti et al. 1993
SB based immunofluorescence	5–10 ng	Biological fluids	*N. kaouthia*	Gao et al. 2008
ELISA	1 ng	Human & rat sera	*B. arientanus, C. maculates, E. carinatus, N. haje, N. nigricollis*	Theakston et al. 1977
ELISA	6 ng	Human serum & wound swab	Australian & other snakes	Coulter et al. 1980
ELISA	5 ng	Human blood	Australian snakes	Chandler and Hurrell 1982
ELISA	1 ng	Rabit blood	*V. ammodytes*	Labrousse et al. 1988
ELISA	1 ng	Human blood & Urine	*V. aspis*	Audebert et al. 1992
ELISA	2 ng	Human & Mice sera	*B. atrox, L. Mutamuta*	Chavez-Olortegui et al. 1993
ELISA	0.8 ng	Human plasma	*V. Berus berus*	Sjostrom et al. 1996
ELISA	0.5 ng	Human serum	*Naja atra* (Taiwan)	Huang et al. 2002
AB-ELISA	14.6 ng	Mouse serum	*B. Jararaca*	Barral-Netto et al. 1990
AB-ELISA	1 ng	Biological fluids	*N. Naja, D. russelii, B. Caeruleus, E. Carinatus*	De 1996

(*continued*)

Table 18.1 (continued)

Assay type	Sensitivity (per ml)	Source	Snake species	Reference
AB-ELISA	0.1 ng	Tissue homogenate	*N. Naja, D. russelii, B. Caeruleus, E. Carinatus*	Selvanayagam et al. 1999
AB-ELISA	0.1 ng	Body fluids	*B. jararacussu* and *B. Alternatus*	Heneine et al. 1999
AB-ELISA	0.2–1.6 ng	Body fluids	*T.* popeorum, *C. rhodostoma*, *N. kaouthia* and *O. hannah*	Dong le et al. 2003
AB-ELISA	0.15 ng	Human serum	*Oxyuranus scutellatus*	Kulawickrama et al. 2010
Optical immunoassay (OIA)	0.4– 08 ng	Body fluids	*T. albolabris*, *C. rhodostoma*, *N. kaouthia* and *O. Hannah*	Dong le et al. 2004
AB-OIA	100 pg	Mice whole blood or plasma	*B. multicinctus*	Dong le et al. 2002

years, this technique is used mainly for demonstration of cross-reactivity between snake venoms and antivenoms rather than detection purpose. Tu et al. (2001) used double immunodiffusion in combination with other techniques to confirm the causes of two reported human deaths in a forensic investigation. The sensitivity of the test is around 10 mg/ml of venom.

Advantages:

- The test is a simple and reliable one.
- It's inexpensive as the reagents required are of low cost.
- The test does not require big and special laboratory equipment.
- It can be done with minimum expertise, thus appropriate for use at rural medical centers in developing countries.

Disadvantages:

- Although it is simple and reliable, the test requires a large sample when compared to other techniques.
- The test is not rapid, and the time required for obtaining results is often too long.

- The high level of common precipitating bands between venoms and antibodies in the case of closely related species limits its use in species-specific diagnosis.

Immunoelectrophoresis

This techniques was first used successfully to detect venoms of *B. arientans, C. maculatus, E. ocellatus*, and *E. colaratus*, the four common snakes of Nigeria, in the wound aspirates, blister fluids, sera, and urine samples from 101 snakebite patients (Greenwood et al. 1974). Tu et al. (2001) used this technique in combination with immunodifussion to confirm the causes of human deaths in forensic investigations. The sensitivity of the test was at 10,000 ng/ml.

Advantages:

- The test is a simple and reliable method.
- It is inexpensive because the reagents required are of low cost.
- Comparatively, big and special laboratory equipment is not required to carry out the test.
- The test can be done with minimum expertise, thus appropriate for use at rural medical centers in developing countries.

Disadvantages:

- Although simple and reliable, the test requires a large sample when compared to other techniques.
- It is not rapid, as the time required for results are often too long.
- The high level of common precipitating bands between venoms and antibodies in the case of closely related species limits its use in species-specific diagnosis.

Hemagglutination Assays

A passive hemagglutination assay (PHA) technique was first used to identify *Crotolous viridis helleri* venom with sensitization of sheep red blood cells (Boche and Russell 1968). In this test, the venom/antigen is estimated by examining the reduction in antibody titre due to the ability of the venom to eliminate or decrease the extent of hemagglutination when mixed with antivenom. Kittugul and Ratanabanangkoon (1993) developed a reverse passive hemagglutination assay (PHA) test for the detection of venoms of six major venomous snakes of Thailand. The sensitivity of PHA and reverse PHA are in the order of 1–10 ng/ml of antigen or antibody, respectively. Thus the sensitivity of these assays are 2–3 orders of magnitude greater than immunodiffusion or immunoelectrophoresis and is

comparable to that of ELISA as described by Theakston et al. (1977). Also, the test takes comparatively less time. It is reported that with avian erythrocytes, which are nucleated and settle faster, it can be read within 30 min.

Advantages:

- The test is a reliable, rapid, and simple method.
- It is inexpensive because the reagents required are of low cost.
- Comparatively, big and special laboratory equipment is not required to carry out test.
- It can be done with minimum expertise, thus appropriate for use at rural medical centers in developing countries.

Disadvantages:

- The main problem associated with this technique is the instability of the coupling agent (as RBCs are prone to hemolysis).
- The test provides imprecise end point determination.

Agglutination Assays

Considering the limitation of hemagglutination assays given the instability of red blood cells, Chinonavanig et al. (1991) developed latex agglutination (LA) assay method for detection of six medically important snakes of Thailand. The sensitivity of this test was recorded between 0.16 and 1.2 μg/ml of crude venom. Also, the sensitized latex particles were able to be stored at 4 °C for more than a year or at room temp for longer time in a lyophilized and desiccated form. A reverse agglutination test using latex particle coated with affinity-purified antibodies was developed for detection of *Micrurus nigrocinctus nigrocinctus* venom in biological fluids with sensitivity at 0.3 μg/ml. Khow et al. (1999), developed an improvised version of a passive latex agglutination kit for detection of Thai cobra venom (*Naja Kaouthia*), and the sensitivity of the test was in the range of 25–50 ng/ml of venom.

Advantages:

- The tests are rapid and simple.
- They are less expensive, as the reagents required are of low cost.
- The tests do not require special laboratory equipment or expertise, making them appropriate for use at rural medical centers in developing countries.

Disadvantages:

- The instability of the coupling agent, although addressed to some extent, still remains a limiting factor for use in developing countries where temperatures are high.
- The imprecise end point determination limits its practical application.

Radioimmunoassay (RIA)

Radioimmunoassay (RIA) was developed in Australia for the detection of snake venom by Coulter and his colleagues in the early 1970s. In an earlier instance, a solid phase competitive assay was developed for detection of venoms of tiger snake (*N. scutatus*) and brown snake (*Pseudonaja textilis*). In experimental animals, 10 min after subcutaneous injection with lethal dose, this technique was capable of detecting 15 ng/ml of venom in circulation (Coulter et al. 1974). Later, this method was improved for its sensitivity to detect as low as 0.4 ng/ml of venom and was used in various forensic analyses (Sutherland and Coulter 1977). RIA was also used for detection of Thai Russell's viper (*Daboia russelii*) venom in body fluids. Sjostrom (1996) used RIA to measure the plasma level of *Vipera berus berus* venom, in comparison with conventional ELISA method, and observed that both assays showed good correlation for detection of snake venom.

Advantages:

- RIA is a highly reliable and sensitive assay for detection of snake venom.
- The competitive RIA requires fewer steps and smaller volumes of sample than the double sandwich antibody technique and could be performed in 1 h.

Disadvantages:

- Its high cost and the hazard associated with handling of radioisotopes.
- The use of elaborate counting equipment limits its application in routine diagnosis of snake envenomation in the field.
- It is expensive and time consuming, and many times false positive detection occurs.

Fluorescence Immunoassay

Immunofluorescence, as an immunohistological technique was used to demonstrate sites of snake venom localization by Tiru-Chelvam (1972). This method provides visual demonstration of the venom localization. The detection limit of this assay is known to be approximately at 1.5 ng/ml. However, Bhatti et al. (1993) showed high level of sensitivity and demonstrated venom detection levels of up to 0.001 ng/ml.

Advantage:

- The technique is simple, rapid, sensitive, and less time consuming.
- It is inexpensive, as the reagents required are of low cost.

Disadvantages:

- It is time consuming, and many times false positive detection occurs.
- There exists some degree of cross-reactions.
- This technique is limited to animal models because the dye has to be injected into subject's body for detection.

Single-Bead Based Immunofluorescence Assay (SB – Immunofluorescence Assay)

A rapid and sensitive immunofluorescence method for the detection of snake venom by using microscale polystyrene beads as platform combined with semiconductor quantum dots Qdots) as fluorescence label was developed by Gao et al. (2008) to detect *N. koauthia* venom. The detection limit was at 5–10 ng/ml, which could be completed within 3 h. Here the antigen–antibody (venom/toxin – antibody) complex can be easily observed under UV microscope. It is also shown that using beads that are dyed with different colors for conjugation of the capture-antibodies or by using different types of Qdot conjugated detection antibody, the assay can also be applied for multiple venom detection.

Advantages:

- The method is simple, sensitive and less time consuming.
- Multiple venoms/toxins can be identified at single time.

Disadvantages:

- The method is expensive and requires expertise to carry out limiting its field application in developing countries.

Enzyme-Linked Immunosorbent Assay (ELISA)

Theakston et al. (1977) first described the use of ELISA for the detection of snake venom and venom antibodies, which was able to detect 1–5 ng of venom/ml within 3 h. Since then, ELISA has been applied for detection of various venoms all over the world (Chavez-Olortegui et al. 1993; Coulter et al. 1980; Dong le 2004; Huang et al. 2002; Kulawickrama et al. 2010; Labrousse et al. 1988). ELISA has been used extensively and has become a useful tool to study the kinetics of snake venom in blood, the severity of envenomation, and adequacy of antivenom serotherapy (Ratanabanangkoon et al. 1987; Selvanayagam and Gopalakrishnakone 1999). ELISA remains the suitable method for the detection of snake venoms, toxins and venom antibodies in body fluids. In many aspects ELISA is considered to be of more practical use than any other immunoassay (Ratanabanangkoon et al. 1987; Selvanayagam and Gopalakrishnakone 1999; Selvanayagam et al. 1999; Theakston 1983).

Over the years several important improvements have been made to increase the sensitivity, rapidity, specificity, and simplicity of ELISA, including (i) application of new immunoabsorbent chromogenic substrates for detection of specific proenzyme activators present in snake venoms, (ii) introduction of enzyme linked

coagulant assay using factor-X activator-antibody conjugates, (iii) the use of avidin-biotin amplification systems, (iv) utilization of lyophilized conjugate within well, where last reaction occurs, and (v) the use of enzyme-labeled immunoreactants in combination with fluorogenic substrates (Bhatti et al. 1993). In most of the snakebite cases, the average venom detected is in the range of few ng/ml showing that the assay should be highly sensitive. In regard to sensitivity of ELISA, considerable improvements have been made in order to detect venom concentrations in the range of picogram levels. Avidin-biotin amplification has been used by many investigators to improve the sensitivity of ELISA (Kulawickrama et al. 2010). Sensitivity of the assay could be further improved by increasing the affinity of the antibodies, which could be achieved by lengthening the immunization period and increasing the frequency of booster injection. Monoclonal antibodies and affinity-purified, venom-specific antibodies often are used to achieve species specificity of ELISA, and the latter seems to be the ideal for venom detection (Dong le et al. 2003, 2004; Kulawickrama et al. 2010). Recently, Kulawickrama et al. (2010) developed an ELISA method that was able to detect *Oxyuranus scutellatus* at 0.15 ng/ml. Different levels of sensitivities achieved by different ELISA methods are shown in Table 18.1.

Advantages:

- The ELISA method's main advantage for detection over other methods includes its relatively high sensitivity and specificity, reproducibility, and ease of sample preparation.
- The test is simple to perform and can be modified for specificity and rapidity.
- It is inexpensive and therefore economical for use in developing countries.
- It is quantitative because it gives a clear picture of the exact quantity of venom present in the biological fluids, which will be of great practical importance for the administration of appropriate quantity of snake antivenom.
- Circulating venom antigens during envenomation can be detected quantitatively, thus helpful in prognosis and administration of appropriate antivenom.
- It can be modified to measure/monitor antivenom levels.
- Species-specific diagnosis is possible.
- This method can be easily adapted into diagnostic kits for field use.

Disadvantages:

- Non-specific reactivity along with cross-reactivity is an important factor that still limits the use of ELISA for species identification, which has been elaborately discussed by Selvanayagam and Gopalakrishnakone (1999).
- The high background absorbance in some cases has led to the incorrect interpretation of nonenvenomed cases. A number of approaches have been developed to reduce background absorbance (Kulawickrama et al. 2010). Limits of

detection (LoD) for venom or toxin assays have been reported between 0.2 and 10 ng/mL (Sjostrom et al. 1996), depending on the use of these methods to reduce background absorbance.

Optical Immunoassay (OIA)

This technique was first applied for the detection of a single snake toxin, β-bungarotoxin, a presynaptic neurotoxin from venom of *Bungarus multicinctus* snake, in experimental envenomation (Dong le et al. 2002). Further, it was shown to semi-quantitatively detect venoms from four medically important snakes of South Vietnam (*Trimeresurus albolabris*, *Calloselasma rhodostoma*, *Naja kaouthia*, and *Ophiophagus hannah*) (Dong le et al. 2002, 2004). This prototype kit was able to detect venom analytes in blood, plasma, urine, wound exudates, blister fluid, or tissue homogenates (Dong le et al. 2004). A highly sensitive avidin-biotin optical immunoassay (AB-OIA) was developed for the detection of beta-bungarotoxin (beta-BuTx), a neurotoxin from the venom of *Bungarus multicinctus*, in whole blood, plasma and urine (Dong le et al. 2002). AB-OIA is known to be simple, requires only 40 microl of biological fluid and can be performed without specialized equipment. It was shown that the assay could detect beta-BuTx levels as low as 16 pg/ml in sample buffer and 100 pg/ml in the whole blood or plasma. The principle on which the test is based is that of detection of physical changes in thickness of molecular thin film resulting from specific binding events on an optical silicon chip. Here, the reflection of white light through the thin film results in destructive interference of a particular wavelength of the light with color change, which depends on the thickness of the thin film formed or the amount of venom in the test sample. The lower detection limit of this assay is at 0.4 and 0.8 ng/ml, whereby in general, OIA is equal or more sensitive than ELISA.

Advantages:

- The test is reliable and rapid (~35 min), which is very advantageous for clinical usage.
- It is a highly sensitive and specific test that can detect venoms in different body fluids, such as whole blood, plasma, urine, wound exudate, and blister fluid, as well as tissue homogenates, without any manipulation. In general, it is equal or more sensitive than ELISA.
- The visual evaluation is simple, rapid, and highly desirable for use in the low-income, low-technology countries where most snakebite victims are relatively poor.
- The final results (appearances of colors) are highly stable and can be kept for months without losing the color.
- The whole assay can be carried out in a field situation and clinics with basic facilities.

Disadvantages:

- Although, it is highly sensitive and stable, it is semi-quantitative when compared to ELISA; therefore, it does not give a clear picture of the exact quantity of venom present in the biological fluids, which influences the administration of appropriate quantity of snake antivenom and subsequent monitoring.

Antigen/Antibody Microarray Immunoassay

This is a protein-microarray-based assay for specific snake venom identification. Antigen/antibody microarrays could be developed based on Specific requirement of the Antigen to be quantified (Dong le et al. 2003), therefore the antibody microarrays are believed to be extremely useful in snake venom identification for prognosis and treatment of snakebite. Antivenom microarray assay is a multianalyte immunoassay. Dong le et al. (2004) first described the fabrication of snake venom and antivenom microarrays and demonstrated its preliminary application in detection of four common venomous snakes of South Vietnam: *T. albolabris, C. rhodostoma, N. kaouthia*, and *O. hannah*. This assay was based on sandwich fluorescent immunoassay technique. Although encouraging results were obtained, no further studies were reported on further development or use of this technique for snake identification for field application.

Advantages:

- The method is a highly reliable, specific, sensitive, and rapid test.
- It is much more powerful than, and can overcome difficulties of, ELISA in terms of sample requirement (small amount) and also addresses the interassay variability.
- Apart from being highly sensitive and less time consuming, the main advantage is its multianalyte nature, i.e., these antibody microarrays can test up to about 500 different venoms in parallel.
- It can be easily developed as kit and does not require high expertise to carry out in the field.

Disadvantages:

- It is mainly qualitative (Dong le et al. 2004) and its use is limited to the identification of snake venom. However, quantification studies on venom have to be optimized for its practical application for simultaneous detection of snake species and the quantification of the venom.

PCR Based Snake Identification Kit

This is a DNA based assay system to identify the snake species involved in cases of envenomation. PCR application in snake detection began when Feng et al. (2006) for the first time successfully applied and develop a convenient and effective method for the identification of *Bungarus multicinctus* snake. Recently, this technique is being tested for its practical application around the world for the identification of snake species from the trace DNA left at bite sites on human skin (swabbing the snakebite area). Recently, it was shown that this technique was applied successfully in the identification of Indian snakes species, viz., *N. naja, D. russelii*, and *B. caeruleus* (Valmiki et al. 2013). Here, the sequence of Cyt b gene fragment that is specific for each species is compared with that of humans. Highly specific primers are designed to distinguish from other species and used for identification.

Advantage:

- It is highly reliable, sensitive, and species-specific.
- The sample is stable and can be stored for long time.
- PCR assay can be used for identification of snake species from trace DNA left at bite site on human skin.

Disadvantages:

- It is time consuming and involves expensive sequencing costs etc.
- Has no relationship to prognosis.
- Can not be developed into simple kit for use in field.

Snake Venom Detection Kit(s) (SVDK) Presently in Field Application

The CSL Snake Venom Detection Kit®

Although a number of tests for snake venom detection have been reported, only a few of them have been developed as diagnostic kit in field use. Considering the specific nature of the antigen–antibody interactions, based on immunological reactions, the commonwealth Serum Laboratories (CSL) Australia issued Snake Venom Detection Kit (SVDK) to detect envenomation of common venomous snakes of Australia notably tiger snake (*Notechis sculatus*), brown snake (*Peudonaja textilis*), king brown snake (*Pseudechis australis*), death adder (*Acanthophis antarcticus*) and taipan snakes (*Oxyuranus scutellatus*), thereby help in administration of the appropriate monovalent antivenom to the affected patients. In 1979, the first version of this kit developed was an enzyme immunoassay. Later a modified version as capillary tube enzyme immunoassay was introduced and later the glass capillary format, which was expensive. Subsequently, it was replaced by a much simpler and

more efficient one. The specificity of the kit to detect all the venoms was at 10 ng/ml and cross-reactions, though present, were considerably weaker than specific reactions. CSL-SVDK is a rapid, freeze-dried, sandwich enzyme immunoassay kit in commercial use. Its sensitivity and specificity has been rigorously characterized in terms of its sensitivity and specificity. The efficiency of this kit has been evaluated in several clinical studies and found to be very successful. However, it is to be noted that CSL-SVDK can only be used in Australia and Papua New Guinea as the snakes there are not encountered in the rest of the tropics. The limited success in developing a detection kit for tropics may also be probably due to the instability of the immunoreagents in field use, especially in tropical countries where the immunoreagents must be stable to withstand transportation and storage conditions. The main advantage of the CSL-SVDK is the usage of lyophilized conjugate within the well so that it does not interfere with the stability of the capture antibody, and at the same time it retains the stability of the enzyme conjugates. Similar approaches would be of great practical use for the modification of the existing ELISA tests, which can be species-specific for the field use for other snake envenomation around the world.

In India, De (1996) developed a rapid, species-specific AB-ELISA kit for detection of four common Indian venomous snakes, viz., *N. naja, B. caeruleus, D. r. russelii*, and *E. carinatus*. The kit was able to detect venoms up to the levels 10 ng/ml, within 30 min. The kit's efficacy for detection was demonstrated in 27 human victims using blood, serum, and swab obtained from the bite area (De 1996). However, since then no further report of the test kit in clinical application is available. Similarly, ELISA based species-specific diagnostic kits were developed for identification and quantification of envenomation by four common venomous snakes of South Vietnam. The test kit utilized SSAbs (species-specific antibodies) purified by immunoaffinity chromatography. The kit was able to identify venom levels as low as 1 ng/ml in body fluids, but its large-scale clinical application has not yet been reported. Thus, use of test kits is limited to Australia, South Vietnam, and India and cannot be applied universally for all snakes around the world due to the variation of the venom components and subsequent clinical effects as observed in other parts of the world. Furthermore, rapid, simple, and more widely available alternative diagnostic tests, such as the 20 min Whole Blood Clotting Test (20WBCT), preclude the need for SVDK. In West Africa, non-clotting blood in the 20WBCT is diagnostic of *Echis ocellatus* envenomation. Similarly, the 20WBCT has been found useful in several other settings (França et al. 2003; Ogunfowokan et al. 2011; Gaus et al. 2013).

Considerations or Challenges in the Development and Application of SVDK

As snakebite is a medical emergency in developing countries of the tropics, where a far greater variety and number of deadly snakes are found. Various important aspects have to be taken into consideration for the successful development and

application of SVKDs. For example, (i) tests must be reasonably cheap relative to the per capita income of the people; (ii) tests must be stable to the climate and be able to withstand transportation and storage conditions that may be far from optimal as observed in developing countries; (iii) tests should be rapid, considering the fact that developing countries lack good transport facilities, resulting in patients arriving at the hospital after considerable amount of time after snakebite, leading to high rates of morbidity and mortality; (iv) tests should be specific, sensitive, and rapid enough; and they should also be simple to perform and require minimal equipment or expertise given that medical persons in developing countries at primary health care centers are less equipped and possess minimal expertise to handle snakebite cases; (v) based on poorer means of transport in developing countries, check the kit should be portable and applicable for the field so it can be carried to the place where the snakebite patient is and then immediate first aid and treatment can be started based on the diagnosis; (vi) country or regional species-specific diagnostic kits ideally should be developed and optimized due to the variation aspects of venoms; they can be customized to include additional species or to delete those unnecessary with in a given area.

Therefore, in the present circumstances, there are several general specific requirements for a kit for the diagnosis of snake envenomation. It should be reliable, highly species-specific, sensitive, rapid, and inexpensive. The reagents should be stable, and it should be simple to carry out without the need of special expertise. Also it should not require sophisticated equipment and should be easily adaptable into a portable version of diagnostic kits for field use. In addition, for effective management of snakebite, it is desirable that each country or region develops and optimizes its own regional species-specific diagnosis kits where no alternative diagnostic tests exist.

Conclusion and Future Aspects

Although there have been several reports on venom detection and assay protocol development from various part of the world, so far only a few have been effectively developed for application as field kits. Several are at the experimental stage, and still more work has to be carried out before they can be used in the field. Due to the specific nature of the antigen–antibody interaction, immunological reactions offer better methods for snake venom detection and ELISA based methods has shown more success than any other tests in clinical, practical use. In recent times, important contributions have been made to improve the specificity, sensitivity, rapidity, and simplicity of the ELISA methods. Monoclonal antibodies and affinity-purified, venom-specific antibodies are being used to achieve species specificity of ELISA, and this seems to be ideal for venom detection (Dong le et al. 2004; Kulawickrama et al. 2010). However, other techniques are also in various developmental stages, such as optical immunoassays, venom/antibody microarray assay, PCR based assays, etc., which are showing much more promise in real-time application for snake venom detection. Recently, an immunoturbidity method was suggested for

venom detection (O'Leary et al. 2013). However its validity and clinical use has to be demonstrated.

The low commercial viability of SVDK currently limits pharmaceutical companies' investment in research and diagnostics of inexpensive venom diagnostic kits tailored to specific countries or regions. Therefore, it is essential that researchers and funding agencies establish more public–private partnerships to collectively develop SVDK. In a related development, a miprolab rapid test for diagnosis of *Daboia siamensis* venom has been developed recently by scientists from the biotechnology company miprolab GmbH and the Biodiversity and Climate Research Centre (BiK-F), Germany, in collaboration with researchers from Myanmar. It is claimed the test is rapid with results being obtained within 20 min. Tests for other relevant species from the region are also underway to validate their application in the field (Dr. Ulrich Kuch, personal communication). The development of this test (miprolab rapid test) is an example of a successful public–private partnership. Therefore, for bringing out an efficient, reliable, species-specific SVDK for its successful implementation in field, such public–private partnerships and/or collaborations have to be developed and nurtured between researchers and entrepreneurs.

Cross-References

- ▶ Complications of Hemotoxic Snakebite in India
- ▶ Developing Snake Antivenom Sera by Genetic Immunization: A Review
- ▶ Hemotoxic Activity of Jellyfish Venom
- ▶ Management of Snake Envenomation in Taiwan
- ▶ Socioeconomic Aspects of Snakebite in Africa and the Tropics
- ▶ Venomous Snakes and Snakebites in India

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Developing Snake Antivenom Sera by Genetic Immunization: A Review

19

Henrique Roman Ramos and Paulo Lee Ho

Contents

Abstract

Snakebite is a common and generally harmful neglected tropical disease that constitutes a highly relevant public health problem with worldwide mortality estimated to be around 50,000 deaths annually.

The only approved and accepted treatment for snakebite envenoming is the use of antivenoms produced by the purification of IgG immunoglobulins from large animals (i.e., horses) immunized against specific snake venoms. Nonetheless, since its conception, by Albert Calmette and Vital Brazil, very little has changed on the way these antivenoms are being produced.

Over the last years, on the other hand, with the advance of molecular biology techniques and the rise of transcriptomic and proteomic analysis, the constitution of different snake venoms has been characterized, leading to an increasing

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demand for the development of new methods of antivenom production, with a more specific immune response and with less adverse effects, such as serum sickness, and even without the need for the collection and maintenance of snake specimens.

DNA immunization, an elegant and robust technique of directly injecting a specific antigen DNA coding sequence directly onto the cells of an immunized animal, seems to be a much easier way of developing specific antibodies without the need for recombinant and frequently laborious protein expression and purification from heterologous organisms (i.e., *Escherichia coli*).

In this chapter, we will discuss the advances on the transcriptomic analysis of venom glands from different snake species with a focus on the efforts to develop antivenom sera by DNA immunization and its efficacy in neutralizing the toxic effects elicited by the envenomation from snakebite.

Introduction

With its importance largely ignored by medical science, the envenomation by snakebite is a common and generally harmful environmental and occupational disease that constitutes a highly relevant public health problem with worldwide mortality estimated to be around 50,000 deaths annually (Chippaux 1998; Kasturiratne et al. 2008). Furthermore, there are victims of snakebites that opt to not seek for an appropriate treatment, either in government health units or in private hospitals. Moreover, there are also those regions, with high incidence of accidents, in which medical posts are unable to keep accurate records with death certification of snakebite being very imprecise (Snow et al. 1994; Fox et al. 2006), turning those numbers underestimated. Moreover, there are much more people affected by severe sequelae derived from snakebite accidents.

Snakebite envenoming creates medical emergencies involving diverse tissues, depending on the species responsible for the bite, and the only accepted treatment for snakebite envenoming is the use of snake antivenom immunoglobulins (antivenoms) whose production dates back to 1894, when Albert Calmette published his early observations that sera from rabbits and guinea pigs, immunized against cobra and viper venoms, showed the ability of neutralizing the toxic effects elicited by those venoms (Calmette 1894). Furthermore, the specificity of antisera to specific snake venoms was later demonstrated by Vital Brazil (Brazil 1901a, b).

Originally, antivenom formulations were constituted by serum from hyperimmunized horses. Later, when it was found that the active molecules responsible for the therapeutic action were, indeed, the immunoglobulins present on the animal's blood, these molecules, rather than crude serum, started being used as antivenin. Different purifications methods were, then, introduced, with the Fc (***F***ragment ***c***rystallizable region) fragments of IgG being removed and F(ab′)2 (***F***ragment ***a***ntigen ***b***inding region) fragments being used in an attempt to reduce serum sickness reactions (Fig. 19.1). Nonetheless, when compared to its original

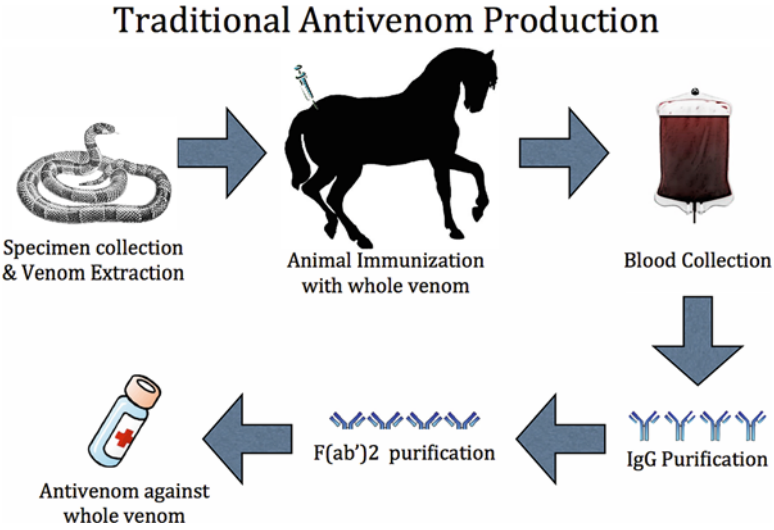

Fig. 19.1 Traditional antivenom production. Briefly, after specimen collection, venom is extracted and then injected into a large animal (e.g., horses). The animal blood is then collected and total IgG is purified. For antivenom formulations, total F(ab′)2 fragments are extracted and used as antivenin. This method requires the capture and maintenance of snakes for venom extraction

formulation, very little has changed on the way snake antivenom is being produced, with traditional antivenom production relying upon snake collection, maintenance, and venom extraction, all being processes which, sometimes (i.e., coral snakes), could be associated with high maintenance difficulties and low venom yields.

The venom glands of snakes produce numerous and different kinds of proteins, enzymes, and biologically active peptides with only a small percentage of those molecules being actually responsible for the extensive spectrum of the biological manifestations produced by envenomation. Consequently, antivenoms do include antibodies against a vast number of different proteins with specificities not confined to the toxic target molecules. Likewise, there are also those toxins that, actually, are not as immunogenic as other nontoxic components of the venom (Schottler 1951), leading to a reduction of the antivenom's efficiency and, thereby, increasing the probability of serum sickness reactions due to large volumes of equine proteins (Ko and Chung 2013; Reid 1980). Moreover, snakebite is a disease that mainly afflicts the poorest regions of the world (Harrison et al. 2009) with the antivenom production holding very limited commercial value which consequently hinders its production by major pharmaceutical companies and leading to an increasing shortage of antivenom (Theakston and Warrell 2000).

The development of an alternative, but still efficient, procedure for the production of snake antivenom, with less reliance upon snake venom extraction, should, therefore, be of great assistance for the future of the global snakebite accident treatment.

Over the last decades, with the recent progress on the biotechnology field, the transcriptomic analysis of venom glands turned out to be a common experimental approach and has already been applied on different snakes from the Viperidae (Junqueira-de-Azevedo Ide and Ho 2002; Junqueira-de-Azevedo et al. 2006; Qinghua et al. 2006; Wagstaff and Harrison 2006; Valente et al. 2009), the Elapidae (Leao et al. 2009; Correa-Netto et al. 2011), and even the Colubridae (Ching et al. 2006, 2012) families. These studies allowed the comprehension, for instance, that snake venom metalloproteinases (SVMPs), a class of enzymes responsible for the proteolytic activity present on viper's venoms (Deutsch and Diniz 1955), are indeed the major constituents of these snake venoms (Georgieva et al. 2008; Sanz et al. 2008; Alape-Giron et al. 2008).

Similarly, it was not until recently that it was described that the main molecules responsible for the neurotoxic effects elicited by venoms from Elapidae snakes, like *Micrurus corallinus* and *Micrurus altirostris*, are, indeed, a family of low-molecular-weight toxins, named three-finger toxins, and a phospholipase A_2 (PLA_2) that may exert neurotoxic action by binding to nerve cell membranes and receptors and also catalyzing phospholipid hydrolysis, with the production of lysophospholipids and free fatty acids in the case of the later toxin (Leao et al. 2009; Correa-Netto et al. 2011).

Recombinant Expression and Immunization

Since the advent of biotechnology and recombinant DNA technology, the insertion of foreign DNA into a heterologous host and consequently the expression and purification of recombinant proteins have become quite trivial with different protein expression systems being used to produce different proteins of biotechnological interest. These recombinant proteins could then be injected on different animals either for the generation of specific antibodies and, as a result, be used as a way of antivenom development or even for the development of vaccines.

One of the most attractive systems for recombinant protein expression is the one constituted by the Gram-negative bacterium *Escherichia coli*, which has one of the most well-characterized molecular biology, displaying a vast and always increasing number of cloning vectors and host strains. The *Escherichia coli* system has been used for the expression of both SVMP and neurotoxins from vipers and elapids, respectively (Selistre-de-Araujo et al. 2000; Carbajal-Saucedo et al. 2013; Jeon and Kim 1999; Gong et al. 1999). Snake toxins, however, are a group of proteins very rich on disulfide bonds that have to be correctly established between the correct cysteine residues to display their toxic activities. As a result, the use of *E. coli* for the production of high quantities of correctly folded and active molecules is a challenge by itself. From all the work available in the scientific literature, very few resulted in toxins that displayed some sort of activity and, sometimes, with only a specific domain being expressed and purified (Selistre-de-Araujo et al. 2000; Moura-da-Silva et al. 1999; Suntravat et al. 2013). The use of eukaryotic systems, like *Pichia pastoris*, on the other hand, has been shown to be more effective on these issues and,

therefore, could be an alternative for recombinant expression of snake toxins (Singhamatr and Rojnuckarin 2007; Schwettmann and Tschesche 2001; Pinyachat et al. 2011). Despite all these works, there is no record on the literature showing the use of recombinant toxins for the development of antivenoms.

DNA Immunization: A Brief History

It was in 1990 that Wolff JA et al. described his observations that the direct gene transfer into mouse muscle in vivo resulted in the expression, by the host, of the respective proteins (Wolff et al. 1990). Later, in 1992, Tang et al. reported that immunization of mice with microprojectiles coated with plasmids containing the genomic copy of the human growth hormone (hGH) gene under the transcriptional control of the cytomegalovirus (CMB) promoter (Tang et al. 1992) generated detectable levels of specific antibodies. This study generated strong enthusiasm along the scientific community for not only that it could be a new approach for generating antibodies against a specific antigen without the need for its expression and frequently laborious purification from heterologous organisms (i.e., *Escherichia coli*), but also that it could be an alternative form of vaccination against a pathogenic infection.

More recently, the use of DNA vaccines has gone through a considerable enhancement in terms of eliciting better immune responses. More efficient gene delivery methods started being applied together with gene optimization strategies like the use of species-specific codon optimization, resulting in an increment on the levels of expressed proteins and leading not only to a better cellular immune response (Ramakrishna et al. 2004; Yan et al. 2007) but also to the induction of high levels of antibodies (Yadava and Ockenhouse 2003; Narum et al. 2001; Smith et al. 2004).

The actual means by which genetic immunization results in the production of antibodies against a specific pathogen are still under investigation. It is thought, however, that when delivered by any of the available methods, exogenous DNA sequence enters the nucleus of antigen-presenting cells (APC) which, in turn, start the production of the coded protein. These foreign antigens are then presented to the host's immune system by either the major histocompatibility complex (MHC) class I or class II molecules, leading to the activation of T cells or even to the release of antibodies by B cells.

Under these circumstances, genetic immunization would, therefore, be a good solution for the development of antivenoms, as it would be an easy way of presenting correctly folded snake toxins to a eukaryotic host with the concomitant generation of possibly neutralizing antibodies.

Development of Snake Antivenom by Genetic Immunization

Harrison SA et al., in 2000, showed for the first time a DNA immunization approach targeting the carboxyl-disintegrin and cysteine-rich domain from the soluble zinc-dependent metalloproteinase named Jararhagin (JD9), of *Bothrops jararaca* venom,

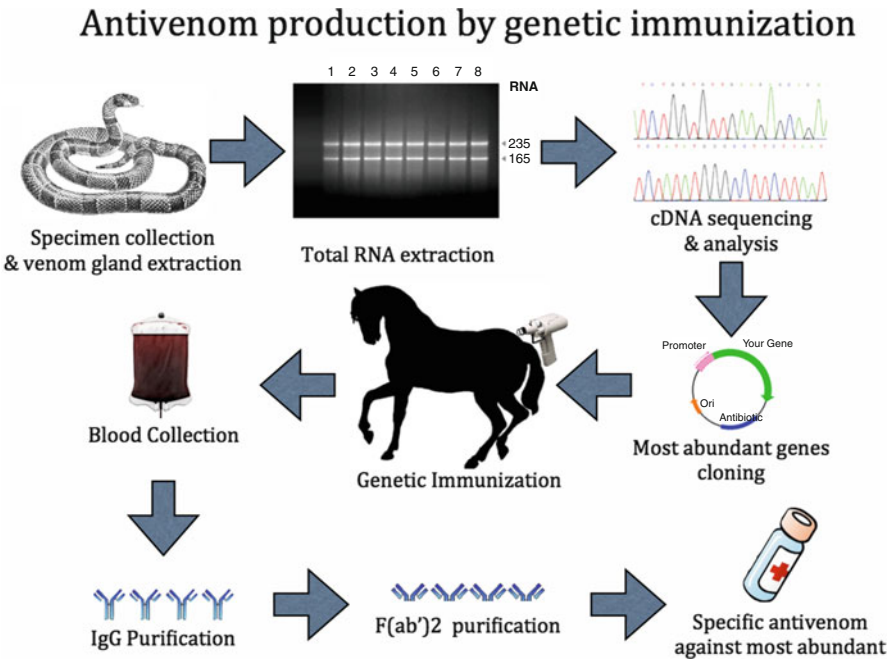

Fig. 19.2 Antivenom production by genetic immunization. Briefly, after specimen collection, venom glands are extracted for total RNA purification. Most abundant toxins are identified by transcriptomic analysis and then cloned into an appropriate cloning vector, which will be used for animal immunization. The animal blood is collected, total IgG is purified for antivenom formulations, and total F(ab′)2 fragments are extracted and used as antivenin. This method does not require the maintenance of snakes in captivity for venom extraction. Snakes are captured only once for RNA extraction and analysis

as an attempt to generate antibodies capable of neutralizing the toxic effects elicited by this toxin (Harrison et al. 2000). In this occasion, DNA was intramuscularly delivered to mice by gene gun immunization in order to exploit its markedly T helper 2-type polarized immune response and promote high levels of antibodies (Feltquate et al. 1997).

In this initial work, the titers of Jararhagin-specific IgG of sera from immunized mice were similar to those observed on a previous work where rabbits were immunized with the same toxin, expressed and purified from *Escherichia coli*, together with Freund's complete adjuvant (FCA) (Moura-da-Silva et al. 1999), demonstrating the potential of gene gun immunization to evoke high titers of antibody responses. Furthermore, when these immunoglobulins were preincubated with the whole *B. jararaca* venom, a reduction of 77 % on the size of the hemorrhagic lesion could be observed, clearly demonstrating the proof of concept that DNA immunization could be a new method for the development of next-generation antivenom (Fig. 19.2).

Two years later, considering the fact that the immune response to gene gun immunization results primarily from antigen presentation by DNA-transfected skin dendritic cells (Akbari et al. 1999; Boyle and Robinson 2000) and that the granulocyte/macrophage colony-stimulating factor (GM-CSF) is a potent activator of dendritic cells (Kim et al. 2000), the same researches have observed that mice which were immunized with JD9 plasmid in coadministration with a GM-CSF plasmid showed a log-fold higher IgG response to Jararhagin than mice immunized with JD9 DNA alone (Harrison et al. 2002). The authors also showed that antibody levels could be raised when mice were subjected to a five-time immunization regimen, with the two last doses with monthly intervals; sera of these animals showed greater Jararhagin-specific IgG antibody titers (1×10^6) than sera of mice immunized only three times (1×10^5). These authors reported later that these immunoglobulins also possess extensive immunological reactivity to venom components in snakes of distinct species and genera, promoting the potential use of DNA immunization to generate toxin-specific antibodies with polyspecific cover (Harrison et al. 2003). These initial works clearly demonstrated to the scientific community the great potential that DNA immunization displays as a rational approach to the design of toxin-specific immunotherapy.

It must be taken into consideration, however, that snake venoms are a mixture of different numbers of toxins. Even with the observation that an antivenom produced against a single specific toxin (e.g., the JD9 domain of Jararhagin) is capable of reducing the toxic effects elicited by the whole venom, there are still those snakes, like *Echis ocellatus*, the most medically important venomous viper in Africa, that display a molecular diversification of SVMPs (Wagstaff et al. 2006) that far exceeds the diversity observed in other viper species, including *B. jararaca* (Junqueira-de-Azevedo Ide and Ho 2002; Francischetti et al. 2004; Kashima et al. 2004). This implicates that an appropriate neutralization of the toxic hemorrhagic activity of *E. ocellatus*' venom would only be accomplished by the generation of antibodies targeting surface-available and antigenic epitopes present in most, if not all, of the numerous and diverse SVMP isoforms. With this rationale, Wagstaff SC et al. reported a comprehensive EST (***e***xpressed ***s***equence ***t***ags) survey from the venom glands of *E. ocellatus* and combined it with a bioinformatics approach to identify those genetic sequences of key structural or functional significance and to create an antiserum that would react with epitopes representative of all the SVMP isoforms (Wagstaff et al. 2006). In this work, six SVMP domains, whose antigenic profiles are similarly conserved among the different isoforms, were identified, and a synthetic DNA immunization construct containing a string of SVMP epitopes that are represented across numerous and diverse SVMP isoforms was designed for the immunization of mice and investigation of its cross-generic and cross-specific antibody responses and its in vivo capability of neutralizing the venom-induced hemorrhage.

The results observed in this work showed that the antibody titers from sera of mice that were immunized with this synthetic string were, indeed, lower than those titers observed in sera from mice immunized with DNA encoding the

metalloproteinase (MP) domain, disintegrin (DC) domain, or metalloproteinase and disintegrin domains (MPDC) from one of the SVMPs from *E. ocellatus* venom. On the other hand, despite these low antibody titers, the authors reported an almost complete reduction in the mean area of hemorrhage induced by *E. ocellatus* venom. The results also clearly demonstrated that, as expected, the antisera raised against a single MP domain was ineffective at neutralizing *E. ocellatus* venom-induced hemorrhage with both DC and MPDC antisera offering limited and also statistically nonsignificant protection against the toxic effects elicited by this viper's venom. This shows that the use of bioinformatics analysis in selecting the epitopes present in different isoforms of SVMP is, undoubtedly, a great way of replicating the toxin-neutralizing capabilities of conventional antivenom.

Following these pioneer works, Azofeifa-Cordero G et al. reported the cloning and sequence analysis of the cDNA encoding a SVMP from the Central American rattlesnake *Crotalus durissus durissus* and showed that DNA immunization in mice using tungsten microparticles as carriers induced the production of antibodies which significantly neutralized the hemorrhagic activity of *C. d. durissus* venom (Azofeifa-Cordero et al. 2008). In this occasion, the use of immunopotentiators, like coadministration of a GM-CSF-encoding plasmid (Harrison et al. 2002), was also assessed; however, in place of GM-CSF, the authors reported the use of IL-2 as a way of increasing the antibody titers. The results clearly demonstrated that mice co-immunized with the plasmid encoding the SVMP from *C. d. durissus*, and the IL-2-encoding plasmid showed statistically significant higher IgG responses to *C. d. durissus* venom antigens than mice immunized with the SVMP plasmid alone. On the other hand, the ability of these different sera to neutralize the hemorrhage induced by *C. d. durissus* venom was statistically the same, with both sera reducing in about 60 % the hemorrhagic lesions. These results indicate that rather than the quantities of antibodies, the quality and ability of these immunoglobulins to neutralize snake venom are far more important when developing antivenoms.

The same group of researchers, later, evaluated the use of DNA immunization with a cDNA encoding an SVMP from *Bothrops asper*. In this work, for the first time, a horse was used for DNA immunization, antisera production, and, here again, the preincubation of this serum with venoms from *B. asper* and *C. d. durissus* reduced in up to 100 % and up to 90 % the hemorrhagic lesions provoked by *B. asper* and *C. d. durissus* venoms, respectively. Nonetheless, the preincubation with venom from *Lachesis stenophrys* did not show any reduction on the hemorrhagic lesions provoked by this snake venom (Arce-Estrada et al. 2009). Neutralization of the lethal activity induced by *B. asper*, *C. durissus durissus*, and *L. stenophrys* snake venoms was also tested, whereas no neutralization of any of the venoms was observed.

For what concerns the elapids, up to now, there is only one work in the literature where DNA immunization was used for an antielapidic serum. On this occasion, four cDNAs coding for different three-finger toxins and one cDNA coding for a phospholipase A2 from *Micrurus corallinus* were intramuscularly injected in the anterior tibia muscle or quadriceps of mice, resulting in low antibody titers. Furthermore, no LD50 assay was performed, and, therefore, it is not possible to

infer if these immunoglobulins are actually able to neutralize the neurotoxic effects of *M. corallinus* venom. On the other hand, it is very clear that the main focus of this work, however, was the transcriptomic survey of the venom gland of *M. corallinus* that resulted in an incredible amount of data and DNA sequences (Leao et al. 2009), which in turn will certainly allow the researchers to investigate whether it is possible or not to develop an antielapidic antiserum by DNA immunization.

Conclusion and Future Directions

The research and development of snake antivenom have experienced very little changes since its first use back on the first decades of the nineteenth century. The earliest antivenoms consisted of pure serum obtained from hyperimmunized horses. Later, with the advent of ammonium sulfate precipitation, the IgG fraction started being separated and used as antivenin. Subsequent modifications included the production of F(ab′)2 antivenoms by pepsin digestion of IgG molecules and the production of Fab fragments by papain digestion followed by the elimination of non-IgG proteins by caprylic acid precipitation. The relatively limited commercial value of antivenom production is one of the major reasons why there has been very little development and research of its production techniques, which culminated in the current crisis in antivenom supply such as that present in sub-Saharan Africa.

The main problem regarding the current antivenom formulations is that they are produced with the use of whole venom to induce immune responses on an appropriate animal. In this context, toxin-specific antibodies are, therefore, diluted among immunoglobulins produced against nontoxic venom antigens that are useless for the snakebite treatment. As a result, large volumes of antivenom are necessary for an adequate treatment, leading to an increased risk of serum sickness reactions development.

With the onset of transcriptomic data of venom glands from different snake species, the major toxins that constitutes the venoms from vipers and elapids has been identified as being metalloproteinases (SVMPs) and neurotoxins, respectively. This information, as a result, allows the research of more specific antivenoms, composed only by immunoglobulins against these toxins instead of the current formulations containing a plethora of different antibodies.

Producing a new antivenom formulation, based on these toxins, however, is not so simple as it may look like. Purifying these toxins from crude venom would demand lots of work, and, still, there would not be enough toxins for the immunization protocols. Furthermore, most elapids possess venom glands that deliver very limited quantities of venom, turning negligible the final toxin concentration for immunization assays. The heterologous recombinant expression of these proteins would, as a result, be a good alternative for the generation of large quantities of these molecules.

SVMP and neurotoxins, however, are a class of toxins that possess a considerable quantity of disulfide that should be correctly folded in order to induce a proper

immunological reaction on an immunized animal. As a result, the recombinant expression of these toxins on prokaryotic systems turns to be very difficult to be achieved. Indeed, the main papers available on the literature that deals with the recombinant expression and characterization of snake toxins make use of eukaryotic systems such as *Pichia pastoris* (Singhamatr and Rojnuckarin 2007; Schwettmann and Tschesche 2001; Pinyachat et al. 2011), which, in turn, demands a lot of work on either upstream or downstream processes.

DNA immunization, on the other hand, induces potent cellular immune responses against infectious pathogens, and since its first appearance back in 1990 (Wolff et al. 1990), there has been a huge progress concerning antigen design, the use of improved formulations with adjuvants that augment the immune response against specific antigens, and also the way DNA is delivered, which greatly improved the immunogenicity of DNA vaccines. This superior performance stimulated numerous clinical trials that explored its benefits both for preventive and for therapeutic purposes. The use of DNA vaccines, as a matter of fact, allows the expression of a specific snake toxin by eukaryotic cells, leading to the assembly of much more accurate proteins with concomitant presentation to the immune system without the need of its purification for subsequent immunization protocols.

It was in the year 2000, when researchers from the Liverpool School of Tropical Medicine first described the use of DNA immunization for antivenom development. On this occasion mice were inoculated with a cDNA coding for a disintegrin domain of a metalloproteinase from *Bothrops jararaca* (Harrison et al. 2000), showing, for the first time, that this methodology is, indeed, capable of eliciting high titers of neutralizing IgG antibodies.

It should not be forgotten, however, that an effective antivenom formulation would be the one composed by antibodies capable of recognizing not a single and specific toxin but the most abundant toxins present on a venom gland. This implicates that different DNA constructs should be injected simultaneously on a specific animal in order to obtain an immunological response against all these toxins. This would, on the other hand, result in increased costs, hindering its large-scale development. Nonetheless, the same pioneer researchers that described the use of DNA immunization for snake antivenom development, with the use of bioinformatics, obtained a hydrophobicity pattern from different SVMPs from the venom of *Echis ocellatus* and selected the most exposed and conserved epitopes shared between the different isoforms. These epitopes were then used for the creation of a DNA string, which would possess immunoreactive sequences from the most important molecules present on the venom gland of this important African viper. The genetic immunization of mice with this string resulted in an antivenom formulation with high levels of neutralization activity (Wagstaff et al. 2006), turning DNA immunization into an incredible tool for the production of new and effective antivenoms formulations.

The total amount of works concerning the use of genetic immunization for antivenom production is, by far, very low. A basic search on the literature would

result in less than ten articles with only part of it being actually original data (Leao et al. 2009; Harrison et al. 2000, 2002; Wagstaff et al. 2006; Azofeifa-Cordero et al. 2008; Arce-Estrada et al. 2009). Future research, as a consequence, should be performed and should also be focused on selecting epitopes from the most abundant toxins present on a specific snake venom gland and designing DNA strings with these sequences. A practical and not expensive way of doing so, for instance, is by probing synthetic peptide arrays coated on membrane supports against antibodies elicited by the main toxins (Frank 2002; Frank and Overwin 1996); as a matter of fact, discontinuous synthetic epitopes from TsNTxP (***T****ityus* ***s****errulatus* ***N****on* ***T****o****x****ic* ***P****rotein), a natural anatoxin from the venom of the scorpion *Tityus serrulatus*, selected by this technique, have already been described as being able to elicit antibodies that induce in vivo protection against the envenomation by *T. serrulatus* venom (Duarte et al. 2010). The use of phage display would also be an elegant way of identifying conformational epitopes and could lead to interesting results.

Independently of the method of research applied from now on and the focus, it is very well established and documented that DNA immunization is a powerful tool that could result in much more specific and efficient antivenom formulations for the treatment of snakebite envenomation.

Cross-References

► Clinical Uses of Snake Antivenoms
► Management of Snake Envenomation in Taiwan

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Snake Venom and Hemostasis

20

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Abstract

Venomous snakebites are a common cause of death globally. Snake venom main targets are neuromuscular and/or hemostatic systems resulting in paralysis and/or bleeding disorders. This review focuses only on the latter effect.

Hemostasis is a complex process keeping the balance between bleeding and thrombosis. Venom genes evolved from a few nontoxic genes, duplicated and recruited to express in venom glands. Subsequently, they undergo accelerated evolution to greatly diversify their toxicity affecting all aspects of hemostasis, including vessel walls, platelets, blood coagulation, natural anticoagulants, and fibrinolysis. The effects can be activating and/or inhibitory.

The major classes of venom proteins affecting hemostasis are reviewed. They include viper venom proteins: snake venom serine proteases, snake venom metalloproteinases, disintegrins, snaclecs, and type II phospholipases A_2, as well as elapid proteins: three-finger toxins, prothrombin activators, Kunitz-type serine protease inhibitor, and type I phospholipases A_2. Moreover, L-amino acid oxidases and nucleotidases are present in both snake families. Although some of these toxins have no clinical significance, they are currently used or potentially useful as diagnostic or therapeutic agents.

From the clinical standpoint, the most common hemostatic defect caused by snakebites is consumptive coagulopathy from venom components that activate the common pathway of blood coagulation: factors X and V, prothrombin, or fibrinogen. This combined with fibrino(geno)lysis, platelet activation, and vessel wall damages results in hypofibrinogenemia, thrombocytopenia, and bleeding. Whole blood clotting time is recommended for diagnosis and follow-up for consumptive coagulopathy after snakebites. Additionally, snakebite-induced anticoagulation syndrome without consumption, thrombotic microangiopathy, and thromboembolism has been occasionally reported.

Introduction

Venomous snakebite is still a major, yet neglected, public health problem worldwide. The exact burden of disease remains uncertain. A previous study estimates that there are 20,000–94,000 deaths globally from snakebites per year (Kasturiratne et al. 2008). However, more recent data suggest that there are up to 46,000 deaths from snakebites annually in a single country of India. Consequently, clinical and basic researches in prevention and treatments for this condition are strongly required.

The significance for snake venom studies is far beyond the clinical managements of snakebites. Different snake venoms have been found to affect a great variety of molecules in humans. Therefore, venom proteins are potentially useful as diagnostic or therapeutic compounds or agents for investigating molecular mechanisms of various physiological and pathological processes.

During over 100 Ma of ophidian evolution, the potentially lethal venoms emerge accompanied with advancing venom-injecting system of the front-fanged snake families. The two major highly poisonous snake families are elapids with short and fixed fangs and vipers with long and folded fangs, as well as protective sheaths. The latter contain the most sophisticated venom-delivering fangs, which can swing backward and forward when they close and open their mouths, respectively.

To prevent their prey from fleeing, the two main targets of snake venoms are the neuromuscular system causing muscle paralysis and blood clotting system resulting in rapid death. In Southeast Asia, these effects are usually caused by elapids and vipers, respectively. However, there are many exceptions as Australian elapids commonly cause bleeding disorders and South American vipers may inflict paralysis.

A subset of vipers has pit organs as the infrared detectors between their eyes and nostrils to located warm-blooded prey in the dark. This subfamily is termed crotalids or pit vipers. All vipers in American continents are pit vipers, but there is no pit viper in Africa. Notably, there is no viper in Australia. In Asia, there are elapids, true vipers, and pit vipers.

The colubrids or rear-fanged snakes are usually considered weakly venomous. Their fangs are not hollowed and their venom glands do not attach with skeletal muscles, resulting in less effective venom delivery compared to those of front-fang snakes. However, some of them have been reported to cause severe or even lethal bleeding (Weinstein et al. 2013).

Snake Venom Components

Evolution studies suggest that a few nontoxic genes of snakes are duplicated and recruited to express in venom glands (Fry 2005). This limited number of genes undergoes a process called "accelerated evolution" to diversify the venom effects on a plethora of preys in a wide variety of geographic locations. Supporting this notion, the venom genes have more nucleotide substitutions in the coding regions than in noncoding regions and more non-synonymous than synonymous substitutions (Nakashima et al. 1995). The molecular mechanism of accelerated evolution is still unclear. Notably, venoms contain a high content of cyteines that form disulfide bonds in secreted proteins. The disulfide bonds probably preserve protein structures when they undergo mutations. In addition, alterations in cysteine residues result in new protein structures during evolution (Fox and Serrano 2008). Furthermore, intermolecular cysteine bonds in venom proteins are essential to produce a myriad of dimeric or multimeric proteins, such as snake C-type lectins (snaclecs).

Active components of snake venoms are mostly proteins in nature. Venomics studies show that venom of a snake comprises 10–100 proteins that belong to 4–20 protein families (Calvete 2013). These families can be enzymes or non-enzymatic proteins. Interestingly, some enzymes in snake venoms, e.g., phospholipase A_2, acquire new toxic functions during evolution by action through non-enzymatic mechanisms.

A very large numbers of snake venom components affecting hemostasis have been reported in literature. The review is intended to group them into major classes and give only a few examples of venom proteins demonstrating their extreme diversity. In the future, poorly characterized classes of proteins and/or unreported venom components still remain to be discovered and defined. Because hemostatic system is a complex process comprising many components, an overview of hemostatic mechanisms in human is summarized in the next section.

Overview of Hemostasis

Hemostasis, the mechanism that maintains vascular integrity, is a very complicated system comprising endothelium, platelets, coagulation factors, coagulation inhibitors, fibrinolysis, and antifibrinolytic factors. Defects in any of these factors can tip the delicate balance causing either bleeding or thrombosis.

To sustain normal blood circulation, the vessel wall provides a barrier to prevent extravasation and endothelium serves as an anticoagulant surface preventing any clotting obstruction of the flow. Normal endothelium provides antiplatelets, anticoagulants, and pro-fibrinolytic factors.

When there is a break in endothelial lining, collagen is exposed to blood. The large multimeric von Willebrand factor (vWF), which is attached to collagen, is subjected to the shear stress from circulating blood flow resulting in a drastic change from globular to extended conformation. These "active" vWF multimers can bind to the glycoprotein (Gp) Ib/IX/V complex and Gp IIb/IIIa (Integrin $\alpha_{IIb}b_3$), the vWF receptors on platelet surface, resulting in platelet adhesion to the injured vascular wall. Collagen, then, activates platelets via integrin α_2b_1 (Gp Ia/IIa) and glycoprotein (Gp) VI, the platelet collagen receptors.

Activated platelets synthesize thromboxane A_2 from membrane arachidonic acid and release their granule contents. These synthesized thromboxane and ADP released from platelet dense granules will further activate platelets via their respective G-protein coupled receptors. These signals inside the platelets finally change the conformation integrin$\alpha_{IIb}b_3$. This "inside-out" signaling enables integrin$\alpha_{IIb}b_3$ to bind outside fibrinogen that forms bridges with other platelets resulting in platelet aggregation. Platelet plug formation is the first mechanism to stop bleeding and hence termed "primary" hemostasis (Fig. 20.1).

Injuries to large vessels require elaboration of stronger blood clots that are composed of fibrin derived from coagulation cascade. This is the second mechanism to stop bleeding and so called "secondary" hemostasis. Current concept visualizes coagulation pathways in the context of phospholipid surface of the cell membrane (Fig. 20.2). The coagulation cascade is initiated by a trans-membrane cofactor called tissue factor (TF) expressed on cells residing outside blood vessels. A small amount of factor VIIa normally present in blood when binds with extravascular tissue factor (TF/VIIa complex) can activate factors X and IX to factors Xa and IXa, respectively. Factor Xa, in turn, generates a small amount of thrombin (factor IIa) from prothrombin (factor II). This thrombin activates clotting cofactors,

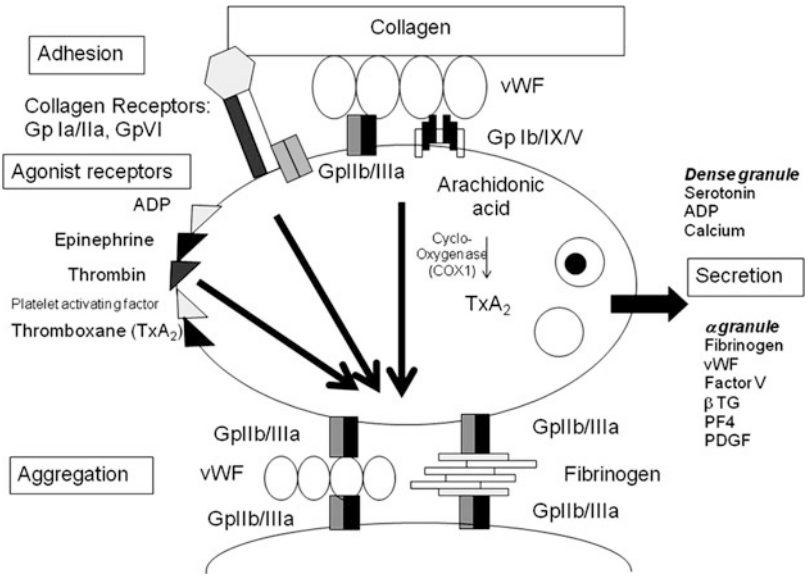

Fig. 20.1 Summary of primary hemostasis

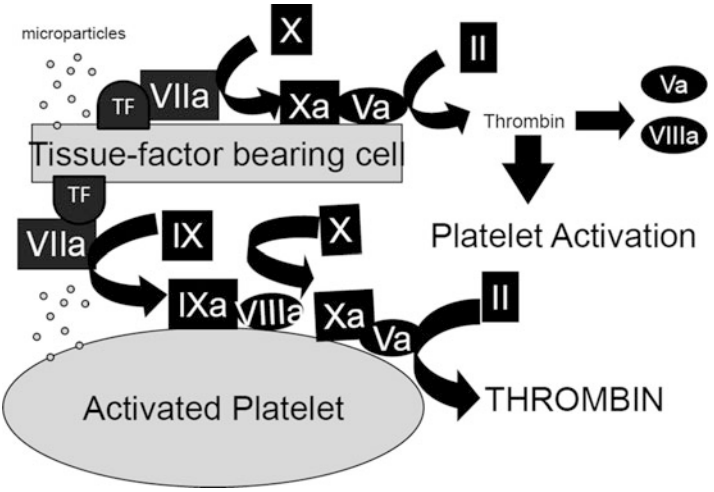

Fig. 20.2 Summary of secondary hemostasis

factors V and VIII, to Va and VIIIa, the active cofactors for factors Xa and IXa, respectively. Thrombin also activates platelets, causing platelets to expose procoagulant surfaces by flipping negatively charged phospholipids from inner to outer membrane leaflet and releasing microparticles that also express these negatively charged phospholipids. These activated platelet and microparticle surface serve as the ground for coagulation factor complexes. The tenase complex (factor

IXa/VIIIa) and prothrombinase complex (factor Xa/Va) are then assembled on these negatively charged phospholipid surfaces for efficient activation of factor X and prothrombin, respectively.

The resultant large amount of thrombin, or thrombin burst , will effectively cleave fibrinopeptide A and fibrinopeptide B from the N termini of Aα and Bb chains of fibrinogen, respectively, resulting in fibrin polymerization and, thus, a clot formation. In addition, thrombin burst activates factors XI and XIII, resulting in factor XIa that further activates factor IX to IXa to amplify the reactions and factor XIIIa, a transglutaminase that cross-links fibrin making it resistant to fibrinolysis.

The contact factors (factor XII, prekallikrein, and high molecular weight kininogen) are activated by artificial surfaces and can catalyze factor XI to XIa in vitro. Deficiency of one of these contact factors shows prolonged activated partial thromboplastin time (APTT) but does not cause any bleeding disorder. Therefore, they are not important for in vivo hemostasis.

These clotting factor enzymes are serine proteases. Vitamin K is the cofactor for post-translational gamma-carboxylation (Gla) of factors II, VII, IX, and X (vitamin K-dependent factors) on their Gla domains. This negatively charged domain enables them to bind negatively charged pro-coagulant phospholipid surface in the presence of positively charged calcium ions as the linkers to form enzyme/cofactors complexes as mentioned above.

The primary and secondary hemostatic mechanisms are not mutually exclusive. Platelets provide phospholipid surfaces for blood coagulation. Additionally, clotting factor V is released from platelet α-granules. On the other hand, thrombin can activate platelets via G-protein coupled receptors called protease-activated receptors (PARs) on the platelet surface.

Coagulation activation needs to be balanced by natural anticoagulants. Tissue factor pathway inhibitor (TFPI) is a Kunitz domain-containing protease inhibitor that inhibits TF/VIIa complex when there is some factor Xa generation. When free thrombin diffuses outside the area of injury, it will contact thrombomodulin (a thrombin modulator) on intact endothelium. Upon the contact, the pro-coagulant thrombin will perform an anticoagulant function by activating a serine protease, protein C. Activated protein C with its cofactor protein S can inactivated factors Va and VIIIa, the two products of thrombin. In addition, protein S is also a cofactor of TFPI. Finally, free thrombin will be trapped by the circulating antithrombin, a *ser*ine *p*rotease *in*hibitor (Serpin), forming an inactive complex. The effects of antithrombin are enhanced in the presence of glycosaminoglycans secreted from intact endothelium or pharmacologic heparin anticoagulants.

Fibrinolytic system is critical to dissolve blood clot, restoring vessel patency. Endothelium normally secretes tissue-type plasminogen activator (t-PA). When fibrinogen is changed to fibrin, it exposes lysine residues that are the binding sites for t-PA and plasminogen. Fibrin formation markedly enhances t-PA action on plasminogen, resulting in plasmin generation on fibrin surfaces. Plasmin digests fibrin into fibrin degradation products (FDPs). The high concentration of thrombin in the presence of thrombomodulin can activate thrombin-activatable fibrinolysis

Table 20.1 The effects of snake venom on platelets showing their protein families in [square brackets] and examples of venom proteins as well as the species of origin in (parentheses)

Enzymatic proteins	Non-enzymatic proteins
1. **Proteolytic digestions of platelet receptors** [SVMP], e.g., kistomin (*Calloselasma rhodostoma*) cleaving Gp Ib, vWF, and Gp VI; alborhagin (*Cryptelytrops albolabris*) cleaving Gp VI	**1. Integrin inhibition** [SVMPs], e.g., disintegrins from many viper venoms [3FTx], e.g., dendroaspin (*Dendroaspis viridis*), gamma-bungarotoxin (*Bungarus multicinctus*) [Snaclecs], e.g., EMS16 (*Echis multiquamatus*) and rhodocetin (*C. rhodostoma*) inhibiting α2beta1 integrin
2. **Nucleotidases** producing adenosine inhibiting platelet function	
3. **Protease-activated receptor (PAR) activators** [SVSP], e.g., thrombocytin (*Bothrops atrox*), PA-BJ (*Bothrops jararaca*)	2. **Platelet activators** [Snaclec], e.g., alboaggregin B (*Cryptelytrops albolabris*) and jerdonuxin (*Protobothrops jerdonii*) binding Gp Ib, trowaglerix (*Tropidolaemus wagleri*) binding Gp VI, alboaggregin D (*Cryptelytrops albolabris*), and convulxin (*Crotalus durissus terrificus*) binding both Gp Ib and Gp VI, aggretin (*Calloselasma rhodostoma*) binding Gp Ib, α2beta1 integrin and CLEC2, botrocetin (*Bothrops jararaca*) and bitiscetin (*Bitis arierans*) binding vWF
4. **Platelet activators via H_2O_2 production** [L-amino acid oxidases]	

SVMP snake venom metalloproteinase, *Gp* platelet glycoprotein, *vWF* von Willebrand factor, *SVSP* snake venom serine protease, *3FTx* three-finger toxin, *snaclec* snake C-type lectin

inhibitor (TAFI). The active TAFI (TAFIa) is a carboxypeptidase that cleaves lysine residues on fibrin, eliminating t-PA/plasminogen binding sites and, hence, inhibiting fibrinolysis. Free serine proteases, t-PA and plasmin, outside an injured area will be inhibited by the molar excesses of serpins, plasminogen activator inhibitor-1 (PAI-1), and $\alpha_2$antiplasmin, respectively.

The effects of snake venoms on various parts of hemostasis are extremely diverse and exemplified in Tables 20.1 and 20.2.

Protein Families in Viper Venoms

Examples of major protein families in viper venoms are listed below.

Snake Venom Serine Proteases (SVSP)

The enzymes are characterized by the active sites with a catalytic triad of histidine, aspartate, and serine. As mentioned above, most enzymes in coagulation and fibrinolysis are serine proteases. However, most SVSPs originated from the glandular kallikrein that later evolves to acquire coagulation or fibrinolytic/fibrinogenolytic activities. The largest subgroup of SVSPs is termed "thrombin-like" enzymes because of their activities, not the evolutionary origins.

Table 20.2 The effects of snake venom on coagulation and fibrinolysis showing their protein families in [square brackets] and examples of venom proteins as well as the species of origin in (parentheses)

Enzymatic proteins	Non-enzymatic proteins
1. **Factor X activators** [SVMP], e.g., RVV-X (*Daboia russelii*), VLFXA (*Macrovipera lebetina*)	**1. FVIIa/TF complex inhibitors** [3FTx], e.g., hemextin A/B (*Hemachatus haemachatus*) [PLA_2], e.g., CM-IV (*Naja nigricollis*)
2. **Factor V activators** [SVSP], e.g., RVV-V (*Daboia russelii*), VLFVA (*Macrovipera lebetina*)	**2. FXa/Va complex inhibitors** [3FTx], e.g., naniproin (*Naja nigricollis*) [PLA_2], e.g., CM-IV (*Naja nigricollis*)
3. **Prothrombin activators** Group A [SVMP], e.g., ecarin A (*Echis carinatus*) Group B [SVMP plus snaclec subunit], e.g., carinactivase B (*Echis carinatus carinatus*), multactivase B (*Echis carinatus multisquamatus*) Group C [Factor X and V homolog], e.g., oscutarin C (*Oxyuranus scutellatus*), pseutarin C (*Pseudonaja textilis*) Group D [Factor X homolog], e.g., trocarin D (*Tropidechis carinatus*), notecarin D (*Notechis scutatus scutatus*), hopsarin D (*Hoplocephalus stephensi*)	**3. Factor IX/X inhibitors** [Snaclec], e.g., factor IX/X binding protein (*Protobothrops flavoviridis*)
	4. (Pro)thrombin inhibitors [Snaclec], e.g., bothrojaracin (*Bothrops jararaca*), bothroalternin (*Bothrops alternatus*)
	5. Plasmin inhibitor [Kunitz-type serine protease inhibitor], e.g., textilinin 1 (*Pseudonaja textilis*)
4. **Defibrinating enzymes** [SVSP], e.g., thrombin-like enzymes from most pit vipers	
5. Fibrinogenolysis [SVSP], e.g., albofibrase (*Cryptelytrops albolabris*)	
6. Fibrinolysis [SVMP], e.g., fibrolase (*Agkistrodon contortrix contortrix*) [SVSP], e.g., GPV-plasminogen activator (*Cryptelytrops albolabris*)	
7. **Protein C activator** [SVSP], e.g., Protac™ (*Agkistrodon contortrix contortrix*)	

SVMP snake venom metalloproteinase, *SVSP* snake venom serine protease, *TF* tissue factor, *3FTx* three-finger toxin, *PLA_2* phospholipase A_2, *Snaclec* snake C-type lectin

Each thrombin-like enzyme does not perform all functions of thrombin. For example, some release only fibrinopeptide A from fibrinogen, leaving fibrinopeptide B intact, e.g., ancrod from Malayan pit viper, *Calloselasma rhodostoma* (Au et al. 1993), batroxobin from lancehead viper, *Bothrops atrox* (Itoh et al. 1987), and GPV-TLs from green pit viper, *Cryptelytrops albolabris* (Rojnuckarin et al. 2006), or release only fibrinopeptide B, e.g., contortrixobin from copperhead viper, *Agkistrodon contortrix* (Amiconi et al. 2000). In addition, they do not activate factor XIII, resulting in non-cross-link blood clots that are easily dissolved. Other functions of thrombin are found in other SVSPs, e.g., PA-BJ from Jararaca snake, *Bothrops jararaca*, activates platelet PAR receptor (Serrano et al. 1995), ACC-C from *Agkistrodon contortrix contortrix* activates protein C

without requirement for thrombomodulin (Kisiel et al. 1987), and RVV-V from Russell's viper, *Daboia russelii*, can activate clotting factor V (Tokunaga et al. 1988). Other SVSPs contain fibrinolytic activities by either direct degradation of fibrinogen/fibrin (Muanpasitporn and Rojnuckarin 2007) and/or activation of plasminogen.

Unlike thrombin, snake-derived thrombin-like enzymes are unaffected by heparin. Therefore, SVSP from *Bothrops atrox* can be used to investigate whether the prolongation of thrombin time is due to heparin or hypofibrinogenemia (Reptilase time™). A protein C activator (Protac™) is used for laboratory tests for activated protein C resistance and protein C activity. A factor V activator is utilized for lupus anticoagulant because it is not interfered with factor VIII inhibitors. As high fibrinogen levels confer a poor prognosis in ischemic stroke, these defibrinating agents, ancrod and batroxobin, had therapeutic potentials. However, a meta-analysis of randomized trials does not find a clear benefit of these SVSPs in ischemic stroke compared with standard therapy.

Snake Venom Metalloproteases (SVMPs)/Disintegrins

SVMPs are multi-domain proteins that require Zn^{2+} for enzymatic activities, and therefore, the catalysis is inhibited by ethylenediaminetetraacetic acid (EDTA), a metal chelator. They are evolutionally originated from nontoxic proteins of an "a disintegrin and metalloproteinase" (ADAM) family. The pro-domain makes the enzyme inactive using a cysteine switch mechanism. They are classified into three classes. The mature P-I class comprises only a metalloproteinase domain. The class P-II contains a following disintegrin domain, while the P-III class is followed, instead, by disintegrin-like and cysteine-rich (CR) domains. The disintegrin domains of SVMP class P-II are often released into free disintegrins, as the name implies, blocking integrin functions. On the other hand, disintegrin-like and CR domains are typically attached to the proteinase targeting the enzymes to the sites of actions. Variations of SVMP structures have been found including attached disintegrin domains, released disintegrin-like domains, or additional disulfide-linked subunits of snaclec proteins (Fox and Serrano 2008).

SVMPs can degrade extracellular matrix and vascular basement membrane contributing to endothelial apoptosis. These activities, combining with the mechanical force of blood flow, result in vascular wall damages and bleeding, termed "hemorrhagic effects" (Gutiérrez and Rucavado 2000). Furthermore, SVMPs are found to activate inflammatory cytokines that play essential roles in local tissue necrosis (Laing et al. 2003).

In addition, SVMPs degrade platelet membrane glycoproteins and their ligands, e.g., glycoprotein (Gp) Ib, von Willebrand factor (vWF), collagen, and P-selectin glycoprotein ligand-1 (PSGL-1). Alborrhagin from *Cryptelytrops albolabris* digests the collagen receptor Gp VI (Andrews et al. 2001).

Furthermore, the SVMPs with attached snalclec subunits can activate prothrombin in the presence of calcium, e.g., carinactivase, a prothrombin activator type B

from saw-scaled viper (*Echis carinatus*), or activate factor X, e.g., RVV-X from *Daboia Russelii*. The 3-dimensional structure reveals that the snaclec domain binds the γ-carboxyglutamic acid (Gla) domain of factor X enhancing its activation (Takeda et al. 2007). Some SVMPs can change prothrombin to thrombin independent of calcium, e.g., ecarin from *Echis carinatus*, and are classified as prothrombin activator type A. Finally, many SVMPs are able to directly digest fibrinogen/fibrin.

SVMPs with clotting activator activities are utilized as diagnostic reagents. The prothrombin activator type A (Ecarin) is used in therapeutic monitoring of a direct thrombin inhibiting the drug dabigatran. RVV-X, together with RVV-V, is employed for lupus anticoagulant assay. Furthermore, a modified SVMP from *Agkistrodon contortrix*, alfimeprase, has been investigated to be a direct thrombolytic agent for peripheral arterial disease. Unfortunately, phase III clinical trials do not show significant clinical benefit of alfimeprase compared with placebo.

Disintegrins are small cysteine-rich venom peptides that bind and inhibit integrins. They are usually the proteolytic products of type P-II SVMPs. However, disintegrin proteins translated from isolated mRNAs are reported. The proteins vary in sizes (40–100 kDa) and numbers (4–8) of cysteine bonds (Calvete 2013). They can also be homo- or heterodimers. The hallmark of disintegrins is the integrin-binding tri-peptide motifs, e.g., the typical RGD (arginine-glycine-aspartate) or KGD (lysine-glycine-aspartate) or other variants, WGD, VGD, MGD, MLD, RTS, and KTS. The sequences of and around these sequences, as well as the non-conserved C-terminal tails, determine the integrin-binding specificity (Marcinkiewicz et al. 1997).

Integrins belong to a family of heterodimeric (α and β subunits) membrane proteins mediating cell-matrix or cell-cell interactions. They are critical for cell survival, proliferation, differentiation, and/or activation, as well as platelet aggregation through platelet integrin $\alpha_{IIB}\beta_3$ or Gp IIb/IIIa. The main action of RGD, KGD, or WGD disintegrins is inhibition of platelet aggregation. Eptifibatide, a cyclic peptide derivative of the disintegrin barbourin from dusky pigmy rattlesnake (*Sistrurus barbouri*), and Tirofiban, a synthetic molecule mimicking the disintegrin Echistatin from *Echis carinatus*, are clinically used as antiplatelets for coronary artery diseases. Additionally, some disintegrins can inhibit integrin-dependent tumor cell migration, metastasis, and angiogenesis in animal models (Calvete 2013). A chimeric echistatin with contortrostatin (from *Agkistrodon contortrix*) disintegrin molecule, vicrostatin, can bind many integrins ($\alpha_V\beta_3$, $\alpha_V\beta_5$, and $\alpha_5\beta_1$) and shows anti-tumor activities in an animal model. The liposomal delivery of disintegrin evades the immune system, avoids platelet inhibition, and targets the drug to tumor cells (Minea et al. 2010).

Snake C-Type Lectins (Snaclecs) or C-Type Lectin-Like Proteins (CLPs)

Snaclecs are evolved from a large family of the C-type lectins that are widely expressed from microbes to humans. However, snaclecs are neither C-type (Calcium dependent) nor lectin (carbohydrate binding) proteins. The basic building block of a snaclec is a heterodimer of one α chain of 14–15 kDa and one β chain of 13–14 kDa

linked by an inter-chain disulfide bond. Snaclec structures may be αβ, $(\alpha\beta)_2$, $(\alpha\beta)_4$, or $\alpha_1\alpha_2\beta_1\beta_2$. X-ray crystallography reveals the domain swap between the αβ dimer, providing a binding surface for various target proteins (Morita 2005).

The common targets of snaclecs are platelet receptors, glycoprotein (Gp) Ib, Gp VI, α2beta1 integrin (Gp Ia/IIa), and CLEC2, as well as vWF. The Gp Ib-binding snaclecs may either agglutinate platelets or inhibit platelet aggregation depending on the stoichiometry of bindings (Arpijuntarangkoon et al. 2007). Some snaclecs are higher multimers and bind also Gp VI activating platelet signal transduction. This high multimeric structure may cluster the receptors, resulting in stronger activation (Navdaev et al. 2001). Furthermore, dimeric botrocetin and bitiscetin target vWF and vWF/Gp Ib complex, respectively, enhancing their interaction (Maita et al. 2003). The antibiotic ristocetin shows similar activity by binding to Gp Ib.

Regarding blood coagulation, bothrojaracin from *Bothrops jararaca* binds and blocks (pro)thrombin exosite. Snaclecs with anti-factor IX/X activities bind the Gla domain of these clotting factors in the presence of Ca^{2+}.

Platelet-targeting CLPs are used to dissect the mechanisms of platelet activation mediated by platelet Gp receptors. A snaclec, aggretin (rhodocytin) from Malayan pit viper (*Calloselasma rhodostoma*), leads to the discovery of the CLEC2 receptor on platelets. Botrocetin, a vWF-activating protein, has been used to test for vWF activity in von Willebrand disease patients. In addition, bothrojaracin can treat a rat model of thrombosis (Zingali et al. 2005).

Phospholipases A_2 (PLA_2)

Viperid PLA_2s originated from type IIA PLA_2s, which release arachidonic acid from membrane phospholipid inducing inflammation. Venom PLA_2s can cause membrane damage in specific cell types probably through specific cell surface receptors (Lambeau et al. 1990). The receptors on muscle cells (M type) or presynaptic neuromuscular junction (N type) are implicated in rhabdomyolysis or paralysis caused by certain viper bites, respectively. Many snake venom PLA_2s have the calcium-binding aspartate at position 49 (D49) mutated to lysine (K49) or asparagines (N49), resulting in the losses of enzymatic activity. However, these enzymatically inactive phospholipases may still contain membrane-damaging properties probably through a phospholipid-binding mechanism.

In addition some PLA_2s show anticoagulant activities, e.g., ammodytoxin from horned viper (*Vipera ammodytes*) can bind to factor Xa (Saul et al. 2010).

Protein Families in Elapid Venoms

Examples of major protein families in elapid venoms are listed below. Interestingly, some components of viper and elapid venoms show similar activities, although they originated from different families of proteins demonstrating convergent evolution (Table 20.3).

Table 20.3 Different protein families are used by elapids and vipers to produce the same venom effects demonstrating convergent evolution

Toxic effects	Elapid protein families	Viper protein families
Tissue necrosis	Three-finger toxin (Cytotoxin)	Snake venom metalloproteinase
Neurotoxin	Three-finger toxin or Phospholipase A_2 (Type I)	Dimeric phospholipase A2 (Type II)
Prothrombin activator	Factor X/V homolog (serine protease)	Snake venom metalloproteinase
Factor X activator	Serine protease	Snake venom metalloproteinase
Anticoagulant	Phospholipase A2 (type I)	Snake C-type lectin (Snaclec)
Disintegrin	Three-finger toxin	A disintegrin domain of snake venom metalloproteinase

Three-Finger Toxins (3FTx)

These non-enzymatic proteins are the major toxin class in elapids and more recently found in colubrid venoms. The name is derived from their three-dimensional structure showing three-finger-like β-stranded loops protruding from a hydrophobic core. Over 500 3FTx have been reported. The toxins originated from nontoxic LYNX/SLUR proteins that bind nicotinic acetylcholine receptor (Fry 2005).

The main 3FTx activity is to bind and block postsynaptic nicotinic acetylcholine receptor at neuromuscular junctions, resulting in muscle paralysis. Venom proteins containing this activity are categorized as α neurotoxins.

The additional activities of 3FTx are diverse. Some of them contain the disintegrin sequence RGD, which can inhibit platelet aggregation through integrin $\alpha_{IIB}\beta_3$. Dendroaspin (mambin) from Jameson's mamba (*Dendroaspis jamesoni*) and many-banded krait (*Bungarus multicinctus*) 3FTx bear this motif on their loop III and loop II, respectively (Kini 2011).

Furthermore, 3FTx with anticoagulant activities have been reported. For example, a heterodimeric 3FTx, hemextin AB complex, from ring-necked spitting cobra (*Hemachatus haemachatus*) inhibits clotting factor VIIa/tissue factor complex (Banerjee et al. 2005). In contrast to human TFPI, it does not depend on the presence of factor Xa. Naniproin from black-necked spitting cobra (*Naja nigricollis*) venom can inhibit factor Xa.

Prothrombin Activators Group C and Group D

In contrast to the viperid prothrombin activators group A and B that are SVMPs, elapid group C and D enzymes are serine proteases. Unlike viperid serine proteases that derived from kallikrein, elapid counterparts evolved from snake coagulation factor X with or without a part of factor V (Joseph and Kini 2001).

Group C prothrombin activators mimic factor Xa/Va complex. For example, pseutarin C from Eastern brown snake (*Pseudonaja textilis*) requires only calcium and phospholipid for its activity. On the other hand, prothrombin activators group D are factor Xa homologs. Trocarin D from rough-scaled snake (*Tropidechis carinatus*) in this class needs factor Va in addition to calcium and phospholipid for prothrombin activation.

Molecular modifications of elapid clotting factor activators are being developed as local or systemic hemostatic agents to stop surgical bleeding (Earl et al. 2012).

Kunitz Proteins

Kunitz domain-containing proteins are serine protease inhibitors, such as bovine pancreatic trypsin inhibitor (BPTI) and tissue factor pathway inhibitor (TFPI).

Textilinin-1 from Eastern brown snake (*Pseudonaja textilis*) is a specific plasmin inhibitor homologous to aprotinin. Systemic aprotinin has been used to reduce blood loss during cardiac surgery, but it is recently found to cause renal dysfunction and increase mortality. The more specific enzyme, textilinin-1, is currently under studied to be a novel hemostatic agent (Earl et al. 2012).

Phospholipase A_2

Elapid PLA_2s evolved from a digestive enzyme in pancreas (type IB PLA_2). They target the presynaptic neuromuscular junction and are classified as β neurotoxin. Due to the irreversible damages, neurologic recovery is usually much slower than that of α neurotoxin.

The reported anticoagulant effects may be either dependent or independent on enzymatic activities. The latter mechanism is probably protein-protein interaction. For example, CM-IV from *Naja negricollis* can inhibit the prothrombinase complex (Kini 2005).

Protein Families in Both Viper Elapid Venoms

Some proteins are found in both elapid and viper venoms. These are two examples of proteins affecting hemostasis. They are usually minor components of venoms, and their clinical significances in snakebite patients are unclear.

L-Amino Acid Oxidase

L-amino acid oxidases (LAOs) are homodimeric enzymes that bind flavin-adenine dinucleotide (FAD). They oxidize L-amino acids to αketo acids, ammonia, and

hydrogen peroxide. Biological activities are believed to depend on the peroxide and free radical formation. Oxidative stress is found to induce platelet activation. However, some LAOs with platelet inhibitory activities have been reported. The formed hydrogen peroxide also shows cytotoxic and/or antimicrobial activities. It is hypothesized that LAO may function as a "preservative" of venoms in snake venom glands (Fox 2013).

Nucleotidases

5′ Nucleotidases are enzymes that digest nucleic acids producing nucleosides. The important reaction is the change of ADP into adenosine, thereby reducing the levels of ADP, a platelet agonist. In addition, adenosine causes smooth muscle relaxation, vasodilation, and platelet inhibition (Hart et al. 2008).

The substrates of 5′ nucleotidases in snakebites patients are probably nucleotides (DNA and RNA) released from necrotic cells that are damaged by other components of venoms. Generated adenosine may contribute to bleeding and hypotension in snakebite victims (Caccin et al. 2013).

Snake-Bite Induced Coagulopathy

Although venom studies discovered innumerable activities of venom proteins, only some of which are clinically relevant. For example, bleeding is not a manifestation of Asian elapids, like cobras and kraits, but their venom may contain anticoagulant effects (Kini 2005; Mitrakul 1979). Investigations on these proteins may not be helpful in managements of snakebites, but can lead to discoveries of useful agents from snake venoms.

Clinically, coagulopathy is merely a part of multisystem manifestations of snakebites. Envenoming causes local and systemic effects. The localized tissue damages give rise to pain, swelling, skin bleb, necrosis, and/or gangrene. Systemic absorption of venoms may cause hemorrhagic disorders with or without paralysis, rhabdomyolysis, acute kidney injury, and/or hypotension (reviewed in Rojnuckarin 2010). This section of the review will focus only on the effects on the hemostatic system.

Sometimes, venomous snakes do not deliver their venoms during bites. These "dry bites" do not cause any clinical effect. If venoms are injected, toxins will rapidly disseminate into circulation within minutes. Venom hyaluronidases, or spreading factors, and proteolytic enzymes enhance diffusion and absorption of venoms.

Coagulopathy after viper or elapid bites may be apparent soon after bites, e.g., within half an hour (Reid et al. 1963). However, in patients bitten by weakly venomous snakes, such as green pit vipers (*Cryptelytrops albolabris*), the prolonged coagulation time may become detectable on day 2–3 after bites (Rojnuckarin et al. 1998). Normal coagulation time on the first clinical visit after bites cannot rule out the later development of coagulopathy.

Elapid venoms, generally, comprise small molecular weight proteins resulting in very rapid absorption and clearance. The half-life of Australian elapid venom is only 1 h as calculated using a mathematical model (Tanos et al. 2008). Therefore, recovery of elapid-induced coagulopathy without antivenom is usually faster than those of viper bites.

On contrary, viper venoms contain higher molecular weight proteins that have longer half-lives (over 24 h). For weak or small amounts of venoms, the toxins that persist in the blood may gradually consume platelets and clotting factors explaining the occasional delay in coagulopathy. The studies on venom levels show that prolonged coagulation time is not correlated with the venom antigen level at the time of blood draw. It is associated with the "venom time" or the product of venom level multiplied by a period of time reflecting the cumulative effects of venoms (Rojnuckarin et al. 1999). Viper venom antigen may be measurable in patient's blood for weeks after bites, if antivenom is not given (Reid et al. 1963). Furthermore, cases with persistently detectable antigenemia for 1–2 weeks are correlated with the coagulopathy, compared with cases with more rapid clearance of the venom, although initial venom levels are similar (Rojnuckarin et al. 2007).

Clinical Syndromes of Coagulopathy

Consumptive Coagulopathy

This is the most common bleeding syndrome caused by venomous snakes. Snake venoms activate the common pathway of blood coagulation followed by fibrinolytic system activation. The fibrin deposition can markedly enhance plasminogen activator activity on its surface. This is probably the main mechanisms of fibrinolysis (Rojnuckarin et al. 1999). Furthermore, several venom components with fibrinolytic or fibrinogenolytic activities are often present (see the above sections on protein families of viper and elapid venoms). The common final results are fibrinogen consumption and elevated levels of fibrin degradation products (FDPs). This may be called "defibrination" syndrome. The sites of coagulation pathway activation are different among various snakes.

(a) Factors X and V activations (mostly true vipers): Russell's viper (*Daboia russelli*), horned vipers (*Cerastes* spp.), some European vipers (*Vipera* spp.)
(b) Prothrombin activators
    (i) Some true vipers: saw-scaled vipers (*Echis* spp.)
    (ii) Australian elapids: brown snakes (*Pseudonaja* spp.), Taipan (*Oxyuranus* spp.), tiger snakes (*Notechis* spp.), rough-scaled snake (*Tropidechis carinatus*), broad-head snakes (*Hoplocephalus* spp.)
    (iii) Colubrids: Boomslang (*Dispholidus typus*), vine snakes (*Thelotornis* spp.), red-necked keelbacks (*Rhabdophis* spp.)
(c) Thrombin-like enzymes (mostly pit vipers): Malayan pit viper (*Calloselasma rhodostoma*), Habu (*Protobothrops* spp.), green pit viper (*Cryptelytrops* spp.),

Halys viper (*Gloydius halys*), American pit vipers (*Bothrops* spp., *Agkistrodon* spp.), rattle snakes (*Crotalus* spp.), bushmaster (*Lachesis muta*)

Apart from the coagulation defects, there is also thrombocytopenia. This is caused by disseminated intravascular coagulation (DIC) and/or venom proteins that activate platelets resulting in accelerated platelet clearance in vivo.

A subgroup of patients displays clinical bleeding. The usual sites of hemorrhage are gum, biting sites, venipuncture sites, and gastrointestinal and urinary tracts. Multiple bleeding sites are not uncommon and fatal intracranial hemorrhage may occur. Russell's viper bite in Myanmar may cause pituitary microvascular thrombosis and bleeding resulting in long-term pan-hypopituitarism (Sheehan syndrome). Bleeding severity depends not only on the procoagulant effects, but also the combined toxins that affect several other components of hemostasis, e.g., vessel wall, platelets, and fibrinolysis, as previously mentioned.

The prominent laboratory findings are non-clotting blood due to hypofibrinogenemia and thrombocytopenia under automated cell counting (CBC). The investigation for coagulopathy after snakebites recommended by the World Health Organization (WHO) is the 20-min whole blood clotting time (20WBCT). The test is simple and can be performed everywhere, even in remote health care facilities, requiring only a clean glass tube and a timer. The 20WBCT has been shown to correlate with severe hypofibrinogenemia after viper bites (Sano-Martins et al. 1994; Pongpit et al. 2012) and should be used for therapeutic decision making. Antivenom is indicated when non-anticoagulated whole blood in a glass tube does not form clot at 20 min. Prothrombin time (PT) is a more sensitive and standardized test, widely available in developed settings. Prolonged PT also indicates severe hypofibrinogenemia requiring antivenom therapy (Pongpit et al. 2012). Additionally, more recent study shows that 20WBCT is less sensitive than PT in detecting Russell viper-induced coagulopathy (Isbister et al. 2013). Notably, the point-of-care PT test measuring thrombin generation is not affected by fibrinogen levels and, therefore, cannot be used for monitoring coagulopathy due to snakebites. Activated partial thromboplastin time (APTT) has lower sensitivity for low fibrinogen levels and is also not recommended for general use. Thrombin time, fibrinogen level determination, FDP, and D-dimer are likely to be the most sensitive tests for consumptive coagulopathy. However, these laboratory tests may not be readily available in emergency settings. Thrombocytopenia is often, but not always, correlated with coagulation defects. There are occasional cases with thrombocytopenia with normal whole blood clotting time and vice versa. Blood smear examination is helpful for platelet number estimation and diagnosis of thrombotic microangiopathy after snakebites (see below).

In some circumstances, clotting factor assays may be helpful to differentiate the species containing thrombin-like effects versus species activating factor X or V or prothrombin. Fibrinogen is the only factor consumed by thrombin-like enzymes, while factor X and V levels are depressed by factor X and V activators in true viper envenoming (Mahasandana et al. 1980). On the other hand, prothrombin activators may depress factors V and factor VIII, the thrombin substrates, as well as prothrombin levels in patients (White 2005).

Anticoagulation Syndrome

Pure anticoagulation with no consumptive coagulopathy may occur after certain Australian elapid bites (White 2005). Similar to clotting factor activation syndrome, PT and APTT may be prolonged, but fibrinogen and FDP levels are normal. The venom components causing these symptoms are probably phospholipases A_2, but the exact mechanisms remain unclear. The clinical importance of the identification of this syndrome, as opposed to consumption, is to suggest the snake species. Anticoagulation syndrome may be due to mulga snake (*Pseudechis australis*), spotted back snake (*Pseudechis guttatus*), Collett's snake (*Pseudechis colletti*), death adder (*Acanthophis* spp.), copperhead (*Austrelaps* spp.), and New Guinea small-eyed snake (*Micropechis ikaheka*). Furthermore, bleeding from defibrination is usually more severe than in anticoagulation syndrome.

Thrombotic Microangiopathy

The syndrome resembles hemolytic uremic syndrome (HUS) consisting of microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. It has been reported in patients bitten by Russell's viper, *Daboia* spp.; Saharan horned vipers, *Cerastes cerastes*; lowland viper, *Proatheris superciliaris*; and a colubrid, Boomslang, *Dispholidus typhus*. Furthermore, it is found in 13% (4/32) of severe brown snakebites, *Pseudonaja* spp. (Isbister et al. 2007). This syndrome may be under-recognized because manifestations are similar to the consumptive coagulopathy as described above. The clinical clues are the hemolytic anemia and thrombocytopenia detectable when coagulation time is normal or near normal. The latter finding does not support the defibrination syndrome. Platelet counts, hemoglobin levels, and renal functions are often decline when fibrinogen levels have been recovered and, sometimes, after antivenom administration. Schistocytes on blood smear, red serum, hemoglobinuria, and elevation of lactate dehydrogenase (LDH) enzyme, as well as unconjugated bilirubin, are seen. Reticulocytes may not appropriately increase due to impaired kidney functions. Renal biopsy may reveal thrombus in glomeruli (Isbister et al. 2007). The pathogenesis is still unknown. A recent study in mice showed that purified RVV-X from Russell's viper venom could cause thrombotic microangiopathy and renal failure (Suntravat et al. 2011). The role of antivenom therapy for this condition is also unclear.

Thromboembolism

Thrombosis is rarely reported after venomous snakebites. It remains unclear whether these are coincidental. Thrombosis occurring very early after snakebite may be a result of rapid and strong procoagulant effects of the venoms prior to fibrinolytic activation. This hypothesis is also used to explain sporadic sudden cardiac death early after the brown snakebites (White 2005).

Thromboembolism is a characteristic feature of bites by two viper species: Martinique viper (*Bothrops lanceolatus*) on the Martinique Island and Saint Lucia viper (*Bothrops caribbaeus*) on Saint Lucia Island. The patients are presented with deep vein thrombosis with or without pulmonary embolism or ischemic stroke. These complications can effectively be prevented using the specific antivenom.

Conclusion and Future Directions

Snake venom studies can be divided in to the basic and clinical science. Current basic researches aim to discover useful agents from snake venoms. This brief review demonstrates the great power of natural selection. Snake venom proteins seemed to "know" human physiology at the molecular level millions of years before we do. As the experiment tools, they have helped us to explore ourselves. Snake venom components are certainly still useful as agents for dissecting molecular mechanisms of hemostasis and/or diagnostic reagents.

On the other hand, therapeutic potentials of snake venom proteins by themselves are limited as they are originally designed by nature for a one-time injection. Repeated administrations may induce antibody. Oral intake is not possible. Notably, they are originally built "not to be safe." Furthermore, industrial recombinant protein expression is technically demanding and expensive. Therefore, snake venom proteins may be utilized for laboratory investigations searching for therapeutic targets. Subsequently, small molecules or modified proteins can be engineered for clinical applications.

For the clinical part, the snakebite-induced consumptive coagulopathy has been well described, but the other uncommon thrombohemorrhagic syndromes required further studies. The key treatment of snakebite is antivenom that is reviewed in details in a separate chapter.

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Clinical Uses of Snake Antivenoms

21

Ponlapat Rojnuckarin

Contents

Abstract

Antivenom is the key treatment for venomous snakebites. It is produced by purification of polyclonal IgG from plasma of large animals pre-immunized by snake venom. Polyvalent antivenoms, which neutralize venoms from many species prevalent in the areas of uses, are preferred over monovalent antivenoms because the snake species are frequently unidentifiable in clinical practice. Antivenom therapy can promptly reverse snakebite-induced coagulopathy and limb edema, but muscular paralysis from presynaptic toxins, tissue necrosis, and renal failure resolve much more slowly, especially when antivenoms are given late after bites. Effective treatments of these latter complications remain to be determined. The anaphylaxis-like early adverse reaction is the major limitation of antivenom uses. It is unpredictable by the immediate hypersensitivity skin test, and therefore, every antivenom administration requires close observation. Highly purified caprylic acid-stabilized IgG antivenoms show significantly lower rates of

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reactions. Clinical judgments to give antivenom should be individualized weighing potential benefits versus risks of antivenoms for the snakes in specific regions. Due to the high cost of antivenom production, this therapy is usually lacking in developing countries where snakebites are very common. Strategies for the adequate supply of good quality antivenoms are strongly needed.

Introduction

Antivenom, the antidote of snake venom, is the main remedy for venomous snakebites. Much research has been performed to determine how to produce effective and safe antivenoms, as well as how to use them efficiently. Nevertheless, randomized controlled clinical trials of snake antivenoms are rare. Moreover, the major global public health problem nowadays is the distribution of antivenoms.

Although it is widely used, good quality antivenoms are still lacking in developing world, e.g., in Africa or India, where snakebites are most prevalent. Antivenom production is costly but not profitable because snakebite is a disease of the poor. In developed countries, such as the United States, antivenom is available but very expensive due to a small number of snakebite patients. In addition, antivenoms cannot prevent and/or reverse all effects of snake venoms, especially when antivenoms are given too late after bites. Rapid transportations of patients in remote rural areas to healthcare facilities are required. Furthermore, other supporting measures, such as assisted ventilation, dialysis, and wound care, may be necessary for certain snakebite patients even after antivenom administrations.

Snake Venom Characteristics

There are some special characteristics of snake venoms to be considered for antivenom therapy.

1. The main toxic components of snake venoms are proteins. Therefore, therapy with antibodies that usually recognize large molecules is an appropriate treatment.
2. Venom of a snake species is a mixture of proteins (Calvete 2013). Therefore, a polyclonal, rather than monoclonal, antibody is often required to neutralize all effects of venom.
3. Different proteins in venoms contain different effects. Some may cause rapid and irreversible damages, such as presynaptic nerve terminal damages or local tissue necrosis. Antivenom administration, which is usually delayed for a period of time in clinical settings, is usually not very helpful for these damages. However, some effects, such as consumptive coagulopathy, can be reversed by antivenom. The body can replenish coagulation factors and platelets within a relatively short period of time (a few hours) after antivenoms stop the consumption process.

4. Although snake venom components display diverse activities, they are evolutionally originated from only a few families of proteins. Consequently, antivenom against one species of venom may be able to neutralize venom from those of others. The phenomenon is called "paraspecificity" (Archundia et al. 2011).
5. Snakes have remarkable capability to adapt their venom compositions to match the prey. This can be done using genetic (accelerated DNA mutations) or epigenetic (microRNA expression) mechanisms (Nakashima et al. 1995; Durban et al. 2013). Therefore, the same snake species in different geographic locations or some snakes at different ages may have dissimilar venom compositions because of the prey variations. For example, Russell's viper bites from different countries were reported to have different clinical manifestations (Warrell 1989). Consequently, the venoms that are used to immunize animals for antivenom production need to be the mixture of all representative venoms in the areas where the antivenoms are intended to be used. Otherwise, antivenom may not be clinically effective.
6. The half-lives of venom components are different. For example, viper venoms contain larger molecular weight proteins compared with elapid venoms, and hence, viper venoms may persist in human blood for weeks. The half-life of cobra, an elapid, venom in human is approximately 7.5 h without antivenom (Hanvivatvong et al. 1988). On the other hand, a kinetics study in green pit viper bite (*Cryptelytrops spp.*) patients shows that venom antigen clearance comprises 2 phases. In the first 3 days, venom half-life is 27.5 h and increases to over 50 h in day 5–7 (Rojnuckarin et al. 2007). If antivenom with a short half-life, such as Fab antivenom, is used, viper venom effects may recur after the antivenom clearance. Repeated administrations of Fab antivenom are usually needed for the treatment of viper bites (Boyer et al. 2001).

Snake Antivenom

Currently, snake antivenom is polyclonal IgG or a part of IgG purified from plasma of immunized animals, such as horses or sheep. Previously, the animal serum separated from clotted blood was used. Currently, blood of these animals is anticoagulated, and plasma is isolated by centrifugation. Red blood cells are, subsequently, returned to the animals. Therefore, higher volume of blood can be taken from them with less harmful effects. The process is termed plasmapheresis.

The immunogen is the snake venom that is derived from 20 to 50 individual snakes. These snakes must be varying in ages and collected from different geographical locations representative of the whole area of antivenom uses. A snake farm is needed for snake husbandry. Antivenom is usually not available for snakes that cannot be raised in captivity, such as many species of sea snakes. In addition, an immunized animal farm is also required. These animals need to be maintained in a pathogen-free environment.

Table 21.1 Comparison of early adverse reaction rates in various venom preparations

Trial Designs	Main results	References
Randomized, double-blind	A. $F(ab')_2$ with NH_4SO_4 36.7 % ($N = 30$)	Otero-Patiño et al. (1998)
Bothrops antivenom	B. IgG with caprylic acid 11.1 % ($N = 27$)	
	C. IgG with NH_4SO_4 81.8 % ($N = 22$)	
Randomized, double-blind	A. IgG with NH_4SO_4 52 % ($N = 25$)	Otero-Patiño et al. (1999
Bothrops antivenom	B. IgG with caprylic acid 25 % ($N = 28$)	
Randomized, double-blind	A. No beta propriolactone 15 % ($N = 34$)	Otero-Patiño et al. (2006)
Bothrops antivenom	B. With beta propriolactone 24 % ($N = 33$)	
IgG with caprylic acid		
Randomized, double-blind	A. $F(ab')_2$ with caprylic acid 28.7 % (N = 38)	Otero-Patiño et al. (2012)
Bothrops antivenom	B. IgG with caprylic acid 20.6 % (N = 34)	

Venom with appropriate adjuvant was injected subcutaneously in to horses using the small volume and multiple site technique. Too much the injected volume may cause tissue necrosis. Horse immunization protocol is recommended in the World Health Organization (WHO) guideline (WHO Expert Committee on Biological Standardization) and needs to be optimized as appropriate. Immunological responses are followed by enzyme-linked immune-sorbent assay (ELISA) for venom antibodies in animal blood. Plasmapheresis will be performed and IgG is then purified from plasma. In vitro and in vivo tests are performed to confirm the ability of antivenom to neutralize snake venoms.

Optimal antivenom production process is important to reduce the adverse reactions in patients receiving antivenoms. The whole animal plasma or serum can induce fatal hypersensitivity reactions, and thus, only the IgG fraction needs to be isolated. The recommended method is to use caprylic acid to precipitate the other plasma proteins. Caprylic acid is inexpensive and yields IgG with a good physicochemical property to avoid IgG aggregation. Randomized controlled clinical trials suggest that caprylic acid-purified antivenom causes lower rates of early adverse reactions compared with those purified using ammonium sulfate precipitation (Table 21.1).

The IgG molecule comprises 2 Fab arms and one Fc portion (Fig. 21.1). The Fc portion is able to activate complement and believed to contribute to severe antivenom adverse reactions. The enzymes, pepsin and papain, are used to cleave IgG Fc portion resulting in $F(ab')_2$ and Fab, respectively (Fig. 21.1). These enzymatic modifications produce smaller molecules with increased renal clearance. The half-lives of IgG, $F(ab')_2$, and Fab are 45–96 h, 28–55 h, and 8–18 h, respectively (Gutiérrez et al. 2003). This may cause recurrences of symptoms of viper bites after fab antivenom uses as previously mentioned.

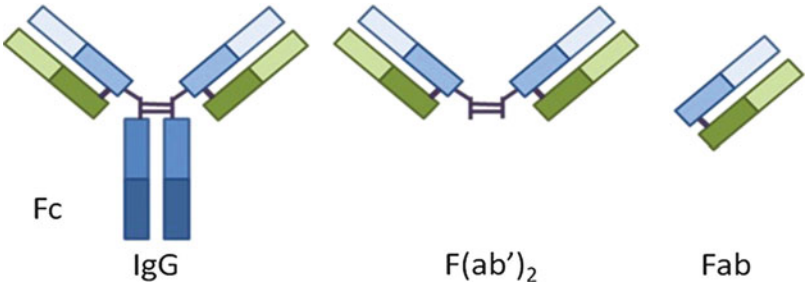

Fig. 21.1 Three types of IgG antivenom

An in vitro study shows that Fc removal results in lower rates of complement activation. In addition, the $F(ab')_2$ antivenom induces lower antibody response in animals compared with the whole IgG (León et al. 2001). $F(ab')_2$ antivenom is found to have lower reaction rate than the whole IgG antivenom in one randomized controlled trial using ammonium sulfate purification. Nevertheless, if both types of the antivenoms are purified using caprylic acid, the patient reactions to $F(ab')_2$ are not lower than that of the whole IgG antivenom (Table 21.1).

Antivenoms can be classified into two types according to their specificity.

1. Monovalent (Monospecific) antivenoms are raised against one species of snake. Therefore, it is effective for only one species or for a few closely related species of snakes. In clinics, the species of snake needs to be identified by snake carcasses brought by patients, patients' identification, epidemiological data, characteristic clinical manifestations, or detectable venom antigens in patients' blood.
2. Polyvalent (Polyspecific) antivenoms are active against more than one species of snake. It is preferably produced by immunization of one animal using several venoms rather than mixing of several monovalent antivenoms (Chotwiwatthanakun et al. 2001). An animal immunized by several venoms may produce antibodies that recognize a common epitope of different species of venom. As a result, the protein load is probably lower than the mixture of monovalent antivenom. In addition, these antibodies to a common epitope may recognize other snake venoms that contain related proteins (paraspecificity).

Polyvalent antivenom is more practical than monovalent antivenom because species of biting snakes are commonly unidentifiable. Polyvalent antivenoms can be designed to cover all common species in a whole country or region. In Thailand, elapid venoms are exclusively toxic to neuromuscular system, and viper venoms are solely toxic to hemostatic system. Queen Saovabha Memorial Institute (QSMI) in Thailand produces two polyvalent antivenoms against elapids and vipers for patients showing neurological signs and coagulation abnormalities, respectively.

Efficacy of Antivenoms

Testing the efficacies of antivenom to prevent deaths in animal models is usually required before clinical uses. However, the results may not be directly applicable to humans. Apart from interspecies (human vs. experimental animals) variations, antivenom administrations in clinical practice are usually much more delayed compared with animal experiments.

As venomous snakebites are potentially lethal, placebo-controlled trials of antivenoms are lacking due to ethical problems. The efficacies are usually deduced from historical controls when antivenoms were not available or when the doctors decided not to give antivenoms (Table 21.2).

1. Snakebite-induced coagulopathy is usually caused by viper bites. Viper venoms have long half-lives. If antivenom was not given, non-coagulable blood may persist up to 2 weeks after bites (Mitrakul and Impun 1973; Visudhiphan et al. 1981). However, antivenom usually corrects coagulopathy within 6–12 h

Table 21.2 Efficacy of antivenom on tissue-damaging effects

Trial designs	Main results	References
Nonrandomized comparison	Time on assisted ventilation	Pochanugool et al. (1997)
Cobra bite and	A. Antivenom (≥100 ml) 10 h ($N = 18$)	
Respiratory failure	B. No antivenom 44 h ($N = 27$)	
In Thailand	$P < 0.002$	
Nonrandomized comparison	A. Early (<6 h) antivenom ($N = 12$)	Hung et al. (2006)
Russell's viper bite and	Oliguria 8.3 %, *renal recovery 7.8 days**	
Acute renal failure	B. Late or no antivenom ($N = 19$)	
In Taiwan	Oliguria 73.7 %, renal recovery 33.4 days	
	$*P = 0.001$, $**P = 0.005$	
Randomized, double-blind	Limb circumference reduction in 1 day	Rojnuckarin et al. (2006)
Green pit viper bites and	A. Antivenom 1.1 ± 0.13 cm ($N = 14$)	
Limb edema	B. Placebo 0.37 ± 0.41 cm ($N = 14$)	
In Thailand	$P = 0.01$	
Nonrandomized comparison	Skin necrosis	Chotenimitkhun and Rojnuckarin (2008)
Green pit viper bites and	A. Antivenom 8.9 % ($N = 80$)	
Dermonecrosis	B. No antivenom 7.3 % ($N = 163$)	
In Thailand	$P = 0.895$	

(Mitrakul et al. 1991; Karnchanachetanee et al. 1994; Rojnuckarin et al. 1998). Therefore, antivenoms are effective for consumptive coagulopathy after snakebites. After stopping the clotting factor consumption, the body can rapidly replenish the deficient clotting factors and platelets. However, a randomized controlled trial in Australia demonstrates that fresh frozen plasma infusion in addition to antivenom can enhance the resolution of coagulopathy as indicated by prothrombin time (PT) prolongation. However, the clinical bleeding symptoms are not different between the two groups (Isbister et al. 2013a).

2. Neuromuscular blockade may be due to presynaptic or postsynaptic neurotoxins. Presynaptic toxins, such as from kraits, are phospholipases A_2 that irreversibly damage nerve terminals resulting in delayed recovery, e.g., 1–2 weeks. Cobra venoms have postsynaptic three-finger neurotoxin with faster spontaneous recovery time. When cobra (*Naja spp*.) antivenom was not available in Thailand, the patients required assisted ventilation for the mean duration of 44 h. On the other hand, the mean recovery time was only 10 h after adequate doses of antivenom administrations (Pochanugool et al. 1997). Therefore, cobra antivenom is probably effective in shortening the recovery when compared with the historical controls. Of note, the effect of cobra antivenom is not immediate. Patients may require several hours of assisted ventilation after antivenom. Therefore, antivenom cannot replace a ventilator. With antivenom therapy, all patients bitten by snakes with neurotoxin still need close observation and assisted ventilation if indicated.

   The efficacy of antivenom for presynaptic neurotoxin is difficult to determine in humans due to the very slow neurological recovery even after antivenoms (Leeprasert and Kaojarern 2007). Because of the high mortality, antivenom is recommended in all patients with suspected krait bites, and it should be given as soon as possible, preferably before neurological symptoms. Antivenom may be able to neutralize circulating venoms in the blood preventing irreversible injury to the nerve terminals.

3. Snakebites may also cause local symptoms around the biting wounds, such as pain, edema, and skin blistering. For limb swelling, a randomized double-blind placebo-controlled trial in patients with green pit viper (*Cryptelytrops spp*.) bites in Thailand shows that antivenom can hasten bitten limb edema resolution on days 1–2 after antivenom with statistical significance. However, the differences in limb circumferences are modest and the pain scores are similar with or without antivenom. Therefore, antivenom is not routinely recommended solely for limb edema. Nevertheless, this study was the proof of concept that antivenom can be helpful when faster recovery of tissue swelling is required, such as the presence of compartment syndrome. Compartment syndrome is a result of muscle swelling under deep fascia markedly increasing subfascial pressure and compromising blood supply. Antivenom therapy, as well as limb elevation, is recommended as the first-line treatment for the compartment syndrome. In addition to enhance edema resolution, antivenom can correct coagulopathy preparing patients for surgical fasciotomy when the medical treatment failed (Gold et al. 2002).

4. Tissue necrosis is a debilitating complication of certain snakebites, such as cobras (*Naja spp*.), king cobra (*Ophiophagus hannah*), or Malayan pit viper (*Calloselasma rhodostoma*). Green pit viper (*Cryptelytrops spp*.) bites more commonly cause dermonecrosis if the bitten sites are fingers or toes (Rojnuckarin et al. 1998). A nonrandomized comparative study in green pit viper bites on digits shows that dermonecrosis still occurs after antivenom administration (Chotenimitkhun and Rojnuckarin 2008). Therefore, antivenom is relatively ineffective in prevention and/or treatment of necrosis in contrast to the efficacy in systemic coagulopathy. Consistent with these data, an animal study shows that the effects of venom on tissue damage are very rapid. Antivenom given later than 15–30 min after venom injection is ineffective (Gutiérrez et al. 1998). The delay time of antivenom is typically much longer in clinical practice as it usually takes a while to bring a victim to a hospital with available antivenoms.
5. Some snakes, such as Russell viper (*Daboia russelli*), may cause renal failure. In addition to the direct toxicity of venoms, reduced renal blood flow, myoglobinuria, and hemoglobinuria from snakebites all contribute to kidney injury. A nonrandomized comparative analysis in Taiwan showed that early (<6 h after bites) antivenom may prevent severe renal damages after Russell's viper bites (Hung et al. 2006). Notably, renal failure is not totally preventable by antivenom. Furthermore, hydration and diuretic medications to promote renal blood flow and to avoid hemoglobin/myoglobin precipitation may also be helpful in prevention for snakebite-induced kidney failure.
6. Sea snake bites often cause systemic rhabdomyolysis. Consequently, myoglobinuria results in acute renal failure. There are many species of sea snakes, but the reported cases are rare, fewer than ten patients per year in Thailand. Furthermore, the snakes cannot be maintained in captivity. Therefore, sea snake antivenom is still unavailable and supportive treatments are needed.

Safety of Antivenoms

Side effects of antivenoms may occur as early reactions (within few hours) or late serum sickness (at 1–2 weeks). Early adverse reactions are rashes, fever, urticaria, bronchospasm, and hypotension that may lead to death. This reaction is the major limitation of antivenom uses. The incidence of early adverse reactions varies from 3.5 % to 85 % depending on the purity and preparation of antivenoms, as well as retrospective versus prospective natures of the studies (Thiansookon and Rojnuckarin 2008; Isbister et al. 2008). The incidence of serum sickness (fever, rashes, arthritis, lymphadenopathy, and/or proteinuria) is unclear due to the lack of prospective trial. However, it is uncommonly encountered in clinical practice.

The mechanism of early adverse reactions is related to the immunoglobulin aggregation. This results in complement, C3a and C5a or anaphylatoxin, activation through the Fc portion of IgG. The hypothesis is supported by the clinical data showing that caprylic acid-purified antivenoms that avoid IgG aggregation or antivenoms that lack the Fc portion has lower rates of reactions (Table 21.1).

However, complement activation has not been demonstrated in patients who develop reactions to antivenoms (Malasit et al. 1986). Furthermore, antivenom production that is added with anticomplement agent, beta propriolactone, cannot reduce the adverse reaction rates compared with caprylic acid-purified antivenom without this agent (Table 21.1).

Because the reactions are IgG-mediated, they are unpredictable using the immediate hypersensitivity skin test that detects IgE-mediated responses. Almost all patients with early adverse reactions had negative skin tests. In addition, patients with positive skin tests did not have reactions after antivenom infusion (Malasit et al. 1986; Thiansookon and Rojnuckarin 2008). Therefore, the antivenom skin test is not recommended before antivenom administrations.

Due to the potentially fatal reactions, close observation is required for all patients receiving snake antivenoms. Approximately 90 % of reactions appeared within 1 h of starting antivenoms (de Silva et al. 2011). However, some reactions may be delayed for a few hours. If there is a severe reaction, such as hypotension, bronchospasm, or hypoxemia, antivenom infusion should be stopped immediately. Adrenaline is the specific medication for severe reactions and antihistamine/steroid may be used for cutaneous reactions, e.g., rashes and urticaria.

Clinical studies on premedications have been conducted to prevent early adverse reactions (Table 21.3). Most trials show that an antihistamine, promethazine, is ineffective. The results of steroid premedication are conflicting. One trial using 1,000 mg hydrocortisone continuous infusion (from 5 min before to 30 min after antivenom) with an antihistamine, chlorpheniramine 20 mg intravenously, shows an efficacy of this regimen (Gawarammana et al. 2004). However, a larger trial of a lower dose of hydrocortisone (200 mg) reveals negative result. Moreover, this dose of steroid may negate the positive effect of adrenaline premedication when they are used in combination (de Silva et al. 2011).

Adrenaline (0.25 mg) subcutaneous injection is found to prevent severe adverse reactions to antivenom in a randomized controlled trial (Premawardhena et al. 1999). However, the safety of adrenaline, especially in elderly patients with preexisting cardiovascular diseases, has been a major concern. A more recent larger study confirms that adrenaline premedication is effective and relatively safe (de Silva et al. 2011). However, the rate of severe reactions was still high in this particular trial even after adrenaline, probably due to the property of the antivenom.

Another endeavor to decrease early adverse reactions is to infuse antivenoms more slowly. However, two randomized controlled trials shows that longer infusion times cannot reduce the incidences of reactions (Table 21.3). Therefore, antivenoms may be given by either injection or slow infusion through an intravenous set. One advantage of antivenom injection is that doctors or nurses must stay with the patients during the time of administration and they can observe for antivenom early adverse reactions.

Therefore, the most appropriate solution for early adverse reactions is to improve the quality of antivenoms and make them widely available. In Thailand, Queen Saovabha Memorial Institute produces caprylic acid-purified $F(ab')_2$ antivenom that shows a very low rate of reactions (3.5 %, Thiansookon and Rojnuckarin 2008). Premedication is not necessary for antivenom therapy in Thailand.

Table 21.3 Comparison of early adverse reaction rates in various premedication regimens and infusion rates

Trial designs	Main results	References
Randomized, double-blind	A. Adrenaline (1: 1,000) 11 % ($N = 56$) (0.25 ml subcutaneously)	Premawardhena et al. (1999)
District general hospital in Sri Lanka	B. Placebo 43 % ($N = 49$) $P = 0.0002$	
Randomized, double-blind	A. Promethazine 24 % ($N = 49$) (0.5 mg/kg or 25 mg in adult IM)	Fan et al. (1999)
Public hospital in Brazil	B. Placebo 25 % (N =52)	
Randomized, double-blind	A. Hydrocortisone(1 g) 80 % ($N = 15$) (IV infusion until 30 min after antivenom)	Gawarammana et al. (2004)
General hospital in Sri Lanka	B. Hydrocortisone (1 g) with Chlorpheniramine (20 mg IV) 52 % ($N = 21$)	
	C. Placebo 81 % ($N = 16$)	
Randomized, double-blind	Total $N = 1{,}007$	De Silva et al. (2011)
Hospitals in Sri Lanka	Overall severe reactions rate 43 %	
2×2×2 factorial design	Odds ratio (OR)	
Adrenaline 0.25 ml SC	A. Adrenaline OR 0.62 ($p < 0.001$)	
Hydrocortisone 200 mg IV	B. Hydrocortisone OR 0.80 (p 0.296)	
Promethazine 25 mg IV	C. Promethazine OR 0.87 (p 0.629)	
Randomized controlled trial	A. Rapid (10 min) 42 % ($N = 33$)	Malasit et al. (1986)
In Thailand	Severe reactions 18.2 %	
Infusion rates	B. Slow (>30 min) 56 % ($N = 33$)	
	Severe reactions 15 %	
Randomized controlled trial	Severe reactions within 4 h	Isbister et al. (2012)
In Sri Lanka	A. Rapid (20 min) 32 % ($N = 104$)	
Infusion rates	B. Slow (2 h) 35 % ($N = 94$)	

Clinical Uses of Antivenoms

The prehospitalization phase is a critical period of snakebite therapy. Many deaths occur before arrival to healthcare facilities. Although pressure immobilization has been recommended as the first-aid measure (Warrell 2010), it is not practical for general uses due to the lack of proper skills and instruments on-site. The most important prehospital management is to bring the victims to hospitals as soon as possible.

There are two strategies of antivenom uses.

1. Antivenom for all snakebite patients: This is suitable for bites by snakes causing rapid, irreversible, and potentially lethal damages, such as kraits (*Bungarus spp.*) that have presynaptic neurotoxin. The burden for this strategy is the side effects and costs of antivenoms.
2. Antivenom only for patients with signs of envenoming: The reason of this strategy is that approximately 50 % of snakebites are dry bites or no-venom injection. Antivenom administrations in this group make the patients at risk for severe early adverse reactions. Using this strategy, the patients need close observation. Antivenom should be given as soon as there are any signs of systemic envenoming because delayed antivenoms may not be as effective.

Regardless of antivenom uses, all snakebite patients need close observations. In particular, patients bitten by snakes with potential neurological toxicity require monitoring for respiratory muscle fatigue. Intubation and assisted ventilation devices must be readily available. Antivenoms do not guarantee that the patients will not progress to respiratory failure. Therefore, patients need further observation even after antivenom administration for both worsening of muscular weakness and early adverse reactions of antivenoms.

Bites by pit vipers usually cause fibrinogen consumption by thrombin-like enzymes resulting in hypofibrinogenemia. Twenty-minute whole blood clotting test (20WBCT) is recommended by World Health Organization (WHO) for the diagnosis of coagulopathy induced by snakebites. In pit viper bites, it reflects severe hypofibrinogenemia requiring antivenom (Sano-Martins et al. 1994; Pongpit et al. 2012). In green pit viper bite patients, 20WBCT is as sensitive as prothrombin time (PT) in diagnosing hypofibrinogenemia (Pongpit et al. 2012). Nevertheless, the coagulopathy may sometimes delay for 2–3 days. A normal 20WBCT at presentation still requires follow-up tests.

Recent data suggests that 20WBCT is not sensitive enough to detect coagulopathy in Russell's viper bites compared with PT (Isbister et al. 2013b). The uses of 20WBCT may result in delayed antivenom. In contrast to the pit viper venoms, Russell's viper venom activates common pathway of coagulation decreasing factor X, factor V, and prothrombin levels, in addition to hypofibrinogenemia. Therefore, appropriate coagulation tests for assessing the effects of snake venom may be different among various species. The 20WBCT is also recommended for follow-up tests after antivenom therapy. Blood coagulation is usually restored within 6 h after antivenom administration. Repeated doses should be given if the clotting time is still prolonged. One dose of antivenom is able to neutralize average venom released from one simulated bite. If the clotting time persistently un-clotted after four doses of antivenom, incorrect species identification should be considered. This problem can be overcome using polyvalent antivenoms. After normalization of clotting time by IgG or $F(ab')_2$ antivenom, recurrences of coagulopathy are rare because of the long half-lives of these antivenoms.

Early antivenom should be considered in Russell's viper bites even without coagulopathy in order to prevent severe renal damages. However, the scientific evidence for this notion is not strong (Hung et al. 2006).

Due to the incomplete reversal of venom effects of venoms, other supportive measures may still be required in conjunction with antivenom administrations. For example, surgical debridement of necrotic tissues, assisted ventilation, or dialysis may be indicated.

In conclusion, decisions to give antivenoms should be individualized depending on the risk benefit of the situations. Different snakes cause effects with dissimilar severity and reversibility. Antivenom availability, reaction rates, and efficacies for certain effects are to be considered. For example, antivenoms with high rates of reactions may be delayed in asymptomatic patients bitten by snakes that cause solely coagulopathy, and their coagulation times are normal. If coagulopathy occurs during observation, it is usually rapidly reversible by antivenoms. On the other hand, good quality antivenom should be strongly considered in presymptomatic patients bitten by snakes that potentially cause severe slow-recovering neuropathy or nephropathy. Therefore, the guidelines for snakebites need to be written specifically for the countries that use them. In addition, they require updates when their situations of antivenom change.

In Thailand, antivenom is indicated in all krait bite cases, but in cobra bite only patients with early signs of muscle weakness, such as mild ptosis. For viper bites, antivenoms are given to patients with prolonged coagulation time or thrombocytopenia or marked limb edema with a concern of compartment syndrome (Rojnuckarin et al. 2012). Due to the low incidence of antivenom reactions, antivenom may optionally be given to asymptomatic patients who are bitten by neurotoxic or nephrotoxic snakes.

Conclusion and Future Directions

At present, the major global problem is the distribution of good quality antivenoms to developing countries (Gutiérrez 2012). Due to the low profit margin of antivenom production, it should be funded by nonprofit organizations that may be governmental or nongovernmental. Technology may be transferred to the countries with capability for the production. Alternatively, a manufacturing center may be responsible for supplying several countries in a region. The third option is using the venoms from one region for the antivenom production in an established facility far away. Furthermore, new modalities are being explored to reduce the cost of antivenom production as described below.

A limitation of antivenom production is the requirement for a large animal farm for immunization. Raising pathogen-free horses or sheep is very costly. Chicken are relatively inexpensive to maintain. Immunized hens secrete the immunoglobulin Y (IgY) antibody into their eggs. The IgY can be easily purified from egg yolks.

Therefore, they have potentials for snake antivenom production (Meenatchisundaram et al. 2008; de Andrade et al. 2013). However, occasional epidemics of avian influenza viruses are major threats to chicken farms.

Camels are the alternative source of antivenom. Dromedaries can tolerate extreme climates, such as in Africa, better than horses or sheep. Immunized dromedaries produce the small single-domain antibody (molecular weight 15 kDa) comprising only one heavy chain, compared with conventional antibodies (molecular weight 150 kDa) comprising two heavy chains and two light chains. Camelid antibody does not contain Fc portion, and therefore, it is less likely to cause infusion reactions. Due to its thermal stability, dromedary antivenom may be heat treated to kill microorganisms and transported at room temperature. These will markedly reduce cost of production and distribution. Furthermore, this smaller structure of antibody may have larger volume of distribution and possibly be more effective for tissue effects of snake venom (Cook et al. 2010a, b).

Another limitation of antivenom production is the requirement of a snake farm as the source of immunogens. An alternative is to use DNA of venom genes for animal immunization (Harrison 2004). The major advantage is that DNA is cheaper than proteins to be amplified and stored. The gene gun delivery system injected DNA-coated beads into the skin dendritic cells resulting in antibody (Th1), rather than T-cell, responses. Common immunogenic epitopes can be predicted in order to produce antivenom that can neutralize several isoforms of toxins present in various species (Wagstaff et al. 2006).

With the advances in proteomics, the technology has been applied to study all components of snake venoms at the same time, termed "venomics." Using this method, venom components are separated on a two-dimensional gel, and each spot identity is determined by mass spectrometry. Complex compositions of snake venom can be identified. Venomic studies are used to investigate venom variations, for example, among ages and geographical locations. Another useful technique is called "antivenomics" as venom components are immunodepleted with antivenoms. The remaining proteins are unlikely to be neutralized by the antivenom. Therefore, this is a useful in vitro test to investigate the antivenom efficacy (Calvete 2010). More cumbersome animal studies may be avoided.

As previously mentioned, antivenom uses need to be customized for each countries or regions because of the wide variety in snake species, antivenom efficacy, and safety. More local or national clinical researches are strongly required to formulate a specific guideline for effective antivenom utilization in each region of the world.

Cross-References

▶ Developing Snake Antivenom Sera by Genetic Immunization: A Review

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Part II

Other Animal Toxins

Scorpion Sting and Envenomation

22

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Contents

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Abstract

Scorpion stings are a major public health problem in many underdeveloped tropical countries, especially Sahelian Africa, South India, the Middle East, Mexico, and South Latin America. Although there are various species of scorpions, only few of these can be potentially lethal to humans. Scorpion venoms consist of a complex of several toxins that exhibit a wide range of biological properties and actions. The venom is variable in chemical compositions, toxicity, and pharmacokinetic and pharmacodynamic characteristics. The envenoming is associated with high morbidity and mortality, especially among children. Victims having envenomed by a scorpion suffer a variety of pathologies, including mainly both sympathetic and parasympathetic stimulation and central manifestations such as irritability, hyperthermia, vomiting, profuse salivation, tremor, and convulsion. Envenoming by scorpion can result in a variable range of clinical features, including cardiotoxicity, neurotoxicity, and respiratory dysfunction. Many health-care providers are unaware of the effects of their stings and scorpions are often feared based on their fatal reputation. Treatment regimen including scorpion antivenom and vasodilators and intensive care management have been tried to alleviate the systemic effects of envenoming. Administration of anti-scorpion venom serum (AScVs) is the only specific treatment available but has many limitations like species specificity, difficulty in availability, affordability, and ideal storage conditions. In spite of advances in pathophysiology and therapy, mortality remains high in rural areas due to lack of access to medical facilities.

Introduction

Scorpions are predatory arthropod animals of the order **Scorpiones** within the class Arachnida. The word *scorpion* is thought to have originated in Middle English between 1175 and 1225 AD from Old French *scorpion* or from Italian *scorpione*, both derived from the Latin word *scorpius*, which is the romanization of the Greek word σκορπίος – *skorpíos*.

A scorpion has a flattened elongated body and can easily hide in cracks. It has four pairs of legs, a pair of claws, and a segmented tail. This long, slender tail is usually arched over the back of the abdomen and contains a bulb-like venom

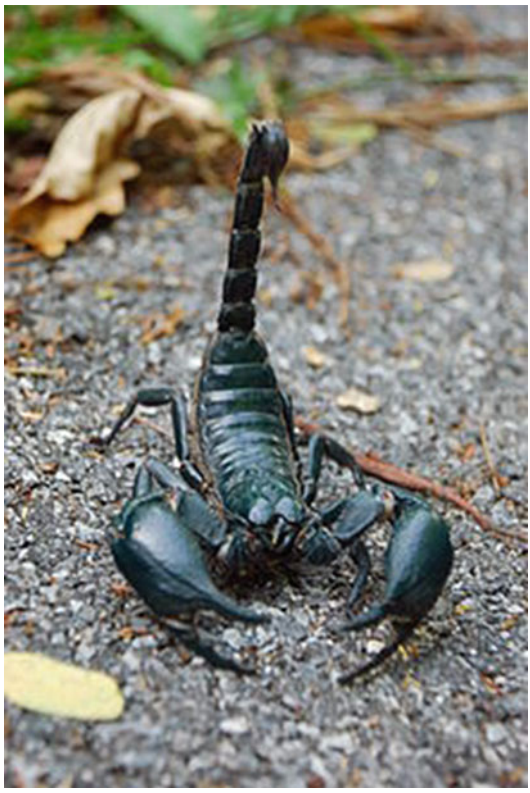

Fig. 22.1 Asian forest scorpion (Russel Wright)

gland and a stinger that has a venomous spike at the end. Scorpions vary in size from 1 to 20 cm in length. Out of 1,752 scorpion species, 50 are dangerous to humans. Scorpion venom has a fatal reputation, but only about 25 species are known to have venom capable of killing a human being. Scorpions are generally found in dry, hot environments, although some species also occur in forest and wet savannas. All species are nocturnal, hiding during the day under stones, wood, or tree barks (Fig. 22.1).

Scorpion stings cause a wide range of clinical conditions ranging from severe local skin reactions to neurologic, respiratory, and cardiovascular collapse. Worldwide, scorpion stings are the most important cause of arachnid envenoming and are responsible for significant morbidity and pediatric mortality in many parts of Central and South America, the Middle East, Asia, and Northern and Southern Africa. Although all scorpions are venomous, the most diverse and widespread family, Buthidae, includes the majority of medically significant scorpion species. Systemic effects include massive autonomic neurotransmitter release (adrenergic or cholinergic) as a result of excitatory neurotoxins and cardiotoxicity characterized by pulmonary edema and arrhythmias. Experience with scorpion

envenoming and its management is greatest in regions of the world where medically significant species are abundant, and antivenom is available in many of these regions.

Geographical Distribution

Scorpions are found on all major land areas except Antarctica. The greatest diversity of scorpions in the Northern area is to be found in the subtropical areas lying between latitudes 23°N and 38°N. Scorpions did not occur naturally in Great Britain, New Zealand, and some of the islands in Oceania, but accidentally introduced in some of these places by human trade and commerce. Today, scorpions are found in virtually every terrestrial area, including high-elevation mountains, caves, and intertidal zones, with the exception of boreal ecosystems, such as the tundra, high-altitude taiga, and the permanently snow-clad tops of some mountains.

In Asia epidemiological data on scorpion stings is scarce. India is the most affected, with a reported incidence of 0.6 %. During hot months March to June and September to October attributed to increase in agricultural activities, daily cases of severe scorpion sting are received at endemic areas western Maharashtra, Karnataka, Andhra Pradesh, Saurashtra, and Tamil Nadu, severe scorpion sting per month reported from Konkan region. There have not been any reports of scorpion envenoming in Bangladesh or other nearby countries of India.

Habits, Habitats, and Foods

Scorpions are ground-dwelling, tree-living, rock-loving, or sand-loving; scorpions usually hide during the day and become active at night when they feed and mate. Most species hide under stones, logs, boards, rubbish, and the loose bark of fallen trees and posts. Newly developed houses may experience an influx of scorpions because construction work has destroyed their natural habitats. Scorpions may also enter older buildings in search of prey or shelter. Rains during late spring and early summer often cause scorpions to seek dryer habitats. Cracks and crevices around the outside of a structure allow scorpions easy access into buildings. As cold weather makes them sluggish, scorpions do not usually enter houses in the winter. Those found inside the house during winter are probably summer visitors that never left.

Inside the home, scorpions are most often found in crawl spaces, wall voids, and attics, but they are also attracted to kitchens, bathrooms, and other areas where water is available. As summer temperatures rise, scorpions move out of the hotter areas of the home and into the cooler living areas. It is during this time that most scorpion stings occur inside the home.

Scorpions feed on spiders, insects, centipedes, and other scorpions. As they have poor vision, they do not stalk or chase their prey but lie standstill waiting for it. They use their pincers to grab the prey, crush it, and draw it to their mouth.

Depending on the toxicity of their venom and size of their claws, they will then either crush the prey or inject it with venom. This will kill or paralyze the prey so the scorpion can eat it. Scorpions have an unusual style of eating using chelicerae, small claw-like structures that protrude from the mouth that are unique to the Chelicerata among arthropods. The chelicerae, which are very sharp, are used to pull small amounts of food off the prey item for digestion into a *preoral cavity* below the chelicerae and carapace. Scorpions can ingest food only in a liquid form as they have external digestion. The scorpion then ingests the body juices of the prey. Scorpions have been reported to survive for almost 2 years without food and water. This survival time depends upon the species and its developmental stage.

Scorpions can consume huge amounts of food at one sitting. They have a very efficient food storage organ and a very low metabolic rate combined with a relatively inactive lifestyle. This enables scorpions to survive long periods when deprived of food; some are able to survive 6–12 months of starvation. Scorpions excrete very little; their waste consists mostly of insoluble nitrogenous compounds such as xanthine, guanine, and uric acid.

Classification

There are 13 families and about 1,400 described species and subspecies of scorpions. In addition, there are 111 described taxa of extinct scorpions. This classification is based on that of Soleglad and Fet (2003) Additional taxonomic changes are from papers by Soleglad et al. (2005).

Medically Important Lethal Scorpions

The lethal members of the Buthidae family include the genera of *Buthus*, *Parabuthus*, *Mesobuthus*, *Tityus*, *Leiurus*, *Androctonus*, and *Centruroides*. These lethal scorpions are found generally in the given distribution:

- *Buthus* – Mediterranean area, from Spain to the Middle East
- *Parabuthus* – Western and Southern Africa
- *Mesobuthus* – Throughout Asia
- *Buthotus* (i.e., *Hottentotta*) – Across Southern Africa to Southeast Asia
- *Tityus* – Central America, South America, and the Caribbean
- *Leiurus* – Northern Africa and the Middle East
- *Androctonus* – Northern Africa to Southeast Asia
- *Centruroides* – Southern United States, Mexico, Central America, and the Caribbean (*Centruroides exilicauda* is found in the Baja California peninsula of Mexico and *Centruroides sculpturatus* is found in the state of Sonora, Mexico, and the Southwestern United States, primarily Arizona and small parts of Utah, New Mexico, Nevada, and California). The accepted taxonomy

of the bark scorpion has changed over time. Either *C. exilicauda* or *C. sculpturatus* have been accepted at various times. However, recent evidence from biochemical, genetic, and physiological characterization of their venom suggests that they are two different species as listed above.

Anatomy

The body of a scorpion is divided into three parts (tagmata): the head (cephalothorax), the abdomen (mesosoma), and the tail (metasoma) (see Figs. 22.2 and 22.3).

Cephalothorax

The cephalothorax, also called the *prosoma*, comprises the carapace, eyes, chelicerae (mouth parts), pedipalps (the pedipalps of scorpions have chelae, commonly called claws or pincers), and four pairs of walking legs. Scorpions have two eyes on the top of the cephalothorax and usually two to five pairs of eyes along the front corners of the cephalothorax.

The pedipalp is a segmented, chelate (clawed) appendage used for prey immobilization, defense, and sensory purposes. The segments of the pedipalp (from closest to the body outwards) are coxa, trochanter, femur (humerus), patella, tibia (including the fixed claw and the manus), and tarsus (moveable claw). A scorpion has darkened or granular raised linear ridges called "keels" or *carinae* on the pedipalp segments and on other parts of the body, which are useful taxonomically.

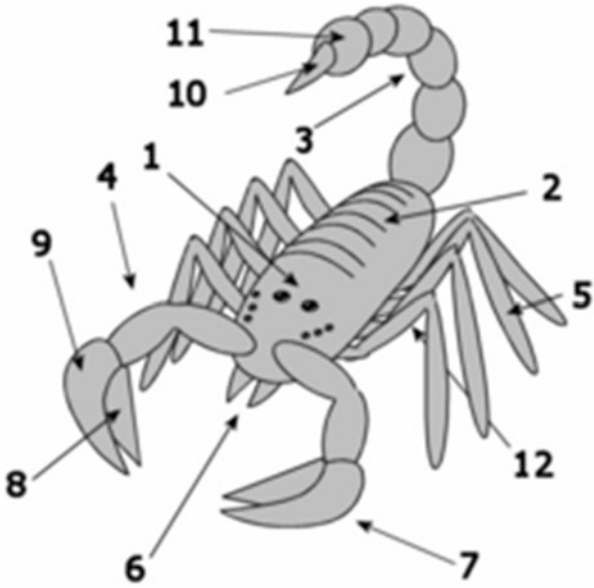

Fig. 22.2 Scorpion anatomy: *1* cephalothorax or *prosoma*, *2* abdomen or *mesosoma*, *3* tail or *metasoma*, *4* claws or *pedipalps*, *5* legs, *6* mouth parts or *chelicerae*, *7* pincers or chelae, *8* moveable claw or *tarsus*, *9* fixed claw or *manus*, *10* sting or *telson*, *11* anus

Fig. 22.3 Barb of an Arizona bark scorpion (Jonathon Leeming)

Mesosoma

The abdomen, also called the *opisthosoma*, consists of seven segments (somites), each covered dorsally by a sclerotosed plate (tergum) and also ventrally for segments 3–7. The first abdominal segment bears a pair of genital opercula covering the gonopore. Segment 2 consists of the basal plate with the pectines. Each of the mesosomal segments 3–7 has a pair of spiracles, the openings for the scorpion's respiratory organs, known as book lungs. The spiracle openings may be slits, circular, elliptical, or oval.

Metasoma

The metasoma, the scorpion's tail, comprises five caudal segments (the first tail segment looks like a last mesosomal segment) and sixth bearing the telson (the sting). The telson, in turn, consists of the vesicle, which holds a pair of venom glands, and the hypodermic aculeus, the venom-injecting barb. The venom glands are composed of two types of tall columnar cells. One type produces the toxins, while the other produces mucus. On rare occasions, scorpions can be born with two metasomata (tails). Two-tailed scorpions are not a different species, merely a genetic abnormality.

Buthidae Family

Almost all of the lethal scorpions, except the *Hemiscorpius* species, belong to the scorpion family called the Buthidae. The Buthidae family is characterized by a triangular-shaped sternum, as opposed to the pentagonal-shaped sternum found in the other five scorpion families (Fig. 22.4).

Fig. 22.4 Triangular sternal plate of Buthidae (copyright – Sean Bush)

Scorpions from the family Buthidae (which includes almost all of the potentially lethal scorpions) generally can be identified by the triangular sternal plate. In other families of scorpions, this feature is more square or pentagonal.

In addition to the triangular-shaped sternum, poisonous scorpions also tend to have weak-looking pincers, thin bodies, and thick tails, as opposed to the strong heavy pincers, thick bodies, and thin tails seen in nonlethal scorpions (Fig. 22.5).

Biology/Life Cycle

Scorpions mate in the fall or early spring. During mating, the male starts locating the female and identifying each other using a mixture of pheromones and oscillatory communication. Once they have satisfied the other and opposite sex of correct species, mating can commence. The male deposits the spermatophore and then guides the female over it. This allows the spermatophore to enter her genital opercula, which triggers release of the sperm, thus fertilizing the female. The mating process can take from 1 to 25 h and depends on the ability of the male to find a suitable place to deposit his spermatophore. If mating continues too long, the female may lose interest, ending the process. Scorpions possess a complex courtship and mating ritual to affect this transfer.

Once the mating is complete, the male and female will separate. The male will generally retreat quickly, most likely to avoid being cannibalized by the female, although sexual cannibalism is infrequent with scorpions.

After an elaborate courtship process, lasting anywhere from 24 to 36 h, it can take from five months to over a year for the eggs to mature within the female. Females do not lay eggs; the young are born alive in semitransparent sacs. A female can produce anywhere from 14 to 100 young in one litter. Once the young scorpions free themselves from their egg sacs, they climb onto the back of the mother and remain there until after their first molt several days later. Young scorpions are

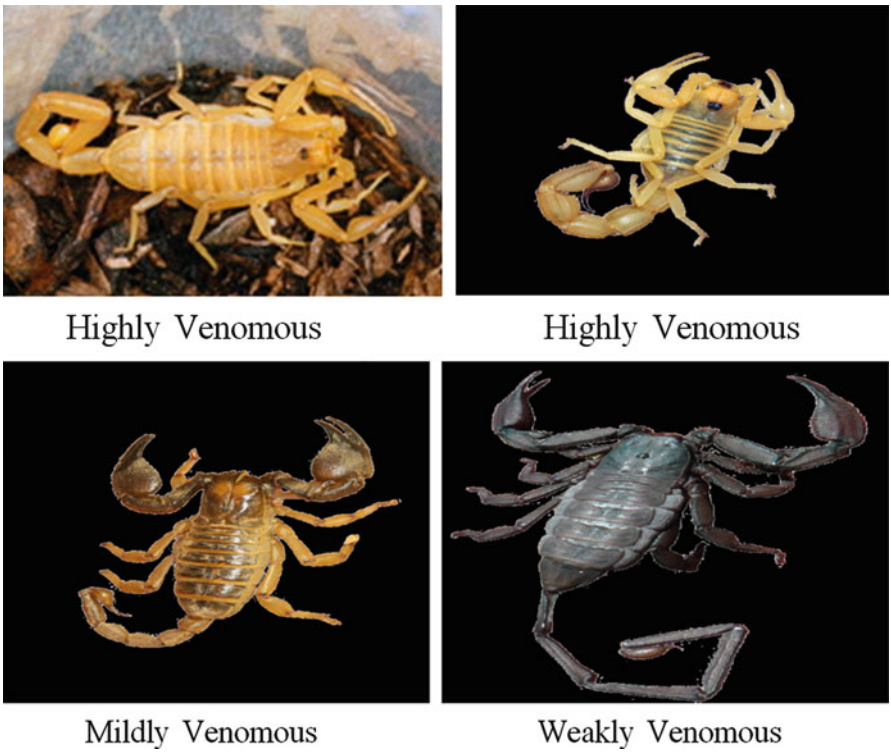

Fig. 22.5 Buthidae family with mild, weak, and highly venomous species (copyright – Jonathon Leeming)

nourished by a yolk material stored in their bodies. After their first molt, young scorpions leave their mother and begin to fend for themselves. At this time, the young scorpions are already capable of stinging. It takes about 1 year for the young to reach maturity. Depending on the species and the environmental conditions, scorpions can live for 3–5 years.

Scorpion Stings

Scorpions are basically shy creatures, aggressive only toward their prey. They will not sting humans unless they are handled, stepped on, or otherwise bothered. For most people, a scorpion sting is slightly more painful than a bee or wasp sting. The venom can produce considerable pain around the area of the sting, but swelling is generally limited. In some people, sensations of numbness and tingling may develop in the area of the sting for 4–6 h. After this time, the symptoms start to regress and will normally disappear within 24 h.

Scorpion Venom

Composition of Scorpion Venom

Scorpion uses its venom for both prey capture and defense. The venom is constituted by mucopolysaccharides, hyaluronidase, phospholipase, serotonin, histamine, enzyme inhibitors, and proteins, namely, neurotoxic peptides. The venom contains neurotoxic peptides which are responsible for the symptoms that present during envenomation by interacting with ion channels and has the potential to cause massive damage to the nervous system of both vertebrates and invertebrates. This gradient across is responsible by excitation of the nerve and muscle, hormonal secretion, cell proliferation, sensory transduction, the control of salt and water balance, and regulation of blood pressure. Scorpion toxins present specificity and high affinity and have been used as pharmacological tools to characterize various receptor proteins involved in normal ion channel functioning, as abnormal channel functioning in disease states. In sodium channel gating, the voltage sensors of the sodium channel activate independently, and at least three of them have to be in an activated position for the channel to open. However, if one of them is activated by the β-toxin, the threshold of activation is unlikely to shift significantly since other voltage sensors remain unaffected.

Sodium Channel Toxins (*NaTx*). Voltage-gated sodium channels are critical for the generation and propagation of action potentials and initiation of action potentials by excitable cells. These channels are targeted by neurotoxins presenting a large variety of chemically distinct compounds that bind to several receptor sites on the pore-forming α-subunit. With respect to scorpions, toxins have been observed that they show a preference for distinct sodium channel subtypes of mammals or insects.

Potassium Channel Toxins (*KTx*). Scorpion toxins that target K + channels (KTx) are short-chain peptides cross-linked by three or four disulfide bridges. The α-KTx family constitute by more than 50 different α-KTx has been reported and listed in more than 18 families. Tenenholz et al. (2000) described that α/β scaffold is formed by an α-helix and a two- or three-stranded β-sheet linked by two bridges. However, the α/β fold is shared by a variety of polypeptides with diverse functions, such as toxins active on Na^+ channels. The neurotoxin α-KTx 12.1 initially named as TsTX-IV was isolated from the *T. serrulatus* venom which is constituted with four disulfide bridges described by several studies. Butantoxin which is present in the venoms of three Brazilian scorpions *T. serrulatus*, *T. bahiensis*, and *T. stigmurus* has shown to reversibly block the potassium channels and inhibit the proliferation of T cells and IL-2 production.

Calcium Channel Toxins. Ca^{2+} ions play important roles in regulating a variety of cellular functions such as second messenger-coupling receptor to many active cellular processes that include cellular excitability, neurotransmitter release, intracellular metabolism, and gene expression. The increment of Ca^{2+} concentration is mediated by voltage-gated Ca^{2+} channels that regulate Ca^{2+} influx across the plasma membrane and control the release of Ca^{2+} from intracellular stores. The Ca^{2+} channels are widely distributed in the body such as heart muscle, smooth

muscle, skeletal muscle neurons, and endocrine cells. Scorpion venom consists of numerous peptides that may interfere with the activity of ion channels and modulate their functional properties. These peptides have different physiological and pharmacological activities. Various studies have been shown that scorpion toxins are used in insecticides, vaccines, cancer treatment, and protein-engineering scaffolds.

Mediators Involved in Scorpion Envenoming

Much evidence supports the role of cytokines in scorpion envenomation; it seems that both pro- and anti-inflammatory cytokine levels are overproduced in sepsis syndrome. Their clinical significance and prognostic value have not been elucidated.

The production of cytokines in the envenomation has previously been referred to as a cascade. Tissue injury occurs during inflammation and is a progressive process which may eventually lead to organ dysfunction failure. The categorization of cytokines into pro- and anti-inflammatory response is essential for structural and functional repair of injured tissue, but excessive generation of proinflammatory signals can aggravate tissue damage because of the products derived from inflammatory cells.

Proinflammatory cytokines. IL-1 is a prototypic proinflammatory cytokine that exists in two forms named as IL-1α and IL-1β. After binding to IL-1R, IL-1 induces the production of a high spectrum of cytokines and chemokines as well as the expression of adhesion molecules on endothelial cells, thus leading to the recruitment of inflammatory cells. In addition, IL-1 also contributes to the development of vascular damage by stimulating cell proliferation and differentiation and the release of matrix-degrading enzymes. The levels of IL-1 in serum from human and mice injected with Brazilian scorpion *T. serrulatus* and/or its major toxins are characterized by rapid increments of this proinflammatory cytokine. High levels of these cytokines were observed in supernatants of macrophage from mice exposed to *T. serrulatus* venom and its major toxins.

Increased levels of IL-1β were determined in plasma from patients moderately or severely envenomed by *T. serrulatus* sting. High levels of IL-1α and IL-1β were observed in sera from mice exposed to Mexican scorpion *Centruroides noxius*. The role of IL-1 in scorpion envenomation has been investigated through influencing its level or activity.

IL-6 is a multifunctional cytokine that regulates various aspects of the immune response, acute-phase reaction, and hematopoiesis. IL-6 has both pro- and anti-inflammatory effects. It downregulates the synthesis of IL-1 and TNF-α and also inhibits the production of GM-CSF, IFN-γ, and MIP-2. High levels of IL-6 were observed in sera from mice exposed to *Centruroides noxius* and *T. serrulatus* scorpion venoms. Increased levels of IL-6 were also observed in plasma from patients with different grade of envenomation by *T. serrulatus*.

Scorpion venoms can stimulate the neuroendocrinal immunological axis by its ability to release catecholamines, corticosteroids, bradykinin, and prostaglandins,

and all these agents proved to induce the release of immunological mediator cytokines. There is now accumulating evidence to suggest a causal relationship between overproduction of certain cytokines such as IL-1 and IL-6 and morbidity and mortality associated with critically ill patients. With respect to the experimental animal, high levels of cytokines were found in serum from mice injected with *Centruroides noxius* and *T. serrulatus* venom. In all works the authors concluded that the activation and release of cytokines may play an important role in the pathophysiology of envenomation after stings and may be responsible for some systemic inflammatory manifestations and organ failure. More human and experimental animal studies are required to determine the contribution of the inflammatory system in the genesis of scorpion envenomation.

Increased levels of IL-8 were observed in serum from patients with different grade of envenomation caused by *T. serrulatus* and *Leiurus quinquestriatus* scorpions.*TNF* is a pleiotropic cytokine that exerts potent proinflammatory effects on envenomed and other metabolic and inflammatory disorders which are also risk factors for cardiovascular diseases. TNF-α is primarily produced by monocytes and macrophages. With respect to TNF-α, IL-1 seems to be important in the pathogenesis of envenoming because of its immunological upregulatory and proinflammatory activities.

IFN-γ. The pleiotropic cytokine IFN-γ is a proinflammatory mediator that is expressed at high levels in envenomation by various cells, including monocytes/macrophages, Th 1 cells, and natural killer T cells (NK). High levels of IFN-γ were observed and documented during the envenomation caused in human and experimental animals by different scorpion venoms from *Centruroides noxius* and *Tityus serrulatus*.

Anti-inflammatory cytokines. *IL-4* is a highly pleiotropic cytokine that is able to influence Th cell differentiation; its early secretion leads to polarization of Th cell differentiation toward Th2-like cells. Overall anti-inflammatory cytokines whose roles are less well characterized in envenomation include IL-4 which has a stimulatory molecule for B and T cells and has known immunosuppressive effects in the intestine. Increment of IL-4 production was observed in serum from rats exposed to *Androctonus australis hector* scorpion.

IL-10 is produced by several cell types including $CD4^+$ and $CD8^+$, T cells, macrophages, monocytes, B cells, dendritic cells, and epithelial cells. IL-10 is the most important anti-inflammatory cytokine found within the human immune response. It is the potent inhibitor of Th1 cytokines, including IFN-γ, TNF-α, IL-1β, IL-2, IL-6, IL-8, and IL-12. In serum from patients envenomed with *Tityus serrulatus* scorpions and in experimental animals exposed to *Androctonus australis hector*, *Centruroides noxius*, and *T. serrulatus* venoms, there were observed modified levels of IL-10.

The pathophysiology of envenoming is complex, but there is little doubt that injection often progresses from systemic inflammatory response to severe envenoming. In envenomed humans or experimental animal exposed to venom, crude and/or purified toxins from different scorpions are the primary event in this sequence. The interaction of venom components with cells and serum proteins to initiate a series of reactions generally may lead to cell injury and death (Table 22.1).

Table 22.1 Pro- and anti-inflammatory cytokines

Scorpion	Cytokines produced
Androctonus australis hector	
Experimental animal (rats)	IL-1β, IL-4, IL-6, IL-10, and TNF-α
Buthus martensi Karch	NO and paw edema
Centruroides noxius	
Experimental animal (mice)	IL-1β, IL-1α, IFN-γ IL-6, IL-10, and TNF-α
Leiurus quinquestriatus	
Human and experimental animal (rabbits)	IL-6, IL-8, NO, and TNF-α
Tityus serrulatus	
Human and experimental animal (rabbits)	IL-1β, IL-6, IL-8, IL-10, NO, TNF-α, IL-1α,
	IL-1β,IFN-γ, and GM-CSF

Systemic effects of the cytokines have been shown to induce fever and increase symptoms. In the local action, the cytokines promote recruitment of inflammatory cells to inflammation sites. In scorpion envenoming, the balance between pro and anti-inflammatory cytokines determines the degree and extent of inflammation and thus can lead to different clinical effects. Anti-inflammatory cytokines counteract the effects of proinflammatory cytokines, and therefore the relative concentration of a cytokine to its inhibitor or antagonist will determine its final effect.

Following the injection of venoms, a variety of proinflammatory cytokines are released, along with counterregulatory or anti-inflammatory cytokines, and the outcome of an inflammatory response is dictated by the duration of the stimulus and the balance between the proinflammatory and anti-inflammatory responses.

An excessive proinflammatory response is thought to be important in the pathogenesis of septic shock. In contrast, an excessive anti-inflammatory response could result in failure to clear a venom action, with equally deleterious effects. However, the prolonged compensating anti-inflammatory response syndrome may be associated with excess mortality and morbidity because of increased risk for envenoming. With respect to the functions of many cytokines and frequently multiple antagonists for any given agonist, the ability to compensate for a certain amount of divergence in production of individual cytokine is significant. Cytokines are important for regulation of inflammatory response.

During the local and systemic responses, the release of proinflammatory cytokines, arachidonic acid metabolites, proteins of the contact phase and coagulation system, and complement factors is observed; it is defined as systemic inflammatory response. However, in parallel, anti-inflammatory mediators which produce an imbalance of these dual immune responses seem to be responsible for organ dysfunction. Tissue injury occurs during inflammation and is a progressive process which may eventually lead to organ dysfunction and failure. The increment of the levels of proinflammatory cytokines leads to activation of macrophages, neutrophils, NK cells, T cells, and B cells. The inflammatory response is essential for structural and functional repair of injured tissue.

Pathophysiology

Scorpions use their pincers to grasp their prey; then, they arch their tail over their body to drive their stinger into the prey to inject their venom, sometimes more than once. The scorpion can voluntarily regulate how much venom to inject with each sting. The striated muscles in the stinger allow regulation of the amount of venom ejected, which is usually 0.1–0.6 mg. If the entire supply of venom is used, several days must elapse before the supply is replenished. Furthermore, scorpions with large venom sacs, such as the *Parabuthus* species, can even squirt their venom.

The potency of the venom varies with the species, with some producing only a mild flu and others producing death within an hour. Generally, the venom is distributed rapidly into the tissue if it is deposited into a venous structure. Venom deposited via the intravenous route can cause symptoms only 4–7 min after the injection, with a peak tissue concentration in 30 min and an overall toxin elimination half-life of 4.2–13.4 h through the urine. The more rapidly the venom enters the bloodstream, the higher the venom concentration in the blood and the more rapid the onset of systemic symptoms.

Scorpion venom is a water-soluble, antigenic, heterogeneous mixture, as demonstrated on electrophoresis studies. Furthermore, the various constituents of the venom may act directly or indirectly and individually or synergistically to manifest their effects. In addition, differences in the amino acid sequence of each toxin account for their differences in the function and immunology. Thus, any modifications of the amino acid sequence result in modification of the function and immunology of the toxin.

Scorpion venom may contain multiple toxins and other compounds. The venom is composed of varying concentrations of neurotoxin, cardiotoxin, nephrotoxin, hemolytic toxin, phosphodiesterases, phospholipases, hyaluronidases, glycosaminoglycans, histamine, serotonin, tryptophan, and cytokine releasers. The most important clinical effects of envenoming are neuromuscular, autonomic, or local tissue effects. Autonomic excitation leads to cardiopulmonary effects observed after some scorpion envenoming. Somatic and cranial nerve hyperactivity results from neuromuscular overstimulation. Additionally, serotonin may be found in scorpion venom and is thought to contribute to the pain associated with scorpion envenoming.

The most potent toxin is the neurotoxin, of which two classes exist. Both of these classes are heat-stable, have low molecular weight, and are responsible for causing cell impairment in nerves, muscles, and the heart by altering ion channel permeability.

The long-chain polypeptide neurotoxin causes stabilization of voltage-dependent sodium channels in the open position, leading to continuous, prolonged, repetitive firing of the somatic, sympathetic, and parasympathetic neurons. This repetitive firing results in autonomic and neuromuscular over excitation symptoms, and it prevents normal nerve impulse transmissions. Furthermore, it results in release of excessive neurotransmitters such as epinephrine, norepinephrine, acetylcholine, glutamate, and aspartate. Meanwhile, the short polypeptide neurotoxin blocks the potassium channels.

The binding of these neurotoxins to the host is reversible, but different neurotoxins have different affinities. The stability of the neurotoxin is due to the four disulfide bridges that fold the neurotoxin into a very compact three-dimensional structure, thus making it resistant to pH and temperature changes. However, reagents that can break the disulfide bridges can inactivate this toxin by causing it to unfold. Also, the antigenicity of this toxin is dependent on the length and number of exposed regions that are sticking out of the three-dimensional structure.

Clinical Manifestations

Clinical effects of the envenoming depend upon the species of scorpion and lethality and dose of venom injected at the time of sting. The toxicity, variation, and duration of the symptoms depend on the following factors:

- Scorpion age, size, and nutritional status.
- Scorpion's stinging apparatus (telson).
- Number of stings and quantity of venom injected.
- Depth of the sting penetration.
- Composition of the venom.
- Site of envenoming – Closer proximity of the sting to the head and torso results in quicker venom absorption into the central circulation and a quicker onset of symptoms.
- Age of the victim.
- Health of the victim.
- Weight of the victim relative to the amount of venom.
- Presence of comorbidities.
- Treatment effectiveness.

Nonlethal scorpion species tend to produce local reactions similar to a hymenopteran sting, while lethal scorpion species tend to produce systemic symptoms. The duration to progress to systemic symptoms ranges from 5 min to 4 h after the sting. The symptoms generally persist for 10–48 h. Clinically "autonomic storm" evoked due to venomous envenoming is characterized by transient parasympathetic (vomiting, profuse sweating, salivation, bradycardia, ventricular premature contraction, priapism in male, hypotension) and prolonged sympathetic (cold extremities, hypertension, tachycardia, pulmonary edema, and shock) stimulation. On the basis of clinical manifestations at the time of arrival to hospital and according to severity, they are graded in four grades:

Grade 1 – Severe excruciating local pain at the sting site radiating along with corresponding dermatomes, mild local edema at the sting site, without systemic involvement

Grade 2 – Signs and symptoms of autonomic storm characterized by acetylcholine excess or parasympathetic stimulation and sympathetic stimulation

Grade 3 – Cold extremities, tachycardia, hypotension, or hypertension with pulmonary edema
Grade 4 – Tachycardia and hypotension with or without pulmonary edema with warm extremities (warm shock)

Sixteen scientists involved in the management of scorpion sting from endemic areas of scorpion sting were invited to attend the meeting (ADELF Congress) at Rabat, Morocco, held on 6 and 7 May 2009, and consensus was reached to include three classes as follows:

Class I – Local manifestations
Class II – Systemic involvement
Class III – Cardiogenic failure, hypotension, ventricular arrhythmia, bradycardia, cardiovascular collapse, respiratory failure, cyanosis, dyspnea, pulmonary edema, neurological failure Glasgow score <6 (in absence of sedation), and paralysis

Local Signs

- Neurotoxic local effects
  - Local evidence of a sting may be minimal or absent in as many as 50 % of cases of neurotoxic scorpion stings. In fact, tissue necrosis is rarely found.
  - A sharp burning pain sensation at the sting site, followed by pruritus, erythema, local tissue swelling, and ascending hyperesthesia, may be present. This paresthesia feels like an electric current, persists for several weeks, and is the last symptom to resolve before the victim recovers.
  - The tap test is administered by tapping at the sting site. A positive result is when the paresthesia worsens with the tapping because the site is hypersensitive to touch and temperature. Wearing clothing over the area and changes in temperature exacerbate the symptoms. Tapping over the injury site (i.e., tap test) may cause severe pain after a sting by *C. exilicauda*.
- Cytotoxic local effects
  - A macule or papule appears initially at the sting site, occurring within the first hour of the sting.
  - The diameter of the lesion is dependent on the quantity of venom injected.
  - The lesion progresses to a purpuric plaque that will necrose and ulcerate.
  - Lymphangitis results from the transfer of the venom through the lymphatic vessels.
- Nonlethal local effects
  - Pain, erythema, induration, and wheal may be present.
  - These are secondary to venom activation of kinins and slow-releasing substances.

Local tissue effects vary among species. Minimal local tissue effects are present with *Centruroides* envenoming. Significant local tissue reaction rules out *C. exilicauda* envenoming.

Neurologic Signs

Most of the symptoms are due to either the release of catecholamines from the adrenal glands (sympathetic nerves) or the release of acetylcholine from postganglionic parasympathetic neurons. However, dual manifestations of the adrenergic and cholinergic signs are possible because of varying organ system sensitivities to these neurotransmitters. There are four grades in neurological manifestation:

- Grade I – Local pain or paresthesia at the sting site (83 %)
- Grade II – Pain or paresthesia that has traveled from the sting site (9.1 %)
- Grade III – Either cranial nerve or somatic neuromuscular dysfunction (4.7 %)
- Grade IV – Both cranial nerve and somatic neuromuscular dysfunction (3 %)
- Central nervous system signs
  - Thalamus-induced paresthesia in all four limbs.
  - Patients experience venom-induced thrombotic strokes.
  - The level of consciousness is altered, especially with restlessness, confusion, or delirium.
  - Ataxia is also a sign.
- Autonomic nervous system signs – Predominately sympathetic signs, parasympathetic signs, or a combination of signs
  - Sympathetic signs
    - Hyperthermia
    - Tachypnea
    - Tachycardia
    - Hypertension
    - Arrhythmia
    - Hyperkinetic pulmonary edema
    - Hyperglycemia
    - Diaphoresis
    - Piloerection
    - Restlessness and apprehension
    - Hyperexcitability and convulsions
- Parasympathetic signs
  - Bronchoconstriction
  - Bradycardia
  - Hypotension
  - Salivation, lacrimation, urination, diarrhea, and gastric emesis (SLUDGE)

- Rhinorrhea and bronchorrhea
- Goose pimple skin
- Loss of bowel and bladder control
- Priapism
- Dysphagia
- Miosis
- Generalized weakness

- Somatic signs
  - Spastic muscle of the limbs and torso
  - Involuntary muscle spasm, twitching, clonus, and contractures
  - Alternating opisthotonos
  - Increased tendon reflexes, especially prolongation of the relaxation phase
  - Piloerection accompanied by goose pimples
- Cranial nerve signs
  - Ptosis, nystagmus, and blurred vision.
  - Mydriasis.
  - Tongue fasciculation.
  - Dysphagia, dysarthria, and stridor occur secondary to pharyngeal reflex loss or muscle spasm.
- Peripheral nervous system signs – Intense local burning pain with minimal swelling at sting site, followed by ascending numbness and tingling, and then paralysis and convulsions

Nonneurologic systemic signs

- Cardiovascular signs – Usually follow of a hyperdynamic phase followed by a hypodynamic phase
  - Hypertension is described as follows:
    - Secondary to catecholamine and renin stimulation
    - Observed as early as within 4 min after the sting
    - Lasts a few hours
    - High enough to produce hypertensive encephalopathy
    - Hypotension – Less common and occurs secondary to excess acetylcholine or catecholamine depletion
- Tachycardia is common, although bradycardia can be observed.
- Transient apical pansystolic murmur is consistent with papillary muscle damage.
- Cardiovascular collapse occurs secondary to toxin-induced myocarditis biventricular dysfunction and profuse loss of fluids from sweating, vomiting, diarrhea, and hypersalivation.
  - Observed in 7–38% of cardiovascular cases
  - Mild envenoming – Vascular effect with vasoconstriction hypertension
  - Moderate envenoming – Left ventricular failure hypotension with and without an elevated pulmonary artery wedge pressure, depending on fluid status of the patient

  - Severe envenoming – Biventricular cardiogenic shock
  - Cardiac dysfunctions attributed to catecholamine-induced myocarditis and as well as to the direct effects of the toxin (leading to myocarditis and myocardial conduction interference)
- Respiratory signs
  - Tachypnea may be present.
  - Pulmonary edema with hemoptysis and a normal-sized heart is observed in 7–32 % of respiratory cases. This is secondary to a direct toxin-induced increased pulmonary vessel permeability effect and is also secondary to catecholamine-induced effects of hypoxia and intracellular calcium accumulation, which leads to a decrease in left ventricular compliance with resultant ventricular dilation and diastolic dysfunction.
  - Respiratory failure may occur secondary to diaphragm paralysis, alveolar hypoventilation, and bronchorrhea.
- Allergic signs
  - Urticaria
  - Angioedema
  - Bronchospasm
  - Anaphylaxis occasionally
- Gastrointestinal signs
  - Excessive salivation.
  - Dysphagia.
  - Nausea and vomiting.
  - Gastric hyperdistention occurs secondary to vagal stimulation.
  - Increased gastric acid output may lead to gastric ulcers.
  - Acute pancreatitis may lead to hyperglycemia.
  - Toxic hepatitis.
- Genitourinary signs
  - Decreased renal plasma flow
  - Toxin-induced acute tubular necrosis
  - Rhabdomyolysis
- Priapism may occur secondary to cholinergic stimulation.
- Hematological signs
  - Platelet aggregation.
  - Disseminated intravascular coagulation with massive hemorrhage may result from venom-induced defibrination.
- Metabolic signs
  - Hyperglycemia may occur from catecholamine-induced hepatic glycogenolysis, pancreatitis, and insulin inhibition.
  - Increased lactic acidosis may occur from hypoxia and increased venom-induced lactase dehydrogenase activity.
  - Patients may have an electrolyte imbalance and dehydration from hypersalivation, vomiting, diaphoresis, and diarrhea.
- Pregnancy signs – Toxin-induced uterine contraction
- Hospital admission criteria

- Priapism
- Vomiting
- Systolic blood pressure (SBP) greater than 160 mmHg
- Temperature greater than 38 ºC
- Heart rate greater than 100 beats per minute

Laboratory Studies

Scorpion envenoming cases vary in presentation from simple pain at local site up to fatality. So the laboratory studies range from those requiring no laboratory tests to scenarios requiring extensive hematological, electrolyte, and respiratory analysis:

- CBC count and peripheral blood film for leukocytosis and hemolysis in patients with stings from the *Hemiscorpius* species. *Hemiscorpius lepturus* has been shown to cause severe hemolysis.
- Glucose levels should be measured to evaluate for hyperglycemia from liver and pancreas dysfunction.
- Electrolyte evaluation is important in patients with venom-induced salivation, vomiting, and diarrhea.
- Coagulation parameters should be measured for venom-induced defibrination.
- Urinalysis and creatine kinase test are helpful to evaluate for venom-induced rhabdomyolysis. Renal failure may occur secondary to hemoglobinuria (after *H. lepturus* sting) or myoglobinuria from rhabdomyolysis.
- Amylase/lipase values to assess for pancreatitis, which is common, from *Tityus trinitatis* stings.
- Increased aspartate aminotransferase and alanine aminotransferase levels as there is venom-induced liver cell destruction.
- Increased catecholamine, aldosterone, renin angiotensin, and antidiuretic hormone levels can be detected within a few hours after the sting.
- Interleukin (IL)-1 level are elevated in all envenomation.
- High levels of IL-6, interferon-gamma, and granulocyte-macrophage colony-stimulating factor are present in severe envenomation.
- Radiolabeled antibodies or immunoenzymatic assays help quantify the serum venom level because an association exists between the clinical signs of envenomation and this level.
- Arterial blood gas measurement measurements as indicated for respiratory distress or to determine acid/base status.
- Electrocardiography
  - ECG changes persist for 10–12 days before normalizing.
  - ECG changes are observed in 63 % of children who have been envenomed.
  - Rhythm disturbances are related to the venom composition and not to dose.
    - Sinus tachycardia – Most common rhythm
    - QTc prolongation – 53 %

  - ST changes – 39 %
  - T-wave inversion – 39 %
  - Ventricular repolarization abnormalities – 15 %
  - Bundle branch block – 12.8 %
  - First-degree block – 10.2 %
- The sequence ECG changes starts with bizarre, broad-notched, biphasic, peaked T waves with a beat-to-beat variation. This bizarre T wave is followed by the appearance of tiny Q waves and then atrioventricular dissociation with an accelerated junctional rhythm.

Imaging Studies

- A chest radiograph should be done in cases of respiratory difficulty. Unilateral pulmonary edema may be seen due to the venom effect on pulmonary vascular permeability.
- Echocardiography findings in envenomation are:
  - Echocardiography is highly sensitive for assessing myocardial compromise after a scorpion sting.
  - Findings may show a diffuse global biventricular hypokinesis with a decreased left and right ventricular ejection fraction of approximately 0.14–0.38. This dysfunction can appear just a few hours after the sting and usually normalizes within 4–8 days.
- Clinical cardiorespiratory improvement correlates with serial echocardiography which may show the return of left ventricular function to a normal state.
- Color-flow Doppler study findings show mitral incompetence due to venom-induced dilated cardiomyopathy.
- Myocardial perfusion scintigraphy can also be used to investigate the contractility and perfusion of the cardiac tissue.

Other Tests

Arterial blood gas determinations show a decrease in arterial oxygenation tension and an increase in PCO_2 – findings consistent with mild metabolic acidosis.

- Pulmonary artery catheterization findings may include the following:
  - Elevated systemic vascular resistance with elevated mean arterial pressure (MAP) of 203 mmHg.
  - Left ventricular failure produces a MAP of 57–69 mmHg.
  - Biventricular failure produces a MAP of 47 mmHg.
  - Low cardiac index occurs with elevated filling pressures.
- Serial spirometry measurements to detect impending venom-induced diaphragmatic failure.

Procedures

CSF study – Cerebrospinal fluid pleocytosis is evident on spinal tap studies.

Histologic Findings

The local sting site shows mixed inflammatory cell infiltrates with eosinophils scattered among collagen bundles in an edematous dermis. Myocardial changes, which are most prominent at the papillary muscle and subendocardial region, include focal myocardial necrosis; myofibril destruction, especially at the I band; fine fatty deposits in the cardiac muscle fibers; interstitial edema; and increased cellularity, mainly lymphocytes and monocytes. Changes resemble interstitial hypoxia-induced myocarditis caused by large doses of catecholamines.

Management

Prehospital Care

Sting Wound Management

Soon after a sting, patient experiences severe excruciating radiating pain from the sting site, usually toes and fingers. Sudden tap at and around the site of the sting induces severe pain and withdrawal is a diagnostic sign of sting called "TAP sign."

Edema and inflammation at the sting site blunt the sodium channel blocker action of lidocaine. Infiltration of single dose 1.5–2 ml of lidocaine without adrenaline around the sting site, oral acetaminophen or NSAID, cold therapy at the sting site, and oral diazepam make the victim more comfortable. Local incision, tight tourniquet, and application of potassium permanganate and herbal remedies lead to local tissue damage, infection, and gangrene.

- Recommended local treatment:
  - Reassure and calm the patient to lower the heart rate and blood pressure, thus limiting the spread of the venom.
  - Immobilize the affected part in a functional position below the level of the heart to delay venom absorption.
  - Use ice bags to reduce pain and to slow the absorption of venom via vasoconstriction. This is most effective during the first 2 h following the sting.
  - Apply a topical or local anesthetic agent to the wound to decrease paresthesia; this tends to be more effective than opiates.
  - A negative-pressure extraction device (i.e., the extractor) may be useful, although the benefit is unproven. The extractor creates a negative pressure of 1 atm. Apply it to the sting site after incision. Oral extraction is contraindicated.

- For community care, consider applying a lymphatic-venous compression wrap 1 in. proximal to the sting site to reduce superficial venous and lymphatic flow of the venom but not to stop the arterial flow. Only remove this wrap when the provider is ready to administer systemic support. The drawback of this wrap is that it may intensify the local effects of the venom.
- Administer local wound care and topical antibiotic to the wound.
- Administer tetanus prophylaxis.
- Administer systemic antibiotics if signs of secondary infection occur.
- Administer muscle relaxants for severe muscle spasms (i.e., benzodiazepines).

General Measures

- Primary assessment of airway, breathing, and circulation takes precedence.
- The utility of negative-pressure extraction devices has not been evaluated for scorpion stings.
- Endotracheal intubation and vascular access as needed.
- Systemic treatment is instituted by directing supportive care toward the organ specifically affected by the venom.
  - Monitor vital signs (e.g., pulse oximetry, heart rate, blood pressure, and respiratory rate monitor).
  - Use invasive monitoring for patients who are unstable hemodynamically.
  - Administration of oxygen.
  - Administration of intravenous fluids to help prevent hypovolemia from vomiting, diarrhea, sweating, hypersalivation, and insensible water loss from a tropical environment.
  - Intubation and institute mechanical ventilation with end-tidal carbon dioxide monitoring for patients in respiratory distress.
  - Administration of a combination of beta-blockers with sympathetic alpha-blockers is most effective in reversing this venom-induced cardiovascular effect. Avoid using beta-blockers alone because this leads to an unopposed alpha-adrenergic effect. Also, nitrates can be used for hypertension and myocardial ischemia.
  - For hypodynamic cardiac changes, a titrated monitored fluid infusion with afterload reduction helps reduce mortality.
  - A diuretic may be used for pulmonary edema in the absence of hypovolemia, but an afterload reducer, such as prazosin, nifedipine, nitroprusside, hydralazine, or angiotensin-converting enzyme inhibitors, is better.
  - Inotropic medications, such as digitalis, have little effect, while dopamine aggravates the myocardial damage through catecholamine-like actions. Dobutamine seems to be a better choice for the inotropic effect.
  - Finally, a pressor such as norepinephrine can be used as a last resort to correct hypotension refractory to fluid therapy.
  - Administer atropine to counter venom-induced parasympathomimetic effects.

- Insulin administration in scorpion envenomation animal experiments has helped the vital organs to use metabolic substrates more efficiently, thus preventing venom-induced multiorgan failure, especially cardiopulmonary failure. Unfortunately, no human studies have been conducted.
- Administer barbiturates and/or a benzodiazepine continuous infusion for severe excessive motor activity.
- The use of steroids to decrease shock and edema is of unproven benefit.
- Antivenom is the treatment of choice after stabilization and supportive care. Because of the heterogeneity of venom composition between different scorpion species, one species antivenom will have limited effect on another scorpion species venom. Thus, correct scorpion species identification is a prerequisite for proper antivenom treatment.
- The antivenom significantly decreases the level of circulating unbound venom within a few hours. The persistence of symptoms after the administration of antivenom is due to the inability of the antivenom to neutralize scorpion toxins already bound to their target receptors or inadequate antivenom amount.
- General time guidelines for the disappearance of symptoms after antivenom administration are as follows:
  - *Centruroides* antivenom: Severe neurologic symptoms reverse in 15–30 min. Mild-to-moderate neurologic symptoms reverse in 45–90 min.
  - Non-*Centruroides* antivenom: In the first hour, local pain abates. In 6–12 h, agitation, sweating, and hyperglycemia abate. In 6–24 h, cardiorespiratory symptoms abate.
- While an anaphylaxis reaction to the antivenom is possible, the patient is at lower risk for this than with other antivenoms for other venom envenomations if there is a scorpion venom that induced large release of catecholamines. Also, animal-derived antivenom increases the risk of hypersensitivity reaction compared to human monoclonal-derived antivenom. Finally, the larger the dose of antivenom, the greater the chance for serum sickness.
- In a prospective, randomized, double-blind study, Boyer et al. compared scorpion-specific $F(ab')_2$ antivenom (Anascorp, *Centruroides* [scorpion] immune $F(ab)_2$ intravenous [equine], Instituto Bioclon) ($n = 8$) with placebo ($n = 7$) in children who developed neurotoxic symptoms following scorpion envenoming. Neuromotor abnormalities were present in all patients at baseline, and respiratory distress was present in 20 %. Beginning 2 h after treatment, symptom resolution differed significantly in the antivenom group compared with the placebo group. Plasma venom concentrations were undetectable and cessation of the neurologic syndrome occurred within 4 h in 100 % of antivenom recipients compared with 1 placebo recipient ($p = 0.001$).
- Thus, the Boyer et al. study suggests that scorpion-specific $F(ab')_2$ antivenom successfully treated the clinical syndrome, reducing the need for concomitant sedation and reducing circulating unbound venom levels for *Centruroides* envenoming.
- For *Mesobuthus tamulus* envenoming, horse-derived antivenom has been developed. Natu et al. compared the newer antivenom treatment versus the traditional

prazosin treatment in their open-label study of 81 envenomated patients and found that antivenom decreased clinical recovery time to 4.14 h ± 1.6 h compared to prazosin's clinical recovery time of 19.28 h ± 5.03 h.

- Natu et al. also found that the antivenom plus prazosin combination group had a recovery time of 3.46 h ± 1.1 h but felt it was comparable to the antivenom group recovery time and recommended that the combination therapy be reserved for patients presenting with pulmonary edema with hypertension.
- Bawaskar et al. compared antivenom plus prazosin versus prazosin in their open-label trial of 70 patients with only grade 2 envenoming (beginning of systemic involvement) and found that 91.4 % of the combination treatment group had resolution of their clinical symptoms within the 10-h mark compared to 22.9 % in the prazosin treatment group. Both the Natu and the Bawaskar studies suggest the utility of the new *Mesobuthus tamulus* antivenom for systemic symptoms envenoming.
- A vaccine preparation was tried in experimental animals but was not pursued because of the need to prepare different antigens according to different geographical areas and to different species of scorpions living in the same area.
- In some cases, one should be aware that meperidine and morphine may potentiate the venom. Also, the concurrent use of barbiturates and narcotics may add to the respiratory depression in patients who have been envenomed.

Prevention

- False ceiling under loose tiles of roof and bamboo cot with scrupulous use of mosquito net protect from scorpion sting.
- In endemic areas of venomous sting, clothing, beddings, shoes, and package should be vigorously shaken out and checked for scorpion without blindly putting hands.
- Pesticides like organophosphates, pyrethrins, and chlorinated hydrocarbons are known to kill scorpions.
- One should not sit touching to mud walls.
- At times of opening the school, the tables and rooms (roof, walls, and floor) should be thoroughly cleaned and washed.

Conclusion and Future Directions

Early hospitalization and administration of accurate dose of scorpion antivenom and prazosin and closely monitoring the victim in intensive care unit will save many lamented lives.

Periodic training for peripheral doctors in endemic areas regarding management of scorpion sting should be arranged. Scorpion sting should be included as notifiable disease. Scorpion sting should be included in a regular medical teaching at least in tropical and subtropical countries.

Cross-References

► Snake Venoms and Scorpion Venom Research in the Middle East: A Review

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Centipede Envenomations: Clinical Effects and Treatment

23

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Contents

Abstract

Centipede bites are reported worldwide, mostly in tropical and subtropical regions. Centipede envenomation can cause different types of local and systemic reactions and can affect all age groups. A diverse mixture of bioactive compounds and digestive enzymes has been found in centipede venom. Patients who encounter centipede envenomation commonly experience localized pain (often severe), localized pruritus, headache, nausea/vomiting, anxiety, palpitations,

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swelling, erythema, and warmth; rare complications can include necrosis, lymph node swelling, ischemia, rhabdomyolysis, ST elevation MI, and death. In most cases, only minor supportive care is required.

Introduction

Centipedes of medical importance are found across three orders (*Scutigera*, *Lithobius*, and *Scolopendra*) within the class Chilopoda. These arthropods have multiple segments, each containing one pair of legs, and a specially developed pair of anterior appendages connected to a muscle-lined venom gland. Some varieties, such as the Amazonian giant centipede (*Scolopendra gigantea*) can grow to rather intimidating lengths of up to 30 cm (Fig. 23.1).

(*Note: Although not anatomically part of the creature's mouth, the punctures caused by the front appendages or forcipules of a centipede will hereafter be termed "centipede bites." This usage is to ensure internal consistency, with the caveat that other articles and sources sometimes refer to the injury as a "sting."*)

Fig. 23.1 Amazonian giant centipedes can grow up to 30 cms. (Photo by Eleanor Hill [User: John Hill] is licensed under CC BY-SA 3.0)

Centipedes are nocturnal carnivores which prefer dark, damp places such as underneath rocks and leaf litter, but they are occasionally encountered indoors, where most reported bites occur. Most centipede bites occur in warm temperate and tropical climates, where centipede populations are known to thrive. Reported clinical effects of centipede envenomation range from mild localized pain to systemic multiple organ failure and even fatalities.

Epidemiology

Multiple case series from several tropical and warm temperate regions have been published, but these injuries, like all arthropod envenomations, are largely underreported (Fung et al. 2011; Mohri et al. 1991; Balit et al. 2004; Tabrah 2007; Medeiros et al. 2008; Uppal et al. 1990; Knysak et al. 1998; Guerrero 2007; Lin et al. 1995; Eitzen and Seward 1988). What these studies do suggest is that the typical injurious encounter with centipedes occurs indoors, sometimes following rainfall. The living room and bedroom are the most common areas where injurious encounters occur. This is probably because of a lack of protective clothing or footwear on the victims while in these spaces, combined with the ready availability of warm, dark spaces for the creatures to sequester before an attack occurs. Studies indicate that both children and adults are at-risk populations, and there is no gender disparity among victims.

Venom Composition, Apparatus and Pathophysiology

Centipede venoms are mixtures of lipids, enzymes, peptides, and non-peptide small molecules. Specifically, centipede species have been found to express venom containing one or more of the following: serotonin, bradykinin, histamine, polysaccharides, cholesterol and free fatty acids, hyaluronidases, phospholipases, cardiotoxins, hemolysins, neurotoxins, myotoxins, proteases, and esterases (Undheim and King 2011; Burnett et al. 1986; Yang et al. 2012; Cherniack 2011). Several unique components of centipede venom include a cardiotoxic component found in *Scolopendra subspinipes* called toxin S, a muscarinic component isolated from *S. morsitans*, and a necrosis factor called cytolysin from *S. heros*. The venom of centipedes is thought to have both defensive and predatory functions in the life cycle of these carnivores. Additionally, a lipid-toxin complex similar to that of scorpion venom is thought to facilitate penetration and absorption into cells. Dried extracts of the Chinese red-headed centipede (*Scolopendra subspinipes mutilans*) have been used in some Far Eastern medicines (Fig. 23.2).

The envenoming apparatus of centipedes is unique: a specialized pair of anterior appendages, called forcipules (which in other arthropods would serve as either legs or antennae), is each connected to a muscular sac that manufactures venom. Sometimes termed “venom claws,” the curved, hollow forcipule appendage bodies each contain pores and grooves through which venom is expressed, and they are sharp

Fig. 23.2 Chinese red-headed centipede (Photo by Yasunori Koide is licensed under CC BY-SA 3.0)

Fig. 23.3 Underside of the centipede showing the sharp anterior appendages (forcipules) connected to bilateral venom sacs (Photo by Fritz Geller-Grimm is licensed under CC BY-SA 2.5)

enough to puncture the skin or external coating of a prey (Fig. 23.3). Toothlike ridges on the sides of the venom claws function to grip and rip the external tissues of a prey. Some reports of envenoming describe an arrow-shaped lesion on the skin at the site of venom claw penetration, approximately 0.3–3 mm wide.

The physiologic effects of venom are diverse and incompletely reported or studied. A major limitation of the literature is the lack of centipede species confirmation in clinical case reports and species. Pain, erythema, local swelling, and necrosis are likely due to small molecules such as histamine and bradykinin as well as cytolytic enzymes and proteases. More systemic clinical effects (e.g., muscarinic receptor activation with nausea, vomiting, bradycardia, and

bronchospasm) are also observed, but the venom components causing these phenomena have not yet been identified. Cardiotoxicity (e.g., arrhythmias, coronary vasospasm, ACS, and STEMI) has also been reported in the context of centipede envenomation, but it is unknown whether or how the cardiotoxin (toxin S) found in some centipede species relates to the cardiac complications observed in some patients. Rhabdomyolysis and myoglobinuric renal insufficiency have also been observed after centipede envenomation, probably due to the presence of as-yet uncharacterized myonecrotic factors.

Clinical Effects

Reports of envenomations suggest that the vast majority of centipede bites present with more benign clinical effects, of which local pain and erythema are the most common. Systemic symptoms associated with centipede ingestion have been documented in pediatric and adult cases. Systemic symptoms such as sweating, fever, and tachycardia appeared in 5–11 % of patients in some series. Effects of envenomation are typically evident within the first few minutes to hours following a bite, but it is important to recognize that the nature of the injury can evolve. Thus, serial examinations are warranted in all but the most minor bites. Centipedes, particularly of the *Scolopendra* genus, frequently leave visible puncture marks at the bite site, but clinically identifiable wounds are often absent. Bleeding from centipede bites has been described, as has the development of both hemorrhagic and nonhemorrhagic vesicles.

More alarming systemic symptoms such as chest discomfort with and without myocardial infarction have also been noted in conjunction with centipede bites. Although rhabdomyolysis with subsequent acute kidney injury and compartment syndrome due to large-scale myonecrosis has been described, these dramatic presentations seem to be the exception more than the rule. The following sections subdivide the effects of centipede envenomations into minor and systemic effects, which can be difficult to distinguish given variation in patient factors and changes in injury symptoms and signs over time. It is also important to recognize that patients with this dynamic injury will often present hours to days later due to symptom persistence or clinical deterioration, at which time complications of bacterial superinfection can be difficult to distinguish from the venom effects per se (Table 23.1).

Minor Effects

The effects of centipede bites can range from a local, mild reaction to a systemic, severe reaction. Most envenomations result in a limited local reaction with pain, swelling, itching, and minor bleeding at the puncture site. Most reactions occur in an area less than 5 by 5 cm, though areas as large as 10×20 cm have been reported. Occasionally, the bite site may become necrotic, with skin desquamation after 48–72 h. Lymphangitis and painful local lymphadenopathy in the axilla or groin

Table 23.1 Reported clinical effects of centipede injuries

Organ system affected	Effects noted
Skin (local)	Swelling, local bleeding, pain, erythema, itching
Skin (systemic)	Eosinophilic reaction, pruritus
GI	Nausea, vomiting
Cardiac	Palpitations, syncope, coronary ischemia
Respiratory	Shortness of breath, wheezing
Renal	Proteinuria, renal failure
Soft tissues	Rhabdomyolysis, compartment syndrome

of the affected limb can also occur, lasting hours to days if the bite occurs on an arm or leg. Pericoronitis has been reported in association with centipede envenomation in a single case (Gelbier and Kopkin 1972).

Mild systemic reactions are reported less frequently. The most common systemic effects reported are nausea, vomiting, palpitations, shortness of breath, wheezing, diaphoresis, headaches, and anxiety. These reactions can last for days, but most resolve within 24 h. Treatments rendered include standard symptomatic care, although there are several case reports where swelling was treated with a short course of systemic steroids. Clinical suspicion for cellulitis or soft tissue infection following any puncture wound should warrant a search for retained foreign bodies (see Section Treatment for Centipede Envenomations below) and consideration of antibiotic initiation.

Major Effects

More serious complications of centipede bites include myocardial infarction, muscle compartment necrosis, and acute renal failure. Although the pathophysiology of severe toxic effects is not well understood, these effects seem to be very rare following injuries from centipedes. The *Scolopendra* species venom contains toxin S, which in amphibian animal models has cardiotoxic effects. Several previously published case reports describe chest pain, acute ST segment elevation myocardial infarction (STEMI), and elevated levels of cardiac troponins in healthy young adults with no coronary risk factors (Yildiz et al. 2006; Ozsarac et al. 2004; Senthilkumaran et al. 2011). In some of these intriguing case reports, cardiac catheterization studies were negative for atherosclerotic heart diseases, suggesting that the venom may cause coronary ischemia from a vasospastic mechanism.

A case report from Arizona described a middle-aged victim of *Scolopendra heros* bite on the toe that led to lower extremity compartmental myonecrosis, rhabdomyolysis, and acute renal failure (Logan and Ogden 1985). Other centipede species have nephrotoxic venom components, with the potential to cause proteinuria and renal failure. Proteinuria generally lasts for several days, and renal failure requiring long-term dialysis is very rare. All reported cases of proteinuria and renal failure made full recoveries with normal renal function.

Table 23.2 Effects of centipede envenomations in children

Organ system affected	Effects noted	Patient age	Effect severity
Neurologic and gastrointestinal	Hypotonia, diffuse weakness, vomiting, pale skin	6 months	Major (but ingested)
Skin/subcutaneous	Local erythema, edema, and pain	16 years	Minor
Skin/subcutaneous	Local erythema, numbness though impressively large at 60 × 20 cm	10 years	Minor
Skin/subcutaneous	Vesicle	11 years	Minor
Skin/subcutaneous, constitutional	Local edema, erythema, pain, hyperoxia, hypersomnolence, hyperesthesia	28 days	Major
Skin/subcutaneous, renal	Local edema, pain, erythema, proteinuria	16 years	Minor

A single case of eosinophilic cellulitis, or Wells' syndrome, has been described following a *Scolopendra* bite, where the patient was biopsied, but outcome was not reported as the patient was lost to follow-up (Friedman et al. 1998).

Although centipede bites occur rather frequently and regularly around the world, there are only three reported deaths. One death occurred in a 7-year-old Filipino girl bitten on the head by *Scolopendra subspinipes* (see below section on Pediatric Considerations) (Remington 1950). The details of the other two deaths are unclear. Though some deaths may have gone unreported, it is clear that death from a centipede bite is a very rare occurrence (Table 23.2).

Pediatric Considerations

There are not many case reports of centipede envenomations in children. Almost all of them describe local edema, erythema, and tenderness at the site of the centipede envenomation, with one that additionally describes a vesicular lesion at the site, and another that describes a rather large, numb area of erythema, approximately 60 by 20 cm.

Additionally, a few reports describe systemic symptoms. Proteinuria was seen after envenomation in a 16-year-old girl who had no history of a renal disorder; levels of urinary protein peaked around week 3 and were present for approximately 15 weeks after the incident (Hasan and Hassan 2005). Envenomation in a 28-day-old infant resulted in local symptoms, poor feeding, and lethargy for 48 h before returning to baseline (Rodriguez-Acosta et al. 2000).

In an unusual case, a 6-month-old ingested a centipede, which was excreted out whole in the infant's stool and appeared to induce generalized hypotonia, diffuse weakness, pallor, and emesis that resolved within 48 h; whether the route of envenomation was gut absorption of leaked toxins or from direct envenomation by centipede within the GI tract is not clear (Barnett 1991).

The most serious adverse pediatric effect is the case of a reported death in a 7-year-old child in the Philippines, who expired 29 h after a centipede bite on the head (Remington 1950).

Based on the available case reports, centipede envenomation in children can be expected to cause local edema, erythema, and tenderness and may present with additional local skin findings in some cases. More rare but serious adverse effects may include loss of appetite, hypersomnolence, hypotonia, emesis, and possibly even death. Due to the infrequency of reported bites, it is uncertain how or whether the child's age affects symptoms.

Considerations in Pregnancy

There are no studies specifically investigating the natural history or course of centipede bites in pregnant patients. Because of the severe pain and other systemic complications which can rarely affect bitten patients, it is prudent to evaluate the whole patient for signs of early labor or muscle contraction. Care for this patient population is otherwise supportive.

Diagnostic Workup

The diagnosis of centipede envenomation is primarily clinical, as most cases can be confirmed using a thorough history and physical examination. In the majority of cases, patients will report local symptoms and demonstrate signs affecting the area proximal to the bite site. When systemic signs are present, diagnostic strategies should be tailored to the effects observed. For example, severe vomiting may require checking and correcting electrolytes. Because of the rare reports of cardiac complications, systemic symptoms or historical elements which suggest thoracic or abdominal complications should prompt a strong consideration for cardiac testing (electrocardiogram, enzyme biomarkers such as troponin and creatine kinase-MB fractions, and, if deemed necessary by a cardiology specialist, imaging workups such as echocardiography and cardiac catheterization). In patients who are suspected of having rhabdomyolysis, serial monitoring of creatine kinase, renal function, urinalysis, and electrolytes is warranted. Although skin and soft tissue infections are not well documented after centipede bites, it is prudent to consider additional diagnostic testing (skin biopsy, ultrasound, computerized tomography) of areas suspected to have deeper necrosis or infectious complications following an injurious encounter.

Treatment for Centipede Envenomations

Local Effects

Little information exists regarding appropriate specific treatments for centipede bites as there is no antivenom available for these envenomation injuries. Because localized pain seems to be the most common complaint and appears to be universally experienced, adequate analgesia is the cornerstone of initial treatment.

Treatment of pain appears to be best accomplished with ice packs and analgesia, based on a single small randomized study of first aid in victims which showed similar effectiveness with the use of ice packs, analgesic medications, or hot water (43–45 °C) immersion (Chaou et al. 2009). Analgesia may be achieved with paracetamol, ketorolac, and opioid narcotics (Balit et al. 2004).

Systemic Effects

When detected, secondary and systemic effects of centipede envenomation should be treated with standard therapy (Bouchard et al. 2004; Bush et al. 2001; McFee et al. 2002; Steen et al. 2007; Veraldi et al. 2010). Standard proper wound care and tetanus vaccination update (if needed) is also indicated. Antihistamines often help with pruritus. Diffuse swelling and urticarial or allergic reactions can be treated with topical and/or systemic corticosteroids, although these agents have not been systematically studied for this indication. Affected areas should be carefully inspected for embedded venom claws or other debris. Elevation of a bitten extremity may help minimize swelling. Systemic antibiotics should be initiated if there is bacterial superinfection (Serinken et al. 2005).

Subacute and Delayed Complications

Use of advanced imaging techniques such as ultrasound or MRI may help with detection of foreign bodies within soft tissue compartments. Although there is no literature describing unique infectious complications of these bites or retained subcutaneous foreign bodies such as venom claws, it is important to recognize that infectious and retained-object complications can potentially occur following these encounters.

Clinical Vignettes

The following clinical case reports provide sketches of actual cases of centipede envenomations where severe complications were documented. As such, these three cases are not meant to be representative of a typical, minor envenomation. The reader is referred to the original publications for more details and descriptions.

Pediatric Case

A 28-day-old girl was bitten while sleeping on the left hand by a “dark brown centipede with yellow extremities” identified as *Scolopendra gigantea*. She began to cry uncontrollably and was examined 6 h after the incident, where she had irritability, local hand edema, and dry blood at the puncture sites. Arm edema and patchy erythema were later notable. The swelling decreased 3 h after hydrocortisone injection and oral acetaminophen, and she was observed to have decreased

Fig. 23.4 *Scolopendra heros* (giant desert centipede) (Photo by John - Flickr: Scolopendra heros is licensed under CC BY 2.0)

feeding and increased somnolence for about 3 days. She recovered uneventfully thereafter (Rodriguez-Acosta et al. 2004).

Adult Case with Complications of Rhabdomyolysis, Renal Failure, and Compartment Syndrome

A 44-year-old woman in the Southwestern United States with a history of allergic reactions to Hymenoptera (bees) was bitten on the dorsal foot in her bedroom by a centipede identified as *Scolopendra heros* (giant desert centipede) (Fig. 23.4). She developed edema of the leg and then a superficial ulcer at the site of the bite. She presented for medical care after 3 days of increased swelling, shortness of breath, and vomiting and failed outpatient care with prednisone and antibiotics. On day 5 she was admitted to a tertiary medical care center where she was noted to have loss of both sensory and motor functions below the ankle. Labs showed proteinuria, leukocytosis, acute transient kidney injury, and rhabdomyolysis. Further investigations of the bitten leg revealed increased anterior compartment pressures and electrical silence of the peroneal compartment muscles. Despite anterior and lateral fasciotomy procedures, her course was characterized by an incomplete recovery at the time of the case report (Logan and Ogden 1985).

Adult Case with Complications of Coronary Ischemia

A 22-year-old man was bitten by an unspeciated centipede of genus *Scolopendra* on the left middle finger and experienced immediate pain and swelling. Within 2 h he began to have central chest pain radiating into the left arm, vomiting, and diaphoresis. He presented to the hospital 14 h later, where he was noted to have a blood pressure of 90/60 mmHg, pulse of 74 beats per minute, and respirations of 22 per minute. Acute ST elevations were noted in the electrocardiogram, and he was given aspirin, clopidogrel, enoxaparin, and nitroglycerin. The troponin level was markedly elevated, and echocardiography demonstrated hypokinesis in the anterior myocardial wall, with an ejection fraction of 35 %. However, cardiac angiography

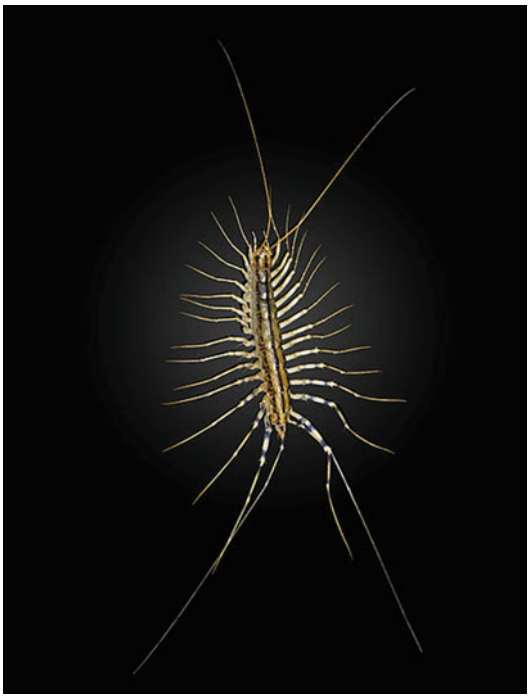

Fig. 23.5 *Scutigera coleoptrata* (the common house centipede) (Photo by Didier Descouens is licensed under CC BY-SA 4.0)

revealed "entirely normal coronary arteries." He was noted to have normalized his wall motion on repeat echocardiography, done 3 days after his initial echocardiogram (Fig. 23.5) (Senthilkumaran et al. 2011).

Conclusion and Future Directions

Centipede envenomation represents a rarely reported injurious encounter that occurs mainly in tropical regions. The majority of bites result in self-limited local injuries, although rare systemic effects and organ injury involving cardiac, renal, and large muscle groups have been described. Diagnosis and care for these injuries is currently symptomatic and supportive, as there is no antivenom available. Because these injuries are relatively underreported, there is a need for increased education about prevention, first aid, and definitive management of these encounters.

There are several future directions in research for these injuries. More clinical epidemiology of these bites in specific countries and populations is needed to better understand the global significance and natural history of these injuries. Additional studies are needed for vulnerable populations such as young children, pregnant patients, and those with comorbid disease states. More basic research about the toxic components and pathophysiology of centipede venoms could help to formulate more effective therapies for envenomations and potentially identify pharmacologically useful molecules.

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Kidney Injury and Animal Toxins

24

Visith Sitprija and Vijitr Boonpucknavig

Contents

Abstract

Animal toxins are complex mixtures of peptides, enzymes, proteins, and chemical compounds. The kidney as a highly vascularized organ is vulnerable to toxin injury. Hemodynamic changes resulting in decreased renal blood flow are the basic finding in severe envenoming and represent the net result of the effect of

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vasoactive substances from toxins, the effect of toxins on vascular or autonomic neuronal ion channels, and the effect of proinflammatory cytokines and vasoactive mediators from the host. Several enzymes in toxins including phospholipase A_2, metalloprotease, and sphingomyelinase are cytotoxic and potentially nephrotoxic. They are also responsible for intravascular coagulation, intravascular hemolysis, and rhabdomyolysis which further enhance renal ischemia. In general, renal injury is attributed to the interaction of inflammatory reaction and renal ischemia. Direct nephrotoxicity is responsible for acute renal injury in some animal envenoming. Immunologic mechanism plays a minor role in renal injury. Clinically, manifestations of animal toxin injury vary from mild urinary sediment changes to nephritic or nephritis syndrome and acute renal failure. Renal pathological changes involve all renal structures which include mesangiolysis, glomerulonephritis, vasculitis, tubular necrosis, interstitial nephritis, thrombotic microangiopathy, and cortical necrosis. Serum electrolyte changes due to effects of toxins on ion transport in renal tubules and cell membranes open a new dimension in animal toxin injury.

Introduction

Injury by animal toxins is one of the important medical problems in the tropics. Severe and fatal envenoming in man and livestock are usually endemic. The incidence is often underestimated due to lack of records. Approximately 5 million snakebites, scorpion stings, and anaphylaxis occur worldwide causing over 100,000 human deaths each year mostly in the tropics. Clinical manifestations in animal toxin injury represent the direct effect of toxin to the tissue and the inflammatory reaction of the host through cytokines and vasoactive mediators. Local symptoms include pain, swelling, redness at the site of injury which may last for a few days. Local bleeding can be observed, and in severe bleeding diathesis, due to toxins mostly of hemotoxic snakes, compartment syndrome can be observed. Systemic symptoms consist of changes in blood pressure. Hypotension is common, but hypertension can also occur. Acute renal failure is a life-threatening complication.

Toxin distribution in each organ is determined by the blood perfusion and the ability of toxin to pass through the vascular endothelium and penetrate the cell. The kidney as a highly vascularized organ receiving 20–25 % of the cardiac output is one of the target organs. The unique ability of the kidney to concentrate urine can raise the toxin concentration in the tubular lumen to the toxic level. The kidney, liver, lung, and spleen are among the organs of high toxin distribution. Distribution is highest in the kidney. The kidney is therefore vulnerable to toxin injury. Glomerular injury may result from positive charge substance or from immune complexes or trapping of large molecules and complement activation. Proximal tubule is an important target of toxin since it is the site for both reabsorption and secretion. Transport of molecule is greater in the proximal tubule especially S_2 segment than in the other segments. Proximal tubule epithelium is leaky in

comparing with the other segments allowing easy flux of toxins into the tubular cells. Proximal tubular epithelial cells are also susceptible to ischemic injury than distal tubules.

Renal injury due to animal toxins is multifactorial. Hemodynamic alterations play the key role in the pathogenesis of renal injury (Sitprija and Sitprija 2012). Besides the proinflammatory cytokines and vasoactive mediators from the host, a number of vasoactive substances are present in the toxin. Toxin effects on vascular ion channels and enzymes with possible nephrotoxicity have been considered. Among several enzymes in the toxins, phospholipase A_2 (PLA_2), metalloprotease, and sphingomyelinase deserve consideration for their toxicity. These topics will be addressed with respect to their roles in the pathogenesis of nephropathy and renal failure.

Mechanism of Toxicity of Animal Toxins

Toxin Effects on Ion Channels

Ion channels are vital for cell function. Ion transport in and out of cells modulates electrical polarity of muscle, nerve, and secretory organ. Depolarization is caused by activation of Na channels, closing of K channels, and opening of Cl channels. Hyperpolarization, on the contrary, is the result of activation of K channels and inactivation of Na and Cl channels. Toxin actions on vascular ion channels and autonomic neuron channels play the role in modulating vascular contraction and neurotransmitter release including catecholamines and acetylcholine which are important in hemodynamics. Depolarization opens Ca channel and increases Ca influx into the cell causing the release of neurotransmitters. In the vascular smooth muscle cells opening of Ca channels, Cl channels, Na channels (ENaC), and closure of K channels (K_{ATP}) cause vasoconstriction. Opening of ENaC causes depolarization and opening of Ca channels with Ca influx. Increased cytosolic Ca results in hypertension. Cellular Na influx upregulates Na K-ATPase to maintain low intracellular Na concentration. Massive Na influx with Na K-ATPase inhibition causes cell swelling which can result in cell death. Closure of Ca channels, ENaC, and opening of K_{ATP} result in vasodilatation and hypotension. L-type Ca channels are abundant on vascular smooth muscle cells and serve important function in regulation of blood flow (Xiong and Sperelakis 1995). Several snake venoms close Ca channels (Shikamoto et al. 2005). Closure of vascular ENaC causes hyperpolarization which closes Ca channel resulting in vasodilatation. ENaC closure also decreases intracellular Na and decreased Na-Ca exchange resulting decreased cytosolic Ca. Although effects of animal toxins on excitable cell Na channels have received much attention, data on animal toxins acting on vascular ENaC are lacking. A number of venoms including those of box jellyfish (*Chironex fleckeri*), scorpions (*Buthidae*), black widow spider (*Latrodectus*), sea anemone (*Actiniaria*), and some crotalids activate Ca channels and cause hypertension (Mebs and Hucho 1990). Extensive Ca influx with massive Ca accumulation in mitochondria can cause cell death.

Studies by isolated renal perfusion technique have highlighted the renal vascular effects of several snake venoms and arthropod venoms. Venoms from *Bothrops moojeni*, *B. jararaca*, *B. jararacussu*, *Crotalus durissus terrificus*, and *Daboia siamensis* cause renal vasodilatation. Venoms from *Crotalus durissus cascavella*, *Polybia paulista*, and *Tityus serrulatus* cause renal vasoconstriction. Renal hemodynamic changes in isolated renal perfusion by venoms are interpreted to indicate direct effect of venoms mediated through ion channels on vascular smooth muscle cells either L-type Ca channels, ENaC or Kca, or K_{ATP}. Further study is required.

Toxin Vasoactive Substances

Several vasoactive substances are present in animal venoms. These include natriuretic peptides, vascular endothelial growth factor, angiotensin-converting enzyme inhibitor, sarafotoxins, proteases, and phospholipases. They are both vasodilators and vasoconstrictors. Hemodynamics can be compromised.

Natriuretic Peptides

Natriuretic peptides have been identified in several venoms including venoms of green mamba (*Dendroaspis angusticeps*), inland taipan (*Oxyuranus microlepidotus*), Brazilian jararaca (*Bothrops jararaca*), habu snake (*Protobothrops flavoviridis*), Japanese mamushi (*Gloydius blomhoffii*), bamboo pit viper (*Trimeresurus gramineus*), crotalid (*Crotalus durissus cascavella*), Southern American coral snake (*Micrurus leminiscaties*), and platypus. A recent report suggested the presence of natriuretic peptides in the venoms of Malayan krait (*Bungarus candidus*), red-headed krait (*Bungarus flaviceps*), and Berg adder (*Bitis atropos*). Hemodynamically, natriuretic peptides decrease systemic and renal vascular resistance and blood pressure. Cardiac output, renal blood flow, and glomerular filtration rates are increased. Natriuretic peptides, binding with receptors linked to cGMP-dependent signal cascade, lead to increased intracellular cGMP which inhibits Na reabsorption in the medullary collecting ducts. cGMP effect through PKG or direct action on cyclic nucleotide channel, amiloride-sensitive Na channels in the medullary collecting duct, results in natriuresis. Natriuretic peptides suppress renin-angiotensin aldosterone axis, thus decreasing angiotensin II and catecholamine release. K channels (BK) are stimulated by natriuretic peptides through cGMP-dependent dephosphorylation (White et al. 1993). At the clinical level, interpretation of data on natriuretic peptides should be done with caution since natriuretic peptides can be generated by the host in response to volume expansion. Scorpion (*Androctonus australis garzonii*) envenomated rats showed natriuretic peptides in the serum (Soualmia et al. 2008).

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF), derived from the venom of viperidae especially viperids, is dimeric glycoprotein. VEGF causes vasodilatation and hypotension, decreases cardiac output, and increases vascular permeability (Ferrara 2004).

Vasodilation is nitric oxide (NO) dependent. It has been demonstrated that VEGF upregulates the endothelial NO synthase leading to increased NO production and hypotension. VEGF induces angiogenesis and can be also produced by the host.

Angiotensin-Converting Enzyme Inhibitor

Angiotensin-converting enzyme inhibitors (ACEI) have been isolated from the venoms of snake and scorpion. Teprotide, an oligopeptide from the venom of Brazilian jararaca (*Bothrops jararaca*), is the prototype of ACEI that inactivates peptidyl dipeptidase which converts angiotensin I to angiotensin II and increases kinin activity by inhibition of kininase. Teprotide decreases systemic and renal vascular resistance, blood pressure, and glomerular filtration rate, but increases renal blood flow. Many snake venoms contain ACEI and bradykinin-potentiating peptides. Scorpion venoms from *Centruroides sculpturatus*, *Buthus occitanus*, and *Mesobuthus martensii Karsh* have ACEI.

Sarafotoxins

Sarafotoxins are polypeptides isolated from the venom of Israel burrowing asp (*Atractaspis engaddensis*). Like endothelins, sarafotoxins increase cytosolic Ca by causing the release of Ca from sarcoplasmic reticulum. Blood pressure and systemic and renal vascular resistance are increased. Cardiac output, renal blood flow, and glomerular filtration rate are decreased (Kon et al. 1989). Sarafotoxins can cause vasoconstriction of coronary arteries and delay atrioventricular conduction.

Serine Proteases

Serine proteases or serine endopeptidases are produced by snakes in the family *Viperidae* and by bees (*Apis mellifera*). The enzymes have thrombin-like action with fibrinolytic activity. Serine proteases activate proteinase-activated receptors (PAR_2) and cause vasodilatation and hypotension through NO-dependent guanylyl cyclase (Gui et al. 2003). Serine proteases also convert kininogen to kinin causing vascular smooth muscle relaxation.

Metalloprotease

Metalloproteases, zinc-dependent endopeptidases, are present in the venoms of snakes of subfamilies Viperinae and Crotalinae. In a recent study in dogs, metalloprotease from Russell's viper (*Daboia siamensis*) venom caused renal vasoconstriction and decreased cardiac output (Mitrmoonpitak et al. 2013). Metalloprotease converts big endothelin-1 to endothelin-1 which is responsible for vasoconstriction and hypertension. Endothelin-1 increases cytosolic Ca and causes vasoconstriction. Decreased cardiac output is due to weakening of cardiac tensile strength (Mujumdar et al. 2001).

Phospholipase A_2

Phospholipase A_2 (PLA_2) are present in several animal toxins including snakes and arthropods. Through mobilization of arachidonic acid from phospholipid and generation of prostaglandins, PLA_2 can cause vasodilatation and hypotension by

decreased phosphorylation of myosin light chain. PLA_2 also stimulates hypothalamus pituitary axis to increase adrenocorticotropic hormone, corticosteroids, arginine vasopressin, and acute-phase response (Chisari et al. 1998). Arginine vasopressin, besides its effect on aquaporin, can also cause vasoconstriction.

Endogenous Proinflammatory Cytokines and Vasomediators

Both Th1 and Th2 are involved in the production of proinflammatory and anti-inflammatory cytokines as innate immune response to toxins. These cytokines include IL-1, IL-2, TNFα, IFNγ, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, and GM-CSF (Petricevich 2010). Th1 cells induce the production of IgG2a opsonizing antibodies. Th2 cells induce IgE and IgA production. IL-1 induces IL-6 and adhesion molecule production and contributes to endothelial injury. IL-6 contributes to acute-phase reaction and hematopoiesis. Both IL-1 and IL-6 are often used as markers for severe systemic reactions. IL-8 is known to mediate brain injury. Anti-IL-8 antibody reduces brain edema. TNFα increases production of IL-1β, IL-6, NO, adhesion molecules, proliferation of fibroblast, and procoagulant factors. PLA_2, cyclooxygenase type 2, and NO synthase induce production IL-1 (Dinarello 2009). IL-4 suppresses IL-1, IL-6, IL-8, TNFα, and macrophage inflammatory protein 1 alpha. IL-10 inhibits production of IFNγ, TNFα NO, IL-1, IL-2, IL-6, IL-8, and IL-12 and also suppresses free oxygen radical and prostaglandin production (Asadullah et al. 2003).

In the inflammatory reactions, generation of arachidonic acid metabolites, toxic oxygen radicals, NO, kinins, prostaglandins, leukotrienes catecholamines, endothelins, thromboxane A2, platelet activating factor, complements, and proinflammatory cytokines are intimately linked (Nouira et al. 2005; Petricevich 2010). Therefore, several vasoactive mediators both vasodilators and vasoconstrictors are released in the inflammatory process and contribute importantly to hemodynamic changes.

Hemodynamic Alterations

Both hypotension and hypertension are observed in animal toxin envenoming. Hypotension attributed to systemic vasodilatation, cardiotoxicity, and hypovolemia is common in animal toxin injury especially in snakebite. Activation and inactivation of ion channel affect blood pressure. Although in most cases of animal toxin envenoming hypotension is presented, hypertension occurs in certain envenoming from burrow or mole vipers, *Vipera berus*, Malayan krait, box jellyfish, scorpion, and spider (Sitprija and Sitprija 2012). The venom of scorpion *Androctonus australis* hector increases mean arterial pressure and pulmonary arterial pressure and decreases cardiac output (Nouira et al. 2005). Isolated renal perfusion is a good technique in assessing the effects of venom on renal hemodynamics and eliminates the endogenous mediators generated by the host. This technique has been used in

several studies using snake venoms, bee venom, and scorpion venom. However, it does not reflect the real renal hemodynamics in the host injured by the toxin.

In most envenoming, hemodynamic studies are incomplete. A number of studies performed in snake and scorpion envenomings use isolated renal perfusion technique focusing only on renal hemodynamics. Hemodynamic study in dogs, in which all parameters including cardiac output, systemic and renal vascular resistance, renal blood flow, and glomerular filtration rate are measured, is a classic model. In Russell's viper envenoming, hemodynamic changes are characterized by decreased systemic vascular resistance with initial increased cardiac output later followed by decreased cardiac output, increased renal vascular resistance, decreased renal blood flow, and decreased glomerular filtration rate (Sitprija and Sitprija 2012). Cobra envenoming showed the same pattern with a shorter duration. Envenomation by sea snake (*Lapemis hardwickii*) did not alter blood pressure, cardiac output, and systemic vascular resistance, but decreased renal blood flow and glomerular filtration rate (Sakwiwatkul et al. 2002). Although complete renal hemodynamic study is not performed in other animal toxin envenoming, they are presumed to be similar to the classic model of Russell's viper envenomation. Hypotension is often observed in severe envenoming. In this respect, hemodynamics in severe envenoming is similar to those observed in sepsis involving the same proinflammatory cytokines and vasoactive mediators including TNFα, IL-1, IL-6, IL-10, INFγ, NO, PGI_2, PGE_2, TXB_2, catecholamines, angiotensin II, and endothelins (Petricevich 2010). Initially, there is hypervolemia due to systemic vasodilatation, but finally hypovolemia develops due to increased vascular permeability with leakage of fluid from the intravascular compartment. Of interest, renal ischemia itself is inflammatory by enhancing synthesis of proinflammatory cytokines including IL-6 and TNFα (Granger 2006). Hemodynamic changes observed in man and animal envenomated by toxin are induced by combination effect of venom with its vasoactive substances and endogenous vasoactive mediators from the host. Disseminated intravascular coagulation, intravascular hemolysis, rhabdomyolysis, hemorrhage, and complement activation due to toxin enzymes further compromise renal blood flow. Pigment nephropathy is attributed to tubular obstruction by hemoglobin or myoglobin casts, decreased renal blood flow, and decreased glomerular filtration.

Direct Nephrotoxicity

Clinically, AKI has been observed following animal toxin envenoming without hypotension and associated insults including intravascular hemolysis, rhabdomyolysis, or disseminated intravascular coagulation. Mesangiolysis, vasculitis, endothelial damage, and extracapillary glomerulonephritis acutely occurring following Russell's viper and Habu snake envenoming would suggest direct nephrotoxicity (Barnes and Abboud 1993; Kanjanabuch and Sitprija 2008). Using cultured renal tubular cells, Russell's viper venom caused nuclear pyknosis, cellular detachment of proximal, distal and collecting tubules, and mesangial disintegration (Willinger et al. 1995). In human proximal tubular cell culture model, Russell's viper venom

decreases cell viability, causes necrosis, and increases LDH. Basic protein isolated from Russell's viper venom (RVV-7) induced tubular necrosis in mice (Mandal and Bhattacharyya 2007). *Bothrops moojeni* venom decreases vero cell uptake of neutral red, causes disarray of the cytoskeleton, and impairs cell to matrix adhesion.

Phospholipase A_{2s} represent families of esterases which hydrolyze the sn-2 ester bond in phospholipids and release free fatty acids and lysophospholipids. Classification of PLA_2 has been made in several ways. PLA_2 has been classified as secretory PLA_2 (s PLA_2) for PLA_2 secreted from the cell, Ca-dependent and cytosolic PLA_2 ($cPLA_2$), and Ca-independent PLA_2 ($iPLA_2$) (Cummings et al. 2000). Involvement of PLA_2 in inflammation is through the ability to mobilize arachidonic acid from phospholipid which results in the production of prostaglandins. PLA_2 therefore has an important role in cellular injury by the ability to create inflammatory response. PLA_2 causes membrane phospholipid hydrolysis, increased membrane permeability, and cell lysis. Arachidonic acid release occurs before cell death and is inhibited by $cPLA_2$ inhibitors $AACOCF_3$ (arachidonyl trifluoromethyl ketone) (Balsinde et al. 1999). Preincubation of Madin Darby canine kidney cells with $AACOCF_3$ decreased toxicity of oxalate (Kohjimoto et al. 1999). Cell expressing $cPLA_2$ are susceptible to H_2O_2 toxicity. Membrane lipid peroxidation, loss of membrane phospholipids, and increased lysophosphatidyl lipids act as detergents decreasing membrane integrity. The role of PLA_2 in apoptosis is debatable. $sPLA_2$ isoforms have low molecular mass and require millimolar amount of Ca for activity; $cPLA_2$ found in cytosol has high molecular mass and requires micromolar amount of Ca. $iPLA_2$ are located both in the cytosol and membrane and do not required Ca for activity. PLA_2 inhibits formation of prothrombinase complex which is composed of factor V, factor X, phospholipids, and calcium ions by degrading phospholipids. PLA_2 can either induce or inhibit platelet aggregation depending upon the enzyme concentration.

Metalloproteases activate complement and glutathione-s-transferase tumal necrosis factor-alpha-fusion protein (GST-TNFα) to generate biologically active TNFα and also induce the release of IL-1, IgE2, and IL-6 (Fernandes et al. 2006). Metalloprotease activates factor X, and prothrombin can cause hemorrhage and endothelial injury. The enzyme degrades extracellular matrix, disrupts cellular matrix and cellular adhesion, cleaves cell surface receptors, and activates chemokines and cytokines (Fernandes et al. 2006). Cell polarity is lost due to disruption of the actin cytoskeleton. Integrin redistributes away from the basal cell surface causing loss of cellular adhesion to the basement membrane. Activation of metalloprotease cleaves glycophorin on erythrocyte surface causing hemolysis. Metalloproteases are also important to tissue remodeling and tissue repair. Metalloprotease and angiotensin II blocker synergistically promote regression of glomerulosclerosis (Hayashi et al. 2010).

Both PLA_2 and proteases are important in causing hemorrhage, coagulopathy, and endothelial injury. Serine protease activates factor V and has thrombin-like effect. Metalloprotease activates factor X and prothrombin and induces endothelial injury. Both metalloprotease and serine protease cause fibrinolysis.

Sphingomyelinase is present in toxins of arthropods. It is a hydrolase which breaks sphingomyelin to phosphocholine and ceramide. By decreasing sphingomyelin and

increasing ceramides, sphingomyelinase predisposes renal tubular cells to ATP depletion during hypoxia and oxidant stress (Zager et al. 2000). Sphingomyelin decreases membrane fluidity and has cytoprotective effect: Ceramide is proapoptotic. In combination with mitochondrial Ca accumulation and oxidative stress, ceramide can cause cell death. Sphingomyelinase can activate metalloprotease, causing lysis of red blood cells through complement activation.

Immune Response

Both innate immune response and adaptive immune response are involved in animal toxin envenoming. Cellular response, release of proinflammatory cytokines and chemokines vasoactive mediators, and complement activation operate initially as innate immune response. Inflammatory reaction at the site of injury and systemic symptoms reflect innate immune response. A number of proinflammatory cytokines especially TNFα, ILS, vasoactive mediators, acute-phase proteins, and leukocytosis are incriminated (Petricevich 2010; Mitrmoonpitak et al. 2013). Local reactions may be less striking in toxin exposure. However, allergic reactions manifested as skin rashes and edema may be observed. Humoral antibody response later occurring as adaptive immune response contributes to the formation of circulating immune complexes. Immune complex deposition in the glomeruli and blood vessels reflecting humoral immune response has been observed in snakebite and insect sting. Deposition of IgM and C_3 in the glomerular mesangium has been demonstrated. Glomerular changes resemble those observed in acute infection. There is evidence of in situ immune complex in viper bite with demonstration of the venom antigen in glomerular mesangium (Sitprija and Boonpucknavig 1983). The size of antigen is important in causing immune response. Toxin components with large molecular weight such as enzymes are more antigenic than small molecule peptides. In this respect, toxins from snakes and arthropods are more antigenic than marine toxins, and immune complex glomerulonephritis is therefore observed in snake and arthropod envenoming. Since animal toxin envenoming is an acute injury and the toxins are rapidly eliminated from the body, renal histological study may not show immune complex deposition. The presence of glomerular immune complex has limitation. Immune complex glomerulonephritis is therefore not a consistent finding. Glomerular changes observed may represent direct toxin injury.

Renal Pathology

Renal pathological changes associated with animal toxin envenoming include glomerulopathies, vasculopathies, acute tubular necrosis, tubulointerstitial disease, thrombotic microangiopaties, and cortical necrosis. Most studies are obtained from envenoming by snakes and arthropods. Few studies are from marine toxins. Table 24.1 shows the causes and renal pathological changes due to animal toxin envenoming.

Table 24.1 Nephropathies due to animal toxins

Animal	Causes	Pathological changes
Snake	Hemodynamic changes	Tubular necrosis
	Direct toxicity	Mesangiolysis
	Immunologic reaction	Cortical necrosis
	Rhabdomyolysis	Vasculitis
	Intravascular hemolysis	Thrombotic microangiopathy
	Disseminated intravascular coagulation	Glomerulonephritis
		Interstitial nephritis
		Renal infarction
Bee, wasp, and hornet	Hemodynamic changes	Tubular necrosis
	Direct toxicity	Interstitial nephritis
	Immunologic reaction	Glomerulonephritis
	Rhabdomyolysis	Vasculitis
	Intravascular hemolysis	Thrombotic microangiopathy
Scorpion	Hemodynamic changes	Tubular necrosis
	Disseminated intravascular coagulation	Glomerulonephritis
	Intravascular hemolysis	Vasculitis
		Thrombotic microangiopathy
Spider	Hemodynamic changes	Tubular necrosis
	Rhabdomyolysis	Glomerulonephritis
	Intravascular hemolysis	
	Direct toxicity	
Caterpillar (*Lonomia obliqua*)	Hemodynamic changes	Tubular necrosis
	Disseminated intravascular coagulation	
Jellyfish	Hemodynamic changes	Tubular necrosis
	Intravascular hemolysis	
Carp (raw bile)	Direct toxicity	Tubular necrosis
Centipede	Hemodynamic changes	Tubular necrosis
	Rhabdomyolysis	
Spanish fly (*Lytta vesicatoria*)	Direct toxicity	Tubular necrosis
		Glomerulonephritis
Fire coral (*Millepora species*)	Direct toxicity	Minimal change lesion
Sea anemone (*Phyllodiscus semoni*)	Direct toxicity by heavy complement activation	Tubular necrosis
		Vasculitis
		Extracapillary proliferative glomerulonephritis
Cowfish (*Lactoria diaphana*)	Rhabdomyolysis	Tubular necrosis

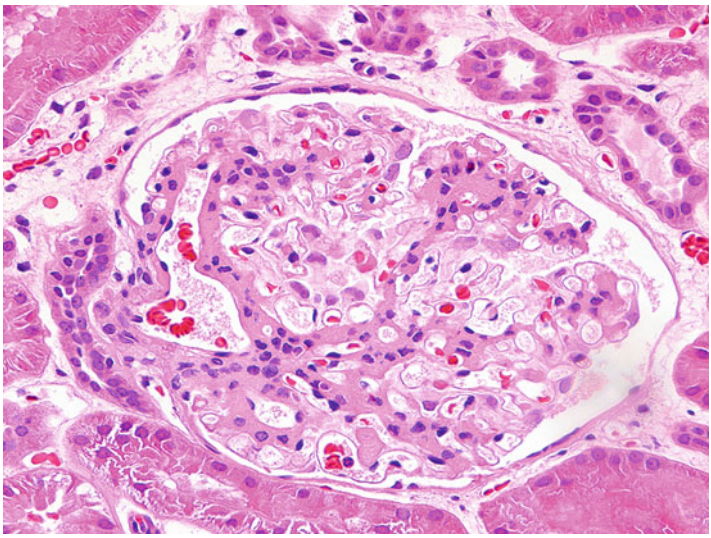

Fig. 24.1 Bee sting: glomerulus showing mesangial hypercellularity and mononuclear leukocyte in some capillary lumens (H&E × 400)

The common glomerulopathy associated with snake and arthropod envenomation is mesangial proliferative glomerulonephritis with glomerular hypercellularity that is mostly confined to the mesangium without definite change of glomerular basement membrane (Sitprija and Sitprija 2012). Mesangial hypercellularity varies from mild to severe. Some glomerular capillary lumens contain mononuclear leukocytes (Fig. 24.1). Immunofluorescence shows either negative or low intensity of IgM and C3 deposits in some mesangial areas. Diffuse proliferative change of endocapillary cells with or without mild neutrophic infiltration is less common, but has been observed occasionally in cases with bee or wasp stings and viper bite (Fig. 24.2). Interestingly, evidence of in situ immune complex glomerulonephritis has been described in *Cryptelytrops albolabris* envenoming. Although membranous glomerulonephritis in bee stings has been described, the evidence for envenoming as the cause was lacking.

Extracapillary proliferative glomerulonephritis is uncommon and has been observed in Russell's viper bite (*Daboia siamensis*) (Sitprija and Boonpucknavig 1983) and in sea anemone (*Phyllodiscus semoni*) contact recently reported (Mizuno et al. 2012). Cellular circumferential crescence is seen in few glomeruli having mild mesangial proliferation (Fig. 24.3). Deposition of fibrin in crescentic areas is demonstrable by immunofluorescence technique. Mesangiolysis, the dissolution of the mesangial cells and matrix, can be observed in viperid and crotalid envenoming. The glomerular capillary walls become unanchored from the underlying dissolving mesangial matrix leading to dilatation with aneurismal change of

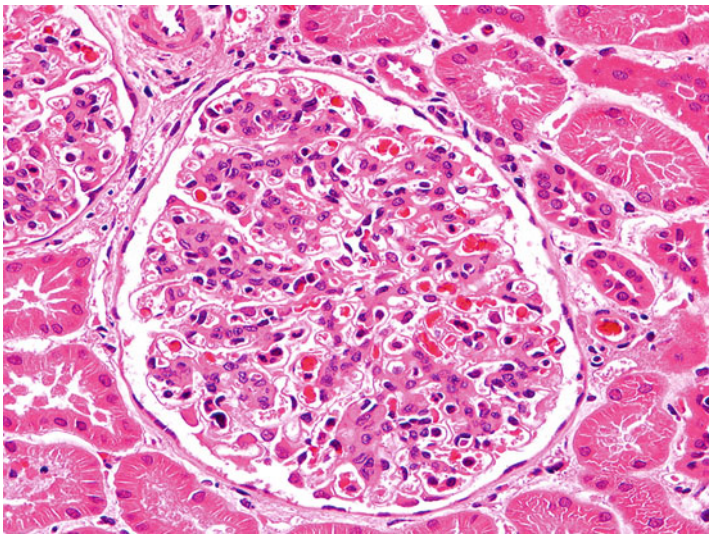

Fig. 24.2 *Cryptelytrops albolabris* envenoming: glomerulus showing endocapillary cell proliferation (H&E × 400)

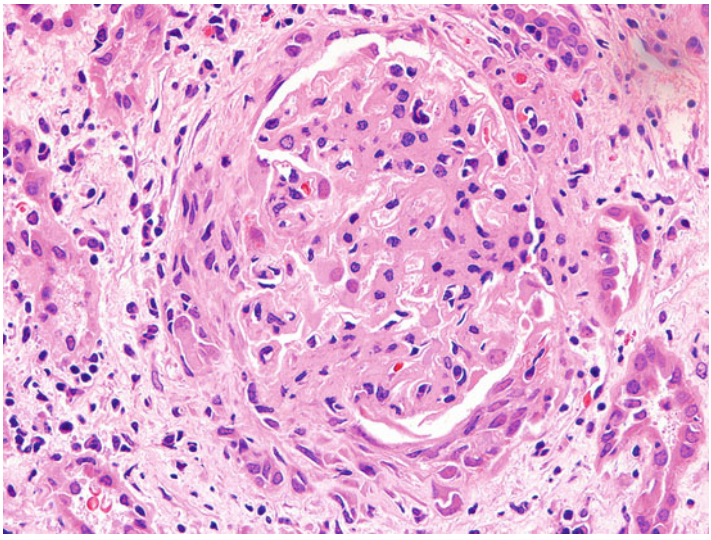

Fig. 24.3 *Daboia siamensis* envenoming: glomerulus showing circumferential crescence with mild mesangial proliferation (H&E × 400)

capillaries. The change may be focal or extensive (Fig. 24.4). Minimal change lesion has been occasionally observed in the patient with nephrotic syndrome in wasp and bee stings (Zaman et al. 2001) and following exposure to fire coral (Ramesh Prasad et al. 2006).

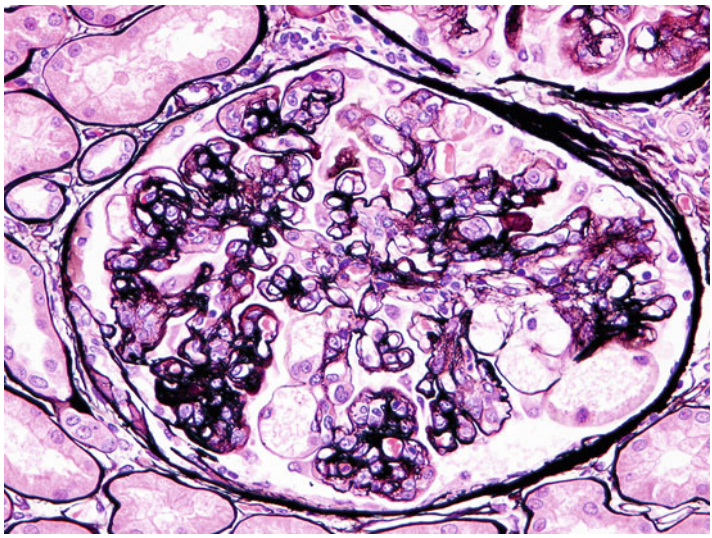

Fig. 24.4 *Daboia siamensis* envenoming: glomerulus showing mesangiolysis with mesangial proliferative change (PASM × 400)

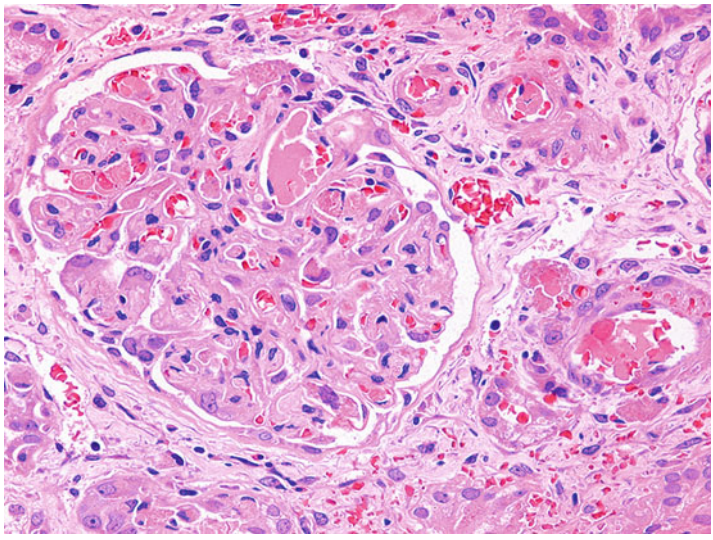

Fig. 24.5 Wasp sting: glomerulus showing thrombotic microangiopathy (H&E × 400)

Among vasculopathies, vasculitis with endothelial cell damage and segmental or diffuse occlusion of glomerular capillaries by fibrin material with areas of fibrinoid necrosis containing fragments of erythrocytes, characteristic of thrombotic microangiopathy, has been observed in snakebites and bee or wasp stings (Fig. 24.5). Necrotizing arteritis is a rare finding. Necrotizing inflammation of the

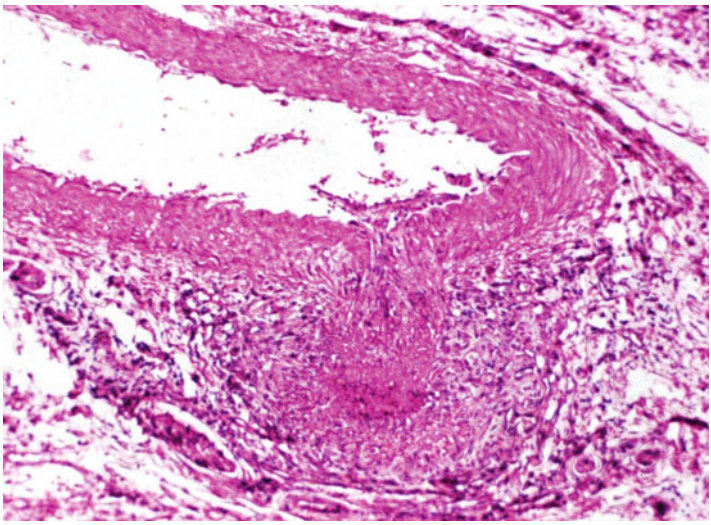

Fig. 24.6 *Daboia siamensis* envenoming showing necrotizing arteritis of medium-sized artery (H&E × 400)

medium-sized artery (interlobular artery) in the kidney has been observed in Russell's viper bite patient (Fig. 24.6). Immunofluorescence microscopy show granular deposition of C3 in necrotic arterial wall without immunoglobulin deposits.

Acute tubular necrosis is considered the most common pathologic entity for acute renal failure associated with animal toxin injury (Sitprija and Sitprija 2012). The morphologic changes of the cortical renal tubules range from minimal alteration to diffuse necrosis and desquamation of epithelial cells. Mild change includes loss of brush border of the proximal tubules with swelling and vacuolation of epithelial cells in focal areas. Extensive apoptosis and necrosis are seen together with the presence of sloughed epithelial cells and different types of casts in tubular lumen. Among those are hemoglobin and myoglobin casts in the patient with wasp, bee, or hornet stings (Fig. 24.7). Dilated tubules with patchy attenuation of epithelial cells are seen in some cases. Moderate to severe interstitial edema is also present with few scattered inflammatory cells.

Tubulointerstitial nephritis with patchy or diffuse interstitial edema and inflammatory infiltrates has been observed in severe animal toxin envenoming. The infiltrate includes varying proportion of lymphocytes, plasma cells, monocytes, macrophages, and small number of eosinophils (Fig. 24.8). Interstitial inflammation is usually accompanied by degeneration, focal necrosis, and regeneration of tubule cells. Occasionally, tubulitis and focal destruction of the tubules are seen (Fig. 24.9).

Cortical necrosis may develop in the patient with severe hemolytic uremic syndrome or following disseminated intravascular coagulation with fibrin thrombi in capillaries and arterioles (Fig. 24.10). Envenoming of viperids or crotalids and arthropods are among the known causes.

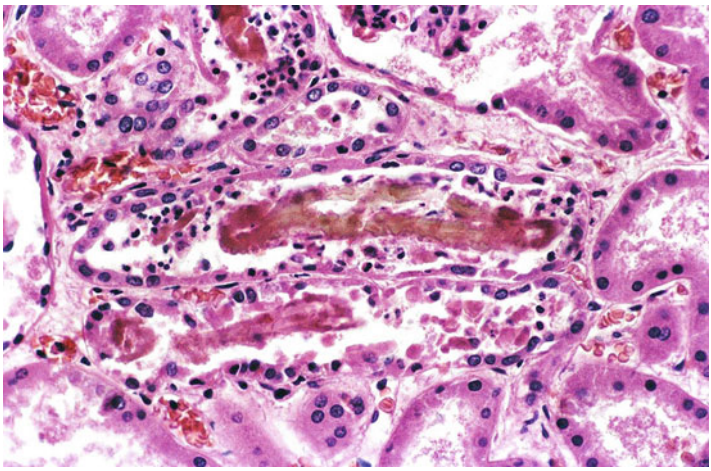

Fig. 24.7 Wasp sting, showing myoglobin cast with polymorphonuclear cells infiltration in tubular lumen (H&E × 100)

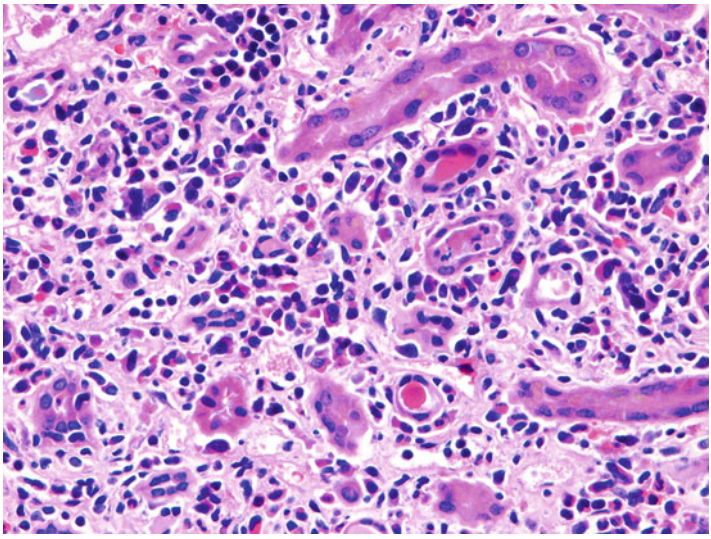

Fig. 24.8 *Daboia siamensis* envenoming: showing tubulointerstitial inflammation (H&E × 200)

Clinical Manifestations

There is a broad spectrum of renal involvement in animal toxin injury ranging in severity from mild to severe.

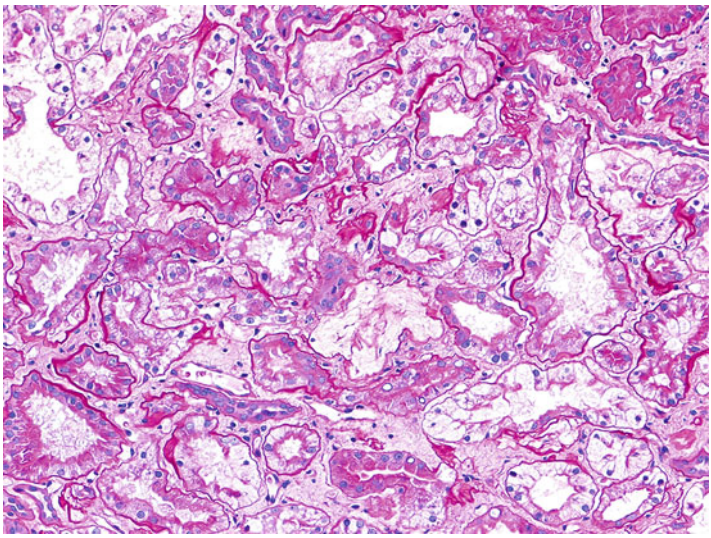

Fig. 24.9 *Daboia siamensis* envenoming: showing tubular epithelial degeneration, necrosis, and regeneration (PAS × 200)

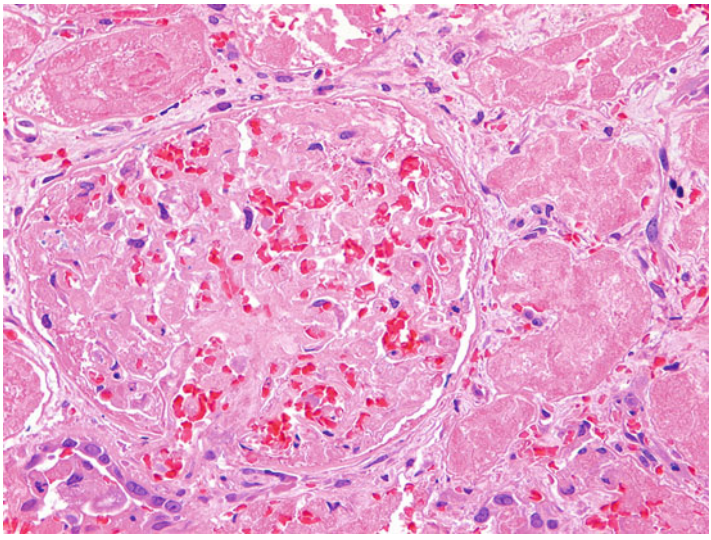

Fig. 24.10 *Daboia siamensis* envenoming: renal cortical necrosis with glomerular and tubular destruction (H&E × 400)

Urinary Findings

In the mild case, there may be a few red blood cells and white blood cells in the urine. Granular casts may be present. Red blood cell casts may be observed with

glomerular involvement and broad casts in the presence of renal failure. Myoglobin casts and heme casts are present in the patients with rhabdomyolysis or intravascular hemolysis. Microscopic hematuria is common in the patient with coagulation defect, thrombocytopenia, and vascular involvement due to the bite by viperid or crotalid. Hematuria in most cases is not serious. Gross hematuria is not common. Renal infarction or renal vein thrombosis as the cause of gross hematuria seldom occurs.

Nephrotic and Nephritic Syndrome

Mild and transient proteinuria can be observed in animal toxin injury. Severe proteinuria with nephrotic syndrome is occasionally reported in arthropod envenoming especially in bee sting (Elming and Sølling 1994). In over 50 % of cases, response to corticosteroid treatment is favorable. Corticosteroid resistant cases require addition of immunosuppressive agent. Relapse of nephrotic syndrome from intrinsic renal disease following a bee sting has been reported. Nephrotic syndrome has been described following fire ant (*Solenopsis invicta*) bite with favorable response to corticosteroid (Swanson and Leveque 1990). Minimal change disease with nephrotic syndrome has been observed following exposure to fire coral (*Millepora* species) (Ramesh Prasad et al. 2006) and wasp sting (Zaman et al. 2001). Besides erythema and blister of the skin, heavy proteinuria, pulmonary edema, and impaired renal function were noted 6 days after exposure. Nephrotic syndrome has been reported following the bite of a brown-colored snake, presumed to be *Demansia textilis*. The cause-effect relationship was uncertain. Heavy proteinuria near the nephrotic range has been observed following the bite of Russell's viper (*Daboia siamensis*). This is an acute phenomenon representing glomerular leakage which disappears with the patient recovery (Tin-Nu-Swe et al. 1993). Nephritic syndrome is less common and has been observed in patients bitten by *Daboia siamensis* and *Cryptelytrops albolabris* (Sitprija and Boonpucknavig 1983).

Acute Kidney Injury (AKI)

Snakebites, especially myotoxic and hemotoxic snakes previously described (Kanjanabuch and Sitprija 2008), are a common cause of AKI. Other reported causes include injury by bee, wasp, and hornet (Bhatta et al. 2005); scorpion (Radmanesh 1990); spider (de Souza et al. 2008; Ramialiharisoa et al. 1994); centipede (Logan and Ogden 1985); Spanish fly (Mallari et al. 1996); caterpillar (*Lonomia obliqua*, *L. achelous*) (Gamborgi et al. 2006); jellyfish (*Chironex*, *Physalia*) (Deekajorndech et al. 2004; Spielman et al. 1982); sea anemone (*Phyllodiscus semoni*) (Mizuno et al. 2012); some marine fish (Shinzato et al. 2008); and grass carp bile (*Cyprinidae*) (Xuan et al. 2003). AKI due to animal toxins is often oliguric and hypercatabolic and can be associated with metabolic

acidosis and hyperkalemia especially in myotoxic snakebite and hemotoxic snakebite in which rhabdomyolysis or intravascular hemolysis is present. Associated symptoms which can be alarming include intravascular hemolysis, rhabdomyolysis, and disseminated intravascular coagulation. This is often the case in the bite by viperid or crotalid. Hemolytic uremic syndrome has been described (Herath et al. 2012). Hepatocellular jaundice can be observed in bee, wasp, and hornet sting, sometimes associated with pancreatitis.

Figure 24.11 shows the mechanism of renal injury induced by animal toxins. AKI in animal toxin injury is due to multiple causes with renal ischemia secondary to hemodynamic changes as an important cause. In most cases of AKI, the pathological counterpart is tubular necrosis.

Snakebite

Among various animal toxins, AKI is most common in snakebites. Bites by hemotoxic and myotoxic snakes including families Elapidae, Viperidae, and Colubridae are important causes (Sitprija and Sitprija 2012). Commonly reported are AKI following the bites of Russell's viper, rattle snake, saw scale viper, *Bothrops jararaca*, puff adder, brown snake, and sea snake. Oliguria usually occurs 24–72 h after the bite. Bites by viperids or crotalids can be associated with intravascular hemolysis or disseminated intravascular coagulation with coagulopathy. Hemolytic uremic syndrome can occur. Muscular pain and myoglobinuria are observed in myotoxic snakebite. Alkalinization of urine in the patient with myoglobinuria or hemoglobinuria prevents the development of AKI (Sakwiwatkul et al. 2002). Early dialysis is life saving. In addition, muscular weakness due to postsynaptic block observed in sea snakebite is ameliorated by hemodialysis (Sitprija et al. 1971). In snakebite early administration of antivenom in AKI is associated with lower mortality. The mortality of AKI in snakebite varies from 1 % to 20 %.

Bee, Wasp, and Hornet Sting

The venom of these arthropods has histamine, 5-hydroxytryptamine, PLA_2, hyaluronidase, α-D-glucosidase, kinins, and peptides. Toxic peptides include apamin and melittin. Apamin inhibits calcium-activated K channels. Melittin enhances the injurious effect of PLA_2 and inhibits the uptake of sodium, phosphate, and α methyl-D-glucopyranoside but increases calcium uptake in proximal renal tubules (Han et al. 2002). AKI developing within 24 h is associated with rhabdomyolysis or intravascular hemolysis. Stings are usually multiple. Thrombocytopenia may be present with or without disseminated intravascular coagulation. Hepatocellular jaundice and pancreatitis may be observed. Nonoliguric renal failure is not uncommon. Oligoanuria is often observed in the elderly. The duration of renal failure is from 1 week to several weeks. The duration is prolonged in the elderly, and residual damage may occur (Bhatta et al. 2005; Sitprija, and Sitprija 2012). Renal tubular acidosis type 1 has been reported in wasp sting (D'Cruz et al. 2008).

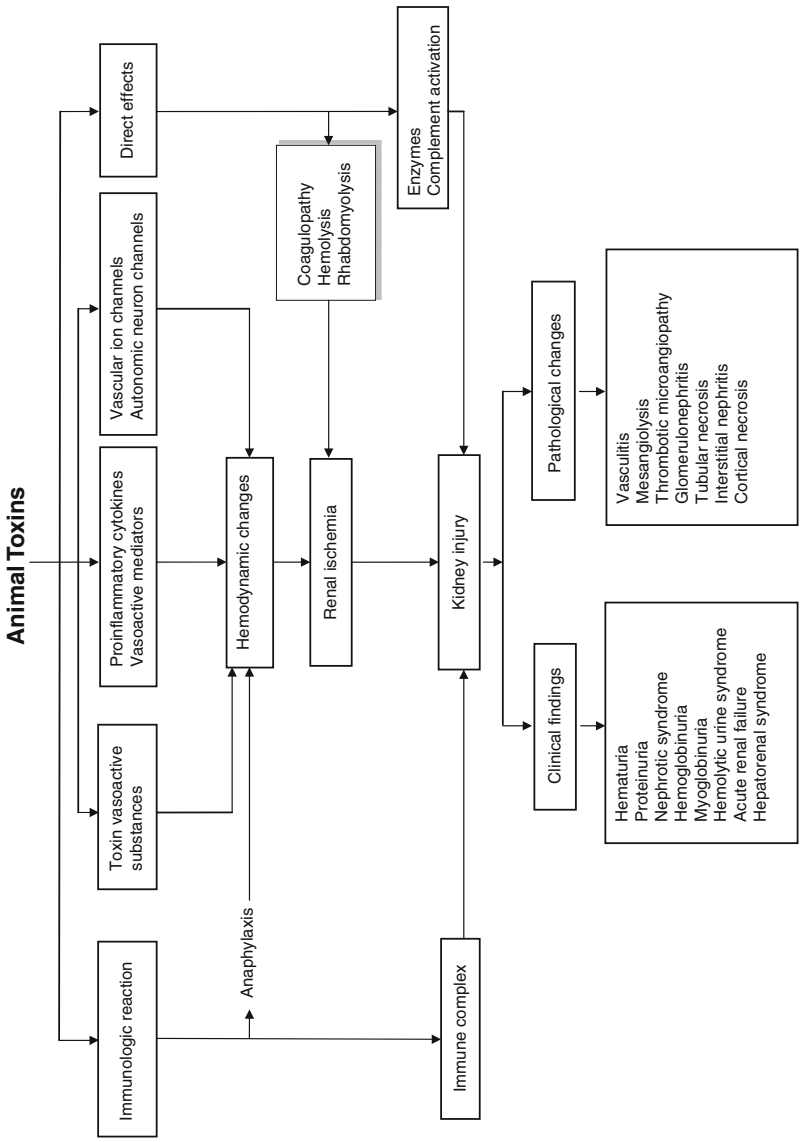

Fig. 24.11 Showing the mechanism of renal injury by animal toxins

Scorpion Sting

Acute kidney injury due to scorpion sting is a common medical problem in the Middle East, South Asia, North Africa, and Eastern Mediterranean region (Pipelzadeh et al. 2007; Visavanathan and Prabhu 2011). Hemodynamic changes are due to venom action on vascular ion channels, autonomic storm induced by Ca channel activation of autonomic neurons, and angiotensin II which result in renal vasoconstriction and renal ischemia. Interaction between renal ischemia and proinflammatory cytokines results in acute kidney injury. Renal involvement includes proteinuria, hematuria, hemoglobinuria, and renal failure (Pipelzadeh et al. 2007). Disseminated intravascular coagulation and hemolytic uremic syndrome have been reported.

Spider Bite

AKI has been reported in *Latrodectus* and brown spider (*Loxosceles*) envenoming. *Latrodectus* bite causes severe pain at the site of bite, nausea, vomiting, salivation, sweating, priapism, headache, hypertension, muscular twitching, respiratory paralysis, myocardial damage, rhabdomyolysis, and renal failure (Ramialiharisoa et al. 1994). *Loxosceles* bite causes local dermonecrosis and hemorrhage. Systemic involvements include intravascular hemolysis, disseminated intravascular coagulation, thrombocytopenia, and acute renal failure (de Souza et al. 2008). The venom has important enzymes including sphingomyelinase and metalloproteases which are important in causing cell injury.

Centipede Bite

Centipede venom contains histamine, five hydroxytryptamine, esterases, hyaluronidase, phosphatasis, proteases, and cardiotoxic proteins. Species in the genus *Scolopendra* are medically important because of their large size and large amount of venom. Proteinuria can be observed following centipede bite (Hasan and Hassan 2005). AKI with extensive rhabdomyolysis and compartment syndrome has been reported following the bite of *Scolopendra heros* (Logan and Ogden 1985). Rhabdomyolysis, AKI, and multiple focal neuropathies following drinking alcohol soaked with centipede have been reported (Wang et al. 2004). AKI induced by *Scolopendra* bite is attributed to hemodynamic charges induced by venom and myoglobinuria.

Caterpillar Contact

The venom consists of histamine, hyaluronidase, proteases, PLA_2, serine, and cysteine proteases which activate factor X, factor XIII, prothrombin, and fibrinolysis. PLA_2 triggers the release of inflammatory and vasoactive mediators which cause hemodynamic alteration. Contact with *Lonomia obliqua* or *L. achelous* caterpillars can cause AKI hemorrhage and disseminated intravascular coagulation (Gamborgi et al. 2006). In a recent report, the incidence of AKI in 2,067 patients who reported contact with *L. obliqua* was 2 %. They had contact with a large number of caterpillars and had AKI with hematuria and proteinuria

(Gamborgi et al. 2006). Full or partial recovery was noted in 90 % of the patient. Chronic kidney disease occurs with delayed treatment, old age, thrombocytopenia, and contact with several caterpillars.

Beetle Poisoning

Beetles can cause vesicular lesion when crushed on the skin. Spanish fly (*Lytta vesicatoria*) has cantharidin, a protein-phosphatase inhibitor, which is nephrotoxic. Consumption of cantharidin in aphrodisiac preparation or Spanish fly contaminated in fried crickets causes hematuria and AKI (Mallari et al. 1996). Cantharidin can cause low-grade disseminated intravascular coagulation. Tubular necrosis and mesangial proliferative glomerulonephritis with IgA deposition are observed.

Grass Carp Bile Ingestion

Ingestion raw carp bile from the carp in the order *Cypriniformes* can lead to acute renal failure. 5-alpha-cyprinol sulfate, a bile alcohol and digestive detergent of the fish, is hemolytic, cholestatic, and toxic (Goto et al. 2003). The amount ingested varies from 15 to 30 ml. Clinical manifestations start from gastrointestinal symptoms with abdominal pain, nausea, vomiting, and diarrhea followed by AKI and jaundice (Xuan et al. 2003). Oliguria noted in 54 % of patients occurs 2–48 h following ingestion. Hematuria is noted in 77 % and jaundice in 62 % of patients. Renal failure lasts from 2 to 3 weeks. Pentoxifylline has been reported to prevent toxicity (Barsoum and Sitprija 2007).

Jellyfish Stings

Stings by box jellyfish (*Chironex* and *Chiropsalmus*) and Portuguese Man of War (*Physalia physalis*) can cause AKI (Deekajorndech et al. 2004; Spielman et al. 1982). The venom from *Physalia physalis* has myotoxin and hemolysin which cause renal failure. Peptides from box jellyfish activate Na channels and voltage-dependent Ca channels and form pores on the cell membrane. Cellular influx of Na and Ca induces the release of catecholamines, 6-hydroxy tryptamine, tetramine, prostaglandins, and kinins resulting in hemodynamic alteration. Massive influx of Na and Ca can cause cell swelling and cell death. Envenoming by box jellyfish can cause nausea, diarrhea, hypertension, hypotension, convulsion, hemolysis, rhabdomyolysis, and renal failure. In a recent study in rats, injection of tentacle extract of the jellyfish *Cyanea capillata* resulted in liver and kidney injury with impairment of renal function (Wang et al. 2013).

Sea Anemone Contact

Sting by sea anemone (*Phyllodiscus semoni*) can cause severe dermatitis, pain, swelling, and ulceration and renal failure. *P. semoni* toxin (P_sTX-T) activates complement and causes mesangiolysis, glomerular endothelial, and epithelial injury with fibrin deposition and tubular necrosis. Thrombotic microangiopathy can occur. Anticomplement agent (sCR1) administration improves renal injury (Mizuno et al. 2012).

Ingestion of Marine Fish

This is indirect toxicity. Consumption of cowfish (*Lactoria diaphana*), parrotfish, mackerel, and serranid fed on palytoxin-producing Zoantharia can cause rhabdomyolysis (Okano et al. 1998; Shinzato et al. 2008; Taniyama et al. 2003). Palytoxin opens Na channel, inhibits Na K-ATPase, and forms pore on the membrane. Activation of Na channels causes membrane depolarization which opens Ca channels. Opening of Ca channels and Na-Ca exchange results in massive Ca influx causing rhabdomyolysis, hyperkalemia, and acute renal failure.

Electrolyte Changes

Animal toxins modulate ion channels of both excitable and epithelial cells. Plasma levels of electrolytes are regulated by the kidney and cellular shift through ion channels. Since most toxins affect ion channels of excitable cells, changes are serum electrolytes therefore have received little attention. Few animal toxins modulate epithelial channels in the renal tubular cells. For example, apamin from *Apis mellifera* inhibits Ca-activated K channels in the renal tubules and melittin inhibits Na and PO4 transport but increases Ca influx in the proximal tubules. Natriuretic peptides from several snakes cause natriuresis by decreasing Na reabsorption in the medullary collecting duct. Scorpion venoms inhibit K secretion in the distal nephron either voltage-gated K channels, Ca-activated K channels or inward rectifying K channels. The best known toxin is charybdotoxin from *Leiurus quinquestriatus* which inhibits several K channels (Angsanakul and Sitprija 2013). Inhibition of Na K-ATPase by toad venom can result in hyperkalemia. Serine protease enhances Na reabsorption in the distal nephron through ENaC activation. Equinatoxin and palytoxin open Na channels, inhibit Na K-ATPase, form pores on the membrane, and cause cytolysis.

Clinically, there have been a few reports on fluid and electrolyte changes in animal toxin envenoming. Some electrolyte changes are indirect effect. Pituitary or adrenal insufficiency due to hemorrhage that resulted from viper envenomation can cause hyponatremia and hyperkalemia. Polyuria with hypernatremia has been observed in the patient with diabetes insipidus secondary to hemorrhage in hypothalamus due to viper bite. Iatrogenically, dilutional hyponatremia has been observed in the patient envenomated by toxin who has fluid overload.

There are few clinical reports representing the true effect of animal toxins on serum electrolyte changes. Hyperkalemia, acidosis, and bradycardia have been observed following ingestion of aphrodisiac pills containing toad venom which has cardiac glycosides that inhibit Na K-ATPase (Chi et al. 1998; Gowda et al. 2003). Hyperkalemia resulted from rhabdomyolysis has been described in a patient consuming cowfish fed on palytoxin in food chain (Shinzato et al. 2008). Recently, hyponatremia has been reported in 40 % of those bitten by *Bungarus*

candidus presumed to be due to natriuretic peptides (Trinh et al. 2010). Hyperkalemia has been observed in scorpion envenoming (Osnaya-Romero et al. 2008). At clinical levels, several uncontrolled factors are in operation which could modify K transport in the renal tubular epithelial cells and in the other cell types. Therefore, hyperkalemia may not be consistent in complicated cases.

Conclusion

Animal toxins cause renal injury by enzymes and peptides. Through enzymatic activity, toxins can induce direct cellular injury and inflammation. The release of proinflammatory cytokines and vasoactive mediators results in hemodynamic changes which culminate in renal vasoconstriction and renal ischemia. Direct cellular injury causes not only nephrotoxicity but also intravascular hemolysis, disseminated intravascular coagulation, and rhabdomyolysis which further compromise renal blood flow. Hemodynamic changes can also be induced by vasoactive substances present in animal toxins. Toxin peptides modulating ion channels in vascular smooth muscle cells and autonomic neurons can alter host hemodynamics through either vascular constriction or vasodilatation. Effects of toxins on ion transport in the epithelial cells, especially K, Na, and PO_4 transport in renal tubules, can result in serum electrolyte changes. Enzymes as big molecules are present in snake and arthropod venoms. They are antigenic and produce antibody response that causes immune complex glomerulonephritis in snakebite and arthropod stings occasionally observed. Clinically, acute renal failure is common especially in myotoxic and hemotoxic snakebites. Myoglobinuria and hemoglobinuria may be observed. Nephrotic and nephritic syndromes are less frequent. Pathologically, all renal structures are affected by animal toxins. Tubular necrosis is the common pathological counterpart of acute renal failure.

Animal toxinology remains a fascinating field that opens for research in transport physiology. Much has been done in excitable cells. Transport in non-excitable cells especially in the renal tubules and vascular smooth muscle cells is a new dimension that needs exploration.

Cross-References

- ▶ Centipede Envenomations: Clinical Effects and Treatment
- ▶ Hemotoxic Activity of Jellyfish Venom
- ▶ Scorpion Sting and Envenomation
- ▶ Viperidae Envenomation in India

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Puffer Fish Poisoning: Bangladesh Perspective

25

Fazle Rabbi Chowdhury and H.A.M. Nazmul Ahasan

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Abstract

As a coastal country, puffer fish (tetrodotoxin) poisoning is quite common in Bangladesh, which sporadically involved many of the rive rain districts and so far brought considerable number of death. Puffer fish is also known as fugu (in Japan), toadfish, globefish, blowfish, balloonfish, etc. around the world. There are nearly 100 different species and 38 of them are found in Japan. Tetrodotoxin (TTX), a potent neurotoxin, was first isolated and named in 1909 in Japan. The fish belongs to the order Tetraodontidae which also includes ocean sunfish and porcupine fish. TTX is also found in the venom on the blue-ringed octopus available around Australia. So far there were eight outbreaks of TTX

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poisoning that happened in Bangladesh from 1988 to 2008 involving 247 people as victims with a death toll of 46 (18.6 %). Twenty (20) species of puffer fish are available in Bangladesh, of which two are freshwater puffer (*Tetraodon patoca* and *Tetraodon cutcutia*) and the rest are marine puffer (mainly *Takifugu oblongus*). *Tetraodon patoca* is commonly found in the southern part and *Tetraodon cutcutia* in the northwest, northeast, and northern part of the country. Out of the eight outbreaks, two were caused by freshwater species, five by marine species, and one by unidentified species. Most common clinical features of TTX poisoning include perioral numbness; tongue, face, and extremity paresthesia; salivation; nausea; vomiting; diarrhea; abdominal pain; vertigo; dizziness; etc. Unfortunately there is no specific antidote for TTX poisoning, and respiratory muscle paralysis is the main cause of death. Building awareness is the main way of preventing this type of poisoning.

Introduction

The pattern of poisoning has been changing in recent years from country to country. The nature of poisoning also varies between countries (Chowdhury et al. 2007b). Acute poisoning is a serious threat to society and one of the most common cause of mortality and morbidity in many communities (Chowdhury et al. 2011). Bangladesh has been known in the global arena as one of the most innocent victims of global warming and indeed facing new challenges in her health sector. Poisoning is one of them. The issue has been seriously addressed by the government of Bangladesh and the toxicology society of Bangladesh (TSB) in the countries which held the first national conference on poisoning and snake bite in March 2011 where the impacts of global warming on increasing trend of different variety of poisoning was pointed out. Other than organophosphate compound (OPC) poisoning and commuter-related poisoning, some forms of unusual poisoning (plant toxin, marine toxin, etc.) also happened in Bangladesh (Chowdhury and Mamun 2004; Amin et al. 2009). Puffer fish or tetrodotoxin (TTX) poisoning is one of them. As a coastal country, TTX poisoning is quite common, sporadically involving many of the rive rain districts (Chowdhury 2007; Islam 2011). TTX poisoning is also a point of concern because historically the country depends on her marine resources as major source of food.

Unfortunately this toxin does not have any antidote and respiratory paralysis is the mode of death (Islam et al. 2011). Considerable amount of work has been done on the chemical structure and pharmacokinetics of TTX in different laboratories; however research on clinical ground is still lagging behind. This might happen because the poisoning exclusively occurred in developing and least developing countries where research facilities were very meager. Most of the valued research on TTX poisoning was done in developed countries like Japan, Taiwan, and the United States.

In this chapter the Bangladesh perspective of TTX poisoning will be discussed, particularly focusing on outbreaks (published data) and offending species. A brief idea on pharmacokinetics and pharmacodynamics of TTX, clinical manifestations, and management of TTX poisoning will also be highlighted.

Poisoning in Bangladesh

Poisoning and snake bite are the commonly encountered emergency situations in Bangladesh. The mortality per annum reported from static primary health-care setup and district hospitals following poisoning is ~0.56 % of the total mortality (Faiz 2007). Reports published from the health directorate in 2001 recorded poisoning as the second most common cause of death. Common poisoning encountered in the community include poisoning with pesticide, copper sulfate, and kerosene; by unknown sedative substances for stupefying purpose; and with methanol, aluminum phosphide, and puffer fish with occasional reports (Chowdhury 2007; Chowdhury and Mamun 2004). In one tertiary care health facility, Chittagong Medical College Hospital (CMCH) alone, >1,000 cases of poisoning and 400 cases of snakebite were reported in 1 year (Year Book of the Department of Medicine, CMCH). One hundred ten cases of death following poisoning were reported from the same hospital in 2002 (Faiz 2007). Mortality due to pesticide poisoning has been found to be 14–15 % in Bangladesh compared to <1 % in the developed world (Faiz 2007).

The Health Bulletin of the health directorate 1998 has tabulated the data of the disease profile including poisoning cases from the year 1988 to 1996 on hospital records at the secondary health-care level and below. It has been observed that the incidence of poisoning increased gradually since 1988–1996 except in the year 1990 and 1993 (Faiz 2007). A retrospective study conducted in Dhaka Medical College and Hospital (DMCH) in 1993 revealed that 2.63 % of total admitted cases were related to acute poisonings (Faiz 2007). The findings from all the hospitals of Dhaka division showed that the most common agents of poisoning were OPC. It is found as the most common (27.3 %) type of poisoning in southern part of the country as well (Chowdhury et al. 2011). Travel-related poisoning was the second leading cause of poisoning (16.03 %) followed by copper sulfate (14.03 %), sedatives (13.35 %), snakebites (12.93 %), and others (Chowdhury et al. 2011). Snakebite is an important issue in Bangladesh. Recently Rahman et al. found that the estimated incidence density of snakebite is around 623.4/100,000 person annually (Rahman et al. 2010). Commuter poisoning or street poisoning with unusual substances is alarmingly increasing throughout the country in recent years (Chowdhury et al. 2011). Among the unusual causes of poisoning, puffer fish or TTX poisoning is the most commonly occurring marine poisoning in Bangladesh. Sporadic incidents happened along the coastal and rive rain belt of the country notably in Khulna, Cox's Bazar, Rajshahi, etc. In fact, unusual poisonings are drawing more attention in recent years throughout the country.

Puffer Fish and Tetraodontidae Fish Family

Regarded by many as a delicacy, puffer fish (*Lagocephalus scleratus*) is a lethal source of food poisoning with a high mortality. Puffer fish is also known as fugu (in Japan), toadfish, globefish, blowfish, and balloonfish (Ahasan et al. 2005). It is also widely known as the blowfish or the puffer fish because it can swell up its belly until it resembles a ball (Chowdhury and Mamun 2004). Puffer fish can be found in the Indian Ocean and in the Pacific Ocean. Some can also be found in North American waters. There are nearly 100 different species of puffer fish worldwide, 38 of them found in Japan (Haque et al. 2008). The highly toxic Red Sea porcupine fish may have prompted the biblical injunction: ".... and whatsoever hath no fins and scales ye may not eat; it is unclean unto you" (Deuteronomy 14:9–10) (Haque et al. 2008). The puffer fish is illustrated on an Egyptian tomb of the fifth dynasty dated 2500 BC (Haque et al. 2008). Captain Cook was poisoned by it in 1774, and the voodoo poisons of Haitian folklore responsible for "zombification" reportedly contain TTX (Haque et al. 2008). Puffer fish, or fugu, is a delicacy in Japan where specially trained chefs prepare it. The small amount of toxin present in correctly prepared fish produces a mild tingling around the mouth, which, together with the thought of sharing a potentially fatal dish with your friends, supposedly adds to the gastronomic experience (Haque et al. 2008). Japanese consumes about 20,000 t of blowfish per year, 6,800 t in imports (Haque et al. 2008). In Japan, 100 annual human fatalities due to ingestion of toxic puffer had been reported until 1960 (Chowdhury 2007; Haque 2008).

The first recorded cases of TTX poisoning were from the logs of Captain James Cook. He recorded his crew eating some local tropic fish (puffer fish) and then feeding the remains to the pigs kept on board. The crew experienced numbness and shortness of breath, while the pigs were all found dead the next morning (Haque et al. 2008). In hindsight, it is clear that the crew received a mild dose of TTX, while the pigs ate the puffer fish body parts that contained most of the toxin, thus killed them. The toxin was first isolated and named in 1909 by a Japanese scientist Dr. Yoshizumi Tahara (Haque et al. 2008). Puffer fish belongs to the order Tetraodontidae which also includes ocean sunfish and porcupine fish (Chew et al. 1983). TTX is also found in the venom on the blue-ringed octopus available around Australia (Haque et al. 2008).

Chemical Structure and Pharmacokinetics of TTX

TTX is a fish toxin with a long history in pharmacology and toxicology. The pharmacology of TTX had been studied for a long period of time, especially in Japan as puffer fish is regarded as the most delicious fish among Japanese. It is a potent neurotoxin of low molecular weight whose unique structure was determined by three groups in 1964 (Arakawa et al. 2010). Various TTX derivatives have so far been separated from puffer fish, newts, frogs, and other TTX-bearing organisms (Yotsu-Yamashita 2001). High-purity TTX is insoluble not only in all sorts of

organic solvents but also in water, though it becomes soluble in water when an acid is added. The toxin is stable in neutral to weakly acidic solutions and does not decompose by cooking (i.e., the application of heat) (Saoudi et al. 2010). TTX inhibits the conduction of action potential by selectively plugging sodium channels on the nerve/muscle membrane at extremely low concentrations (Arakawa et al. 2010). Low threshold currents through TTX-sensitive Na+ channels contribute to the action potential generation and control of peripheral nerve excitability (Saoudi et al. 2010). Blockade of these voltage-sensitive Na^+ channels at the nodes of Ranvier affects both action potential generation and impulse conduction and leads to conduction failure in severe cases (Saoudi et al. 2010). TTX thus has effects on both action potential generation and impulse conduction.

The lethal potency is 5,000–6,000 MU/mg [1 MU (mouse unit) is defined as the amount of toxin required to kill a 20 g male mouse within 30 min after intraperitoneal administration], and the minimum lethal dose (MLD) for humans is estimated to be approximately 10,000 MU (≈2 mg) (Arakawa et al. 2010). However, since the pioneering discovery of the selective and potent blocking action of TTX on the sodium channel, extensive investigations by a number of scientists into its cellular and molecular mechanism of action have been launched. Equally important is the fact that TTX has since then been used extensively as a chemical tool in the laboratory for the purpose of studying the sodium channel, other ion channels, and various aspects of membrane excitability and synaptic transmission (Saoudi et al. 2010). Voltage-gated sodium channels are sensitive to a variety of pharmacological agents, some of which, such as TTX, can block these channels with a high degree of selectivity (Saoudi et al. 2010). Because TTX is known to suppress action potentials in axons and to reduce ectopic peripheral nerve activity, a few studies have investigated its potential role in blocking pain while causing minimal side effects at low doses in rodent models of neuropathic pain (Saoudi 2010; Marcil et al. 2006). Furthermore, in patients, it was reported to reduce neuropathic pain associated with cancer (Hagen et al. 2008). In addition to that based on clinical response, another concept of TTX action is that it causes a competitive reversible block at the motor end plate as well as at the motor axon and muscle membrane. This blockage can be reversed by increasing the quantal release of acetylcholine at the neuromuscular junction by anticholinesterase drugs (Chew et al. 1983). This pharmacological effect was further supported by few clinical incidences (Chowdhury 2007; Ahsan et al. 2005).

Puffer Fish Poisoning in Bangladesh

Epidemiology of TTX Outbreaks in Bangladesh

TTX poisoning is probably the most common fish poisoning along the coasts of Asia for quite a long time (Chew et al. 1983). Deaths have been regularly reported from Japan, Australia, Taiwan, Singapore, Hong Kong, Thailand, and Cambodia (Chowdhury and Mamun 2004; Yang et al. 1996; How et al. 2003; Ngy et al. 2008b;

Ngy et al. 2009). In Japan the fish is popularly known as "fugu." Fugu flesh is edible and is a delicacy in Japan costing about $400 (£230; 330€) a meal (Centers for Disease Control and Prevention (CDC) 1996). There is provision of special license for the chefs in Japan who prepare and cook fugu fish. Despite all precautions every year, Japan experiences around 100 deaths (Chowdhury and Mamun 2004). Deaths were also reported from the United States (US) in 1996 due to personal importation of fish from Japan to California by a family and after that incident entry of puffer fish into the United States is strictly prohibited (CDC 1996). Spain, Brazil, and Lebanon also reported cases due to TTX poisoning with fatality (Fernandez-Ortega et al. 2010; Silva et al. 2010; Chamandi et al. 2009).

In Bangladesh the fish is popularly known as potka fish (local name, dora potka or badami potka) or tepa fish. So far eight reported outbreaks of TTX poisoning had been found in Bangladesh after an extensive search.

Between the years 1988 and 1996, Ahmed S and Mahmud Y et al. reported ten poisoning incidents due to consumption of freshwater puffer fish, involving 55 patients with 16 deaths (Table 25.1) (Ahmed 2006; Mahmud 2000). The authors collected the data from a thesis paper done in Kagoshima University, Japan, where the sites of outbreaks were not mentioned (Ahmed 2006). Mahmud Y et al. reported the second outbreak where eight people consumed the fish; five of them died (Mahmud et al. 1999c). The incident happened at Cox's Bazar a coastal district of Bangladesh where fishing is the main profession of the people. The fish were caught concomitantly with other commercially important fish in seine nets from the offshore area of the Bay of Bengal (Fig. 25.1; Mahmud et al. 1999c).

The third outbreak was a large one involving 37 victims (19 men and 18 women) and of whom eight people died. It happened in Khulna, another coastal district situated in the southern part of Bangladesh (Chowdhury 2007; Ahmed 2006; Ahasan et al. 2004). Totally eight families were affected. They bought the fish from a nearby village market and had no past experience in preparing, cooking, and eating puffer fish.

Next outbreak in a small scale occurred in Khulna in July 2005 where six people from a single family were affected (Chowdhury et al. 2007). Three were males and three were females. All of them fortunately survived. The fifth and sixth outbreaks happened only within a day interval at Kishoreganj and Narsingdi districts in April 2008, respectively (Humaira et al. 2010; Islam 2011). At Kishoreganj, three people were affected (two males and one female) whereas 45 people were affected at Narsingdi (Humaira et al. 2010; Islam 2011). Among them 25 were males and the rest were females (Humaira et al. 2010; Islam 2011). Out of them five victims died. Narsingdi and Kishoreganj are two inland districts situated near the capital and the two outbreaks were the first occurrence of TTX poisoning incident within inland districts in Bangladesh.

People of Narsingdi and Kishoreganj reported that they last saw the fish in their locality 20–30 years ago and at that time they knew it as poisonous (ICDDRB 2008). After a long hiatus, when they again saw the fish at local market, they thought that the fish was no longer poisonous. The two people who died at Kishoreganj belonged to the fish seller's family.

Table 25.1 Epidemiologic data of different TTX poisoning outbreaks occurred in Bangladesh as of December 2013

Outbreak	Year (month)	Responsible species	Identified toxin(s)	Place of outbreak	Number of victims	Death	Report published
1	1988–1996 (not specified)	*Tetraodon patoca and Tetraodon cutcutia* (fresh water)	Not identified	Not specified	55	16	Ahmed (2006), Mahmud (2000)
2	1998 (November)	*Takifugu Oblongus* (marine)	TTX	Cox's Bazar	8	5	Mahmud et al. (1999c)
3	2002 (April)	*Takifugu Oblongus* (marine)	TTX, anhydrotetrodotoxin, 11-deoxytetrodotoxin, and trideoxytetrodotoxin	Khulna	37	8	Ahasan et al. (2004), Chowdhury (2007), Ahmed (2006), Diener (2007)
4	2005 (July)	*Tetraodon patoca* (freshwater)	TTX analogue: purified compound PFT-1 and PFT-2	Khulna	6	0	Chowdhury (2007), Hasan (2007)
5	2008 (April)	*Takifugu stellatus* (marine)	Not identified	Kishoreganj	3	2	Islam (2011), Homaira (2008)
6	2008 (April)	*Takifugu stellatus* (marine)	Not identified	Narsingdi	45	5	Islam (2011), Homaira (2008)
7	2008 (June)	Not identified	TTX , 4-epiTTX, and 4,9-anhydroTTX	Dhaka	10	3	Islam (2011), Homaira (2008)
8	2008 (June)	*Takifugu Oblongus* (marine)	TTX (in the blood and urine sample of patients)	Natore	83	7	Islam (2011), Homaira (2008)

Fig. 25.1 The map showing the cluster of outbreaks (in *black circle*) in Bangladesh

On 3 June 2008 another outbreak occurred in the heart of the capital, which involved ten (10) people of a single family (six were males and four females) (Humaira et al. 2010; Islam 2011). Three of them died. The family lived in a slum area of Dhaka and got the fish by their relative who was a community waste cleaner (ICDDRB 2008). The fish were collected from a nearby dust bin. The family and the cleaner were completely unaware of the potential toxicity of the fish.

Bangladesh experienced her last biggest outbreak of TTX poisoning on 8 June 2008 where 83 people were affected (50 males and 33 females) (Humaira et al. 2010; Islam 2011). This is possibly the biggest reported outbreak of TTX poisoning ever in the world in terms of the number of cases. It happened in Natore district situated in the northern part of Bangladesh. This is an inland district too. The victims said that they ate the freshwater species of the fish before and the fish was widely available in their village rivers and beels (water body), especially after the rainy season (April to June), but they never became ill (ICDDRB 2008). They did not believe that it would be poisonous.

In Bangladesh the mating and spawning seasons of puffer fish usually range from March to July (Galib 2011). During this time the fish remains very toxic as the TTX is principally concentrated in the gonads, intestine, and liver. The abovementioned epidemiological trend suggested that TTX poisoning is a common form of poisoning for the coastal belt of the country and frequently affects the inland districts as well, which reflects the alarming phenomenon of wide epidemic involvement of that poisoning in Bangladesh.

Species of Puffer Fish and Toxins Responsible for the Outbreaks in Bangladesh

Diener M et al. identified 13 species of puffer fish in Bangladesh, of which two are freshwater puffer (*Chelonodon patoca*/*Tetraodon patoca and Tetraodon cutcutia*) and the rest are marine puffer (mainly *Takifugu oblongus*) (Diener et al. 2007). But recently Bangladesh Fisheries Information Share Home declared the presence of 20 species of puffer fish in the surrounding marine environment and two abovementioned species in the freshwater (Galib 2011). *Tetraodon patoca* (Fig. 25.2) is commonly found in the southern part of Bangladesh and *Tetraodon cutcutia* (Fig. 25.3) is common in the northwest, northeast, and northern part of the country (Galib 2011).

Puffer fish shows wide individual, regional, and seasonal variation in toxicity (Arakawa et al. 2010). In marine species the toxic parts can be categorized into three groups. Group one includes the species in which the skin, muscle, and testis are nontoxic (less than 10 MU/g) and edible, whereas the rest are toxic. *T. rubripes*, *T. xanthopterus*, *Lagocephalus wheeleri*, etc. fall within this group, the dish of which is most popular in Japan (Arakawa et al. 2010). The second group contains *T. snyderi*, *T. porphyreus*, *T. vermicularis*, etc. in which the skin is toxic, but the muscle and testis are edible (Arakawa et al. 2010). In the third group only the muscle is edible but the testis is toxic like *T. niphobles*, *T. poecilonotus*, *T. pardalis*,

Fig. 25.2 Common species in Bangladesh – *Tetraodon patoca* (© Chowdhury FR)

Fig. 25.3 Common species in Bangladesh – *Tetraodon cutcutia* (© Chowdhury FR)

etc. (Arakawa et al. 2010). So in general, the viscera, especially the liver and ovary, are considered highly toxic (the toxicity often exceeds 1,000 MU/g) (Arakawa et al. 2010). On the other hand toxicity of the skin is higher than that of the viscera in small puffer fish inhabiting freshwater available in the Southeast Asian region including Bangladesh (Mahmud et al. 1999a, b; Ngy et al. 2008a).

The biosynthesis of TTX is actually a wide area of research where many things are still unknown. The research done so far suggested that the precursor of TTX comes from the amino acid L-arginine and the remainder of the carbon skeleton most likely comes from a C5 isoprene unit, probably supplied by isopentenyl diphosphate (IPP) (Kotaki and Shimizu 1993; Dewick 2001; Saoudi 2010). TTX has been isolated from several different phyla, including fish, chaetognaths, gastropod mollusks, octopus, echinoderms, horseshoe crabs, amphibians, nemerteans, dinoflagellates, algae, and annelids (Saoudi 2010; Ngy et al. 2008a; Miyazawa and Noguchi 2001). Matsui et al. and Mosher et al. in two of their studies first described that TTX-bearing organisms were infected by TTX-producing microorganisms living symbiotically within their bodies (Saoudi 2010; Matsui et al. 1981; Mosher et al. 1964). Among the microorganisms *Vibrio species* was the first to be identified. *Vibrio I* and *Vibrio VIII*, *Vibrio alginolyticus*, *Shewanella algae*, and *Alteromonas tetraodonis* have been successfully isolated from TTX-bearing organisms such as puffer fish, toxic starfish, the xanthid crab *Atergatis floridus*, and the red alga *Jania* sp. (Arakawa et al. 2010; Saoudi 2010; Williams 2010). Endogenously TTX is also produced within the fish by some intestinal organism, but the amount of production is so small that it cannot play a

major role in the accumulation of toxin. *Vibrio species* belongs to that group of endogenous microorganism (Saoudi et al. 2010). Researchers already established that the toxicity of the gonads and liver is more potent than that of other organs, but whether the anatomical variation of toxicity is due to the different distribution of TTX-producing bacteria or not is not known (Saoudi et al. 2010). Seasonal variation (more toxic in reproductive season that is in rainy season) of toxicity is probably related to this organ variation. The toxic profile or characteristics of the toxin also vary between the fish (marine versus freshwater). In this chapter detailed search was made to identify the species of puffer fish and toxic profile responsible for the outbreaks in Bangladesh.

Mahmud Y et al. extensively reviewed various published newspaper articles, visited the affected area, took interview of the victims, identified specimen, and reported at least ten incidents of TTX poisoning between the years 1988 and 1996 (Mahmud et al. 2000). Two freshwater species *Tetraodon patoca* (Fig. 25.3) and *Tetraodon cutcutia* (Fig. 25.2) as the offending fish were identified, but details of toxicological investigation were not done. Second outbreak was also investigated and marine variety of *Takifugu Oblongus* was identified as the culprit. At that time a total of 336 samples of three marine puffer fish species were collected and examined. Except *T. Oblongus*, very low toxicity (less than 10 MU/g) was found in the other two species, *Lagocephalus wheeleri* and *L. lunaris* (Mahmud et al. 1999). High-pressure liquid chromatography (HPLC) was done on different tissues of three species and identified TTX as the offending agent.

The third outbreak happened in Khulna in the year 2002 where again marine variety of *Takifugu Oblongus* was found to be responsible (Table 25.1). Diener M and his group later collected the marine *T. oblongus* fish from Khulna. Hydrophilic interaction chromatography and mass spectrometry were done and identified TTX and its analogues anhydrotetrodotoxin, 11-deoxytetrodotoxin, and trideoxytetrodotoxin in different tissues of these fish (Diener et al. 2007). TTX was predominant in the skin, muscle, and liver, whereas trideoxytetrodotoxin was present in the ovary (Diener et al. 2007). Mouse bioassay was used to determine the various toxicities of the tissues (Diener et al. 2007).

The next outbreak happened in Khulna caused by *Tetraodon potoca*, the freshwater species (Hasan et al. 2007). Hasan S et al. collected similar specimen from the affected area later and did the toxicological analysis through ^{1}H nuclear magnetic resonance spectroscopy (H-NMR), ^{13}C-NMR, and infrared spectroscopy (IR) (Hasan et al. 2007). He reported the presence of TTX analogues purified compound puffer fish toxin (PFT)-1 and PFT-2 in different tissues of the fish. PFT-1 is quite similar in structure to TTX but PFT-2 is a derivative of TTX which contains an alcoholic side chain (Hasan et al. 2007).

Takifugu stellatus (marine species) was responsible for the Kishoreganj and Narsingdi outbreaks. Unfortunately the details of toxicological analysis were not done due to unavailability of the samples. In the Dhaka outbreak the exact species could not be identified but the sample of cooked fish was collected and liquid chromatography-fluorescence detection (LC-FLD) was done (Islam et al. 2011).

LC-FLD detected TTX and its analogues 4-epiTTX and 4,9-anhydro TTX as culprit toxins (Islam et al. 2011).

Takifugu Oblongus (marine species) was responsible for the eighth outbreak where blood and urine samples of the patients were collected for analysis. Enzyme-linked immunosorbent assay (ELISA) was done and TTX was identified in both the samples (Islam et al. 2011). Besides the outbreaks, Zaman L et al. detected saxitoxin, decarbamoylsaxitoxin, gonyautoxins 2 and 3, decarbamoylgonyautoxins 2 and 3, and three other unidentified components from *Tetraodon cutcutia* species (Zaman 1998; Hasan 2007). The authors also reported the presence of methyl derivatives of saxitoxin in the same species (Zaman et al. 1998). Electro-spray ionization mass spectrometry, ^{1}H-NMR, and conversion experiments were done to delineate the toxicological profile (Zaman et al. 1998). Moreover Taniyamma et al. in another study detected palytoxin (PTX) or PTX-like substance from the freshwater puffer of Bangladesh (Hasan 2007; Taniyamma et al. 2001). The study was based on delayed hemolytic activity, inhibited by anti-PTX antibody and ouabain (*g*-strophanthin) (Hasan 2007; Taniyamma et al. 2001).

Clinical Manifestations of TTX Poisoning

Clinical features of TTX poisoning depend upon the amount of toxin ingested. Most common clinical features includes perioral numbness or paresthesia, tongue paresthesia, facial and extremity paresthesia, salivation, nausea, vomiting, diarrhea, abdominal pain, vertigo, dizziness, etc. (Haque et al. 2008). Motor dysfunction like paralysis of extremities, speech difficulties, etc. can also occur (Haque et al. 2008). At the terminal stage cardiac dysfunction with hypotension and arrhythmias and nervous system dysfunction such as coma and seizures can develop (Haque et al. 2008). Death usually occurs due to respiratory muscle paralysis. Some authors tried to categorize the features sequentially into three forms. In mild stage only sensory features and few gastrointestinal features develop (Saoudi et al. 2010). Moderate poisoning includes vertigo, dizziness, distal muscle weakness, and weakness of bulbar and facial muscles followed by incoordination and ataxia with intact reflexes. Severe poisoning includes generalized flaccid paralysis, respiratory failure, bradycardia, hypotension, various arrhythmias, and coma (Saoudi et al. 2010).

The poisoning can also be categorized into four clinical grades or degrees based on the symptoms and signs. First degree includes oral numbness and paresthesia, sometimes accompanied by gastrointestinal symptoms like nausea; second degree includes numbness of the face and other areas, advanced paresthesia, motor paralysis of extremities, incoordination, and slurred speech, with normal reflexes. Third degree includes gross muscular incoordination, aphonia, dysphagia, dyspnea, cyanosis, drop in blood pressure, fixed/dilated pupils, and precordial pain, but the victims still remain conscious. Fourth degree includes severe respiratory failure and hypoxia, severe hypotension, bradycardia, cardiac arrhythmia, and eventually asystole.

Treatment of TTX Poisoning

There are no antidotes for TTX poisoning, and treatment is predominantly supportive and symptomatic. Good cardiovascular and respiratory support is critical and the prognosis is excellent if supportive care is instituted early. Activated charcoal can be administered after ingestion of the toxin, especially within 1 h of ingestion (Lehane 2001). Most patients will recover with supportive care alone, but they should be monitored for signs of respiratory depression and neurotoxicity and may require endotracheal intubation and mechanical ventilation (Saoudi et al. 2010). Electrolytes should be replaced, and fluids should be regulated according to arterial blood pressure and urinary output (Saoudi et al. 2010). Adrenergic antagonists may prolong the neuromuscular blockade of TTX and are not recommended (Kohane et al. 2001). Atropine can be given for a systolic cardiac arrest.

Treatment with cholinesterase inhibitors has been attempted for TTX-induced muscle weakness with successful outcome in a limited number of poisoned patients (Chew et al. 1983; Ahasan et al. 2004; Chowdhury 2007). One study showed an improvement of muscle weakness after TTX ingestion using IV edrophonium (10 mg) or intramuscular neostigmine (0.5 mg) (Chew et al. 1983). The effectiveness of cholinesterase inhibitors however remains unsubstantiated, and large-scale prospective studies are required to prove its usefulness in the treatment of TTX poisoning.

Hemodialysis was attempted in one instance because TTX is made up of low-molecular-weight, water-soluble molecules that are significantly bound to protein; but there were little data concerning the effectiveness of this treatment (Lan et al. 1999). Interestingly, numerous traditional herbal medicines are used to treat many cases of TTX poisoning. *Artemisia campestris*, a medicinal plant often included in a variety of traditional medicine applications (as antibacterial and antifungal; for radical scavenging, gastric disturbances, diarrhea, abdominal cramps, hypertension, rheumatism, envenomation, etc.), was also tried in rats with a good result (Saoudi et al. 2010).

Conclusion and Future Direction

TTX poisoning due to puffer fish or gastropod or shellfish toxin has already become a significant issue in the southeast and eastern countries of Asia particularly in Bangladesh (Arakawa et al. 2010). So, regional networking or collaboration among these countries is very important. Comprehensive monitoring of the diversity of TTX-bearing organism can give clues for future prediction of poisoning incidents. More national and regional collaborative research is needed for better understanding of TTX biosynthesis and to explore new treatment options for the better management of poisoned cases. Bangladesh has its own "national poisoning management guideline" where a separate module was put on TTX poisoning. Government has to play a pivotal role by arranging training programs for the physicians and regularly

updating the guideline. Enactment and enforcement of law and provision of cooking guideline came out as a very effective approach of preventing TTX poisoning in Japan. In Bangladesh there is "Fish and Fish products (Inspection and Quality control) Ordinance1983 and Fish and Fish products (Inspection and Quality control) rules 1997" (Hoque and Chowdhury 2008; Fish and fish products rule 1997). The rules of 1997 have specific provisions for the catching, processing, transporting, importing, and selling of "unhygienic fish" with provision of punishment for the offenders (Hoque and Chowdhury 2008; Fish and fish products rule 1997). The definition of "unhygienic fish" covers puffer fish as well. Still, the amount of punishment mentioned in the rules is inadequate (maximum fine not exceeding 285 USD) (Fish and fish products rule 1997). Bangladesh government needs to amend the rules immediately for better control of this poisoning because it is almost impossible for everyone to comprehend the sophisticated processing of the fish before consumption. So the best way is to target the fish sellers and importers and bring them under the legal frameworks. Law enforcement mechanism also needs to be strengthened by increasing the number of health and food inspectors and giving them proper training.

Capacity development is an important issue in this regard. Knowledge about the fish and detailed training of the health personnel regarding the presentation and management of TTX poisoning is vital. Bangladesh still does not have any laboratory facility to any of her tertiary institutes for toxicological analysis. Government should come forward for immediate establishment of a well-equipped laboratory for specific identification of the toxin. Mass awareness campaign through print and electronic media (TV, newspaper, poster, leaflets, miking in local village market, special play show, etc.) is the most effective and vital means of preventing TTX poisoning. After the 2008 outbreaks, the Ministry of Health and Family Welfare and the Ministry of Fisheries and Live Stock in Bangladesh launched extensive awareness program for the general public especially in the coastal districts. These forms of extensive dissemination program (particularly during rainy season) should be continued for future prevention. It is encouraging that after 2008 Bangladesh did not face any outbreaks of TTX poisoning.

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Hemotoxic Activity of Jellyfish Venom

26

Dibakar Chakrabarty and Akriti Rastogi

Contents

Abstract

Jellyfish are marine invertebrates of the phylum Cnidaria. All jellyfish species are venomous. Human victims of jellyfish sting each year are 120 million. However, most victims do not require hospitalization. Severe cases of envenomation may sometimes be fatal. Among the symptoms of envenomation, hemotoxicity constitutes a small fraction. Hemolysis has been reported in severe envenomation cases. On the other hand, coagulopathy in jellyfish envenomation is almost absent in scientific literature. Some hemolytic pore-forming toxins have been isolated from venom and tentacle extracts of a few jellyfish species. These toxins show some degree of variation in size and structure. However, many of them cause hemolysis by disturbing the transmembrane ion concentrations. It is also claimed that lipid peroxidation

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in the membrane is another mechanism of hemolysis. There is no report on anticoagulant or procoagulant toxins isolated from jellyfish venom, although strong fibrinogenolytic and platelet-inhibiting activities have been shown in the tentacle extracts of moon jellyfish. Isolation and characterization of hemolytic and anticoagulant toxins from marine venoms is expected to provide novel molecules of therapeutic interest.

Introduction

It is one of the most surprising facts in science that in spite of their abundance and variety, marine venomous organisms attracted so less interest compared to the terrestrial animals from toxinologists, till recently. Although investigations on marine envenomation have a long history, particularly in Australia and the USA, research on the use of marine venoms and toxins for therapeutic use was rare. The good news is extremely specific activities of certain marine toxins on different physiological systems have finally succeeded in attracting scientific attention. Several marine compounds are now recognized to be active against pathological conditions involving the cardiovascular, endocrine, immune, and nervous systems (Table 26.1). Marine toxins with anti-inflammatory, antiplatelet, antitumor, or cytotoxic activities are now being reported at regular intervals. A few of these toxins are active against infectious diseases also. A good number of these compounds are currently in preclinical phase trials and/or under phase I and II clinical studies. One of the least explored of marine group of animals falls under the phylum Cnidaria.

Table 26.1 Therapeutic agents from marine animals

Source animal	Active compound	Therapeutic use
Cone snail, *Conus magus*	ω-conotoxin	Analgesic – binds to voltage dependent ion channels
Ascidian, *Ecteinascidia turbinata*	ET-743	Soft tissue sarcoma, ovarian cancer
Sea fan, *Pseudopterogorgia elisabethae*	Pseudopterosin A	Anti-inflammatory
Caribbean sponge, *Cryptotethya crypta*	Cytarabine (Ara C)	Acute myeloid leukemia (AML) and non-Hodgkin lymphoma
Bryozoan, *Bugula neritina*	Bryostatin 1	Anticancer, Alzheimer's
Ascidian, *Aplidium albicans*	Dihydrodidemnin B	Antitumor, antiviral, and immunosuppressive
Dogfish shark, *Squalus acanthias*	Squalamine	Cancer, macular degeneration, diabetic retinopathy, and fibrodysplasia ossificans progressiva

Cnidaria (formerly, Coelenterata) is a diverse phylum of basal metazoans, comprising over 10,000 species, predominately marine organisms (Daly et al. 2007; Zhang 2011). Cnidaria is also the largest phylum of generally toxic animals. The phylum has two major lineages: Anthozoa (sea anemones and corals), which live as sessile polyps, and the Medusozoa (jellyfish and hydra), comprising the classes Cubozoa, Hydrozoa, Scyphozoa, and Staurozoa. **Polyps** have a tubelike body with an opening on top that is surrounded by outward and upward facing tentacles. **Medusae** are usually bell-shaped animals with a concave oral surface, or mouth, and tentacles that dangle downward from the rim of an umbrella-like body. Cnidarian polyps and medusae have a single body opening that acts as both mouth and anus and is generally surrounded by tentacles lined with the unique venom apparatus, the *nematocytes*. The nematocytes or stinging cells (sometimes called **cnidocytes**) are a special type of cnidae and constitute the defining synapomorphic trait of the phylum Cnidaria (Marques and Collins 2004).

Species of these four classes have a free-swimming or attached medusa stage and many retain the ancestral stage of sessile polyps during their life cycles. Cnidarians have external radial symmetry, although many species are either asymmetric or bilateral in their internal anatomy (Marques and Collins 2004).

Cnidarians are animals living on this planet since Precambrian or Cambrian era. These animals are believed to have evolved the first toxin-injecting mechanism. While they do not have the macro-morphological apparatus such as the fangs of snakes to deliver its venom, cnidarians have unique secretory organelles (*nematocysts*) within their stinging cells. There have been numerous studies characterizing the venoms and toxins of many poisonous animals such as cone snails, scorpions, snakes, and spiders, but by comparison very few cnidarian venoms and toxins have been examined in detail (Turk and Kem 2009).

The term "jellyfish" is commonly used to describe members of Cubozoa, Hydrozoa, and Scyphozoa. The box jellyfish, a scyphozoan, is considered as one of the most venomous animals in the world. All jellyfish have a very soft body with jelly-like appearance, and the body is divided in two main parts: a bell and tentacles (Fig. 26.1). All jellyfish are carnivorous. They eat zooplanktons, small fish, and marine invertebrates. Some species of jellyfish are extremely venomous and may cause fatalities in sea bathers. Being carnivores, they had to develop a system of prey – the nematocyst or cnidocyst developed from Golgi bodies.

The nematocysts are encased in cells called nematocytes or cnidocytes. Each cell contains one cnidocyst. A cnidocyst is a capsule attached to a coiled threadlike structure at the bottom. The cnidocyte on its external surface has a hairlike structure, which acts as a trigger. On activation of the trigger, the cnidocyst can be shot out and can penetrate the prey or predator organism like a harpoon (Fig. 26.2). Cnidocyst shooting is an extremely fast mechanism. It is interesting to note that TRPA1 (transient receptor potential ion channels), a pain and mechanoreceptor in vertebrates, also occurs in the hair bundles of sensory neurons associated with nematocytes in sea anemones as stress sensor (Holstein 2012). In case of hydra *stenoteles*, the barbed part of the tubule is discharged with acceleration greater than 5,000,000 g in less than 700 ns and a pressure of up to 7 GPa. This

Fig. 26.1 Moon jellyfish under artificial lighting in a marine aquarium (Photo by authors)

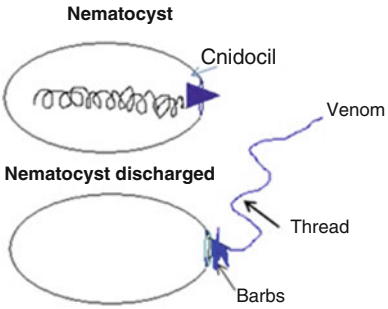

Fig. 26.2 Schematic diagram of nematocyst of a jellyfish before and after discharge (Sketch by authors)

creates sufficient force to penetrate even the hard cuticles of crustaceans (Holstein 2012). On penetration of the body of the prey, the cnidocyst remains attached with the skin by the help of barbs on the cnidocyst.

Nematocyst discharge can be caused by several mechanical and chemical stimuli. Recently, the stimuli which give rise to the discharge and the cell structure of cnidarian capsules have been reviewed, showing that nematocyst can trigger either independently or under the influence of adjacent cells (Turk and Kem 2009). The discharge could be caused by mechanical stimuli, with and without chemo-sensitization, and by minor vibration. Each nematocyst can be used only once. The nematocyst attached to the skin can discharge its venom content on contact with alcohol or fresh water. Even pressure immobilizing bandages are capable of nematocyst activation (Cegolon et al. 2013). Cnidarian nematocysts are mainly targeted at their natural prey or predator. One interesting symbiotic relationship between clown fish or anemone fish and some sea anemone species casts light on the presence of natural inhibitors of nematocyst discharge (Mebs 2009). Research on several species of clown fish has suggested secretion of specific inhibitory compounds in the fish mucus, as well as coating of the fish body by anemone mucus. Many crustaceans also can escape nematocyst discharge by secreting

Fig. 26.3 Sea anemone with two clown fish (Photo by authors)

inhibitory substances (Fig. 26.3). However, these specific inhibitors have not been adequately characterized (Roopin and Chadwick 2009).

Nematocysts can discharge venom even after death of the jellyfish. Sun-dried jellyfish lying on the beach can also be dangerous. Each year about 150 million jellyfish sting cases are reported around the world with some Pacific areas reporting a very high number. However, most victims do not require to be hospitalized. It should be kept in mind that jellyfish venom is not meant for use on humans. The venom probably cannot pass through the thickness of human skin. The severity of envenomation depends on the number of discharged nematocytes. The envenomation symptoms can vary from simple itching, severe pain, inflammation, severe hemolysis, paralysis, and death.

Composition of cnidarian venom depends on the stinging species, but in general the venoms are composed of proteins, peptides, and many other substances of pharmacological importance. The venom components act as antigens and evoke a defense response with consequent production of specific antibodies and activation of the "memory" phenomenon (Mariscal 1974). Bioactive substances discovered in cnidarians include prostaglandins (15R)-PGA2 (15(R)-15-methyl prostaglandin A2) in the gorgonian *Plexaura homomalla*; palytoxin, the local anesthetic and vasoconstrictive agents from the zoanthid *Palythoa toxica*; pseudopterosin, sarcodictyns, and eleutherobin; cytolytic and antitumoral substances from the anthozoan *Clavularia viridis*; equinatoxin extracted from *Actinia equina*; and many others (Weinheimer and Spraggins 1969; Moore and Scheuer 1971; Kohl and Kerr 2003; Honda et al. 1985; Orduña-Novoa et al. 2003; Tabrah et al. 1972; Giraldi et al. 1976).

A recent investigation of cnidarian toxins revealed 29 unique sequences homologous to toxins from a variety of animal phyla, including cone snails and terrestrial venomous animals. It is interesting to note the presence of some very rare toxins like sphingomyelin phosphodiesterase B (SMase B) and prepro-haystatin (Weston et al. 2013). Using, high-throughput proteomics technique, the toxins present in the venom of the jellyfish *Olindias sambaquiensis* collected from the Brazilian coast

were studied. It is noteworthy that the venom contains representative toxins of all major toxin superfamilies, including snake venom metalloproteases (SVMPs) and ion channel blockers. Among the ion channel blockers, toxins from Mediterranean black widow spider, Chinese earth tiger, tarantula, and snakes were identified. The toxins include postsynaptic neurotoxins from elapid snakes and selective antagonists of nicotinic acetyl choline receptors. One of the important findings is the presence of SMase B known to be responsible for tissue necrosis associated with certain spider envenomations. The authors expressed surprise by the presence of SMase B as necrosis is not reported in envenomation cases attributed to *Olindias sambaquiensis*. However, an interview with fishermen exposed to jellyfish in Goa, India, revealed incidences of severe dermonecrosis.

Among all the jellyfish species, the Portuguese man-of-war and the box jellyfish have attracted most scientific attention, as envenomation by these two may cause serious injuries and fatalities around the world. Apart from neurological and gastrointestinal disturbances, hemolysis leading to renal complications is noted in severe envenomation by these two species.

Hemotoxicity in Jellyfish Envenomation

Hemotoxicity mainly involves hemolysis (lysis of red blood cells) and coagulopathy in victims of envenomation. Envenomation by jellyfish of some species is known to cause hemolysis in severe sting cases. Some hemolytic toxins have also been isolated from jellyfish venom or tentacle extracts. Coagulopathy in case of jellyfish envenomation is also rarely noted and least studied. Literature on hemotoxic components of jellyfish venoms is scanty.

Hemolysis

Hemolytic action is brought about by digging pores on the RBC membrane. These hemolytic toxins are therefore called pore-forming toxins. The pore-forming action is not restricted to RBC membrane only. These toxins can form pores on a variety of cell membranes.

The hemolytic, pore-forming toxin equinatoxin III (a cardiotoxic protein from the sea anemone *Actinia equina*) has demonstrated the ability to pass through a large tissue mass in an animal model of envenoming (Suput et al. 2001). Pore-forming toxins in the venom of the box jellyfish *C. fleckeri* have been demonstrated to form pores of 50–80 nm on cell membranes of myocytes and are very similar to those observed for *Physalia physalis* venom-treated cells (Edwards et al. 2002). The modes of action differ among hemolytic toxins of different species of jellyfish. Variation in modes of action is also noted among different toxins from the same venom.

Edward and Hessinger reported in 2000 that box jellyfish (*Physalia physalis*) venom induces rise in Ca^{2+} concentration in different types of cultured cells in vitro

prior to pore formation. They showed that the nematocyst venom does not act on Na^+/K^+ ATPase or any other ATPase. The venom directly acts on the plasma membrane to increase permeability for Na^+ and Ca^{2+}. Based on the data on inhibition of pore formation and cytolysis, the authors speculated that cations, namely, Zn^{2+} and La^{3+}, blocked anionic sites on the cell membrane required for oligomerization of hemolytic proteins. Some workers believe that the metal-induced inhibition of pore-forming toxins is due to reduced membrane fluidity caused by cations. Similar inhibition is also noted with exposure to low temperatures. Low temperature has been observed to reduce membrane fluidity as well as hemolysis of rat RBCs. Based on these observations, it may be speculated that at least in some cases, nematocyst venom-induced hemolysis may be caused by reduced membrane fluidity.

The hemolytic activity of *Cyanea nozakii* Kishinouye nematocyst venom also showed a dose-dependent increase in activity with rising concentration of Ca^{2+}. However, several other divalent cations inhibited the hemolytic activity with varying degrees. High concentration of EDTA caused total loss of hemolytic activity (Feng et al. 2010).

Reports of hemolytic activity in some jellyfish venoms initiated attempts to purify the responsible toxins. Brinkman and Burnell (2007) reported purification of two of the most abundant hemolytic toxins from the nematocysts of box jellyfish, *Chironex fleckeri*. They were named *C. fleckeri* toxin-1 (CfTX-1) and *C. fleckeri* toxin-2 (CfTX-2). Both toxins have similar molecular weights of around 43 kDa. The amino acid sequences of the mature CfTX-1 and CfTX-2 share homology with the CrTXs, CaTX-A, and CqTX-A isolated from the deadly Okinawan sea wasp *Chiropsalmus quadrigatus* (also known as *Chironex yamaguchii*). The secondary structure predictions showed presence of α-helices, β-strands, and loops. The N-terminal regions of these toxins harbor amphipathic α-helices. These amphipathic regions are thought to be important for hemolytic action of the venom, as such structures present in some cytolytic protein toxins are known to promote formation of pores in plasma membranes. A secondary structure analysis of the CfTX-1 and CfTX-2 has identified a putative transmembrane region, designated TSR1. This region is common to cubozoan toxin sequences CqTX-A, CrTXs, and CaTX-A. This common transmembrane region empowers the toxins with pore-forming ability through disruption of transmembrane ion gradients. Tertiary structure prediction of CfTx-1 and CfTx-2 suggested the presence of a central domain with some structural similarity with the members of cytokine superfamily. Weak homology between this central domain and interleukin-1 was indicated. It is suggested that CfTX-1 and CfTX-2 are probably a "newly emerging family of unique jellyfish toxins." The existence of two more hemolytic protein toxins of about 40 kDa mass has been reported in the nematocyst of *C. fleckeri*. However, precise characterization of these toxins has not yet been made. CARTOX, a larger protein toxin of 102 kDa, isolated from *Carybdea marsupialis* was also found to possess significant hemolytic activity. However, the hemolytic activity of CARTOX is restricted "to" sheep RBCs only and unable to hemolyze rabbit or human RBCs. CARTOX is a heat-labile and highly unstable protein and does not

possess phospholipase or sphingomyelinase activity found in many pore-forming toxins. Molecules with diameters 1.8 nm or more provided osmotic protection against CARTOX-induced hemolysis. Glucose having a molecular diameter of 0.84 nm was unable to provide osmotic protection. The authors suggested that CARTOX-induced pores have diameters between 0.84 and 1.8 nm. Pore formation was also found to be dependent on Ca^{2+} concentration, but not on K^{+} concentration. It is suspected that pore formation probably requires dimerization of the toxin. The authors suggested that some specific glycosidases, β-galactopyranoside, and n-acetylneuraminic acid moieties present on RBC membrane may act as receptors for CARTOX. Molecular structure of CARTOX has not yet been characterized (Rottini et al. 1995). Earlier, Chung et al. (2001) characterized a partially purified a hemolysin CAH1 from *Carybdea alata* box jellyfish inhabiting both Pacific and Atlantic waters. This strong hemolysin is similar to CfTX-1 and −2 in size. The hemolytic activity of CAH-1 could be completely inhibited by d-lactulose. *p*-Nitrophenyl-α-d-galactopyranoside and *p*-nitrophenyl-β-d-galactopyranoside were also able to inhibit hemolysis.

The partial sequence obtained for CAH1 is suggestive of divalent cation-dependent hemolytic activity. The aspartate residues seen in the N-terminus sequence may act as a cation-binding site. Hydrophobic and hydrophilic residues in the N-terminus are consistent with an α-helical structure. It is the N-terminal α-helix in the hemolytic proteins from the cnidarians *Stichodactyla helianthus* and *Actinia tenebrosa* that are believed to initiate hemolysis by contacting the lipid membrane (Macek et al. 1994).

Recently, in early 2013, Wang et al. proposed that lipid peroxidation may be another potential mechanism underlying hemolysis besides pore formation by tentacle extract from the jellyfish *C. capillata*. They suggested that in view of jellyfish having highly efficient toxic strategies for prey and defense, lipid peroxidation might be another potential mechanism of hemolysis, which can cause loss of polyunsaturated fatty acids, inactivation of membrane enzymes and cytoplasmic proteins, alteration in ion transport, and generation of lipid hydroperoxides. It has been attributed as one of the major pathways for explaining the toxicity of many xenobiotics (Stark 2005; Rice Evans 1994). In addition, several sea anemone venoms, for example, those from *Actinia equina* and *Bartholomea annulata*, can induce intracellular reactive oxygen species (ROS) formation in cultured cells (Bartosz et al. 2008; Santamaria et al. 2002). Some digestive enzymes, acting as toxins, from box jellyfish, sea anemones, and corals induce ROS or lysophospholipid formation thus damaging target cells of small prey or contributing to human envenomation (Butzke and Luch 2010).

Maisano et al. recently reported the hemolytic activity of nematocyst venom from *Pelagia noctiluca* (Cnidaria: Scyphozoa). The results of their experiments indicated that *Pelagia noctiluca* venom induced hemolysis and lysosomal membrane destabilization, but there were no significant differences in glutathione (GSH) levels between control and treatments; consequently, the toxins do not cause the oxidative stress but possibly recognize specific targets (i.e., sphingomyelin) in the plasmatic membrane of red blood cells.

Coagulopathy

Venoms of many terrestrial animals contain toxins capable of acting as either a procoagulant or an anticoagulant. Procoagulants directly help in immobilizing prey by sudden closure of intravascular circulation by clot formation, whereas anticoagulants facilitate hemorrhage (caused through hemorrhagins) by blocking the blood coagulation process. In case of venomous snakes, this is an efficient method to make a small prey "bleed to death." Human victims of snake envenomation, particularly viper envenomations, also suffer from this so-called dramatic hemorrhage. Apart from clinical interest, these anticoagulants have attracted researchers for their possible use against thrombosis. Many such anticoagulants have been isolated and characterized from snake and other hematophagous (blood-consuming) animals. However, coagulopathy is not a frequently reported phenomenon in marine envenomation. Reports on coagulopathy in jellyfish envenomation are not available. However, intracranial hemorrhage is one of the symptoms in severe Irukandji syndrome, named after the Irukandji tribe who live in north Queensland, Australia. However, whether this hemorrhage is due to action of hemorrhagic components of the venom or due to severe hypertension is not clear.

The most predominant toxins found in jellyfish venom are members of SVMP disintegrins. SVMPs share ancestral genetic relationship with endogenous matrix metaloproteinases (Moura-da-Silva et al. 2007). The members of this family are known to disrupt blood vessel integrity and cause blood coagulation and local tissue damage.

Lee et al. (2011) reported the proteolytic activity in the venoms of four scyphozoan jellyfish species, including *Nemopilema nomurai*, *Rhopilema esculenta*, *Cyanea nozakii*, and *Aurelia aurita*. Each of these venoms contained multiple protein components ranging between molecular weight of 17 and 130 kDa. All four jellyfish venoms showed gelatinolytic, caseinolytic, and fibrinogenolytic activity. These activities varied qualitatively and quantitatively based on the species. All venoms except *Rhopilema esculenta* did not show any hyaluronidase. It also suggested that all of these proteases in the venoms were metalloproteinases as 1,10-phenanthroline could inhibit them. They demonstrated that the relative cytotoxic potency of jellyfish venom appears to be closely associated with their proteolytic activity, suggesting the metalloproteinase in jellyfish venom may contribute to its cytotoxicity. When 1,10-phenanthroline inhibited the venom protease, the venom cytotoxicity was significantly diminished.

The first report of anticoagulant activity by a jellyfish tentacle extract was made in 2011 by Rastogi et al. The authors found significant fibrinogenolytic and fibrinolytic activity in the tentacle extracts of moon jellyfish (*Aurelia aurita*) collected from the Goan coast in western India (Fig. 26.4). The whole extract was found to digest Aα, Bβ, and γ chains of bovine fibrinogen in vitro in less than 3 h (Fig. 26.5). The rate of digestion was also faster than some snake venom-derived anticoagulants. The digestion starts from Aα and slowly digests the other two chains. The tentacle extract also digested fibrin clots made in vitro. Apart from direct digestion of fibrinogen or fibrin, the tentacle extract also significantly

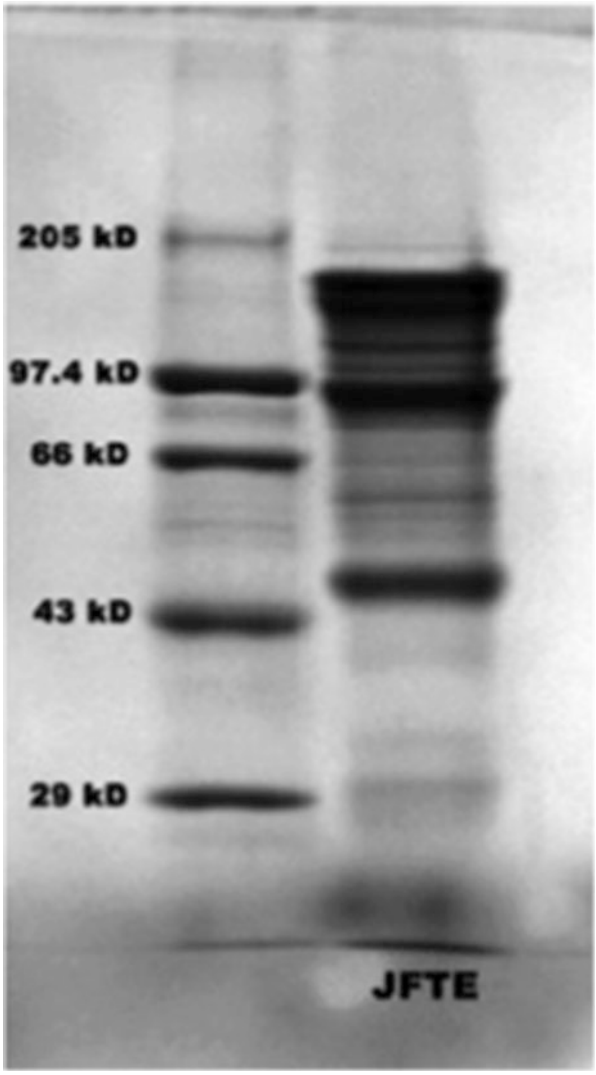

Fig. 26.4 Band pattern of moon jellyfish tentacle extract on 12 % SDS-PAGE gel

inhibited ADP-induced platelet aggregation. The degree of inhibition was more than 80 % with 40 μg of tentacle extract. However, the anticoagulant toxin in this extract is not yet purified. The tentacle extract contains a very high number of proteins of molecular weights ranging from 43 to 200 kDa. Very few protein bands are seen below 40 kDa region in SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). The fibrino(geno)lytic and platelet-inhibiting toxin(s) are yet to be purified. The authors have also shown one important parameter – *autodegradation* of tentacle extract proteins. This phenomenon is one of the biggest hurdles in purification and characterization of jellyfish

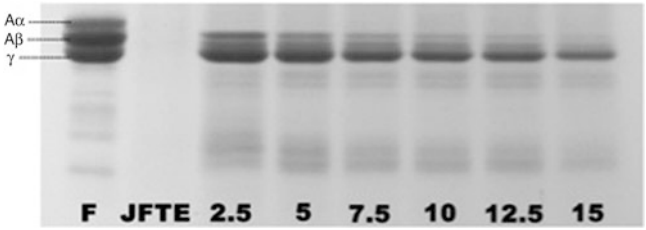

Fig. 26.5 Fibrinogenolytic activity of moon jellyfish tentacle extract. *F* = fibrinogen only; Aα, Bβ, and γ bands are marked; numbers at the *bottom* of lanes indicate the dose of tentacle extract protein in μg/ml

toxins. The authors could inhibit autodegradation by phenylmethylsulfonyl fluoride (PMSF) and ethylenediaminetetraacetic acid (EDTA). However, EDTA protected the venom proteins without affecting fibrinogenolytic activity. This suggests that these fibrinogenolytic protein(s) are not metallo-protease(s) (Rastogi et al. 2012). The strong anticoagulant toxins present in moon jellyfish tentacle extract needs to be purified and characterized for their possible use in therapeutics.

Conclusion and Future Directions

Conclusion

Jellyfish envenomation causes mostly neurological and gastrointestinal disturbances. Hemotoxicity of jellyfish venom is mainly restricted to hemolysis. The hemolytic toxins purified from several jellyfish species have shown considerable variability in structure and modes of action. These pore-forming hemolytic toxins in many cases change the ionic equilibrium of the cell membrane through different mechanisms or may act as phospholipases. Clinically, coagulopathy is rarely encountered. However, strong fibrinogenolytic and platelet aggregation-inhibiting activities observed in vitro raise question about their utility in venom. It will be interesting to find out why such molecules are incorporated in the cnidarian venoms. Whatever may be the reason, it is indeed important to study these molecules in depth. It is also observed that volume of literature on pharmacologically active components from cnidarian venoms is very small. One of the reasons expressed by many researchers is rapid degradation of such toxins in laboratory conditions (Williamson and Burnett 1995), which restricts attempts at purification and characterization of the active components. Many researchers have prescribed various methods to increase shelf life of the venom and its components. But the number of techniques adopted for stabilizing the toxins also reflects that a single method cannot be adopted for all jellyfish venoms/toxins. The development of newer resins and chromatography equipments for protein purification will probably help solve this long-standing problem soon.

Future Direction

Jellyfish envenomation in most cases is not dangerous to humans. However, considering the huge number of sting victims, it is important to understand the envenomation symptoms clearly for formulating standardized protocols for better clinical intervention. Due to global warming or some other unknown cause, jellyfish population has increased almost all over the world. This increase has also contributed to the increasing numbers of sting victims. This may act as a deterrent for tourism industry in many parts of the world. Efforts directed to characterization of toxic components will help in the development of specific antiserum and other antidotes. Lessons in this regard should also be learnt from anemone fish and crustaceans capable of inhibiting certain cnidarians venoms and toxins. It is also observed that jellyfish blooms are hazardous for edible fish population. Apart from clinical and economic interest, venoms of jellyfish and other marine creatures need to be researched more aggressively to find novel molecules as future drugs. Considering the huge biodiversity in our seas, the effort given to look into these potential sources of advanced drugs is very small. With more number of researchers on marine venoms and toxins, we can only hope to see a much better therapeutic intervention against life-threatening diseases in the near future.

Cross-References

▶ Complications of Hemotoxic Snakebite in India
▶ Envenoming and Natural Toxins in New Zealand

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Part III

Plant, Herb, and Mushroom Toxins

Envenoming and Natural Toxins in New Zealand

27

Robin Slaughter, Wayne Temple, and Leo Schep

Contents

Abstract

New Zealand is only home to a small number of venomous creatures. This chapter aims to examine New Zealand's venomous creatures, including their habitats and distribution, the clinical effects that might arise following envenoming, and the appropriate medical treatment required. The hazardous creatures discussed include spiders (katipo and redback spiders, white-tailed spiders), other insects (bees, wasps, and centipedes), and marine animals

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(stingrays, scorpion fish, sea urchins, bluebottle jellyfish, and sea snakes). These animals are found in a range of different habitats throughout the country and, depending on the animal encountered, can produce a wide array of adverse effects following bites or stings. Overall, envenoming from venomous creatures is not common in New Zealand. If envenoming does occur, it is rarely fatal; however, it does have the potential to cause significant morbidity.

Introduction

New Zealand is home to a small but varied group of terrestrial and marine venomous creatures. These animals range from those that typically cause only minor injuries to creatures which can cause more significant envenoming. Medically important venomous animals found in New Zealand include stingrays, a range of other venomous fish, sea urchins, bluebottle jellyfish, a small number of spiders, and some venomous insects including bees, wasps, and centipedes. Additionally, although rarely encountered, sea snakes are occasionally found along the northern coast. New Zealand is one of the few places in the world that has no native or introduced terrestrial snakes or scorpions.

Envenoming by these venomous creatures is relatively rare in New Zealand, although occasional bites and stings do occur and in some circumstances there is the potential for prolonged morbidity. Fatalities from venomous animals are extremely rare in New Zealand. The number of inquiries the New Zealand National Poisons Centre received regarding venomous creature bites or stings between 2003 and 2012 is presented in Table 27.1. This chapter aims to examine New Zealand's venomous creatures, including their habitats and distribution, the clinical effects of envenoming, and the appropriate medical treatment required.

Table 27.1 Number of inquiries received by the New Zealand National Poisons Centre regarding venomous creature bites or stings between 2003 and 2012 (©National Poisons Centre)

Common name	Species name	Number of enquiries
White-tailed spider	*Lampona cylindrata* and *L. murina*	655
Wasps	*Vespula germanica* and *V. vulgaris*	204
Bees	*Apis spp.* and *Bombus spp.*	170
Bluebottle jellyfish	*Physalia utriculus and P. physalis*	124
Centipede	*Various*	41
Stingray	*Various*	37
Fish stings	*Various*	33
Katipo spider	*Latrodectus katipo*	32
Sea urchins	*Evechinus chloroticus*	21
Redback spider	*Latrodectus hasseltii*	16
		1,333

Spiders

The major spiders in New Zealand that potentially could lead to human envenoming are two species of spider from the genus *Latrodectus*. These include the native katipo spider (*Latrodectus katipo*) and an Australian import, the redback spider (*Latrodectus hasseltii*). The katipo was named by the indigenous Maori and translates to "night stinger"; the name is derived from two words, kakati (to sting) and po (the night) (Hornabrook 1951). Additionally, the black widow spider (*Latrodectus mactans*) has occasionally been discovered in New Zealand, typically in grapes imported from California. However, it is not thought that this spider has become established in New Zealand (Ministry for Primary Industries 2001). The only other spider which has been of medical interest in New Zealand is the white-tailed spider, which describes two species, *Lampona cylindrata* and *L. murina*. White-tailed spiders are native to Australia and were introduced into New Zealand in the late nineteenth century (Platnick 2000). New Zealand has no spiders from any other medically significant genera of spider (*Atrax*, *Hadronyche*, *Loxosceles*, and *Phoneutria*) (Slaughter et al. 2009).

Katipo and Redback Spiders

The katipo is distributed throughout most of New Zealand's coastal regions including the North Island and most of the South Island (excluding the southernmost portions). Katipos are specialized in their habitat and are only found among sand dunes in coastal areas. Redback spiders were accidentally introduced into the South Island of New Zealand in the 1980s (Forster and Forster 1999). Both spiders are quite distinctive in their appearance. The katipo is slightly smaller than the redback (~10 mm body length), but otherwise, their appearance is similar. They are dark brown to shiny black in color, and they typically have an orange-red stripe running down the length of the dorsal abdomen; however, some katipo spiders may lack this stripe. Like many other *Latrodectus* spiders, both the katipo and redback typically have a red hourglass marking on their ventral surface (Forster and Forster 1999).

Katipo or redback spider bites are not common in New Zealand (Slaughter et al. 2009; Crook et al. 2010; Thatcher and Janes 2012). Historically, severe effects from katipo bites have been occasionally reported (Hornabrook 1951), and more recent literature has also reported cases of significant envenoming (Crook et al. 2010; Thatcher and Janes 2012). Bites are uncommon as these spiders are typically shy and nonaggressive, although the female may act aggressively when protecting an egg sac. Furthermore, diminishing populations of katipo and their narrow habitat only provides minimal interaction between the spider and human populations. Intentionally antagonizing a spider or disturbing a web may result in a bite (Slaughter et al. 2009).

Bites from both the katipo and redback spiders produce a similar toxic course known as latrodectism. In general, the most common characteristic symptom

displayed following a bite from either species is pain. Initially there may only be minimal effects associated with a bite before a gradual onset of local redness, sweating, and pain. The pain may become more intense and radiate from the bite site. Further systemic effects can include abdominal and back pain, gastrointestinal disturbance, generalized sweating, headache, fatigue, hypertension, and, less commonly, other neurological and autonomic symptoms. Symptoms may persist for hours or days, although very rarely symptoms lasting weeks or months have been reported (Sutherland and Trinca 1978; Isbister and Gray 2003a; Slaughter et al. 2009; Isbister and Fan 2011). The two recently reported cases of katipo bite in New Zealand showed a similar profile with signs and symptoms of pain at the bite site, migrating pain, chest and abdominal pain, diaphoresis, nausea, vomiting, fever, headache, photophobia, tachycardia, and in one case, myocarditis (Crook et al. 2010; Thatcher and Janes 2012).

The treatment for these bites consists of initially applying a cold pack to the area while simple analgesia may be beneficial for pain. Medical assessment is recommended for those with local pain unresponsive to simple analgesia or if clinical features of systemic envenoming such as sweating, back or abdominal pain, myalgia or arthralgia, or vomiting develop. Several treatments for latrodectism have been proposed although evidence for efficacy is limited. Oral non-opioid and/or opioid analgesia is deemed appropriate for the symptomatic relief of pain. If this is unsuccessful, parenteral opioids such as morphine may be required. Benzodiazepines may be helpful as an adjunct treatment for muscle spasm. Administration of calcium or magnesium does not appear significantly beneficial and is not recommended (Slaughter et al. 2009; Isbister and Fan 2011). Redback antivenom has been widely used in Australia for treatment of latrodectism, and while there is some concern over its effectiveness (Isbister and Fan 2011), it is indicated in cases where pain is refractory to analgesia or where there are clinical features of systemic envenoming (Murray et al. 2011). It is likely to be effective for both redback and katipo spider bites. Case reports from New Zealand using redback spider antivenom for katipo bites have shown an improvement in, or resolution of, signs and symptoms following antivenom administration (Crook et al. 2010; Thatcher and Janes 2012). Adverse reactions to this antivenom are possible although relatively uncommon; anaphylactoid reactions may occur in 0.5–0.8 % of patients while serum sickness occurs in less than 5 % of patients (Isbister et al. 2003). Severe anaphylaxis or death has not been reported.

White-Tailed Spiders

White-tailed spiders are now found throughout New Zealand and may shelter in and around residential homes. Typically active at night, they hide during the day. When found inside the home, they are normally discovered sheltering in shoes, in bed linen, or under clothes. White-tailed spiders have a distinctive appearance; they have a dark gray- to black-colored cylindrical-shaped body and are normally between 12 and 17 mm in length. The major distinguishing feature is a small

white patch at the end of the abdomen. White-tailed spiders are typically not aggressive and only tend to bite if they are threatened, startled, or provoked. Bites are more common in the warmer months; normally occurring indoors, they are more likely if a spider is caught up in bedding or clothing or disturbed in some way (Isbister and Gray 2003b; Slaughter et al. 2009; Victoria Museum 2013).

These spiders have only become a medical concern in New Zealand in the last 15–20 years, as they have been linked to the disease of necrotizing arachnidism. Medical reports and media attention both in Australia and New Zealand have suggested bites from these spiders may cause necrotic ulcers (Spring 1987; St George and Forster 1991).

Studies have shown that necrotizing arachnidism is an uncommon outcome following bites from these spiders (Isbister and Gray 2003b; Banks et al. 2004). One prospective cohort study of 130 cases of white-tailed spider bite in Australia showed there was no link between white-tailed spider bites and tissue necrosis. In this study patients were only included if they were clearly bitten by a spider and the spider in question was caught and identified by an expert arachnologist. Of the 130 cases reported, none of the patients developed a necrotic ulcer (Isbister and Gray 2003b). A study in New Zealand consisting of nine patients with suspected white-tailed spider bite (although with no confirmation of a spider bite or identification of the spider) also found no evidence of necrotizing arachnidism (Banks et al. 2004).

White-tailed spider bites are, however, known to produce painful local effects in most cases. Signs and symptoms most commonly reported following a bite have included local pain, pruritus, swelling, redness, and possibly the formation of a lump. Other less common effects may also occur including nausea and vomiting, headache, and malaise. The local effects are normally relatively rapid in onset. Pain may persist for up to 2 h or longer; redness and swelling usually resolve over 24 h but may persist for days in some cases. Overall, the symptoms displayed are usually mild and self-limiting (Isbister and Gray 2003b). Possible further risks may include local secondary infection.

Treatment for a white-tailed spider bite consists of routine first aid including cleaning the wound, disinfection with a mild antiseptic, and application of an ice or cold pack to reduce pain and swelling. Symptomatic care with oral analgesia and/or antihistamines may be required (Slaughter et al. 2009). Further treatment is unlikely to be required. If a patient does present with a necrotic lesion thought to be due to spider bite, it should be thoroughly investigated to ensure there is no other treatable cause (Isbister 2004). A robust history and physical examination should be performed. There are numerous alternative diagnoses to spider bite necrosis. Microbial investigation should include cultures for organisms such as fungi and unusual bacteria.

Insects

New Zealand is also host to some venomous insects and centipedes. Medically significant species encountered are typically hymenoptera (bees and wasps). New Zealand has a number of native wasps and bees, although most are solitary

species and are not responsible for many stings or adverse medical outcomes. Of more toxinological concern are the introduced species of hymenoptera including German and common wasps (*Vespula germanica* and *V. vulgaris*), honeybees (*Apis spp.*), and bumblebees (*Bombus spp.*). These are social species and may be encountered in large numbers. The majority of medically important incidents involve few stings and anaphylactic shock. Multiple stings (numbers measured in hundreds or higher) are rare in New Zealand; however, there is a risk of multiple stings and systemic envenoming if a nest is disturbed (Allen 2012; Fuller 2013). For example, a man in the Marlborough region of New Zealand was killed after disturbing a nest and receiving multiple wasp stings (Allen 2012). Systemic effects following multiple stings can include nausea and vomiting, dizziness, fever, hypertension, seizures, unconsciousness, hemolysis, rhabdomyolysis, disseminated intravascular coagulation (DIC), hypoglycemia, hyperkalemia, liver dysfunction, and acute kidney injury (Vetter et al. 1999; Watemberg et al. 1995).

Centipedes are also found in New Zealand, and while encounters are rare, they may cause envenoming following a bite. The symptoms are normally mild and typically consist of pain, weakness, itching, paresthesias, erythema, and edema (Balit et al. 2004). The clinical toxinology of hymenoptera and centipede envenomings are discussed in chapter "► Centipede Envenomations: Clinical Effects and Treatment," respectively.

Stingrays and Marine Puncture Wounds

Stingrays

Stingrays are common throughout New Zealand's coastal waters, especially in sheltered bays, river mouths, and other sandy regions. Stingrays have a distinctive round, flattened body and a thin tail. The tail additionally has at least one serrated spine on the dorsal surface. The spines vary in size as well as position on the tail. The spine is lined with short barbs and has two grooves on the underside which house the venom glands (Acott and Meier 1995).

Stingrays are not aggressive and generally do not attack humans; strikes tend to occur to the lower limbs and typically occur when divers or swimmers accidentally step on stingrays. In this situation the stingray has no means of escape and will defensively react by quickly raising its tail and accurately stinging the offending person. Fishermen also may be injured when they find stingrays in their nets or on their lines (Barss 1984; Acott and Meier 1995).

The most important concerns following a stingray strike are the risk of traumatic injury, envenoming, and bacterial wound contamination. The stingray's tail has a wide range of movement and can produce a deep laceration or a penetrating puncture wound which may involve direct injury to muscles, nerves, tendons, blood vessels, or internal organs. The wound may have a bluish-white appearance, while the spine can break off and may be retained in the wound.

If envenoming occurs additional local complications may include intense local pain, edema, muscle cramps, and localized tissue necrosis (Hawdon and Winkel 1997; Slaughter et al. 2009).

Systemic envenoming is relatively uncommon but may occur following stings to either central or peripheral sites. Symptoms reported have included gastrointestinal upset, syncope, hypotension, tremor, or, rarely, seizures, cardiac dysrhythmia, and circulatory collapse (Fenner et al. 1989; Weiss and Wolfenden 2001). Serious injury or death may occur due to exsanguination or direct trauma to vital organs in the thoracic or abdominal cavities or from complications due to poor wound management such as septicemia or tetanus infection. Secondary infection is a significant cause of morbidity (Fenner et al. 1989). Most serious injuries occur when a spine penetrates the thoracic or abdominal cavity and deaths have been recorded around the world including in New Zealand (Liggins 1939). In this case from New Zealand, an 18-year-old female was swimming in the Hauraki Gulf and was stung in the leg and chest by a stingray. She died before reaching medical attention with the cause of death determined to be hemorrhage from a wound penetrating the heart.

The initial treatment for a stingray strike includes ensuring the area is flushed with fresh water (seawater if fresh water is unavailable). Flushing helps to remove any barb fragments or venom in the wound. If there is hemorrhage, it needs to be controlled with local pressure. In significant cases, exsanguination, including internal hemorrhage, may occur, and standard trauma protocols should be followed. The recommended treatment for local pain is hot water immersion; an optimum temperature of approximately 45 °C for 15–20 min is recommended, taking care not to cause a thermal burn. The water temperature should always be checked by the aid provider to ensure it is not too high. If possible it is recommended that the non-injured limb also be placed in the hot water; thus, if the water temperature is too high, the non-injured limb will let the patient know if the water temperature is too high. If it is not practical to immerse the area (the wound is to the abdomen or chest), a hot pack or soak can be applied. If hot water provides pain relief, it can be continued for up to 2 h; however, if no pain relief occurs in the first 15–20 min, the procedure should be abandoned (Slaughter et al. 2009; White 2013). Refractory or severe pain may require further treatment including infiltration of the wound area with local anesthetic, regional nerve block, and/or parenteral opioids (Slaughter et al. 2009).

It is important that all wounds are explored and debrided as soon as practicable. Wound management in hospital is recommended as all foreign matters, including retained sting fragments and any nonviable tissues, must be removed (Flint and Sugrue 1999). If local or regional anesthesia is required, adrenaline should be avoided as this delays microvascular clearance of venom, thereby aggravating necrosis. In the case of deep wounds, debridement under general anesthesia may be required. It is important that all wounds have an X-ray examination to ensure removal of all stingray barb fragments. As not all remnants may be radiopaque, ultrasound can be useful if there is suspicion about further retained material (Acott and Meier 1995; Slaughter et al. 2009). The wound needs to be thoroughly cleaned

following debridement and left open to granulate and heal by secondary intention (Fenner et al. 1989). Systemic effects are uncommon but if present, should be treated supportively as there is no specific antivenom available (Slaughter et al. 2009).

A further concern is the risk of bacterial infection. Following a sting the spine may introduce a range of marine bacteria into the wound. Prophylactic antibiotics are not necessary for minor wounds, but they are indicated in the situation of deep wounds, if there is considerable foreign material present, or if there is a delay of 6 h or more before wound debridement can occur (Fenner et al. 1989; Isbister 2001). Infection should initially be managed with a broad-spectrum parenteral antibiotic regimen. Culture should be used to determine the best antibiotic(s) for continued management. It is important to specify seawater involvement when submitting specimens for microbiological analysis (Lehane and Rawlin 2000). Additionally, it is necessary to ensure that tetanus prophylaxis is up-to-date. Medical follow-up at 24–48 h is required to detect any evidence of infection or necrosis (Slaughter et al. 2009).

Other Venomous Marine Punctures

Other organisms found in New Zealand's coastal waters that can cause puncture wounds and envenoming include a range of venomous fish and sea urchins; the majority of venomous fish in New Zealand are from the Scorpaenidae family (scorpion fish). Other venomous fish that may be encountered in New Zealand include the spiny dogfish (*Squalus acanthias*), elephant fish (*Callorhinchus milii*), and the brown bullhead catfish (*Ameiurus nebulosus*) (Slaughter et al. 2009). All these fish have external venomous spines located on different regions of the body. Spines are most commonly found on the dorsal region, but they may also be located on pectoral, shoulder, pelvic, opercular, anal, and caudal regions. The toxinology of the different venoms from these fish have not been studied comprehensively, but they are all thought to produce similar toxic effects in humans following envenoming (Church and Hodgson 2002).

Venomous puncture wounds can also be caused by sea urchins (Echinodermata phylum) which are distributed around New Zealand. One sea urchin, *Evechinus chloroticus* (known locally as kina), is commonly collected and consumed in New Zealand. This sea urchin is typically responsible for most injuries, which are normally to the hands or feet following victims stepping on or handling sea urchins. Sea urchins have two venom apparatuses consisting of external spines and small organs named pedicellariae which are small grasping organs covered by venom-producing glandular tissue (Mebs 1995).

Symptoms following fish or sea urchin stings produce a similar toxic course; most injuries are not severe but they can produce significant local pain, which may spread to involve the whole of the limb. The pain is typically more severe than would be expected from the trauma alone. Systemic effects may also occur and can include nausea, sweating, syncope, hypotension, and respiratory distress. Wound

contamination may lead to secondary infection, and if multiple stings are present, infection may be severe (Cracchiolo and Goldberg 1977; Kizer et al. 1985; Trestrail and al-Mahasneh 1989; Aldred et al. 1996; Wu et al. 2003).

While more commonly seen with sea urchins, both fish and sea urchin spines can break off and remain lodged in the wound. If not removed, sea urchin spines can cause a foreign body or a sarcoid-type granuloma. Additionally, when spines become embedded within joints and over bony prominences or in contact with neurovascular bundles, they may lead to arthritis, fasciitis, tenosynovitis, and bursitis (Guyot-Drouot et al. 2000; De La Torre and Toribio 2001). Rarely sea urchin stings induce a delayed hypersensitivity reaction consisting of erythema, pruritus, vesicular eruptions, paresthesia, myalgia, and malaise (Kane 1982; Burke et al. 1986).

The initial treatment for fish and sea urchin stings, similar to that for stingrays, consists of thorough flushing of the wound, control of any hemorrhage, and hot water immersion. Spines need to be removed, preferably with forceps. If there is evidence of a retained spine, imaging with X-ray or ultrasound can help identify any foreign material. Surgical exploration and debridement is necessary if there is evidence of retained fragments. This is especially important for sea urchin stings to prevent long-term lesions such as chronic granulomas. Adequate pain relief is important, particularly if hot water immersion is unsuccessful in relieving pain. Simple oral analgesics, parenteral opioid analgesics, or local or regional-block anesthetic may be required if pain is persistent. There is no antivenom available for envenomings by these creatures, and symptomatic and supportive care is required for any further systemic symptoms. As outlined for stingray injuries, control of infection and tetanus prophylaxis may also be required (Slaughter et al. 2009).

Jellyfish

Bluebottle Jellyfish

New Zealand has a number of jellyfish in its coastal waters; the only two species (within the same genus) considered medically important are the bluebottle jellyfish (*Physalia utriculus*) and the Pacific or Portuguese man-of-war jellyfish (*Physalia physalis*) (Slaughter et al. 2009). These animals are siphonophores, an order of the Hydrozoa which is a class of marine animals belonging to the phylum Cnidaria. The phylum Cnidaria also includes a number of other classes, regarded as true jellyfish, including the Scyphozoa (true jellyfish) and the Cubozoa (box jellyfish).

The two *Physalia* species are distinctive jellyfish, both having a bright blue floating bladder. The bluebottle is the smaller of the two, with the bladder typically measuring 2–15 cm in length (Nimorakiotakis and Winkel 2003). The Pacific or Portuguese man-of-war is larger and its floating bladder may be up to 25 cm long (Fenner et al. 1993). These jellyfish are made up of a colony of symbiotic animals. Subpopulations within the animal are responsible for different functions (e.g., capturing food, reproduction, and digestion) (Williamson and Burnett 1995).

Jellyfish have numerous nematocysts (microscopic stinging cells) located on the tentacles or body of the animal which are used for capturing prey. Each nematocyst contains a small dose of venom and is normally discharged in response to mechanical or chemical stimulation (Williamson and Burnett 1995). Different species of jellyfish have characteristic nematocysts and they can be used to identify a particular species of jellyfish (Currie and Wood 1995). New Zealand is fortunate in that the two jellyfish that are thought most dangerous to humans, the box jellyfish (*Chironex fleckeri*) and the Irukandji jellyfish (*Carukia barnesi*), are not found around the New Zealand coast (Slaughter et al. 2009).

A large number of people are stung each year by *Physalia* jellyfish; stings typically do not cause any major effects. Common effects displayed following envenoming include localized skin pain with itching and a burning sensation; a characteristic local reaction that is encountered is the formation of linear collection of elliptical blanched weals with a surrounding red flare (appearance resembles a "string of beads") (Hawdon and Winkel 1997). Systemic symptoms are rare but may occur if there is extensive stinging from a large specimen. These may include nausea, vomiting, chills, drowsiness, headache, breathing difficulties, and cardiovascular collapse. Rarely, deaths have been reported following extensive stinging by the Atlantic species (*Physalia physalis*) (Stein et al. 1989). No deaths have been reported in New Zealand.

The treatment for all jellyfish stings in New Zealand is similar. Initially patients should be prevented from any vigorous activity or rubbing of the sting area; this helps to prevent further discharge of attached nematocysts and venom movement into the general circulation. Initial on-site first aid consists of removing adherent tentacles by flushing with seawater (or fresh water, if seawater is unavailable). If tentacles are still adherent, careful removal with forceps may be required. In tropical waters of Australia, vinegar is used to treat box jellyfish stings (Fenner et al. 1993). Vinegar is not thought to be beneficial for *Physalia* stings and may actually increase discharge of *Physalia* nematocysts; its use is therefore not recommended in New Zealand (Slaughter et al. 2009). Other techniques including application of urine or methylated spirits have not been shown to be beneficial and are similarly not recommended.

Pain at the sting site can be relieved with the use of hot water immersion. A randomized trial investigating the preferred treatment for *Physalia* stings in Australia found that there is significant benefit of hot water over cold packs for alleviating pain and that hot water immersion or a hot shower should be the treatment of choice following *Physalia* envenoming (Loten et al. 2006). The technique is similar to that described for stingray stings. If pain persists, further care with simple oral analgesics (e.g., paracetamol) is recommended; in severe cases topical anesthetics or parenteral opioids may be considered. Antihistamines can be administered for pruritus. Further care may be required if systemic symptoms such as vomiting, drowsiness, or breathing difficulties are present. There is, however, no antivenom and any systemic symptoms that develop should be treated supportively. Hypersensitivity or infection is rare but may require symptom-directed care (Slaughter et al. 2009).

Snakes

Sea Snakes

Sea snakes are not commonly found in New Zealand's waters but there are occasional reports of specimens being beached around the northern coast of New Zealand. Overall, sea snakes are a diverse group of snakes in the Elapidae family. Most species are 1.2–1.5 m in length and are found in a range of colors. Their distribution includes the warm tropical waters of the Pacific and Indian Oceans; typically their habitats would be around coral reefs or close to the shore (White 1995).

Some species are pelagic and these are more likely to be encountered around northern New Zealand. Species which have been found in New Zealand coastal waters include the yellow-bellied sea snake (*Pelamis platurus*) and the banded or yellow-lipped sea krait (*Laticauda colubrina*). It is not thought that any cases of envenoming from these snakes has been reported in New Zealand; however, there is a risk that envenoming could occur if a beached snake is handled (Slaughter et al. 2009).

All sea snake venoms are thought to be similar and produce a similar toxic course following envenoming in humans. While about 80 % of bites from sea snakes do not result in envenoming, there is the risk that severe, potentially life-threatening, effects could occur. Pain at the bite site itself is normally felt but is unlikely to feature major bruising, swelling, or necrosis. If envenoming occurs there is typically an asymptomatic time interval following the bite, ranging from one to several hours, before the onset of systemic toxicity. Initial signs and symptoms are normally related to myolysis, with the development of muscle aches and pain, occasionally in association with nausea, vomiting, dizziness, and weakness. Complications of myolysis may include acute kidney injury, hyperkalemia, and secondary cardiotoxicity. Neurological toxicity includes the development of a flaccid paralysis with depressed or absent deep tendon reflexes, dysarthria, difficulty swallowing, ptosis, and ophthalmoplegia. This may potentially advance to more severe paralysis and respiratory arrest. Coagulopathy is not seen following sea snake envenoming (Marsden and Reid 1961; White 1995; Slaughter et al. 2009).

The initial first aid treatment following a sea snake bite is the application of a pressure immobilization bandage. This is an effective method of retarding venom transport via the lymphatic system thereby minimizing the venoms systemic spread (Sutherland et al. 1979). Pressure immobilization consists of applying a broad bandage over the bitten area followed by a second bandage which covers the entire limb. The second bandage should be applied from the tip of the limb proximally toward the body covering as much of the limb as feasible. The area should then be immobilized with a splint or sling. The patient should remain immobilized while being transported to hospital (White 2013).

If systemic envenoming becomes apparent, the recommended treatment consists of administration of appropriate antivenom. Sea snake antivenom is produced by CSL Limited in Australia. This antivenom is recommended for any sea snake bite occurring in New Zealand. Other CSL antivenoms have in the past also been

thought suitable for sea snake bites including CSL Tiger Snake or CSL Polyvalent Snake Antivenom. However, due to a change in the production of these antivenoms, they are now not expected to provide useful antivenom activity against sea snakes. If sea snake antivenom was unavailable, tiger or polyvalent antivenoms could be considered; however, their efficacy for this envenoming is unknown and there are known risks with the administration of any antivenom including severe allergic reactions and serum sickness (White 2013).

Further supportive care may be required and can include management of paralysis and myolysis and their complications. Urine output should be maintained with IV fluids. Should myolysis be apparent, urinary alkalinization may additionally be helpful. Renal failure or hyperkalemia may require hemodialysis. In the short term, paralysis may be reversed with atropine and neostigmine. Respiratory failure may require intubation and artificial ventilation (White 1995; Slaughter et al. 2009).

Conclusion and Future Directions

New Zealand has a very small number of venomous creatures and significant envenoming is rare. However, there exists the potential for prolonged morbidity if suitable treatment is not provided. Because envenoming is uncommon, this chapter is designed to provide information on the most common envenomings in New Zealand along with guidelines for the successful management of these cases.

Cross-References

▶ Centipede Envenomations: Clinical Effects and Treatment
▶ Hemotoxic Activity of Jellyfish Venom

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Anticholinergics Syndrome Related to Plants and Herbs

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Abstract

Anticholinergic poisoning is one of the typical toxidromes. Plant tropane alkaloids – atropine, scopolamine and hyoscyamine – are the common causes of this toxidrome when they enter the human body through GI absorption and/or skin contact. Because the toxins mimic to the essential neurotransmitter acetylcholine, they cause antagonist effects on the parasympathetic nerves with specific receptors that control mucous secretion, sweating, and heart rate. The plants that containing tropane alkaloids are Solanaceae, such as *Atropa*, *Datura*, and *Mandragora*, with the distribution to the individual plant and the ratio of toxins different from species to species. Although the clinical presentations may be slightly different, the antidotes of all the intoxications are physostigmine. Decontamination and supportive care may be required.

Introduction

A 40-year-old woman was admitted to the emergency room because she was found crying and gesturing by her husband. She had no family history of similar problems, but she often went to see the Chinese medicine practitioner recently because of pains in the loins and back. The clinical manifestations appeared 10 min after she drank the Chinese herbal soup. According to the description provided by her family, the patient had 1½ bowls of the Chinese medicine concentrated from the herbs plus three bowls of water. She complained about the headache and dry mouth 5–10 min after she drank the soup; later she acted abnormally as described previously and then was sent to the emergency room. The doctor found the patient was moving restlessly and could not cooperate. She could sometimes answer the questions but most of time she had wrong answers, or murmured something that was not clear. Also, she was disorientated to people, events, time, places and things. Physical examination discovered her temperature was 37.9 °C; breath sounds were normal; bowel sound was hypoactive and no pressed abdominal pain; the center of lower abdomen had a tight, ball-like mass that could be felt; and finally there was no abnormal muscle twist. Under the impression of anticholinergic poisoning, the patient was given 2 mg of physostigmine via slow IV injection. Two minutes later the patient recovered to normal consciousness without any other side effect. Her family brought the rest of the herbs and found about six pieces of dry datura. It was determined that the patient suffered from datura poisoning. Although the herbs were not under further species identification, it was guessed to be *Datura stramonium*.

This is a typical anticholinergic syndrome due to datura misuse and poisoning in Taiwan. “Blind as a bat, hot as a hare, dry as a bone, red as a beet and mad as a wet hen” (Sands and Salen 1976; Trabattoni et al. 1984) describes the general presentations of anticholinergic poisoning syndrome, although these presentations might be slightly different in different cases. Anticholinergic syndrome is not a rare toxidrome for clinical toxicologist. More than 600 compounds have

anticholinergic properties, including drugs of antihistamine, antidepressant or even over-the-counter medications, and plants. Many plants with tropane alkaloid, often also known as belladonna alkaloids, serve as the source of anticholinergic medicines and may also produce anticholinergic toxicity. Some plants such as *Datura* have been noted to be abused (ingested or, less commonly, smoked) by adolescents in North America for their hallucinatory effects (Richardson et al. 2007; Ramjan et al. 2007). According to the American Association of Poison Control Centers (AAPCC), there were approximately 20,000 exposures to anticholinergic drugs and plants with 46 major outcomes in 2009 and was noted to increase in 2011 (Bronstein et al. 2009, 2012). But there were only a few systemic clinical studies about plant-induced anticholinergic syndrome in addition to sporadic cases reports in literature. One of the largest cases series analysis came from an abstract report at an annual meeting of the North American Congress of Clinical Toxicology, with 1,458 cases of exposure to *Datura* reported to American poison centers (Krenzelok et al. 1995). This report revealed that adolescents accounted for more exposures than adults (46.2–41.2 %), and most of them (72 %) resulted from intentional abuse for recreational purpose. An examination of those whose purpose was to take advantage of the psychoactive properties of the *Datura* species confirmed the widely held perception about *Datura* abuse in North America. The clinical severity of *Datura* abuse seemed to be less than other drugs abused due to no fatalities in this case series. Fatalities involving *Datura* are rare. Another series involving the analysis of 8 years of American poison center data identified only 30 botanical-related fatalities, and *D. stramonium* accounted for 17 % (5) of the deaths (Krenzelok 2002). To put this in perspective, the fatalities related to *Datura* exposures represented only 0.1 % of all fatal poisonings that were reported to American poison centers.

In Taiwan (Lin et al. 2009), the PCC data showed that more than one-third of plant poisonings resulted from anticholinergic plants, including plants of the genus *Atropa*, *Brugmansia*, *Datura*, *Hyoscyamus*, *Solandra*, and *Solanum*. But the poisonings in Taiwan were different from poisonings in North America in that adults (more than 80 %) and not adolescents accounted for the majority of exposures. Only mild to moderate poisonings without organ failure were noted in these cases. One-fourth of *Datura* species exposures received physostigmine antidote treatment with fair outcome.

Pharmacology of Anticholinergics

Cholinergic nerves are parts of autonomic nervous system. They pass the action potential by acetylcholine as the neurotransmitter at synaptic sites of the central and peripheral nerves. There are two types of acetylcholine receptors: muscarinic and nicotinic receptors. Nicotinic receptors appear at the skeletal muscle and neuromuscular junctions, spinal cord, and ganglia. On the other hand, muscarinic receptors are located in the cerebrum, parasympathetic postganglionic neurons, and some parts of sympathetic postganglionic neurons, such as sweat glands. Antimuscarinic

agents, such as tropane alkaloids, have little effect on the actions of acetylcholine at nicotinic receptor sites of autonomic ganglia or skeletal muscle junctions. However, higher concentrations of scopolamine in plants may cause competitive blockade of these receptors and lead to curare-like action of muscle weakness and flaccidity (Hall et al. 1977; Smith et al. 1991).

There are five types of muscarinic receptors (M1-5); all of them are G-protein bound transmembrane proteins. Acetylcholine binds to the muscarinic receptors, generating stimulation or inhibition of cell function. This is different from the cation channels controlled by acetylcholine binding to the nicotinic receptors. Anticholinergic drugs or alkaloids are competitive inhibitors of muscarinic receptors. This inhibition and the clinical symptoms are dose related, but different organs with different parasympathetic tones have different sensitivities to the inhibitors. Also, the levels of clinical responses would not be the same. Moreover, other neural transductions may affect organs differently. Even the ability of drugs to reach the responding organs is different. Usually, low-dose exposure of anticholinergic chemicals mainly affects the sweat glands, salivary glands and bronchial secretion. Pupil dilation or tachycardia due to the inhibition of vagus nerves require larger dose of exposure.

Tropane alkaloids in anticholinergic plants are atropine, scopolamine, and hyoscyamine, with scopolamine (L-hyoscine) being present in higher concentration than hyoscyamine in most *Datura* spp. Atropine is a racemic combination of the D- and L-forms of hyoscyamine, and the L-isomer (L-hyoscyamine) possesses greater antimuscarinic potency (O'Grady et al. 1983; Krenzelok 2010). Tropane belladonna alkaloids in plants rapidly enter the human body through gastrointestinal tract and skin (Pereira and Nishioka 1994; Janes and Stiles 1959). Symptoms usually onset within 5–10 min after ingestion of tea and occur 30 min to 2 h after leaves or seeds are ingested and may continue for 24–48 h because tropane alkaloids delay gastric emptying and absorption. Atropine, as the example compound, is metabolized by the liver into atropine-N-oxide and noratropine while it is excreted by the kidneys without biotransformation. The half-life of varied alkaloids is 2–8 h (Hayden et al. 1979; Schneider et al. 1996), but it differs depending on the plant species that the patients are exposed to. Although both atropine and scopolamine will produce central nervous system (CNS) effects that include delirium, hallucinations, agitation, and excitation (Gowdy 1972), especially with an increasing dose, the hallucinogenic effects are attributed largely to scopolamine (Krenzelok 2010). Scopolamine also may produce euphoria, which is a basis for some abuse potential. Scopolamine exhibits greater brain penetration ability than atropine and is a depressant even in therapeutic doses. Scopolamine also possesses amnestic properties and has been used as a preanesthetic medication to reduce vagal effects secondary to visceral manipulation during surgery. Higher doses of scopolamine can antagonize central muscarinic neurotransmission, resulting in depression of the reticular activating system (Hall et al. 1977). The ratio of atropine to scopolamine differs between species and parts of plant, which explains the difference in severity of patients' presentations.

Plants with Anticholinergic Alkaloids

Alkaloids are plant metabolites that have a nitrogenous bicyclic ring structure, alkali-like chemical reactivity, and pharmacologic activity. The highly anticholinergic tropane alkaloids are one of the three major pharmacologic groups of alkaloid amines in plants. Plants that contain the tropane (also called *belladonna*) anticholinergic alkaloids atropine, scopolamine, and hyoscyamine are members of the Solanaceae family. This family includes a wide variety of other plants, such as tobacco, ornamentals, and foods (eggplants, paprika, potatoes, red pepper, tomatoes) (Müller 1998). These toxic plants include the genera *Datura*, *Hyoscyamus*, *Atropa*, mandrake, *Duboisia*, and many other families. All of these plants have long histories of hallucinogenic use and have been connected with sorcery, witchcraft, native medicine, and magico-religious rites dating back to 1500 B.C. Table 28.1 lists the anticholinergic plants of the Solanaceae family, including their common names and distributions.

The anticholinergic syndromes caused by belladonna alkaloids have been known for a long time. Mandrake, one of these types of plants, was an anesthetic used by the Romans (Hanus et al. 2005); it was also used as a fertility drug, aphrodisiac and hallucinogen in Europe during medieval times and the Renaissance (Aziz et al. 2000). Belladonna – probably the most famous plant that causes anticholinergic poisoning – is a well-known pupil dilator in cosmetic and medical uses. Jimsonweed might be found in teas during Indian Rites and tribal ceremonies in North America (Shultes 1969); it was also used to treat wounds, ulcers, hemorrhoids, and other health problems by Mayans in South America (Litzinger 1994). Moreover, it was discovered to have an antiasthmatic property. Acute datura poisoning is often caused by drinking tea containing the plant (Klein-Schwartz and Oderda 1984). In addition, datura preparations are commonly found in powders used to treat asthma and in Chinese herbal treatments, and exposure to these compounds leads to the risk of intoxication (Cohen 1996).

Atropa belladonna

Atropa belladonna, (Fig. 28.1a, b) or deadly nightshade, belongs to the family Solanaceae and is found in Europe, Western Asia, and North Africa. Belladonna is an herb that has been used for centuries for a variety of indications, including headache, menstrual symptoms, peptic ulcer, inflammation, and motion sickness. Due to wide distribution, several synonyms can be found in literatures and different languages, such as belladonna, deadly nightshade, devil's cherries, devil's herb, naughty man's cherries, poison black cherries, and so on. The leaves and berries of this plant contain anticholinergic alkaloids, which may be extremely toxic or can be used pharmaceutically (Barceloux 2008). "Atropa" comes from the Greek name Atropos, one of the three fates in Greek mythology, and "belladonna" originally means "beautiful woman" in Italian because of the dilated pupils caused by alkaloids make the women more attractive (Tombs and Silverman 2004).

Table 28.1 Plants with anticholinergic tropane alkaloids – Solanaceae Family (nightshade)

Scientific name	Common name	Alkaloids	Distribution
Atropa			
A. belladonna	Belladonna; deadly nightshade	Atropine, scopolamine, hyoscyamine	Europe, North Africa, Western Asia
A. acuminata[a]	Indian Belladonna		Himalayas from Kashmir to Baluchistan
A. komarovii	Turkmenistan Belladonna		Central Asia
Brugmansia			
B. arborea	Angel's Trumpet	Atropine, scopolamine, hyoscyamine	Tropical area of globe
B. aurea			
B. insignis			
B. sanguinea			
B. suaveolens	Aromatic angel's trumpet		America, Asia, Africa, Australia
B. versicolor			
B. vulcanicola			
Datura			
D. stramonium	Moonflowers, Jimsonweed	Atropine, scopolamine, hyoscyamine	The temperate and tropical regions of the globe
	Jamestown weed		
	Thorn Apple		
	Downy Thorn apple		
	Devil's Trumpet		
	Angel's Trumpet		
	Mad Apple		
	Stink Weed		
	Tolguacha		
D. ceratocaula	Torna loco, desert thorn-apple, long-spined thorn-apple		
D. discolor			
D. ferox L.			
D. inoxia Mill.	Thorn-apple, downy thorn-apple, Indian-apple, moonflower, sacred datura, toloatzin, toloache		
D. leichhardtii (*D. pruinosa*)	Leichhardt's datura		
D. metel L.	Devil's trumpet		
D. quercifolia	Oak-leaf thorn-apple		
D. wrightii	Sacred datura, sacred thorn-apple		
Mandragora	Mandrake	Atropine, scopolamine, apoatropine, hyoscyamine	Southern and central Europe, Mediterranean and the Himalayas

(*continued*)

Table 28.1 (continued)

Scientific name	Common name	Alkaloids	Distribution
M. officinarum	True mandrake		Mediterranean region and the Himalayas
M. turcomanica	Iranian Mandrake		USSR, the western Kopetdag
M. caulescens	Himalayan mandrake	Hyoscine and anisodamine	Southwest Asia, Himalayan
Duboisia			
D. arenitensis	Pituri, Pitchuri	Scopolamine	Australia
D. hopwoodii	Thorn apple	Hyoscyamine	
D.leichhardtii	Emu bush, emu poison bedjeri	Nicotine	
D.myoporoides			
Henbanes-20 species			
Hyoscyamus niger	Black henbane	Hyoscyamine, Scopolamine	Europe, America
H. albus	White henbane		Southern Europe
H. aureus	Golden henbane		S. Europe to W. Asia
H. muticus	Egyptian henbane		Tropical areas of Africa. Egypt
Scopolia-5 species			
S. carniolica	Belladonna Scopola	Scopolamine	Europe, Asia
S. japonica	Japanese belladonna	Hyoscyamine	
		Cuscohygrine anisodamine	

[a]Ashtiania and Sefidkonb (2011)

Atropa belladonna has remarkable flowers in the shape of a bell and in purple with green tinges (Barceloux 2008). Its fruits are sweet, even though they contain alkaloids, in order to attract animals that can disperse their seeds. *A. belladonna*, which is not commonly used in gardens, is usually found in moist, dark places in soils rich with limestone.

The whole plant of *A. belladonna* contains tropane alkaloids, including atropine, scopolamine and hyoscyamine (Barceloux 2008). The root may be the most toxic part, but the berries and leaves may cause most of the poisoning cases. The symptoms of belladonna poisoning include pupil dilation, sensitive to light, tachycardia, flushing, dry mouth, urinary retention, confusion, hallucination, and convulsion (Barceloux 2008). The alkaloids disrupt the parasympathetic nervous system, making it unable to control involuntary activities and leading to death. The antidotes for *A. belladonna*, same as atropine overdose, is physostigmine.

Since the extract of *Atropa belladonna* can interfere with the muscarinic receptors in the muscles that control pupil size, it was once used in cosmetics (Barceloux 2008).

Fig. 28.1 (**a**, **b**) The flowers and fruits of *A. belladonna* (http://www.dr.hauschka.com/en_DE/knowledge-base/medicinal-plant-facts/deadly-nightshade)

However, due to the adverse effects, such as increased heart rate and minor visual distortions, it is no longer used in cosmetics. On the other hand, belladonna was known as an herbal medicine used for pain and muscle relaxation and as an antiinflammatory. It might also be used for the treatment of motion sickness and mental diseases. In addition, belladonna powder, tinctures, and salt mixtures are still used pharmaceutically. With phenobarbital the alkaloids can treat various gastrointestinal disorders.

Datura: *Datura stramonium* as the Representative

Datura is a well-known anticholinergic plant with several common names, such as jimsonweed, angel trumpet, or devil's weed due to its morphological, phytochemical, and pharmacological properties. It is estimated that there are 15–25 different Datura species native to many parts of the world, including Europe, North America, North Africa, and eastern and southwestern Asia (Barceloux 2008). *Datura stramonium* is an annual weed with rank odor, a large white taproot, and a strong stem. The dark green leaves are waxy and large with scalloped borders. The flowers are white and tubular, bloom to a trumpet shape, and vary in length in late spring; the fruits mature in late fall (Fig. 28.2a, c). The seed pods or capsules may be

Fig. 28.2 The plants of Datura and Brugmansia with flowers and fruits (**a**) *D. stramonium* (On the courtesy of Dr. Jung-Kun Lu) (**b**) *B. suaveolens* (On the courtesy of Dr. Jung-Kun Lu) (**c**) *D. metel* (On the courtesy of Dr. Jung-Kun Lu) (**d**) spinous fruits of Datura

50–60 mm in length and are covered with small spines that are soft and feathery when the capsules are immature, but the spines become rigid and "needle" sharp as the capsules develop (Fig. 28.2d).

The tropane alkaloids primarily distribute in seeds and flowers of the *Datura* plants (DeFrates et al. 2005). In general, the ratio of tropane alkaloids varies among *Datura* species. Therefore, it might be important to recognize which part(s) of the plant was ingested. Moreover, the toxicity of an individual plant might depend on the growing environment, location, and age, making *Datura* poisoning unique in clinical settings (Adams and Garcia 2005). Similar to other plant species that contain the alkaloids, the signs and symptoms of *D. stramonium* poisoning include dry mouth, dilated pupil, hallucinations, tachycardia, fever, flushing, fever, ataxia, disorientation, and amnesia (Gowdy 1972). Also, it can be distinguished from adrenergic excess by the absence of bowel sounds and diaphoresis. The plant's toxicity is not very lethal; deadly results are usually caused by trauma or drowning (Coremans et al. 1994; Gowdy 1972).

Other than giving the antidote physostigmine, administration of active charcoal can effectively reduce absorption through the GI tract (Barceloux 2008). Also, benzodiazepines can be used to treat agitation. Later, patients may require supportive care with oxygen, hydration, and other treatments of symptoms (Bliss 2001).

Mandrake

The plant *Mandragora officinarum*, with the common name of Mandrake, is a solanaceous plant that is native to southern and central Europe and in lands around the Mediterranean Sea, as well as on Corsica. It is a perennial herb with oblong ovate leaves, blue violet flowers, and globular, orange to red berries resembling small tomatoes (Piccillo et al. 2002). All parts of *Mandragora* species (leaves, seeds, berries, and roots) are poisonous. Mandrake contains deliriant hallucinogenic tropane alkaloids, such as atropine, scopolamine, apoatropine, hyoscyamine; and the roots sometimes contain bifurcations causing them to resemble human figures. Due to the "human" shape of the Mandrake root and its narcotic and poisonous effects, it was also known as a magic plant that could be used as an aphrodisiac and hallucinogenic, and is closely associated with witchcraft and was sometimes called the "Devil's herb" (Piccillo et al. 2006; Hanus et al. 2005). This plant dates back thousands of years, induces a state of oblivion, and was once used as an anaesthetic for surgery when available in sufficient quantities. It was also used as an emetic and as antidote for snakebites (Ramoutsaki et al. 2002). Mandrake roots have long been used in magic rituals. The berries of the plant are also called love apples because they were believed to increase fertility (Nikolaou et al. 2012).

Others

In addition to *Datura* or *Atropa*, there are some other minor plants containing similar or related toxic alkaloids in some parts of plants distributed in the world.

Brugmansia (Fig. 28.2b) are also called angel's trumpets due to their large, fragrant flowers. They are different from *Datura* in that they are woody trees or shrubs with no spine on the fruit; but they are poisonous in all parts of tree due to rich in scopolamine, hyoscyamine, and several other tropane alkaloids. The clinical toxic effects include paralysis of smooth muscles, confusion, tachycardia, dry mouth, diarrhea, migraine headaches, visual and auditory hallucinations, mydriasis, rapid onset cycloplegia, and death (Evans and Lampard 1972). *Brugmansia* are native to tropical regions of South America, along the Andes from Venezuela to northern Chile, and also in southeastern Brazil.

Duboisias also belongs to the Solanaceae family and occurs in Australia. Their leaves contain alkaloids of nicotine, scopolamine, and hyoscyamine and have been used for stimulant, euphoric, antispasmodic, and analgesic effects by indigenous peoples of central Australia (Pearn 1981; Pellowe and Poncia 2013).

Henbane, *Hyoscyamus niger*, known as stinking nightshade, is also a plant of the family Solanaceae and is globally distributed. Henbane ingestion can induce hallucinations, dilated pupils, restlessness, flushed skin, tachycardia, convulsions, vomiting, hypertension, hyperpyrexia, and ataxia in humans due to hyoscyamine, scopolamine, and other tropane alkaloids found in the plant's foliage and seeds (Doneray et al. 2007).

Cases of anticholinergic syndrome resulting from ingestion of yellow lupine seeds have been reported in literature (Pingault et al. 2009; Di Grande et al. 2004) The toxic principle was noted to be lupanine, the origin of bitter taste of lupine seeds.

Scopolia is also a genus of five species of flowering plants in the family Solanaceae, native to Europe and Asia. Its root and root-like stem (rhizome) have been used as medicine. *Scopolia* is used for spasms of the digestive tract, bile ducts, and urinary tract. It is also used to increase urine production, induce muscle relaxation and sleep, dilate eye pupils, and relieve pain. *Scopolia* contains several chemicals, such as hyoscyamine, atropine, and scopolamine; an overdose can cause anticholinergic syndrome (Cheng et al. 2002).

Clinical Manifestations of Anticholinergic Syndrome

Anticholinergic syndrome is not a rare toxidrome for clinical toxicologist. The principle chemicals in those plants causing anticholinergic syndromes are tropane belladonna alkaloids, mainly scopolamine and hyoscyamine (Hudson 1973). The ratio of these compounds varies from species to species, leading to slight differences in the signs of the intoxication (Miraldi et al. 2001). The proportion of each alkaloid present varies among species, time of year, location, and part of the plant. As little as 1½ teaspoon of *Datura* seed, equivalent to 0.1 mg of atropine per seed, has caused death from cardiopulmonary arrest. There are also chemicals playing minor roles, just like atropine. The distribution of toxins in plants may be different, too. Therefore, there is no standard concentration when eating the plants (Barceloux 2008).

The strong anticholinergic alkaloids are competitive antagonists to acetylcholine on the specific receptors. Those receptors sit at peripheral muscarinic nervous terminals that control cardiac muscle, smooth muscle, and exocrine glands; in the CNS, they can be found in the cortex and subcortical regions of the cerebrum, which are related to mental status (Barceloux 2008). The induced anticholinergic syndromes are dose related. At low doses, patients develop peripheral signs such as mydriasis, which is due to the blockade of papillary sphincter muscle and iris muscle (Tombs et al. 2004); dry mouth, secondary to parasympathetic blockade of salivary secretion and sinus tachycardia caused by competition at muscarinic receptors in postganglionic parasympathetic neurons and blockade of receptors in the SA node; and fever and erythema because of vasodilation and inhibition of sweating. At higher doses, the central nervous system effects start to appear, from confusion and agitation to delirium and hallucination. Coma may also be a severe CNS effect. These effects are dose-dependent. Although both alkaloids of atropine and scopolamine are noted to produce CNS effects that include delirium, hallucinations, agitation, and excitation, the hallucinogenic effects are attributed largely to scopolamine.

Altered mental status is usually the most frequent and prominent clinical manifestation. Hallucination occurs in more than 50 % of patients. Auditory

Table 28.2 Peripheral and central nervous system effects due to anticholinergic plants exposure

Peripheral signs/symptoms	Central nervous system s/s
Blurred vision	Agitation, irritable
Decreased GI motility	Amnesia
Dizziness	Ataxia
Dry mouth & mucous membranes	Coma
Dry and hot skin	Mental confusion
Erythema or flushed	Delirium
Fever	Excitation
Mydriasis	Hallucinations
Palpitation & tachycardia	Myoclonus
Urinary retention	Seizure

hallucinations are less common. Frequently, you can see the patients characteristically pick at imaginary objects in the air, clothing, and bed sheets. The patients can have rapid, mumbling, fragmented, and unintelligible speech. They may also be able to answer questions appropriately with some words, but disorientation and confusion are not unusual. Patients with severe intoxication may be mute (Furbee and Wermuth 1997). Convulsion, generalized seizure, and flaccid paralysis might develop in severe cases, especially reported in cases of *D. suaveolens* due to its high scopolamine content (Hall et al. 1977). Symptoms usually occur 5–10 min after tea or broth ingestion and may continue for 24–48 h due to tropane alkaloids delaying gastric emptying and absorption. The peripheral and central anticholinergic effects are list in Table 28.2.

Aspiration pneumonia due to loss of airway protection is one of the most frequently encountered complications in patients with severe anticholinergic plant poisoning, particularly in patients complicated with seizure and respiratory failure (Schneider et al. 1996; Chang et al. 1999). Acute renal failure resulting from severe agitation and seizure has been noted as a consequence of the combination of dehydration and rhabdomyolysis (Burns et al. 2000). Death from anticholinergic plant poisoning is rare. There were few mortality reports; most deaths resulted from severe hyperpyrexia, prolonged seizure, and ventricular arrhythmias (Urich et al. 1982; Steenkamp et al. 2004).

Differential Diagnosis

The diagnosis is generally made from the patient history and clinical presentation. No routine analysis or test is available to confirm diagnosis on an emergent basis. Plant exposure history is the basic source of diagnosis. In cases of obscure exposure history with an alteration in mental status, tachycardia, urinary retention, or seizure, several conditions or substance exposure should be included in the differential diagnosis. A wide range of medical conditions and drugs can cause agitated delirium. Some organic causes should be considered, including other toxic

exposure, meningitis and sepsis, and metabolic or psychiatric causes. The timing of onset of delirium may help differentiate toxin-induced causes from organic ones, as delirium from anticholinergic poisoning usually begins more abruptly than that from organic causes, such as sepsis or uremia.

Some other classes of drugs and toxins have anticholinergic effects in addition to their major pharmacological characters. Clinicians must differentiate pure anticholinergic poisoning from poisonings in which anticholinergic toxicity is but one aspect. Tricyclic antidepressants can produce prominent effects from quinidine-like sodium channel blockade and alpha-blockade in addition to anticholinergic effects. Sympathomimetic overdose, withdraw from alcohol and sedatives, and serotonin syndrome may cause agitation, tachycardia, and hyperthermia but can usually be differentiated from anticholinergic toxicity. Sympathomimetic overdose, withdraw from alcohol and sedatives, and serotonin syndrome generally cause diaphoresis, in contradistinction to the dry and hot skin symptomatic of anticholinergic overdose. In agitated, hyperthermic patients with altered mental status, salicylate overdose should also be considered.

Some toxic mushrooms also are abused for hallucinogenic purposes and may be difficult to differentiate from the use of anticholinergic plants. *Amanita muscaria, A. pantherina*, and *A. gematta* contain ibotenic acid, muscimol, and muscazone, which have anticholinergic effects or agonistic action at gamma-aminobutyric acid (GABA) receptors, thus explaining the hallucinogenic manifestations. These isoxazole derivatives are present in various concentrations, depending on environmental conditions, the maturity of the fungus, and the season of the year. The *Psilocybe* (magic mushroom), *Gymnopilus*, *Panaeolus*, and *Psathyrella* species contain the indoles psilocybin and psilocin and also induce hallucinations and alterations in perception (Spoerke and Hall 1990). However, hallucinogenic mushrooms rarely cause severe thirst or dry and hot skin, which are characteristic of anticholinergic plants poisoning. In general, plant exposure history might be the key role in differential diagnosis.

Laboratory Examination

Diagnostic studies may be helpful in the differential diagnosis of anticholinergic plant poisoning, and these should include a drug abuse urine screen, blood glucose test, electrolyte measurement, electrocardiogram recording, and complete blood cell count. The diagnosis of anticholinergic toxicity due to plants is based on medical histories, clinical findings, and occasionally the results of a diagnostic/therapeutic trial of physostigmine. Serum creatine kinase level is important to rule out the possibility of rhabdomyolysis in patients with severe psychomotor agitation and seizures. Serum levels of atropine or scopolamine are neither helpful nor readily available in the clinical setting and might be only of forensic interest (Steenkamp et al. 2004; Papoutsis et al. 2010). Qualitative and quantitative assays of blood, urine, and vomitus for atropine, scopolamine, and hyoscyamine by using thin-layer chromatography, high performance liquid chromatography (HPLC), or

mass spectrometry may be applied but are time-consuming and not routinely available (Schneider et al. 1996; Schönberg et al. 2007). We have experience in using an automated high performance liquid chromatographic system REMEDi (Rapid EMErgency Drug identification; Bio-Rad) to detect scopolamine in 0.5–1.0 mL of patients' urine or plasma/serum to help definitely identify cases of *Datura* intoxication.

Management

Initial Treatments

Management of the poisoned patient must always begin with stabilization and maintenance of the airway, breathing, and circulation. Patients should have intravenous access, supplemental oxygen, cardiac monitoring, and continuous pulse oximetry.

Decontamination

Although systemic toxicity has been reported from cutaneous and ocular absorption, most anticholinergic toxicity due to plants results from oral ingestion. Depending on the form of plant toxin and the route and timing of exposure, gastrointestinal decontamination should be performed as early as possible. If the patient's mental status is still intact, activated charcoal (1 g/kg; maximum 50 g) should be given under airway protection. Charcoal should be withheld in patients with an agitated mental status who may not be able to protect their airway, unless tracheal intubation is performed first. However, tracheal intubation should not be performed for the sole purpose of giving charcoal. External decontamination may be necessary for topical plant extracts.

Supportive Treatment

Agitation and seizures should initially be treated with benzodiazepines. Benzodiazepines are effective, have a very high safety threshold, and can be used in high dose to control symptoms. In our experience, dosages of 5–10 mg of diazepam or 2–5 mg of midazolam or 2 mg of lorazepam intravenously are effective and can be repeated at about 10–20 min intervals as needed. Benzodiazepines have the advantage of being a nonspecific means of controlling agitation when the diagnosis is unclear. Neuroleptics, such as phenothiazines and butyrophenones should not be used to calm down patients with anticholinergic plant poisoning; they are themselves anticholinergic, and may exacerbate rather than improve symptoms. Moderate to severe cases of hyperthermia should be treated by evaporative cooling.

Dehydration and hypotension can develop secondary to severe agitation, vomiting, high fever, and decreased intake and should be treated aggressively with adequate intravenous fluids. Rhabdomyolysis also requires vigorous hydration and urinary alkalization. In cases of seizure, patients should be treated aggressively with benzodiazepines. Phenytoin is not likely to be useful and is not recommended.

Antidotal Therapy with Physostigmine

Physostigmine may beneficial as an antidotal therapy and to help confirm clinical diagnosis (Beaver and Gavin 1998). Physostigmine is an active alkaloid isolated from the African tropical vine *Physostigma venosum*, known as the Calabar bean. It is a carbamate that reversibly binds and inhibits acetylcholinesterase in both the peripheral and CNS. For acetylcholinesterase inhibition, physostigmine is much weaker than other carbamates or organophosphates that are used as insecticides. Chemically, it is a tertiary amine and can cross the blood–brain barrier. Once physostigmine blocks acetylcholinesterase, the concentration of acetylcholine at muscarinic receptors increases and usually overcomes any anticholinergic blockade. Thus, physostigmine is extremely useful in reversing the peripheral and central effects of anticholinergic toxicity. Treatment with physostigmine may clarify the diagnosis of anticholinergic poisoning. In the absence of a clear plants exposure history, patients who present with fever, agitation, and delirium usually require diagnostic testing (for example, computerized tomography of the head and lumbar puncture). In such patients, if physostigmine leads to dramatic clinical improvement, further workup is often unnecessary (Glatstein et al. 2012).

In using physostigmine, the patient should be placed on a cardiac monitor and resuscitative equipment should be available at the bedside. Physostigmine should be administrated through intravenous route due to its poor absorption orally.

Due to its potential toxicity and severe adverse side effects (Schneir et al. 2003), physostigmine might be reserved for cases of anticholinergic plants intoxication with CNS and cardiotoxicity. Caution should be exercised when giving physostigmine to patients with reactive airway disease, intestinal obstruction, epilepsy, and cardiac conduction abnormalities, as these are relative contraindications. Most patients with plant-induced anticholinergic poisoning do well with supportive care alone.

In adults, the recommended dose of physostigmine is 0.5–2 mg (0.02 mg/kg IV, up to a maximum of 0.5 mg per dose in pediatric patients). The drug should be given by slow IV push generally over 5 min. Overly rapid infusion may result in cholinergic symptoms or seizures. The half-life of physostigmine is approximately 15 min, but its effects often last significantly longer. Additional, smaller doses may be repeated after 20–30 min if agitated delirium recurs.

Extracorporeal elimination and forced diuresis of the tropane belladonna alkaloids are not viable options.

Conclusion and Future Directions

Anticholinergic syndrome due to plants is not rare. Several species of plants in the Solanaceae family contribute to this toxic effect. The typical examples are, *Atropa*, *Datura, Brugmansia* species and henbanes that contain abundant amounts of tropane alkaloids that can produce both local and systemic anticholinergic toxicity. Most exposures are the result of *Datura* use for its substance abuse properties. Fatalities due to anticholinergic plants exposure are rare, but adverse effects arecommon. Conservative treatment is the mainstay of clinical management and may include the judicious use of the cholinesterase inhibitor physostigmine.

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Poisonous Mushrooms

29

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Abstract

Mushrooms are the sexual organs or fruiting bodies of fungi. Although some mushrooms are considered to be a rich source for nutrients and biologically active compounds, some species are known because of their toxicity that may cause fatalities every year generally due to misidentification. Among thousands of mushroom species, fewer than a hundred are toxic. Mushroom poisoning is associated with different signs and symptoms that are mainly attributed to some active substances belonging to poisonous mushrooms. Most mushroom toxins cause mild or moderate signs and symptoms such as nausea, vomiting, abdominal pain, fever, and headache. However, some species result in severe poisoning. Renal failure, neurotoxicity, hepatotoxicity, rhabdomyolysis, and other toxic effects were identified in toxicity studies with various species.

The toxicity of mushroom is influenced by many factors including genus and species, geographic location, preparation prior to ingestion, and the human's susceptibility. This chapter is aimed to address various types of mushroom toxins, thus providing some information about their toxic mechanisms, a brief description of the toxicokinetics (absorption, distribution, metabolism, excretion) of mushroom toxins, and management of mushroom poisonings.

Introduction

Mushrooms are large and highly diverse group of organisms called fungi which are similar in many aspects to the plants. Among thousands of mushroom species in the world, fifty to a hundred are known to be toxic (Brent and Palmer 2007). It is very difficult to verify exposure to mushroom toxins, because clinical reports of mushroom poisoning are uncommon and there are many unreported cases (Beuhler and Graeme 2005). Serious poisoning and lethality induced by some mushroom species along with the misidentification of toxic species have greatly raised fear in clinicians (Beuhler and Graeme 2005). Proper identification is very important to avoid accidental mushroom poisoning. Diagnosis of toxic signs and symptoms provides

the success of treatment. Recently, modern technology of intensive care medicine has reduced the mortality and morbidity of mushroom toxicity (Table 29.1).

It is very important to consider the time period for exhibition of clinical presentations rather than the time of consumption in patients with suspected mushroom poisoning. Based on these findings, toxic mushrooms are generally classified as follows (Beuhler and Graeme 2005):

1. Mushrooms which exhibit symptoms within 4 h. Generally they do not induce serious toxicity.
2. Mushrooms which show symptoms more than 6 h following ingestion. Typically these mushrooms induce life-threatening syndromes.

Based on the mechanism of toxicity and clinical presentations, poisonous mushrooms can be categorized as follows.

Mushrooms-Induced Delayed Nephrotoxicity

Species of genus *Cortinarius* (including *C. splendens*, *C. orellanus*, *C. gentilis*, and *C. speciosissimus*) and *Amanita smithiana* cause delayed renal toxicity (Danel et al. 2001; Beuhler and Graeme 2005) (Fig. 29.1).

Cortinarius spp.

Cortinarius spp. poisoning is characterized by a delayed acute renal failure (Danel et al. 2001).

Toxicology

The genus *Cortinarius* contains the cyclopeptide orellanine (Danel et al. 2001), which is a heat-stable bipyridine N-oxide (3, 3′, 4, 4′-tetrahydroxy-2, 2-bipyridine-*N*, *N*′-dioxide). Orellanine chemically resembles the pyridine herbicides paraquat and diquat. In vitro studies revealed that orellanine produces oxygen-free radicals at the target site through redox cycling and/or redox activation of iron. Furthermore, it is indicated that a metabolite of the toxin can inhibit protein synthesis (Nilson et al. 2008).

In addition to orellanine, cortinarins A and B are cyclopeptides which may involve in *Cortinarius*-induced renal toxicity (Beuhler and Graeme 2005).

Clinical Presentations

The signs and symptoms of *Cortinarius* poisoning may appear between 2 and 14 days after the ingestion of mushrooms (mean 6 days and maximum 17 days) (Beuhler and Graeme 2005). The signs and symptoms include nausea, vomiting, abdominal pain, intense thirst, chills, headache, myalgia, paresthesia, polyuria or oliguria, and possibly anuria. Hemodialysis may be necessary until kidney function

Table 29.1 Main toxins, clinical features, and treatments related to mushroom poisonings

Mushrooms	Toxins	Clinical presentations	Treatments
Cortinarius spp., *Amanita smithiana*	Orellanine, cortinarins A and B, and aminohexadienoic acid	GI disturbances, chills, headache, myalgia, paresthesia, and renal dysfunction	Hemodialysis, hemoperfusion, plasmapheresis, and kidney transplantation
Clitocybe and Inocybe	Muscarine	Bradycardia, miosis, salivation, lacrimation, diarrhea and bronchospasm	Supportive, anticholinergic agents such as atropine in the presence of severe toxicity
Coprinus atramentarius	Coprine	Flushing, headache, dyspnea, sweating, arrhythmia, hypotension, and confusion	Propranolol, fomepizole
Amanita gemmata, *Amanita pantherina*, *Amanita muscaria*	Ibotenic acid and muscimol	Continues periods of excitation and inhibition in the nervous system	Supportive, sedative, and hypnotic agents
Gymnopilus spectabilis, *Panaeolus foenisecii*, *Conocybe cyanopus*, *Psilocybe caerulescens*, *Psilocybe cubensis*	Psilocybin and psilocin	Euphoria, hallucinations, tachycardia and blood pressure, mydriasis, tremors, and fever	Supportive, diazepam
Omphalotus olearius, *Chlorophyllum molybdites*		Gastroenteritis	Supportive
Tricholoma equestre, *Russula subnigricans*	Russuphelins	GI disturbances, weakness, myalgia, rhabdomyolysis and renal failure	Supportive
Amanita verna, *Amanita virosa*, *Amanita phalloides*, *Lepiota helveola*, *Galerina marginata*	Amanitin	Initial phase: latency 2nd phase: GI disturbances 3rd phase: recovery 4th phase: liver and renal failure	Silibinin, PCN and NAC
Gyromitra esculenta *Gyromitra californica*	Gyromitrin	GI irritations, neurotoxicity, liver/ renal failures, and hemolysis	Methylene blue, pyridoxine, folinic acid, NAC, vitamin K

Supportive management includes gut decontamination, fluid therapy, cardiac monitoring, etc.
NAC N-acetyl cysteine, *PCN* penicillin, *GI* gastrointestinal

Fig. 29.1 *Cortinarius speciosissimus* (Photo by Eric Steinert is licensed under CC BY-SA 3.0)

has returned (Michelot and Tebbett 1990; Tegzes and Puschner 2002; Beuhler and Graeme 2005; Brent and Palmer 2007).

Although it was shown that renal dysfunction may be improved several weeks following poisoning, chronic renal failure had been observed in nearly 30–46 % of population who were poisoned by *Cortinarius* (Beuhler and Graeme 2005; Brent and Palmer 2007).

Treatment

Because the onset of toxicity is delayed, patients usually do not present symptoms early after the ingestion. It is unlikely that gastric lavage with activated charcoal is helpful (Brent and Palmer 2007). Hemodialysis is indicated in cases of acute renal failure. There is currently no specific treatment for such mushroom poisoning; however, hemoperfusion and plasmapheresis have been recommended (Beuhler and Graeme 2005). At the late stage of chronic renal failure, kidney transplantations might be carried out (Michelot and Tebbett 1990; Danel et al. 2001).

Amanita smithiana

Amanita smithiana is a mushroom which is also found to induce delayed nephrotoxicity (Goldfrank 2009; Fig. 29.2).

Toxicology

It is established that aminohexadienoic acid (allenic norleucine) present in *Amanita smithiana* is responsible for toxicity. Mechanism of *A. smithiana* toxicity is similar to that of orellanine (Beuhler and Graeme 2005; Goldfrank 2009).

Clinical Presentations

Usually the patients experience asymptomatic period which ranges from 30 min to 12 h (mean 6 h). The onset of symptoms is earlier than orellanine toxicity. Toxic

Fig. 29.2 *Amanita smithiana* (Photo by Nathan Wilson is licensed under CC BY-SA 3.0)

ingestion causes a syndrome of gastroenteritis including nausea, vomiting, abdominal pain, lethargy, headache, and myalgia followed by delayed onset renal failure within 1 week (2–5 days) with increase in BUN and creatinine. ALT and LDH are found to be increased as well, which may be due to liver damage (Beuhler and Graeme 2005; Goldfrank 2009; Brent and Palmer 2007; West et al. 2009). However, the increase in amylase, ALP, and bilirubin is uncommon (Donnelly and Wax 2005).

Treatment

No specific treatment has been reported. In most patients, renal failure would be recovered after 4 weeks (80 %), but sometimes dialysis is recommended (Beuhler and Graeme 2005).

Mushroom-Induced Muscarinic Syndrome

Species of genus *Clitocybe* (including *C. illudens* and *C. dealbata*) and *Inocybe* (including *I. geophylla* and *I. iacera*) cause muscarinic syndrome (Young 1994; Beuhler and Graeme 2005; Goldfrank 2009; Fig. 29.3). Also, *Rhodophyllus*

Fig. 29.3 *Inocybe geophylla* (Photo by Luridiformis is licensed under CC BY 3.0)

rhodopolius which is found in Japan causes muscarinic syndrome (Beuhler and Graeme 2005; Goldfrank 2009). The amount of muscarine in *Amanita pantherina* and *Amanita muscaria* is not sufficient to induce toxicity in human (Michelot and Melendez-Howell 2003).

Toxicology

Muscarine is a quaternary ammonium compound which is found in the abovementioned mushrooms. Similar to acetylcholine, muscarine activates postganglionic muscarinic receptors and produces cholinergic syndromes (Young 1994).

Clinical Presentations

Clinical signs develop within 30 min to 2 h of ingestion (Lima et al. 2012). Because of its quaternary structure, muscarine does not cross the blood–brain barrier, and its cholinergic effects are entirely peripheral. The signs and symptoms include bradycardia, miosis, salivation, lacrimation, diarrhea, nausea, vomiting, and bronchospasm (Stallard and Edes 1989; De Haro et al. 1999; Salhab 2007). Muscarine is poorly absorbed after oral exposure; therefore, the severity of cholinergic syndrome due to the consumption of these mushrooms is less than organophosphate compounds. Moreover the stimulation of nicotinic receptors was not observed (Beuhler

and Graeme 2005; Goldfrank 2009; Brent and Palmer 2007). Muscarine is not susceptible to inactivation by acetylcholinesterase, so the duration of its effect could be more prolonged compared to acetylcholine. Clinical signs are usually self-limited and would last for about 6–24 h if large amounts were consumed (Brent and Palmer 2007).

Treatment

Sever poisoning is rare. Treatment includes early decontamination, administration of activated charcoal, and fluid therapy. If life-threatening clinical signs are present, atropine should be administered. The dose in adults and children are 1–2 mg and 0.02 mg/kg via continuous i.v. injections, respectively (Goldfrank 2009; Beuhler and Graeme 2005). The best criteria for therapeutic endpoint with atropine include ease of respiration and lack of respiratory secretions. It has been shown that glycopyrrolate is safer than atropine because it does not cross the blood–brain barrier. The doses in adults and children are 0.05–0.1 mg and 0.005–0.01 mg/kg via continuous i.v. injections, respectively. In patients with bronchospasm, inhalation of ipratropium bromide is also recommended (Beuhler and Graeme 2005; Goldfrank 2009; Brent and Palmer 2007).

Mushroom-Induced Disulfiram-Like Syndrome

Coprinus atramentarius is a well-known mushroom which produces disulfiram-like syndrome (Carlsson et al. 1978; Fig. 29.4).

Toxicology

Coprinus atramentarius contains amino acid coprine. Coprine is metabolized to the active compound named as aminocyclopropanol (ACP). ACP inhibits aldehyde dehydrogenase which is responsible for acetaldehyde hydrolysis. As a result of coprine ingestion, acetaldehyde accumulates in the body. When ethanol is consumed simultaneously or during 24–72 h after coprine, disulfiram-like syndrome will occur. Although coprine acts similar to that of disulfiram, it is an alkylating agent and mutagenic (Beuhler and Graeme 2005). Coprine is heat stable and remains toxic even after being cooked (Carlsson et al. 1978; Michelot 1992; Beuhler and Graeme 2005; Brent and Palmer 2007).

Clinical Presentations

Signs and symptoms begin within minutes of ethanol ingestion and include flushing (face and neck), headache, metal taste, nausea/vomiting, dyspnea, chest pain,

Fig. 29.4 *Coprinus atramentarius* (Photo by Markus Hagenlocher is licensed under CC BY-SA 3.0)

sweating, tachycardia, premature ventricular contraction, atrial fibrillation, hypotension, hypothermia, confusion, and coma. The symptoms could improve within 24 h (Michelot 1992; Beuhler and Graeme 2005; Goldfrank 2009; Brent and Palmer 2007).

Treatment

Treatment is supportive. The effectiveness of activated charcoal is not established. There is no evidence regarding the beneficial use of antihistamines to reduce flushing. Propranolol is recommended to treat sympathomimetic syndrome but may be dangerous in severe toxicity (Beuhler and Graeme 2005). Fomepizole, known as an alcohol dehydrogenase inhibitor, may reduce toxicity due to the decrease in acetaldehyde production. Fluid therapy is recommended for hypotension, and vasopressors may be required for patients in shock status. Dopamine can be preferentially used because coprine does not inhibit dopamine β-hydroxylase. In life-threatening poisoning, hemodialysis might be recommended to remove ethanol and acetaldehyde (Michelot 1992; Beuhler and Graeme 2005; Goldfrank 2009; Brent and Palmer 2007).

Fig. 29.5 *Amanita muscaria* (Photo by Onderwijsgek is licensed under CC BY-SA 3.0 NL)

Mushrooms-Induced Isoxazole Syndrome

Species of genus *Amanita* including *A. gemmata*, *A. pantherina*, and *A. muscaria* cause neurotoxicity. The major toxins of these mushrooms are ibotenic acid and muscimol (Beuhler and Graeme 2005; Tsujikawa et al. 2006; Lima et al. 2012; Fig. 29.5).

Toxicology

Ibotenic acid and muscimol are pseudoneurotransmitters. Ibotenic acid is a potent agonist of *N*-methyl-D-aspartic-acid (NMDA) receptor, and muscimol is a powerful GABA (gamma-aminobutyric acid) agonist (Tsujikawa et al. 2006). After rapid absorption, muscimol and ibotenic acid cross the blood–brain barrier via an active transport system. Ibotenic acid decarboxylates to form muscimol in the stomach, liver, and brain (Nielsen et al. 1985). Therefore, the main toxin resulting in clinical signs of poisoning is muscimol. Muscimol and ibotenic acid can be detected in urine within 1 h of exposure. Lethal dose of muscimol in rats is 25 mg/kg (Beuhler and Graeme 2005).

Clinical Presentations

Muscimol is a potent agonist of $GABA_A$, which is an inhibitory neurotransmitter. Typical clinical signs and symptoms of muscimol toxicity begin within 30 min to 2 h after ingestion and include mydriasis, dry mouth, ataxia, confusion, euphoria, dizziness, and drowsiness. Vomiting is not consistently seen in cases of isoxazole poisoning. Ibotenic acid activates glutamate receptors. After a brief period of sedation, glutamatergic manifestations appear and include muscle spasms and seizures. Continuous periods of excitation and inhibition in the nervous system could be seen during poisoning. Recoveries are recorded within 6–12 h (Stormer et al. 2004; Beuhler and Graeme 2005; Tsujikawa et al. 2006; Brent and Palmer 2007; Tsujikawa et al. 2007).

Although patients may manifest features similar to cholinergic or anticholinergic toxidromes, it is not known whether these symptoms are due to the presence of large amounts of muscarine, an unidentified compound, or due to the isoxazoles (Brent and Palmer 2007; Beuhler and Graeme 2005).

Treatment

Treatments are mainly symptomatic and supportive. Gastric decontamination should be considered. In human poisoning, the use of atropine is contraindicated because of the absence of cholinergic-like symptoms. Furthermore, physostigmine is rarely recommended because humans do not manifest true anticholinergic symptoms.

Patients with unstable clinical signs or obvious mental disorders should be admitted to intensive care unit until complete recovery (Beuhler and Graeme 2005; Brent and Palmer 2007).

Mushroom-Induced Hallucination

Mushrooms including *Gymnopilus spectabilis*, *Panaeolus foenisecii*, *Conocybe cyanopus*, *Psilocybe caerulescens*, *and Psilocybe cubensis* are known as mushrooms that induced hallucinogenic manifestations (Fig. 29.6).

Toxicology

Active toxic substances in these mushrooms include psilocybin which is an indole-like serotonin similar to LSD (lysergic acid diethylamide) and biologic amines, namely, baeocystin and norbaeocystin. Psilocybin is 50 % absorbed via orally and is rapidly dephosphorylated to psilocin. According to the literature, the main toxin is psilocin because it is lipid soluble and crosses blood–brain barrier. Psilocin is

Fig. 29.6 *Psilocybe cubensis* (Photo by Wowbobwow12 is licensed under CC BY-SA 3.0)

responsible for the central nervous system toxicities (Beuhler and Graeme 2005). Psilocin is excreted unchanged or as psilocin glucuronide in urine and to some extent unchanged via the bile (Hasler et al. 2002).

Clinical signs and symptoms of hallucinogenic mushroom poisoning are attributed to the indole–tryptamine derivatives including psilocybin and psilocin which are chemically similar to LSD and serotonin. These agents are $5HT_{2A}$ agonists, thus inducing hallucinogenic effects (Musshoff et al. 2000; Brent and Palmer 2007).

Clinical Presentations

The most common manifestation is euphoria. In humans, the psychoactive effects of psilocin are similar to those induced by LSD. The clinical manifestations are observed within 20–30 min following ingestion and include visual and auditory hallucinations. Visual hallucinations are more common. Other autonomic nervous system effects are increased heart rate and blood pressure, mydriasis, tremors, and increased temperature. The effects can last up to 8 h, but hallucinogenic activity rarely exceeds 1 h. It is reported that difficulty in concentration and visual and auditory hallucinations could occur 2 weeks after ingestion due to neuronal demyelinization (Beuhler and Graeme 2005; Goldfrank 2009; Brent and Palmer 2007).

Treatment

The management of hallucinogenic mushrooms poisoning is primarily supportive, and in most cases, treatment is not necessary. Agitated patients should be maintained in a calm environment. Gastric lavage is not recommended. The beneficial effect of activated charcoal in such poisonings is not approved, but its

Fig. 29.7 *Chlorophyllum molybdites* (Photo by Nathan Wilson is licensed under CC BY-SA 2.5)

administration can be considered. If severe neurologic signs such as seizures occur, diazepam is recommended (Beuhler and Graeme 2005; Brent and Palmer 2007).

Mushrooms-Induced Gastrointestinal Irritation

This group includes species such as *Omphalotus olearius* and *Chlorophyllum molybdites* which result in gastroenteritis as the primary clinical sign (Fig. 29.7).

Toxicology

Although muscarine is not found in *O. olearius*, sometimes it is considered as muscarine-containing mushrooms. Similar to the muscarinic symptoms, clinical features of poisoning are vomiting, nausea, diarrhea, abdominal pain, lethargy, and blurred vision (French and Garrettson 1988). The most common signs of muscarinic syndrome are shown except salivation and lacrimation (Beuhler and Graeme 2005).

Clinical Presentations

These mushrooms exhibit gastroenteritis early after ingestion. It was shown that ingestion of only part of one *C. molybdites* may result in gastroenteritis

along with clinical signs and symptoms of nausea, vomiting, and diarrhea which occur 1–2 h after ingestion. Diarrhea is a common sign. Moreover some cases exhibited bloody diarrhea (Blayney et al. 1980). Other clinical signs and symptoms include electrolyte abnormalities, abdominal pain, sweating, and dizziness. Although most patients could recover after 4–6 h, complete recovery had been shown to be as late as 24–48 h post-ingestion in severe cases (Beuhler and Graeme 2005).

Treatment

Treatment is nonspecific and should focus on rehydration and correction of serum electrolyte abnormalities. Antiemetics should be recommended. Vasopressors are often useful in hypotensive patients. Vomiting is a hallmark of poisoning by gastrointestinal irritant mushrooms. Thus, emetics are not recommended. Activated charcoal is thought to have benefit when administered within 1 h after ingestion (Beuhler and Graeme 2005; Goldfrank 2009).

Mushroom-Induced Rhabdomyolysis

Tricholoma equestre and *Russula subnigricans* are known as mushrooms that induced rhabdomyolysis (Beuhler and Graeme 2005; Brent and Palmer 2007; Fig. 29.8).

Fig. 29.8 *Tricholoma equestre* (Photo by Matthias Renner is licensed under CC BY-SA 3.0)

Toxicology

Although the mechanism of toxicity is not fully known, it is indicated that russuphelins is probably responsible for *R. subnigricans* toxicity (Beuhler and Graeme 2005; Goldfrank 2009; Brent and Palmer 2007).

Clinical Presentations

Clinical signs and symptoms in humans appear 2 h after ingestion and include diarrhea, nausea, and vomiting. Complete recovery could occur after 24 h. In severe poisoning, muscular weakness, fatigue, myalgia, and rhabdomyolysis have been reported. Renal failure and metabolic acidosis could also be present (Bedry et al. 2001; Karlson-Stiber and Persson 2003).

Treatment

Treatment is completely supportive and is recommended as soon as possible (Beuhler and Graeme 2005).

Mushroom-Induced Delayed Gastroenteritis and Liver Failure

Most fatalities are reported by exposure to cyclopeptide-containing mushrooms. Species of genus *Amanita* (including *A. verna*, *A. virosa*, and *A. phalloides*), *Lepiota helveola*, *and Galerina marginata* are known as mushrooms that contain cyclopeptides (Donnelly and Wax 2005; Goldfrank 2009; Fig. 29.9).

Toxicology

The most toxic cyclopeptide-containing mushroom is *A. phalloides*, the ubiquitous "death cap." Amanitin was isolated from *A. phalloides* in 1940 by Hallermayer. There are three groups of cyclopeptides, including the amatoxins, phallotoxins, and virotoxins. Amatoxins are bicyclic octapeptides and include the amanitins (α, β, γ,). Severe poisonings and lethality are mainly attributable to the amanitins. Phallotoxins are bicyclic heptapeptides and potent hepatotoxin. Phallotoxins are thought to be the cause for gastrointestinal toxicity (Barbato 1993; Donnelly and Wax 2005). Because of heat stability of amatoxins, poisoning could and usually occur after cooking (Jaeger et al. 1993; Donnelly and Wax 2005). Amanitins exert their toxicity by the inhibition of nuclear RNA polymerase II (Lindell et al. 1970; Wieland 1983), which results in impaired protein synthesis and cell death. Other

Fig. 29.9 *Amanita phalloides* (Photo by Archenzo is licensed under CC BY-SA 3.0)

toxic mechanisms include the induction of apoptosis (Leist et al. 1997), production of reactive oxygen species (ROS), and depletion of hepatic glutathione (Enjalbert et al. 2002). Amanitins are poorly but rapidly absorbed from the gastrointestinal tract. α-Amanitin may be enterohepatically recirculated (Goldfrank 2009). Excretion is particularly renal, but significant amounts are also excreted in bile and feces (Jaeger et al. 1993).

Clinical Presentations

The clinical signs and symptoms can be divided into four phases: The initial phase or the latency period lasted for about 6–24 h (mean 8–12 h). The second phase is recognized by severe gastrointestinal manifestations including nausea, vomiting, bloody diarrhea, and severe abdominal pain. Gastrointestinal signs improved after 60 h (Vogel et al. 1984). The gastroenteric phase is often followed with a lag time of several hours to a few days. During this phase, the patients will seem to have recovered. Within the third phase, monitoring of liver and kidney functions is recommended to avoid misdiagnosis. The final stage or fourth phase

begins approximately 36–84 h after exposure to amanitins. In this stage, liver, renal, and multiorgan failure may occur. Elevation in serum AST, ALT, ALP, and bilirubin are commonly observed (Vogel et al. 1984; Mas 2005). Coagulopathy, encephalopathy, and coma are also present with liver failure (Vogel et al. 1984). If patients survive beyond hepatic failure, renal failure may happen as a result of proximal and distal tubular necrosis (Tegzes and Puschner 2002). Clinical signs of renal failure include polyuria, polydipsia, vomiting, and anorexia (Santi et al. 2012). Severe hypoglycemia may occur after the gastrointestinal phase and is accompanied with the breakdown of liver glycogen (Donnelly and Wax 2005; Mas 2005). Death usually occurs due to cyclopeptide-containing mushrooms at days of 6–16 after ingestion (Donnelly and Wax 2005; Brent and Palmer 2007).

Treatment

The use of activated charcoal may be beneficial in adsorbing toxins within the gastrointestinal tract as well as those that reenter it due to enterohepatic recirculation. The recommended dose is 1 g/kg every 2–4 h for speeding the rate of toxin elimination (Goldfrank 2009). Also a number of decontamination procedures have been applied in humans and include hemodialysis, hemoperfusion, plasmapheresis, forced diuresis, and nasoduodenal suctioning. Close monitoring, fluid replacement, and supportive care nevertheless are the essential parts of the treatment of amanitin poisoning. As part of vigorous supportive care, i.v. fluids, correction of hypoglycemia and electrolyte abnormalities, vitamin K1, and plasma transfusions should be considered dependent on the clinical presentations of each poisoned patient. Liver transplantation has been used successfully in patients with severe liver failure (Donnelly and Wax 2005; Goldfrank 2009; Brent and Palmer 2007).

Some antidotes are available to treat amanitin poisoning. These include silibinin, penicillin, and *N*-acetylcysteine (NAC) which are most commonly recommended along with decontamination procedures and supportive care (Ward et al. 2013). Although the exact mechanisms of silibinin and penicillin are not fully understood, both compounds reduce the uptake of amanitins into hepatocytes. Silibinin (also known as silybin) is the main component of silymarin and provides most of the hepatoprotection that is related to milk thistle *(Silybum marianum)*. Silibinin is a free radical scavenger and has immunostimulatory and iron-binding properties (Mayer et al. 2005; Karimi et al. 2010). It is reported that silibinin could interact with the enterohepatic recirculation of amanitin. Experimentally, silibinin was shown to be effective. The recommended i.v. dose of silibinin in humans is an initial bolus infusion of 5 mg/kg followed by a continuous infusion of 20 mg/kg/day for a minimum of 3 days (Karlson-Stiber and Persson 2003; Donnelly and Wax 2005; Brent and Palmer 2007). The recommended dose for oral preparations is 140 mg, two to three times per day. Side effects of silibinin administration are rare but may include anaphylactic reactions, mild laxative effects, and interactions with

certain phase I and phase II metabolic enzymes (Venkataramanan et al. 2000). Recently, the efficacy of penicillin G alone, not in combination with silibinin, was shown to be ineffective in humans with amanitin poisoning (Enjalbert et al. 2002). Based on animal and retrospective human studies, the recommended dose of penicillin G is 300,000–1,000,000 IU/Kg/day (Brent and Palmer 2007). Research has shown that the administration of silibinin appears to have greater therapeutic benefit than penicillin G at least in humans (Enjalbert et al. 2002). The use of antioxidants in amanitin poisoning was also established. It was shown that NAC is as useful as silibinin in reducing mortality in humans after amanitin poisoning (Enjalbert et al. 2002). It is believed that NAC could be beneficial to reduce the development of encephalopathy, renal failure, and coagulopathy. Thioctic acid is also used in the treatment of amanitin poisoning and could increase the rate of survival of poisoned patients. Steroids, hyperbaric oxygen, cimetidine, ascorbic acid, D-penicillamine, and diethyldithiocarbamate are also recommended (Donnelly and Wax 2005; Brent and Palmer 2007). Controversy still remains about the efficacy of many of these procedures as specific data do not exist.

Mushroom-Induced Central Nervous System Abnormalities and Hemolysis

Gyromitrin is found in species including *Gyromitra esculenta* and *Gyromitra californica*. It is reported that *G. esculenta* is thought to be one of the mushroom species which induce severe poisoning (Brooks and Graeme 2005; Goldfrank 2009; Fig. 29.10).

Toxicology

This group of mushrooms produces gyromitrin (acetaldehyde *N*-methyl *N*-formylhydrazine). It is a toxin which can be partly removed by boiling and/or drying. After hydrolysis of gyromitrin in the stomach, methylformylhydrazine and monomethylhydrazine are formed. Hydrazines caused toxicity similar to that of isoniazid. As soon as hydrazines reach the liver, they are further metabolized to reactive intermediates, such as methyl cations and free methyl radicals (Gannett et al. 1991). It is established that monomethylhydrazine can inhibit pyridoxal phosphokinase, an enzyme responsible for the formation of pyridoxal phosphate. Enzyme inhibition results in decreased pyridoxal 5-phosphate concentration (Lheureux et al. 2005). The inhibition of glutamic acid decarboxylase, an enzyme responsible for the formation of γ-aminobutyric acid (GABA), was also reported. Depletion of GABA and an increase in glutamic acid concentration lead to seizures (Michelot and Toth 1991; Brooks and Graeme 2005; Goldfrank 2009; Brent and Palmer 2007).

Fig. 29.10 *Gyromitra esculenta* (Photo by Severine Meißner is licensed under CC BY-SA 3.0)

Clinical Presentations

Gyromitrin is considered as a gastrointestinal irritant which leads to clinical signs of vomiting, abdominal pain, and diarrhea 6–48 h after ingestion of the poisonous mushroom. Most patients exhibit only mild gastrointestinal symptoms and recover fully within several days after exposure. However, in some cases, especially in patients with severe poisoning, neurotoxicity, liver and renal failures as well as hemolysis could also occur (Brooks and Graeme 2005; Goldfrank 2009; Brent and Palmer 2007). Other clinical signs and symptoms that may be present include vertigo, sweating, diplopia, headache, dysarthria, incoordination, ataxia, seizures, coma, hemolysis, methemoglobinemia, rhabdomyolysis, myalgia, hypoglycemia, and electrolyte abnormalities (Berger and Guss 2005). *N*-methyl-*N*-formylhydrazine is found to inhibit cytochrome P450 and glutathione-metabolizing enzymes (Braun et al. 1979) and can lead to liver necrosis. Moreover, the highly reactive metabolites, such as methyl cations generated in the liver, may significantly contribute to hepatic injury (Toth and Gannett 1994).

Treatment

Management is mainly supportive. Because of the delayed onset of clinical symptoms, early decontamination is not possible. Activated charcoal has been recommended, although its efficacy has not been proved. Fluid therapy and correction of electrolyte imbalances are also important. In patients with methemoglobinemia, methylene blue should be provided. The recommended dose is 1–2 mg/kg via i.v. injection (maximum 7 mg/kg). Caution is recommended because higher doses of methylene blue could induce oxidative stress. In patients with G6PD deficiency, methylene blue is contraindicated (Brooks and Graeme 2005). Administration of pyridoxine is also provided. The recommended dose in humans is 25–50 mg/kg i.v. over 15–30 min. The dosing can be repeated in patients manifesting coma or recurrent seizures but should not exceed 20 g/day. While pyridoxine can effectively control seizures, it has no benefit in preventing liver injury (Brooks and Graeme 2005; Goldfrank 2009; Brent and Palmer 2007). Pyridoxine can be used alone or in combination with diazepam for the control of seizures. It is believed that the efficacy of combination therapy is better than that of pyridoxine alone (Villar et al. 1995; Goldfrank 2009; Brent and Palmer 2007). Phenobarbital is not recommended for the management of seizures because of its cytochrome P450-inducing capability (Brooks and Graeme 2005). Administration of folinic acid has been recommended in humans because hydrazine inhibits the formation of activated folate. The recommended dose is 5–15 mg/kg (i.v., i.m., or orally) for 5–7 days. The cytochrome P450 inhibitors such as cimetidine and also NAC should be considered to prevent hepatic injury (Brooks and Graeme 2005). Thioctic acid is also recommended due to its antioxidative property. Vitamin K (0.5–10 mg/kg, i.v., i.m., or orally) could be used in the presence of liver injury (Brooks and Graeme 2005).

Conclusion and Future Directions

Toxicities induced by some commonly consumed mushrooms are described with more emphasis on the mechanisms of toxicity, main toxins, clinical signs, and therapeutic approaches. Because of the occurrence of many accidental poisonings, proper identification is very important to avoid accidental mushroom poisoning. Furthermore, the prompt identification of signs and symptoms of mushroom poisoning provides the success of treatment. In severe poisoning induced by agents such as amatoxins, intensive care is necessary to save lives, and rapid toxin identification would help clinicians to confirm the diagnosis earlier and then commence proper management. Currently, therapeutic approaches are primarily based on both the mechanisms of toxicity and clinical signs of mushroom poisoning. Our knowledge regarding appropriate analytical techniques for specific mushroom toxins should be updated frequently, and further research is necessary in order to develop timely analytical techniques that are based on toxin characterizations.

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Plants and Herbal Poisoning in Bangladesh 30

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Abstract

Acute poisoning caused by plant is not uncommon in Bangladesh. Intentional or accidental plant poisoning sometimes results in morbidity and significant death. People have been traditionally using medicinal plant from the very early period of civilization, and this practice is still continued in a well-established manner, especially for the treatment of some chronic non-curable disease; medicinal plants are considered nontoxic by the general public due to their natural origin. The mixture of different plants and herbs made by a traditional healer and their canvassing on the street magnetize a wide range of people. The easy low-cost availability and the consumption of unknown and inappropriate mixture of mysterious medicinal plants sometime cause hepatic and renal toxicity, even deaths which are mostly unreported. Inadvertent deaths do occur in children in Bangladesh due to plant poisoning. Food scarcity during a disaster also drives poor people to deviate their usual consumption practices and take immature or nonprocess plants which are occasionally fatal. Plant poisoning for suicidal attempt is now declining mostly due to the availability of other easy method like agrochemical and pharmaceutical drug. Suicidal attempt by yellow oleander is infrequently reported in different hospitals. In Bangladesh, once *Datura* plant seeds were popularly used by miscreants as a stufying agent offered through different food items to a bystander to make them stupor in order to rob the capital. A separate group of poisonings with significant mortality and morbidity occurs after women use plants to induce abortions. Treatment for most plant poisoning is symptomatic, and specific antidotes are used in only a very few cases.

Introduction

Poisonous plants have been the subject of practical tradition since ancient times. Thus, it is very difficult to draw a distinct boundary between the poisonous plants and the medicinal plants as most of these plants qualify for both categories. Acute poisoning induced by plant, plant product, or its derivatives which produce deleterious effects on human and other animals' body and sometimes causes their death (Banglapedia 2013). Plant exposures rank sixteenth in adults in the list of the exposures most commonly reported to poison control centers in one of the largest medical college hospitals in Bangladesh (Basher et al. 2011).

Plant poisoning is common but unaddressed cause of death in South Asian countries like India, Bangladesh, and Sri Lanka. An estimated 5,000 species of phanerogamous plants grow in Bangladesh, about 500 of which are regarded as medicinal plants as they possess therapeutic properties. Some of these medicinal

plants (about 50) are also classified as poisonous plants as they produce toxic effects on the animal system, if they are not used carefully or in regulated amount (Banglapedia 2013).

A particular plant acquires the poisonous property when it accumulates some special types of chemical substances like alkaloids, glycosides, toxalbumins, and the like in substantial quantities in its cells. Indigenous systems of medicine in this subcontinent had studied and recorded detail accounts of poisonous plants way back in 1500 BC (Banglapedia 2013). Some of them are, of course, drastically poisonous, i.e., causes death immediately after administration. They are often used for suicidal and homicidal purposes. Many of them are also used for criminal abortions and for other similar purposes (Eddleston and Warrell 1999).

However, in parts of the developing world, plant poisoning is a major clinical problem. Poisoning with *Thevetia peruviana* (yellow oleander) and *Datura stramonium* causes numbers of deaths each year in Bangladesh (Eddleston and Persson 2003). Almost all deaths result from suicide or homicide. Sometimes it is accidental also. A separate group of poisonings with significant mortality and morbidity occurs after women use plants to induce abortions – e.g., *T. peruviana* in Bangladesh and India (Modi, NS. Modi's 1988). Unintentional deaths do occur in children in the developing world – for example, *Xanthium strumarium* poisoning has a reported case fatality rate greater than 25 % (Gurley et al. 2010).

There are only a few studies investigating the epidemiology, and these publications of human exposure to plants indicate that accidental exposure is the most frequent circumstance of poisoning, closely followed by abuse. Many cases of intentional and accidental poisoning of humans are known (Langford and Boor 1996). The following text concentrates on a comprehensive review of plants that cause serious poisoning and their management.

Yellow Oleander (*Thevetia peruviana*), an Ornamental Tree

The actual incidence of plant poisoning in Bangladesh is not known. Sri Lanka has the highest reported incidence of yellow oleander poisoning because the seeds of yellow oleander are known there as "lucky nuts" and every year many people ingest them (Bandara et al. 2010).

Ingestion of oleander seeds, which contain highly toxic glycosides including thevetins A and B and neriifolin, hampers the cardiac muscle and autonomic nervous system resulting in cardiac dysrhythmias, sinus and AV node block, bradycardia, vomiting, diarrhea, hyperkalemia, etc.

Thevetia peruviana is an evergreen tropical shrub or small tree. Its leaves are willow-like, linear-lanceolate, and glossy green in color. They are covered in waxy coating to reduce water loss (typical of oleanders). Its stem is green turning silver/gray as it ages (Kemper Center 2013).

Flowers bloom from summer to fall. The long funnel-shaped sometimes fragrant yellow (less commonly apricot, sometimes white) flowers are in few-flowered terminal clusters (Kemper Center 2013). Its fruit is deep red – or black – in color.

Toxic Component

Thevetia peruviana plants are toxic to most vertebrates since their milky sap contains cardiac glycosides, as are all parts of the plants. The toxins are cardenolides called thevetin A and thevetin B (cerebroside); others include peruvoside, neriifolin, thevetoxin, and ruvoside. These cardenolides are not destroyed by drying or heating, and they are very similar to digoxin from *Digitalis purpurea*. They create gastric and cardiotoxic effects.

Mechanism of Toxic Effects

Symptoms may occur following chewing or ingestion of the fresh or dried plant. The sap may be irritating to the skin and eye and cause dermatitis in some individuals.

Cardiotoxicity appears to follow inhibition of Na^+-K^+-ATPase similar to the digitalis glycosides. Animal studies suggest *Thevetia cardenolides* bind with increased potency but that other mechanisms of toxicity may also be present. An increase in vagal tone may contribute to some of the toxicity noted (e.g., abdominal colic and bradycardia). Gastrointestinal effects are secondary to local effects, although central stimulation may also contribute to this.

The fatal dose is uncertain. Ingestion of only 8–10 seeds can cause death of an adult within 24 h, but it is reported that ingestion of only one seed resulted in death of a 4-year-old child (Greenberg 1957).

Signs and Symptoms

Symptoms usually appear within 1–2 h of ingestion but may be delayed up to 6 h. Gastrointestinal symptoms predominate initially, followed by CNS and other symptoms. Cardiac symptoms usually develop after a delay of at least 6 h (Linden 2001).

The early clinical presentation of yellow oleander poisoning is burning sensation in the mouth with tingling of the tongue and dryness of the throat; along with these, vomiting, diarrhea, headache, dizziness, and dilated pupils may also occur. Initially, gastrointestinal symptoms (nausea, vomiting, abdominal pain) predominate, followed by disturbed color vision and CNS effects (weakness, drowsiness, dizziness, confusion, delirium). Cardiovascular effects generally occur lately few hours after ingestion; automaticity of the heart and different types of arrhythmia may develop. Electrolyte abnormalities, especially hyperkalemia, are the hallmark of cardiac glycoside poisoning and may result in severe toxicity (Taboulet et al. 1993a). Bradycardia, sinus arrest, and various degrees of AV block are characteristic of cardiac glycoside poisoning. However, almost every kind of cardiac dysrhythmia, except supraventricular tachydysrhythmias, may occur (Ma et al. 2001). Preexisting heart disease, ECG changes, hyperkalemia, or the

presence of other symptoms in the patients may indicate toxicity (Haddad et al. 1998). Diuresis, tachypnea, and hypoxia are also less commonly observed.

Ventricular arrhythmias and hyperkalemia are associated with a poor prognosis. Without appropriate treatment, ventricular tachycardia carries a 50 % mortality rate, and bidirectional tachycardia is almost always fatal (Fauci et al. 1998).

Symptoms commonly persist for 4–6 days due to the long half-life of the toxins in this plant. Due to enterohepatic recycling, recurrence of toxicity may occur 3–24 h following treatment or up to 8 days later in patients with renal failure (Linden 2001).

Severity of Poisoning

The presence of any symptoms may indicate severe toxicity (source: www.toxinz.com).

Mild toxicity	Moderate toxicity	Severe toxicity
Nausea	Visual disturbances	Cardiac dysrhythmias
Abdominal pain	Dizziness	Particularly ventricular dysrhythmias
Vomiting	Fatigue	Hyperkalemia
Anorexia	Confusion	Syncope
	Delirium	Coma
		Death

Treatment

For acute (or acute-on-chronic) overdoses, decontamination with activated charcoal is recommended within 4 h of ingestion. Avoid gastric intubation as this may result in vagal stimulation and ventricular fibrillation or asystole (Taboulet et al. 1993a).

The administration of digoxin-specific Fab antibody fragments (digoxin Fab) is the mainstay of treatment in cardiac glycoside poisoning. With appropriate digoxin Fab treatment, clinical improvement is seen within 1–4 h, but the patient may take 13 h to stabilize.

Indication of digoxin Fab (source: toxinz.com):

Life-threatening dysrhythmia
Hemodynamic compromise
Serum potassium >6 mmol/L (>6 mEq/L)
Plasma digoxin level >20 nmol/L (15.6 ng/mL) 6 h after acute overdose
Plasma digoxin level >10 nmol/L (7.8 ng/mL) in chronic toxicity

If digoxin Fab is unavailable, close monitoring and meticulous supportive care are essential. Atropine is indicated for bradydysrhythmia, and magnesium sulfate is

indicated for tachydysrhythmia and ventricular dysrhythmias – even with a normal serum magnesium level. Further antidysrhythmic drugs include lidocaine (lignocaine) may be considered and, for resistant dysrhythmias, phenytoin can be cautiously tried. Avoid class IA and IC antiarrhythmic agents, due to adverse impact on AV-nodal conduction.

The use of temporary cardiac pacing for severe bradycardia or ventricular dysrhythmia carries significant risk of adverse effects and should only be undertaken in dire cases when digoxin Fab is unavailable (Taboulet et al. 1993b).

Potassium abnormalities are common. Hyperkalemia can be delayed up to 12 h, and the patient may initially present as normo- or hypokalemic. Hyperkalemia (>6 mmol/L [6 mEq/L]) should be managed with digoxin Fab, or in the absence of Fab, the administration of glucose (0.5–1.0 g/kg) and insulin (1 unit regular insulin per 3 g glucose) may be used. Similarly, sodium bicarbonate (1–2 mEq/kg) may be used in acidosis and polystyrene sulfonate may be used (Douglas 2001). Hypokalemia, usually associated with chronic overdose, will predispose to dysrhythmia and should be cautiously corrected (beware of rebound hyperkalemia) as should hypomagnesemia. Calcium is contraindicated, as this may induce dysrhythmia.

Maintain good renal output and consider hemodialysis in the renally compromised if suffering acidosis or hyperkalemia. Correct metabolic acidosis with sodium bicarbonate; monitoring of serum potassium is essential.

Datura (*Datura stramonium*), Jimson Weed

Datura stramonium, known by the common names, is a plant in the Solanaceae (nightshade) family, which is believed to have originated in the Americas but is now found around the world (Duke et al. 2002).

For centuries, *Datura* has been used as a herbal medicine to relieve asthma symptoms and as an analgesic during surgery or bone setting. It is also a powerful hallucinogen and deliriant, which is used spiritually for the intense visions it produces. However, the tropane alkaloids which are responsible for both the medicinal and hallucinogenic properties are fatally toxic in only slightly higher amounts than the medicinal dosage, and careless use often results in hospitalizations and deaths.

Datura stramonium generally flowers throughout the summer. The fragrant flowers are trumpet shaped, white to creamy or violet, and $2^1/_2$–$3^1/_2$ in. (6–9 cm) long and grow on short stems from either the axils of the leaves or the places where the branches fork (Fig. 30.1). The calyx is long and tubular, swollen at the bottom, and sharply angled, surmounted by five sharp teeth. The corolla, which is folded and only partially open, is white and funnel shaped and has prominent ribs (Fig. 30.2). The flowers open at night, emitting a pleasant fragrance, and is fed upon by nocturnal moths (Grieve 1971).

Datura is very popular among the criminals for robbing purposes. It causes CNS depression and retrograde amnesia. Most of the victims failed to recall what had happened. It was observed that *Datura* was used with different food items and

Fig. 30.1 *D. stramonium* var. tatula, flower (*front*) (Photo by Skäpperöd is licensed under CC BY-SA 3.0)

Fig. 30.2 *Datura* seedpod, opening up to release seeds inside (Photo by Nova is licensed under CC BY 3.0)

offered to the travelers during journey by bus or train. The victim became unconscious and the criminal raid all the belongings and valuables (Jain and Bhatnagar 2000).

Toxic Component

All the parts of the *Datura* plants contain the tropane alkaloids atropine, hyoscyamine, and scopolamine which are classified as deliriants or anticholinergics. There is a high risk of fatal overdose among uninformed users, and many hospitalizations occur among recreational users who ingest the plant for its psychoactive effects. The amount of toxins varies widely from plant to plant. There can be as much as a 5:1 variation across plants, and a given plant's toxicity depends on its age, where it is growing, and the local weather conditions (Preissel and Preissel 2002; Fig. 30.3).

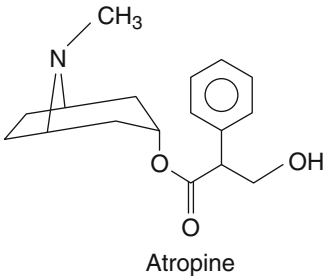


Fig. 30.3 Structure of atropine

The concentrations of toxins are higher in certain parts of the plant than others and can vary from leaf to leaf. When the plant is younger, the ratio of scopolamine to atropine is approximately 3:1; after flowering, this ratio is reversed, with the amount of scopolamine continuing to decrease as the plant gets older. This variation makes *Datura* exceptionally hazardous as a drug. An individual *Datura* seed contains about 0.1 mg of atropine, and the approximate fatal dose for adult humans is >10 mg atropine or >2–4 mg scopolamine (Arnett 1995).

Mechanism of Action

Atropine and scopolamine (both of which are found in very high concentrations in *Datura*) are muscarinic antagonists which can be used to treat Parkinson's disease and motion sickness and to inhibit parasympathetic stimulation of the urinary tract, respiratory tract, GI tract, heart, and eye.

Signs and Symptoms

Symptoms are dose dependent and with large overdoses usually last between 2 and 7 days (Stern 1983; Fahy et al. 1989) but have persisted for up to 1 month (Morgenstern and Trihexyphenidyl 1962).

Symptoms may occur following eye contact, ingestion of the fresh or dried plant or tea made from infusion of plant material in water, injection of a similar infusion, or inhalation of smoke from burning plant material. Symptoms may very rarely occur from prolonged skin or eye contact.

Onset of anticholinergic toxicity is usually within 30 min to 2 h. Tachycardia, dry mouth, and ocular symptoms usually occur first. Symptoms may be significantly delayed due to decreased gastric motility (especially with large ingestions) or with skin exposures.

Datura intoxication typically produces delirium (as contrasted to hallucination), hyperthermia, tachycardia, bizarre behavior, and severe mydriasis with resultant painful photophobia that can last several days. Pronounced amnesia is another commonly reported effect. The onset of symptoms generally occurs approximately

30 min to an hour after smoking the herb. These symptoms generally last from 24 to 48 h but have been reported in some cases to last as long as 2 weeks.

In very large overdoses, a small degree of neuromuscular blockade may be observed causing postural hypotension. Respiratory paralysis is unlikely but may occur secondary to circulatory failure. Death may be the result of circulatory collapse, hyperthermia, cardiac depression, respiratory arrest, or environmental hazards secondary to delirium (e.g., drowning) (Hardman et al. 1996). A range of complications may occur, generally related to prolonged coma, excessive muscle activity, or hyperthermia. These can include metabolic acidosis, rhabdomyolysis, and renal failure. Circulatory collapse, respiratory arrest, and cardiac depression are common causes of death. Cerebral edema may be indicative of an anoxic episode.

Severity of Poisoning

Mild	Moderate	Severe
Dry mouth	Hypertension	ECG abnormalities
Flushed, dry skin	Hyperthermia	QRS widening
Mydriasis	Anticholinergic delirium	QT prolongation
Blurred vision	Decreased gastric motility	CNS depression
Elevated body temperature	Urinary retention	Seizures
Tachycardia	Headache	Ileus
		Hypotension
		Rhabdomyolysis
		Circulatory collapse
		Postural hypotension

(Source: www.toxinz.com)

Treatment

Patients presenting prior to the onset of significant symptoms should receive activated charcoal, provided that gastrointestinal ileus is not present. The patient's psychological state may impede decontamination measures. Immediately ensure cardiorespiratory adequacy and commence pulse oximetry and cardiac monitoring. Management of agitation and delirium is usually the most pressing concern following anticholinergic intoxication. However, close monitoring is required for associated life-threatening conditions such as seizure and hyperthermia.

Commonly, observation in a calming, darkened, environment is all the management required. Agitation, delirium, or seizure generally responds to a benzodiazepine; a barbiturate may be given if this is ineffective. Sinus tachycardia does not generally require specific intervention in isolation but usually responds to crystalloid infusion. Decreased gastric motility is very common and contributes to

prolonged duration of symptoms, although ileus is rare. Urinary retention may require catheterization. Respiratory paralysis is rare and should be managed with mechanical ventilation if it occurs.

Children may require emergency stabilization due to rapid-onset coma, hypotension, or seizure. Seizures must be immediately controlled with a benzodiazepine. A barbiturate may be given if seizures are still refractory. Emergency airway management may be required. Close monitoring of levels of consciousness and cardiorespiratory function is required in children.

Physostigmine may reduce supportive care requirement but should be used cautiously. It should be administered to severely poisoned patients, i.e., those with seizure, delirium, narrow QRS, supra-ventricular tachydysrhythmias, hemodynamic deterioration, or ischemic pain.

Cannabis

Cannabis is believed to be native to Asia but is widespread throughout most tropical and subtropical countries and prefers moderate to high rainfall and well-drained soils of moderate to high fertility (Everis 1981). It is commonly grown for its use in textiles, as well as for recreational drug use. Cannabis can be grown hydroponically and primarily used for the psychoactive effects. As a herbal medicine, it has also been used as an analgesic and sedative/hypnotic (Grieve 1984).

Signs and Symptoms

The correct identification of the substance is important. If the symptoms are inconsistent with those described or the history is considered unreliable, other substances may need to be considered.

Cannabis products are generally used by smoking or ingestion.

Plant material is often smoked from cigarettes and various types of pipes or bongs (water cooled pipe). Hashish or hash may be added to tobacco cigarettes before smoking. Ingestion of cannabis is often in the form of confectionary, e.g., biscuits, cookies, and cake, in which the plant or hash has been incorporated.

Plant, hash, or hash oil may also be administered by "spotting," which involves contact of material with heated knives and subsequent inhalation of the smoke produced. Other routes of exposure include injection or consumption of aqueous extracts.

Adult patients may appear stimulated or sedated (Williams and Keyes 2001). Mild poisoning generally causes laughing, increased appetite, tachycardia, and altered perception of mood including euphoria and relaxation.

Moderate intoxication leads to hypotension (more commonly than hypertension), tremor, muscle weakness, bronchodilation, and urinary retention.

CNS symptoms of hallucinations, paranoia, short-term memory loss, and ataxia also commonly occur. Following ingestion or inhalation, death in adults is usually secondary to impaired judgement or motor skills. However, severe consequences of IV use include cardiovascular collapse, disseminated intravascular coagulation, and, rarely, death.

Children are more susceptible to toxicity, and ingestions can be life threatening; rapid-onset sedation, opisthotonic movements, hypotonia, apnea, cyanosis, bradycardia, right bundle branch block, and seizures can occur (Macnab et al. 1989).

Cannabis cigarettes may be soaked in formaldehyde to increase their effect. In these patients, salivation, sweating, tremor, and difficulty in moving may occur (Otten 2002).

If ingested, onset of symptoms is generally within 30–60 min and persists for 6 or more hours. If inhaled or smoked, symptoms occur immediately, peak in 20–30 min, and persist for about 3–4 h.

Severity of Poisoning

Mild	Moderate	Severe
Mild euphoria	Short-term memory impairment	Decreased motor coordination
Increased sensory awareness	Mild confusion	Decreased muscle strength
Somnolence	Impaired judgement	Tremor
Relaxation	Depersonalization	Sedation
Reddening of eyes and conjunctiva	Mood alterations	Slurred speech
Minor distortions of time perception	Depression	Ataxia
Dry mouth and throat		Respiratory depression
Tachycardia		Coma
		Death

(Source: www.toxinz.com)

Treatment

Decontamination is not recommended as the risk of aspiration due to CNS depression or seizure outweighs benefit. Enhanced elimination is not recommended. There are no antidotes.

Children may require emergency stabilization due to rapid-onset coma, hypotension, or seizure. Seizures must be immediately controlled with a benzodiazepine. A barbiturate may be given if seizures are still refractory. Emergency airway management may be required. Close monitoring of levels of consciousness and cardiorespiratory function is required in children.

Adults seldom require acute supportive management beyond observation and monitoring. Mild to moderate acute psychological effects may be managed with a quiet environment and a benzodiazepine if necessary. Patients with chest pain should be given oxygen and investigated for pneumothorax and pneumopericardium (Caravati 2004).

Intubation and ventilation may be required for significant CNS depression, respiratory distress, or seizure.

Tobacco Leaf (*Nicotiana tabacam*)

Tobacco within the genus Nicotiana included product of cigarettes, pipe, snuff, and chewing tobacco; are manufactured from dried tobacco leaves. The addictive alkaloid nicotine is considered the most characteristic constituent; it also contains beta-carboline which inhibits monoamine oxidase. Tobacco itself is an annual plant, about 2 m high, and has large leaves and pink blossoms.

Nicotine poisoning has been most frequently described in children due to consumption of tobacco products like cigarette ends or medication prepared from tobacco. A syndrome term "green leaf tobacco sickness" is caused by dermal absorption of nicotine while harvesting wet tobacco leaf without skin protection (Davies et al. 2001).

Betel nuts are widely used in Southeast Asia and are commonly mixed with lime and nicotine as a mixture called "pan." Both contain cholinergic alkaloids: nicotine and arecoline, respectively.

Signs and Symptoms

Gastrointestinal symptoms usually develop soon after ingestion and include nausea, vomiting, abdominal pain, and diarrhea. Tremor, dizziness, and symptoms of cholinergic discharge, i.e., diaphoresis, pallor, and salivation, are also frequent early signs of intoxication.

In severe cases neurological and cardiovascular dysfunctions become apparent. The symptoms may follow a biphasic pattern in which there is initial stimulation of nicotinic cholinergic receptors followed quickly by inhibition. This produces restlessness, seizures, muscle fasciculation, hypertension, tachycardia, and tachypnea, followed by hypotension, bradycardia, and dyspnea, finally leading to coma, paralysis, respiratory failure, dysrhythmias, and cardiovascular collapse.

Severity of Poisoning

Mild	Moderate	Severe
Nausea	Severe GI effects	Bradycardia

(*continued*)

Mild	Moderate	Severe
Vomiting	Abdominal pain	Hypotension
Sweating	Tremor	Convulsions
Pallor	Headache	Muscular paralysis
	Dizziness	Respiratory failure
	Drowsiness	Coma
	Hypertension	Cardiovascular collapse
	Tachycardia	
	Tachypnea	

(Source: www.toxinz.com)

Treatment

The administration of activated charcoal may be considered within one hour of ingestion of nicotinic plant material; however, benefit is unproven, and risks of seizure, vomiting, and aspiration potentially outweigh gain. In symptomatic patients, general supportive measures should take precedence over decontamination (Schep et al. 2009).

Pronounced agitation or seizures may present early and will respond to a benzodiazepine. Severe cases will, however, progress to muscular weakness/paralysis and coma. Loss of muscular function, combined with bronchoconstriction and increased airways secretions, makes close monitoring of airway function essential. Intubation and ventilation together with atropine (to reverse constriction and reduce secretions) may be required.

Supportive care is the mainstay of management with an emphasis on respiratory and cardiovascular support. While transient hypertension may occur, hypotension is more common and generally responds to fluids – pressors may be required. Atropine is indicated for bradycardia. Other cardiac dysrhythmias should be treated following standard protocols. Severe vomiting requires an antiemetic and attention to fluid and electrolyte balance; atropine is indicated to settle gastrointestinal hyperactivity.

With rapid and full supportive care, the prognosis is good and recovery expected in the majority within 24 h.

Xanthium strumarium, the Local Name Ghagra Shak

Ghagra shak is used in the treatment of various ailments including asthma, urinary disorder, impotence, skin disease, burning foot sole, dental pain, allergy, ear infection, body muscle pain, jaundice, diabetes, blood purification, rheumatism, and gastritis (Fig. 30.4). People in Bangladesh reported the use of ghagra shak as ingredient of other vegetables because of its green mango-like flavor (Islam et al. 2009). Single use of ghagra as vegetable is toxic. Occurrences of toxic incidents were reported in 12 localities throughout Bangladesh, particularly in

Fig. 30.4 Ghagra shak (Source: M. A. Faiz)

Companigonj, Guainghat, Nroshindi, Tejgaon, Bakergonj, Barguna, Barisal, Mehendigonj, Chittagong, Comilla, Patuakhali Bhola, and Sylhet (Gurley et al. 2010). Overdosage of seedling and juvenile plants of ghagra shak may pose severe toxic effect on human health.

Toxic Component

It is an erect herb. The leaves are alternate and broadly ovate to cordate in shape. Male heads are globose and florets are numerous and whitish green. The plant possesses toxic effects on the liver cell of experimental rats, which might also be toxic to human subjects. This hepatotoxicity can be correlated to the death occurring after the administration of extracts of mature plant and seedlings of *X. strumarium* (Islam et al. 2009).

The main toxic compound isolated from *X. strumarium* has been identified as carboxyatractyloside (CAT), a kaurene glycoside. When ingested in sufficient quantities by animals, CAT by itself or *Xanthium* that contains CAT produces hypoglycemia and hepatic damage, the latter possibly due to increased vascular permeability in response to severe hypoglycemia. In addition to CAT, potentially toxic ingredients of *Xanthium* include several sesquiterpene lactones (e.g., guaianolides, germacranolides, and elemanolides) that can cause vomiting, weakness, tremors, weak pulse, a loss of appetite, and convulsions in high doses (Omar et al. 1984; Fig. 30.5).

Signs and Symptoms

The majority of cases reported vomiting, fever, and altered mental status. In severe case, vomiting of blood, abdominal pain, shortness of breath, excessive salivation, frothy discharge in mouth, stiff neck, and convulsion followed by death may ensure.

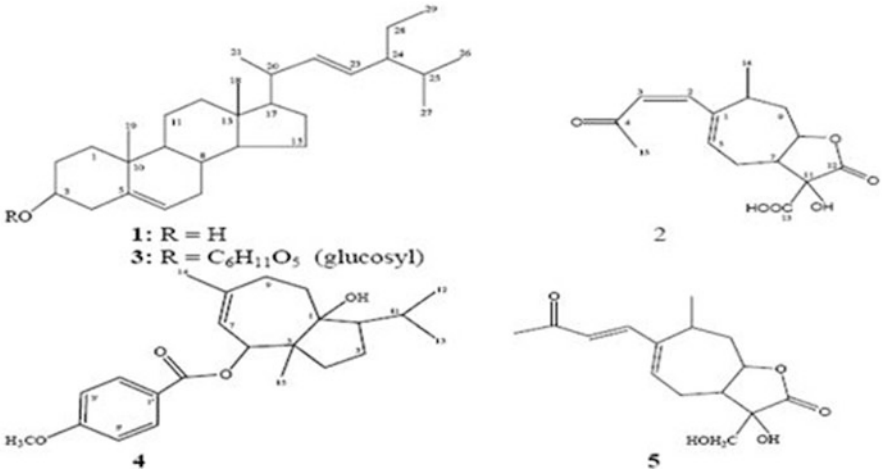


Fig. 30.5 Carboxyatractyloside (CAT), a kaurene glycoside

Women and children were likely at increased risk of poisoning because of their relatively lower body weight (ICDDR,B 2008). The clinical syndrome of cases is consistent with toxic poisoning; onset of symptoms to death was rapid, and liver function tests were distinctly abnormal in many patients.

Treatment

Treatment should be supportive and symptomatic. Decontamination is not recommended. There are no antidotes.

Children may require emergency stabilization due to rapid-onset coma, hypotension, or seizure. Emergency airway management may be required. Close monitoring of levels of consciousness and cardiorespiratory function is required in children.

Adults seldom require acute supportive management beyond observation and monitoring. Mild to moderate acute psychological effects may be managed with a quiet environment and a benzodiazepine if necessary.

Intubation and ventilation may be required for significant CNS depression, respiratory distress, or seizure.

Unknown Medicinal Plant Poisoning

In Bangladesh, traditional medicine like herbs and plants are used since at the early period of civilization and still practiced in the same manner. Though it is used all over the world, in this subcontinent, its use is much more because of its easy accessibility, there is no expert consultation required, and it is considered safe to

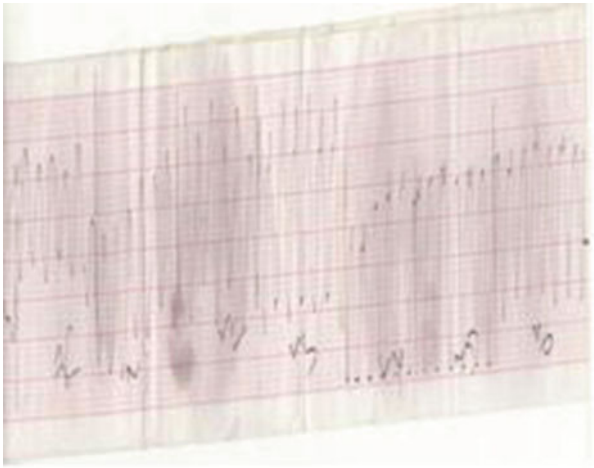


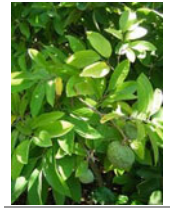
Fig. 30.6 Ventricular tachycardia developed following ingestion of unknown glycoside (Source: A. Basher)

Fig. 30.7 Different ingredients used for the preparation of tonic (Source: A. Basher)

use and also because primary health care services fall short of peoples' need both in qualitative and quantitative terms.

Traditional healers used herbs only after processing to reduce the amounts of toxic alkaloids. Faulty processing after harvest or during decoction preparation and the use of a greater than recommended dose will increase the risk of acute poisoning. Recently, experienced two fatal cases developed ventricular tachycardia following ingestion of mixed herbs for masculinity. Analysis of ingredients revealed individual component was not toxic. Santalum album (chandan wood) contains santalol and other etheric oils; *Plantago ovata* (ispaghula husk) contains diverse alkaloids, phenols, etc.; *Mimosa pudica* is the common *Mimosa* and contains the alkaloid mimosine, all of which can be toxic by oral uptake only in large dose (Figs. 30.6 and 30.7, Table 30.1).

Table 30.1 Some important poisonous plant in Bangladesh

Local name	Scientific name	Poisonous parts	Poisonous effects	Treatment	Prognosis
Kunch 	*Abrus precatorius*	Seeds, roots	Abortifacient, emetic, cathartic, cattle poison	Activated charcoal, supportive care	Life threatening if untreated
Ata 	*Annona squamosa*	Roots, seeds	Roots are drastic purgative; seeds are strong eye irritant	Supportive care	Not life threatening
Shialkanta 	*Argemone mexicana*	Seeds, latex	Seeds cause severe dropsy, vomiting, and diarrhea; latex is irritant	Supportive care	Most exposure chronic
Hijal 	*Barringtonia acutangula*	Fruits	Fruit causes severe vomiting	Supportive	Not fatal

(*continued*)

Table 30.1 (continued)

Local name	Scientific name	Poisonous parts	Poisonous effects	Treatment	Prognosis
Fire lily 	*Gloriosa superba*	Roots, seeds	Nausea Vomiting Abdominal pain Hematemesis Diarrhea	Activated charcoal, supportive	Fatal
Batul 	*Sapium indicum*	Leaves, fruit	Nausea, vomiting, diarrhea	Supportive	Not fatal
Akanda 	*Calotropis gigantea*	Latex, leaves	Latex is violent purgative, abortifacient, and infanticide; leaves are homicidal poison	Supportive	Not fatal
Bhanga 	*Cannabis sativa*	Latex, leaf, flower	Loose motion	Supportive	Not fatal

Papaya 	*Carica papaya*	Latex of young fruit	Latex is intestinal irritant and induces abortion	Observation	Not fatal
Makal 	*Citrullus colocynthis*	Fruits	Powerful hydragogue and cathartic	Supportive	Not fatal
Swarnalata 	*Cuscuta reflexa*	Whole plant	Antifertility, decoction causes nausea, vomiting, and abortion	Supportive	Not fatal
Datura 	*Datura inoxia*	Seeds	Dryness of the mouth, delirium, fever, convulsion	Activated charcoal. Physostigmine, supportive	Fatal if untreated
Dundul 	*Luffa cylindrica*	Fruits	Fruit juice of wild plant, causes severe purgation	Supportive	Not fatal

(*continued*)

Table 30.1 (continued)

Local name	Scientific name	Poisonous parts	Poisonous effects	Treatment	Prognosis
Ghora neem 	*Melia sempervirens*	Fruits	Fruits produce nausea, spasm, and choleric symptoms	Supportive	Not fatal
Rakta karobi 	*Nerium indicum*	All parts	Cause death on ingestion; roots cause abortion on local application	Activated charcoal, digoxin Fab	Fatal if not properly treated
Chita 	*Plumbago zeylanica*	Whole plant	Extract; causes paralysis	Supportive	Not fatal
Palik 	*Ranunculus sceleratus*	Whole plant	Highly acrid, causes violent irritation, paralysis, and convulsion, slows respiration, depresses heart's action	Supportive	Not fatal

Kuchila 	*Strychnos nux-vomica*	Seed	Respiratory failure, nausea, muscle twitching	Activated charcoal, supportive	Fatal if not properly treated
Tagor 	*Tabernaemontana divaricata*	Fruits, seeds	Fruits are deadly poisonous; seeds are narcotic and poisonous and produce delirium	Supportive	Fatal if not properly treated
Helde karobi 	*Thevetia peruviana*	Bark, seeds, latex	Cause serious depression, paralysis, and death	Activated charcoal, digoxin Fab	Fatal if not properly treated
Antamul 	*Tylophora indica*	Leaves, roots	Plant juice causes vomiting, unconsciousness, and death	Activated charcoal, supportive	Fatal if not properly treated

Conclusion and Future Directions

Plants are used worldwide for a wide variety of indications. In health care, health professionals, quacks, and other nonmedical professionals, such as witch doctors, dispense herbs for either therapeutic or tonic purposes. This coupled with lower costs compared with conventional medications is the major attraction to these treatments. Despite the general belief, upon exposure, the clinical toxicity may vary from mild to severe and may even be life threatening. Herbal medicines are associated with a wide spectrum of toxicities. In most instances, treatment includes stopping the offending agent together with supportive care. In industrialized nations, herbal medicine is now a multibillion dollar industry, and in developing countries, up to 80 % of people rely on plant-based medicines (Inamul Haq 2004). Plant poisoning is not well studied and possible targets for antitoxins have not been explored.

The identity, authenticity, and quality of crude plants are often uncertain and difficult to assess. The quality control is virtually nonexistent; government agencies seem unwilling to adopt any guidelines.

Cross-References

- ▶ Anticholinergics Syndrome Related to Plants and Herbs
- ▶ Poisonous Mushrooms

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