R. C. Sobti
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Aastha Sobti *Editors*

Delineating Health and Health System: Mechanistic Insights into Covid 19 Complications

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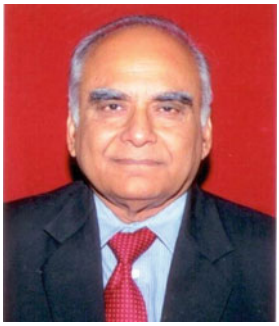
The book is dedicated to Dr. N. K. Ganguli, Former Director General of Indian Council of Medical Research who is an internationally renowned scientist and par excellence administrator.

Biography of Prof. Nirmal Kumar Ganguly



Nirmal Kumar Ganguly, M.D., Ph.D., is a Former Director of the Indian Council of Medical Research, the National Academy of Medical Sciences and the National Institute of Biologicals. He is a microbiologist and Chairman of the Advisory Committee to the Minister of Health on COVID-19. He has recently been appointed the Chairman of the MoHFW (Disaster Management Cell), Govt. of India Technical Committee for "India Covid-19 Emergency Response and Health Systems Preparedness Package-Phase II" (ECRP-Phase-II), Chairman of the Institutional Ethics Committee of Adamas University (AU-IEC) for the Subhash Mukhopadhyay Centre for Stem Cell Biology and Regenerative Medicine (SM-SCBRM), Chairman of Monitoring Committee for NMITLI project on "Development of dental implants for Advanced and Critical Care Applications" of CSIR and Member of Typhoid Working Group of National Technical Advisory Group on Immunization (NTAGI) of National Institute of Health and Family Welfare (NIHFW), Govt. of India.

He is also the Chairman of the Research Council of the Institute of Advanced Virology, National Academy of Medical Sciences, Chairman of Indian Pharmacopeia Commission, Expert Committee of the National Institute of Biologicals, guiding R&D activities and President of Immunology Foundation of India. Prof. Ganguly is at present the Senior Scientific Advisor, Global Health Strategies, New Delhi, and President, Apollo Hospitals Educational and Research Foundation. He is Emeritus Professor and Director of PGIMER, Chandigarh. He is on the Advisory Board of NIH Fogarty International Center, the Health Vaccine Center, the U.S. Centers for Disease Control (CDC) Advisory Board for Global Health, and the

Public Health Advisory Board of the University of California (UCLA). He is a Senior Scientific Advisor to the Bill and Melinda Gates Foundation and a Fellow of Imperial College and the Royal College of Pathologists and the Tropical School, London, and on the Board of the Public Health Innovative Fund, the Canada Innovative Fund, and Grand Challenges, Canada. Prof. Ganguly is Chairman of the Advisory Committee for Health Research of the World Health Organization-SEARO, the International Vaccine Institute Cholera Board (CHOVI), and United Nations Children's Fund SAG TDR. Currently, he is President of the Gut Microbiota and Probiotic Science Foundation (India), Immunology Foundation of India and the Indian Society of Translational Research.

He has published more than 775 research papers and supervised or co-supervised 130 Ph.D. candidate dissertations and more than 20 book chapters. Prof. Ganguly has been honoured with 7 international and 113 national awards along with the prestigious "**Padma Bhushan"** Award in the field of "Medicine" for the year 2008.

Preface

The current rampant pandemic of COVID-19 disease, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to an unprecedented global socio-economic disruption and tremendous loss of human lives worldwide. This affliction and its magnitude are mainly related to the interaction of receptor binding domain (RBD) of the SARS-CoV-2 with human angiotensin-converting enzyme 2 (hACE2) receptors located on cells on different tissues. The structural images of hACE2 following cryo-EM potentially formed the basis for its being receptor for SARS-CoV-2 that has also shown some structural similarity with SARS-CoV. However, the variation in structure actually conforms to the difference in binding affinity of RBD between SARS-CoV and SARS-Cov-2. As a result, about 10- to 20-fold higher affinity for binding of SARS-CoV-2 to hACE2 than SARS-CoV has been reported. This binding affinity measures the susceptibility of host to virus, thus partially explaining the increase in transmissibility of SARS-CoV-2.

Mutation in the RBD of the SARS-CoV leading to amino acid substitution (K479N) increased the binding affinity between RBD and hACE2 that may, as reported by few, have caused the spill from the civet over to humans. Similarly, another mutation (S487T) in the RBD may have facilitated transmission from human to human. The delta variant arising from Pango lineage B.1.617.2 is still regarded as the most contagious form of the SARS-CoV-2 coronavirus so far.

Other mutations at 5 positions in RBD have been found to be present in variants of the SARS-CoV, resulting in enhanced binding to hACE2 or civet ACE2 or enhanced replication rate or transmissibility of the virus. The mutations in the RBD also influence host immune responses and therefore, its structure, eventually became the basis and encouraged scientists to design vaccines against coronaviruses. The efficacy and longevity of vaccine-induced immunity are under active investigations suggesting booster dose(s) in view of the decline in its efficacy.

Involvement of the lung as a respiratory organ is the most common severe manifestation of the disease, ranging from asymptomatic disease or mild pneumonia to severe illness associated with hypoxia, acute disease related to shock, respiratory failure, and multiorgan failure or death. Although COVID-19 presents primarily as a lower respiratory tract infection transmitted via air droplets, however, increasing pieces of data now suggest the involvement of multiorgans in the infected patients. The key mechanisms that may have a role in the pathophysiology of multiorgan

injury secondary to infection with SARS-CoV-2 include direct toxicity of virus, endothelial cell damage and thrombo-inflammation, dysregulation of the immune response, as well as of the renin–angiotensin–aldosterone system (RAAS) leading to cytokine storm. Recently, the mechanistic aspects of a broad spectrum of neurological symptoms affecting the central nervous system, peripheral nervous system, and skeletal muscles including anosmia and ageusia due to COVID-19 infection have been delineated. Patients with depleted levels of certain species of biota are associated with elevated concentrations of inflammatory cytokines and blood markers, including C-reactive protein, lactate dehydrogenase, and aspartate aminotransferase. It has been seen that there is an increased incidence of rhino mucormycosis in patients with COVID-19, particularly for those in the post-COVID stage, and this has been ascribed to various factors including steroids therapy for COVID-19. Besides there are reports of other impacts of COVID-19 on health including effects on fertility in some males.

The book *Delineating Health and Health System: Mechanistic Insights into Covid 19 Complications* discusses organ-specific systemic manifestations of COVID-19. The initial chapter conveys the current knowledge on the origin and evolution of the pathogenic coronaviruses, followed by pathogenesis and immune response during COVID-19 infection. This volume also provides insights into the role of hACE2 in the onset of severe acute respiratory syndrome coronavirus 2 pathogenesis, detection of SARS-CoV-2 and its variants, recently coded as alpha, beta, gamma, and delta by the WHO in human samples for guiding management strategies, as well as the appearance of disease in fresh waves. Every country has passed through multiple waves while the scenario is still fluid. It also discusses COVID-19 as a multiorgan syndrome, besides highlighting the strategies of development of various vaccines, particularly mRNA vaccines. A detailed discussion on rhino mucormycosis has also found place in the volume. Currently, the infection has moved to children and ICUs in the affected countries are mostly full with children. The good news is the US FDA has approved vaccine for children from 6 to 11 years old that will cover nearly 28 million children in the US alone.

All the relevant aspects have been discussed in individual chapters of this book.

The editors are thankful to all the contributors who have spared their time to pen down their experiences comprising the respective chapters of this book.

Dr. R.C. Sobti would like to express gratitude to his wife Dr. (Mrs.) Vipin Sobti for her encouragement and patience.

Chandigarh, India — R. C. Sobti
Winnipeg, Canada — Naranjan S. Dhalla
Kurimamachiya-cho, Japan — Masatoshi Watanabe
Lund, Sweden — Aastha Sobti

Contents

Editors and Contributors

About the Editors

R. C. Sobti former Vice Chancellor, Panjab University, Chandigarh, and Babasaheb Bhimrao Ambedkar University (a central university), Lucknow, is a scientist, an able administrator and a dynamic institution builder.

Dr. Sobti, an active researcher, is also steadfastly committed to the popularization of science in the community through popular lectures and community engagement programmes.

He is a Fellow of the Third World Academy of Science, National Academy of Sciences, Indian National Science Academy, National Academics of Medical Sciences and Agricultural Sciences, the Canadian Academy of Cardiovascular Diseases and few others. Dr. Sobti is the recipient of many prestigious awards like the INSA Young Scientist Medal, UGC Career Award, Punjab Rattan Award, JC Bose Oration and Sriram Oration Awards and Lifetime Achievement Awards of the Punjab Academy of Sciences, the Zoology Society of India and the Environment Academy of India, besides many other medals and awards of national and international levels. He has published more than 350 papers in the national and international journals of repute and has also published 50 plus books.

Dr. Sobti, deeply influenced by the philosophy of Dr. Babasaheb Bhimrao Ambedkar, has now emerged as a very vocal advocate and meticulous champion of social transformation of the deprived/underprivileged/backward classes of the society. He is constantly endeavouring to bring these special sections of the society into the mainstream and works for their upliftment both at the professional and personal levels through active community engagement programmes.

Keeping in view his contributions to education, he was bestowed with one of the highest civilian awards Padma Shri by the Government of India in 2009.

Naranjan S. Dhalla Principal Investigator, Experimental Cardiology, Institute of Cardiovascular Sciences; Distinguished Professor, Max Rady College of Medicine; Professor of Physiology and Pathophysiology, University of Manitoba; Senior Fellow, Centre for the Advancement of Medicine, Dr. Naranjan S. Dhalla has received more than 170 honours and awards from all over the world. These include the Order of Canada, Order of Manitoba, Order of the Buffalo Hunt from the

Province of Manitoba, Fellowship in the Royal Society of Canada, Medal of Honour of the Canadian Medical Association, Research Achievement Award of the Canadian Cardiovascular Society, Chair in Cardiovascular Research and Honorary Professorship at different universities.

Dr. Dhalla was elected 2nd Greatest Manitoban of All Time and was featured in "Greatest Manitobans", a book published by the Winnipeg Free Press. Dr. Dhalla has published a large number of research papers and book chapters. He is a widely travelled scientist and has addressed various national and international conferences.

Masatoshi Watanabe graduated from Yokohama National University, Faculty of Engineering (1983), and Mie University, Faculty of Medicine (1989). He obtained a Doctor of Medicine from Mie University (1993). He was a professor at the Graduate School of Engineering, Yokohama National University (2003–2017), and also a guest professor at the School of Medicine, Yokohama City University (2011–1017). He is now a professor at the School of Medicine, Mie University (2017–). He has published more than 130 scientific publications. He is a member and councillor in the Japanese Society of Pathology and in the Japanese Cancer Association.

Aastha Sobti is currently a doctoral student at the Department of Immunotechnology, Lund University, Lund, Sweden, under CanFaster, Marie Skłodowska-Curie COFUND programme. She has a background in dental sciences and did a specialization in master's in clinical dentistry (oral surgery) from Eastman College, UCL, London. Her core interests include head and neck cancer-based clinical research and in entirety aiding in the field to bring reforms that are required in the present multifarious surgical as well as translational research areas. She has teaching and research experience of about 8 years and published a few papers in international journals of repute. She is a recipient of a large number of prizes and medals for her exceptional work, namely the academic certificate of excellence from BAOS, UK, and IADR Hatton award (India). Additionally, she has attended and presented papers in international conferences in Hong Kong, Croatia, Brazil, Sweden, the UK, Japan, and other countries.

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Corona Viruses: Emergence, Evolution, and Recurrence

1

R. C. Sobti, Bharti Kotarya, Abhishek Pandeya, Raj Kumar Khalko, Neelam Yadav, Sudipta Saha, Y. Vasudeva Rao, and Sunil Babu Gosipatala

Abstract

Corona viruses (CoVs) are enveloped RNA viruses that infect a broad array of avian and mammalian species, including humans. The existence of these viruses is believed to have occurred thousands of years ago as animal CoVs; bats, birds, rodents were reported to be natural reservoirs. They garnered scientific attention after their emergence as human pathogens, till date, seven corona viruses were reported to infect humans, with mild to moderate and/or severe respiratory illness. The ongoing pandemic COVID-19 is caused by one of such Corona viruses named Severe Acute Respiratory Syndrome Corona Virus -2 (SARS-CoV-2), which surprised all with its unprecedented transmission dynamics and etiology. This virus surged twice within a gap of a year all over the world and became a major health concern to many nations. Most of these Corona viruses transferred to

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humans through intermediate hosts. Here, in this chapter, we summarized the structural and genomic features of the Coronaviruses in general and emphasizing the SARS CoV-2 and added an account of the different vaccines and their production platforms in combating the pandemic. We briefly discussed the evolution of new variants of SARS-CoV-2 and their role in the surge of COVID-19 infections. We tried to give a brief account of the historical aspects, cross-species transmission, mutations/recombinations scenarios of CoVs with a note on their emergence as human pathogens and future prospects of recurrence.

Keywords

Corona viruses · Cross-species transmission · Evolution · Mutations/recombinations

1.1 Introduction

The Corona viruses (CoVs) are animal and human pathogens having positive-sense RNA as their genetic material (McIntosh 1974). Initially, from the phylogenetic dating of RNA-dependent RNA polymerases sequence divergence, it was suggested that the most recent common ancestor of mammalian and avian CoVs appeared around 7000–8000 years ago and 10,000 years ago, respectively. The present estimates show that the dispersal of the human population and different CoVs worldwide about 15,000–100,000 years ago; and enormously increased in the last 10,000 years during the first historic transition. Thus, we can say that human travel and trading augmented and resulted in spreading these viruses to distant and isolated places (Chan et al. 2013). The first isolated animal CoVs was the Infectious bronchitis virus (IBV), from the chicken embryos by Beaudette and Hudson in 1937 (McIntosh 1974; Woo et al. 2009). While the first Human CoVs (HCoVs) were identified in the mid-1960s, the history of the virus began with the passage of B814 (obtained from the human upper respiratory tract in organ cultures of the human embryonic trachea in 1965 by the Scientists named Tyrrell and Bynoe). However, this strain was lost in the laboratory, so, it cannot be compared with upcoming identified strains (Kahn and McIntosh 2005; Yang et al. 2020; Hierholzer and Tannock 1988). At present, seven HCoVs have been reported: 229E, OC43, NL63, HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2, and all of them belong to the subfamily *Coronavirinae*. In 1966, the first HCoV known as HCoV-229E was isolated and reported to cause common cold symptoms in healthy adults, lower respiratory tract infections in younger children and elderly people. After a year (1967), another human corona virus, HCoV-OC43, was isolated and reported to cause mild upper respiratory tract infections. Later on, HCoV-NL63 was identified in a 7-month-old baby in late 2004 in the Netherlands. In 2005, HCoV-HKU1 was reported in 71-years old patients of Hong Kong (Li et al. 2020a, b; Lau et al. 2006). These four HCoVs are less pathogenic than MERS-CoV, SARS-CoV, and

SARS-CoV-2 that have different pathogenicity, and lead to higher mortality rates in the human population (Kasmi et al. 2020; Yang et al. 2020).

In mid-November 2002, SARS was first reported in Guangdong province in southern China, and by the end of July 2003, a total of 8098 confirmed cases were recorded in more than 30 countries on different continents with 774 deaths (Wang and Eaton 2007).MERS first appeared in Saudi Arabia in 2012, as a result of another corona viral outbreak, and spread to 26 countries, infecting 1698 people and killing 609 between 2012 and March 23, 2016. Initially, this virus was known as HCoV-EMC, later it was renamed as Middle East Respiratory Syndrome CoVs (MERS-CoV) because all the individuals diagnosed with this disease were linked to one of four countries in the Middle East either directly or indirectly (Gao et al. 2016). In December 2019, a new human CoV known as SARS-CoV-2 (novel CoVs), emerged in Wuhan, China. This novel CoV belongs to the genus *BetaCoVs*, and subgenus *Sarbecovirus* (Zhang and Holmes 2020). Phylogenetic analysis reveals that SARS-CoV-2 shows more similarity to SARS-CoV than MERS-CoV with high transmission efficiency and lower fatality rates compared to SARS and MERS-CoVs. According to the World Health Organization (WHO), 125,781,957confirmed cases have been reported in 217 countries and territories around the world with a total number of 3,264,143 deaths, while 156,496,592 recoveries as of 5 May 2021, and these numbers are expected to increase as time passes.

1.2 General Classification and Distribution of Corona Viruses (CoVs)

Corona viruses (CoVs) are members of the order *Nidovirales* that contains four families:*Arteriviridae, Coronaviridae, Mesoniviridae* and *Roniviridae*. The family *Coronaviridae* has 2 subfamilies (*Torovirinae and Coronavirinae)* and 5 genera (Table 1.1). The subfamily *Torovirinae* has two genera: *Torovirus* and *Bafinivirus.* Mostly Toroviruses have been isolated from mammals having stomach flu as well as from horses, pigs, sheep, cattle, goats, and some from humans also. Culturing this subfamily of viruses was difficult; that's why they have not been studied immensely (Payne 2017). Subfamily *Coronavirinae* has four genera: *AlphaCoVs, BetaCoVs, GammaCoVs,* and *DeltaCoVs* (International Committee on Taxonomy of Viruses). AlphaCoVs and *BetaCoVs* are transmitted among mammals and cause respiratory illness in humans. All seven known HCoVs (OC43, NL63, HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2) belong to these two genera, whereas the *Gamma CoVs* and *Delta CoVs* infect mammals as well as birds (Kumar et al. 2020).

HCoVs are distributed throughout the world, and the 229E, NL63, HKU1, and OC43 cause mild acute respiratory illness and become fully adapted to humans; as a result, they are less pathogenic compared to SARS and MERS-CoVs 1 and 2. The SARS and MERS CoVs are highly pathogenic, infecting the lower respiratory tract, causing severe pneumonia that can even lead to death in many cases, and were responsible for the pandemics. However, these viruses are not fully adapted to humans yet (Renu et al. 2020; Yang et al. 2020). Among the less pathogenic

Table 1.1 Classification of corona viruses (Order Nidovirales)

Order	Family	Subfamily	Genus	Strains
Nidovirales	*Coronaviridae*	*Coronavirinae*	*AlphaCoVs*	CCoV, FCoV, FIPV, PRCV, TGEV, HCoV 229E, HCoVNL63, Mi-BatCoV 1A, Mi-BatCoV 1B, Mi-BatVoV HKU8, PEDV, Rh-BatCoV HKU2, Sc-BatCoV
			BetaCoVs	BCoVMebus, ECoV NC99, HCoV OC43, PHEV, HCoV HKU1, MHV, RCoV Parker, Pi-BatCoV HKU5, Ro-BatCoV HKU9, Ty-BatCoV HKU4, SARS-CoV, MERS-CoV, SARS-CoV-2
			GammaCoVs	IBV, BWCoV SW1, TcoV
			DeltaCoVs	Night heron CoV HKU19, Bulbul CoV HKU11, Munia CoV HKU13, Thrush CoV HKU12, Wigeon CoV HKU20, Common, moorhen CoV HKU21, White-eye CoV HKU16, Porcine CoV HKU15
		Torovirinae	*Torovirus*	BToV, EToV, HToV, PtoV
			Bafinivirus	White bream virus (WBV)
	Arteriviridae		Arterivirus	PRRSV, EAV
	Mesoniviridae		Alphamesonivirus	DKNV
	Roniviridae		Okavirus	YHV

[As per International Committee on Taxonomy of Viruses; Wang et al. 2020; Cong et al. 2017)]
The green color indicates human Corona viruses

HCoVs most commonly detected are HCoV-OC43 followed by HCoV-NL63 and HCoV-HKU1 (with similar frequencies) and then HCoV-229E having lower detection frequency (Poutanen 2012). HCoV-NL63 epidemic was observed to peak during spring and summer. HCoVs: HKU1 and 229E peaks are observed in winters, while OC43 causes infections throughout the year. Infections caused by these HCoVs also vary by age. For example, HCoV-HKU1 primarily infects elderly people (50–65 years), NL63 primarily infects people of 35–50 years, 229E primarily infects 7–15 years old children, and OC43 mostly infects infants and toddlers aged

less than 3 years (Zhnag et al. 2018). The high pathogenic strains SARS-CoV-1 & 2 and MERS-CoV are reported to be biased towards sex. Males were more infected when compared to females (Hafeez et al. 2020; Petersen et al. 2020). The spread of MERS infection in the population is enhanced by low relative humidity and higher temperatures and mostly infects people above 45 years of age (Alghamdi et al. 2014; Channappanavar et al. 2017). Children are rarely shown severe illness from these highly pathogenic strains of CoVs.

1.3 Structural and Genomic Features of Corona Viruses

1.3.1 Structural Features

The *Coronaviridae* family of viruses shares many structural features: the diameter of the virion is 120–160 nm, roughly spherical, club-shaped spike projections (formed by spike protein) on the surface, giving them the appearance of the solar corona, hence they are named as Corona viruses. The four major viral structural proteins are spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (Fig. 1.1a). Spike protein (~150 kDa) is a class I fusion protein, modified by N-linked glycosylation, and is a major therapeutic target because of its significance in binding the host cells. They are expressed on the viral envelope as a trimeric form; containing two subunits, S1 (head) and S2 (stalk). In many CoVs, "**S**" protein is usually cleaved by host proteases resulting in two subunits S1 and S2 (Fig. 1.1b). The S1 subunit has a receptor-binding domain (RBD) while membrane fusion is mediated by the S2 subunit. However, it was reported that further priming of the S2 subunit is necessary before the fusion of the virus to the cell membrane. "**M**" protein (~25–30 kDa) is an integral type III membrane protein, essential for membrane curvature and assembly of CoVs particles and also interacts with other structural proteins. "E" protein (~8–12 kDa) is necessary for electrochemical balance in the subcellular compartment because they form ion channels via assembling in the membrane. "**N**" protein (349–470 amino acids) binds to genomic RNA for RNA packaging and is also necessary for viral genome replication, budding, and RNA synthesis.

Toroviruses have some additional/different features, such as doughnut-shaped nucleocapsid and hemagglutinin-esterase (HE) glycoprotein (~48 to 65 kDa) projected outward from the virion. HE glycoprotein is responsible for binding with sialic acid residue and also acts as a receptor destroying enzyme which is necessary for releasing the viral progeny from infected cells. HE glycoproteins are also present in some beta CoVs, HCoV-OC43, and HCoV-HKU1. The members of the *Coronavirinae* subfamily have flexible nucleocapsid (Baric et al. 2005; Langereis et al. 2009; Fehr and Perlman 2015; Li 2016; Payne 2017; Song et al. 2019; Tortorici et al. 2019; Widagdo et al. 2019).

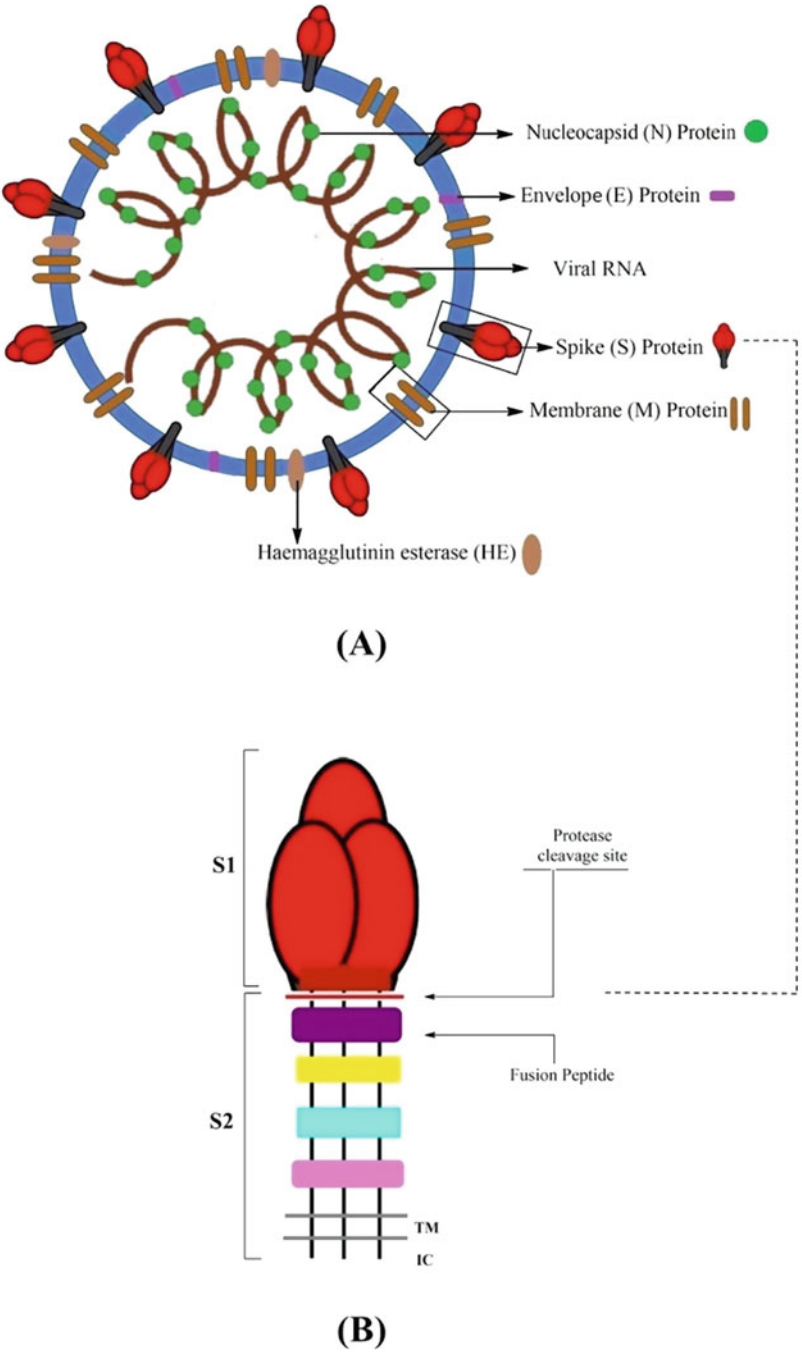

Fig. 1.1 (**a**) CoVs Virion Structure: Haemagglutinin esterase (HE) glycoprotein is only encoded by Toro viruses and some beta CoVs (HCoV-OC43 and HCoV-HKU1). (**b**) S1 & S2 subunits 1 & 2 of spike protein, *TM* Transmembrane, *IC* Intracellular tail Spike (S) protein is expressed as a trimeric form; containing two subunits, S1 (head) and S2 (stalk). S protein is cleaved by host

1.3.2 Genomic Features

Genomic features of all HCoVs are common in many ways, such as they all have a large, positive-sense RNA genome of ~29.7 to 32 kb (largest viral RNA genome), contains at least six open reading frames (ORF). At 5′ end, a single ORF, which is two-third of their genome consists of ORF1a and ORF1b. ORF1a and ORF1b are translated into two large polyproteins, pp1a and pp1ab, that are autoproteolytically cleaved and lead to the generation of 16 nonstructural proteins (Nsp1–16) (except *Gamma CoVs* which does not have nsp1). The remaining genome (ORFs from 3′ end) encodes four major viral structural proteins: membrane (M), spike (S), nucleocapsid (N), and envelope (E) proteins and other associated proteins (Fig. 1.2c). Multiple enzymes essential for genome replication, nucleic acid metabolisms such as viral RNA-dependent RNA polymerase, endo, and exonuclease, and so on, consist of this16-protein replication-transcriptase. The gene order is **5′**-replicate (rep gene), spike (S), envelope (E), membrane (M), nucleocapsid (N)-**3′**, and short untranslated regions UTR at both termini (Fig. 1.2a). Apart from these structural proteins, other structural proteins are also encoded by different CoVs such as hemagglutinin esterase (HE), 3a/b, 4a/b protein (Fig. 1.2b). ORF1a and ORF1b genes, that encode nonstructural proteins are highly conserved throughout the CoVs (Song et al. 2019; Fehr and Perlman 2015; Liu et al. 2020a, b; Chen et al. 2020a, b; Su et al. 2016; Yang et al. 2020).

1.4 Evolution of Corona Viruses

1.4.1 Animal Origin of Human Corona Viruses (HcoVs)

Since the later 1930s, animal CoVs have been reported to cause infections in a variety of animals including, cow, pig, dog, cat, turkey mouse, and others. However, the human corona viruses (HcoVs) were first characterized in the mid-1960s after the passaging of the first HCoV B814. All HCoVs are zoonotic pathogens—originated from animals, transmitted from their natural reservoirs to intermediary host (s) and then from the intermediary host (s) to humans (Shi and Hu 2008; Azhar et al. 2014; Ye et al. 2020a, b).

1. The Animal origin of HCoVs which are fully adapted to humans: From all known HCoVs, four CoVs: HCoV-NL63, HCoV-229E, HCoV-OC43, and HCoV-HKU1 arise in the general population. They are community-acquired corona viruses and have become fully adapted to humans. Bats are considered as the natural hosts for HCoV-NL63 and HCoV-229E, whereas rodents are considered

Fig. 1.1 (continued) proteases resulting in two subunits S1 and S2. The S1 subunit has a receptor-binding domain (RBD) while membrane fusion is mediated by the S2 subunit (Source: Malik et al. 2020a, b)

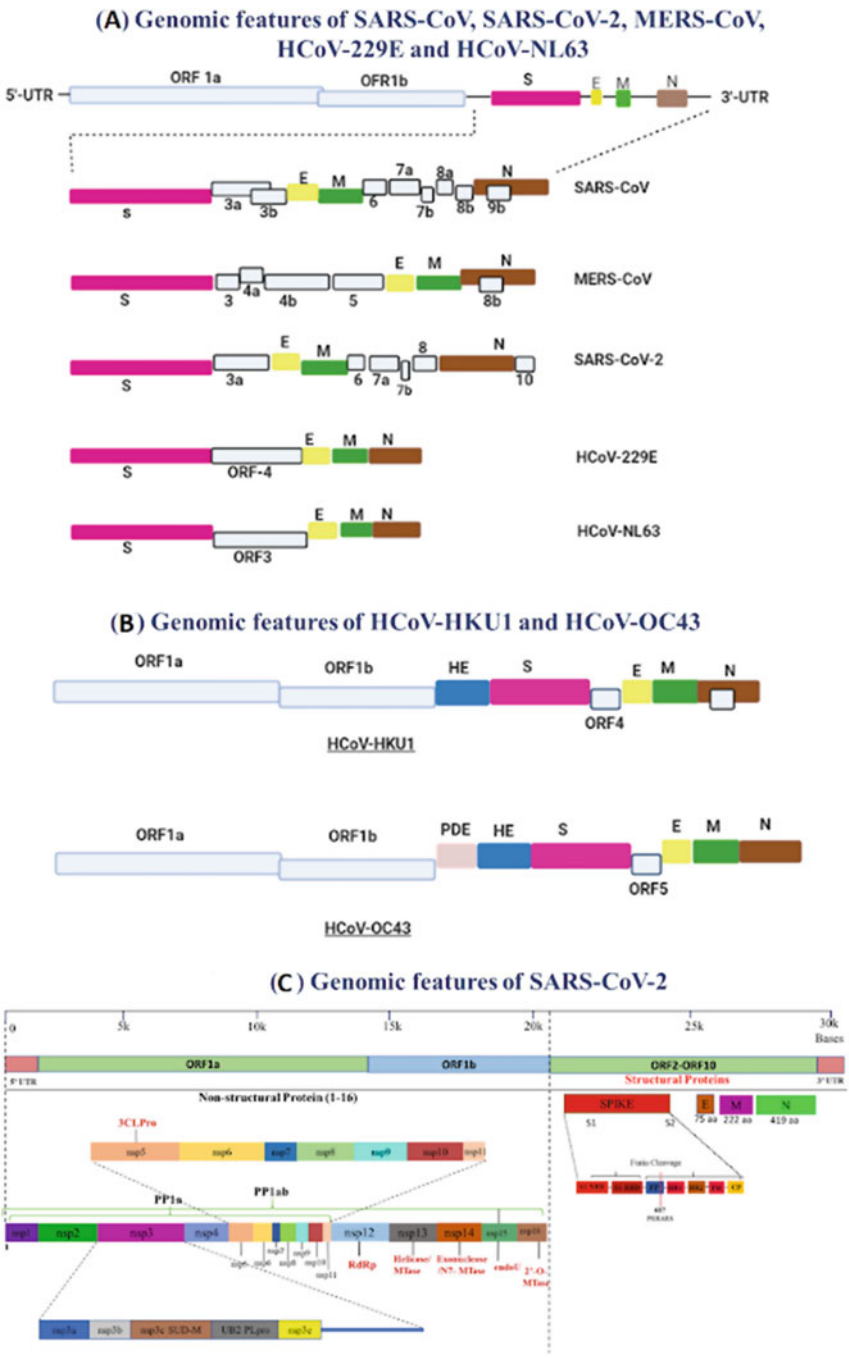

Fig. 1.2 Schematic representation of the genomic features of HCoVs. *ORF* Open reading frames, *S* spike, *E* envelope, *M* membrane, *N* nucleocapsid, *PLpro* papain-like protease, *RdRp* RNA-dependent RNA polymerase, *EndoU* endoribonuclease, 2′-O-MTase 2′ -O-ribose methyltransferase, *NTD* N terminal domain, *RBD* receptor binding domain, *FP* fusion peptide,

to be the natural hosts for HCoV-OC43 and HCoV-HKU1. Intermediate hosts for HCoV-HKU1 and HCoV-NL63 are not yet known, while the intermediate hosts of HCoV-229E and HCoV-OC43 are camelids and bovines respectively (Fig. 1.3) (Su et al. 2016; Ye et al. 2020a, b).

2. The Animal origin of HCoVs which are not well adapted to humans: SARS-CoV, MERS-CoV, and SARS-CoV-2 are not well adapted to humans. However, there is a chance of SARS-CoV-2 becoming fully adapted, as it infects more and more people because the pandemic caused by this virus is ongoing since 2019. Horseshoe bat (s) species of the genus *Rhinolophus* within the family *Rhinolophidae* were the natural reservoirs of the SARS-CoV-like viruses, which later passed through Civets before reaching humans. The natural reservoir and intermediate hosts of SARS-CoV are bats and Palm Civet respectively (Shi and Hu 2008; Cyranoski 2017; Ye et al. 2020a, b). Bats are also reservoir hosts for MERS-CoVs as well as SARS-CoV-2.

Camels act as intermediate hosts of MERS-CoV (Fig. 1.3) that transmit the virus from its reservoir to humans who had close contact with Camel's nasal secretions (Azhar et al. 2014; Yin and Wunderink 2018). The intermediate host (s) of SARS-CoV-2 has not been confirmed yet. However, people are believing the pangolins might be its intermediary hosts (Zheng 2020; Sen et al. 2020).

1.4.2 Genetic Diversity and Possible Reasons Behind the Interspecies Transmission

All the CoVs possess many genetic similarities. Many evolutionary changes have also occurred among them. As we read the above subheadings, that all seven known HCoVs are of zoonotic origin. These viruses infected humans after crossing the species barrier (s), being transmitted from their natural host(s), and then being transmitted from humans to humans (Shi and Hu 2008; Azhar et al. 2014; Su et al. 2016; Cyranoski 2017; Ye et al. 2020a, b). During evolution, mutations and recombinations occurred as a result of the receptor-binding domain (RBD) of the spike protein of these CoVs adapted to bind to human receptors, resulting in the interspecies host jump (Lau et al. 2011). RNA viruses are known for their high mutability; in particular, CoVs have a higher mutation(s)/recombination(s) rate than other single-strand RNA viruses. ~6.410.58 $\times$ 10^{-4} and ~3 $\times$ 10^{-4} substitution/site/

Fig. 1.2 (continued) HR heptad repeat, *TM* transmembrane domain, *CP* cytoplasmic domain (**a**) Genomic features of SARS-CoV, SARS-CoV2, MERS-CoV, HCoV-229E, HCoV-NL63 show the organization in Structural and nonstructural proteins(nsp). (**b**) Genomic features of HCoV-HKU1 and HCoV-OC43 show one additional protein hemagglutinin esterase (HE). (**c**) Complete Genomic organization of SARS-CoV2. At 5′ end ORF 1a gene encodes for PL protease and 3CL protease and ORF 1b gene encodes for RdRp, helicase, exo, and endo nuclease. ORFs from 3′ end encodes major viral structural proteins: S, E, M, and N proteins (Source: Lim et al. 2016; Romano et al. 2020)

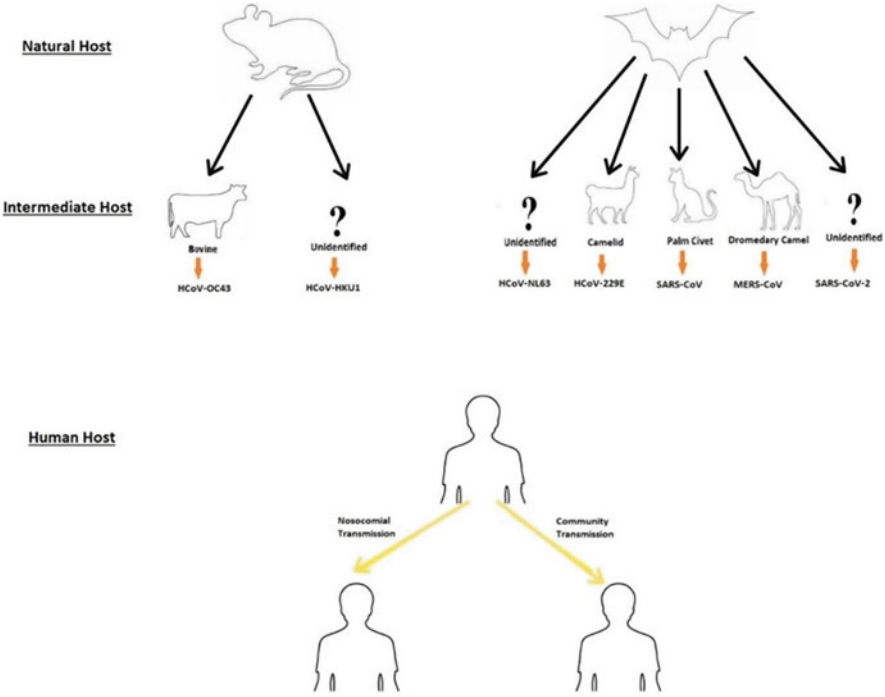

Fig. 1.3 Mode and host for transmission of different HCoVs: Black arrows represent the transmission of CoVs from the natural host (bat and rodent) to their respective intermediate hosts. Orange arrows represent the transmission of CoVs from intermediate hosts to humans and yellow arrows represent human to human transmission (Source: Sunny 2020)

year has been reported in the "Spike" gene of HcoVs OC43 and 229E respectively. 0.80–2.38 $\times$ 10^{-3} nucleotide substitutions/site/year mutation rates had been estimated in the whole genome of SARS-CoV. 1.12 $\times$ 10^{-3} substitutions/site/year for MERS-CoVs (Su et al. 2016). Furthermore, 6.57 $\times$ 10^{-4} substitutions/site/year evolutionary rate and 93 mutations have been estimated in the entire genome of SARS-CoV-2. Three mutations are present in spike surface glycoprotein RBD (Phan 2020; Castells et al. 2020). Furthermore, a hypothesis exists on the origin of Civet SARS-CoV called SZ3, which is raised due to the recombination of two bat strains, WIV 16 and Rf4092 (Cui et al. 2019). Thus, continuous evolution in the viral species is a reason for interspecies transmission and making these viruses capable of escaping from host defense, leading to the increased pathogenicity and higher spreading rate (Stadler et al. 2003; Lu et al. 2015; Su et al. 2016). The mutation scenario that makes these viruses adoptable to the new host (s) and responsible for their increase in pathogenicity is summarized in Table 1.2.

Table 1.2 Genetic diversity and mutation scenario in HCoVs

	HCoV-NL63, -OC43, -HKU1, -229E	SARS-CoV	MERS-CoV	SARS-CoV-2
Evolution rate	~6.410.58 $\times 10^{-4}$ and ~3 $\times 10^{-4}$ substitution/ site/ year has been found in the S gene of HCoV-OC43 and HCoV-229E	0.80–2.38 $\times 10^{-3}$ nucleotide substitutions/site/ year mutation rate	1.12 $\times 10^{-3}$ substitutions/ site/year	6.57 $\times 10^{-4}$ substitutions/ site/year
Genome size	~27.5 kb genome size of HCoV-229E and - NL63. ~30 kb of - HKU1 and -OC43	29.7 kb	30.1 kb	29.8 kb
Mutations in the CoVs strains isolated from humans	• Deletions in spike genes of 229E viral strain isolated from humans and camelids were as deletion has not found in bat-associated 229E strain • A deletion in HCoV-OC43 in downstream of the spike gene, which is believed to be responsible for host switching or adaptation to humans	• Deletion of 29-nucleotides in the 3′ end of ORF8a • 82 nucleotide deletion inORF8 • Deletion of 415 nucleotides (loss of entire ORF8) • Ser487Thr and Lys479Asn amino acid substitution in S protein. • Asp^{77} to Gly (in S protein), Glu^{1389} to Asp (in nonstructural polyprotein), Thr^{244} to Ile (in S protein), and Arg^{17} to Cys (in ORF 8a) amino acid substitution	• Two mutations: Ser746Arg and Asn762Ala at S1/S2 boundary of "**S**" protein make them able to infect humans • Arg911Cys amino acid substitution in nsp3	• Presence of an additional cleavage site for furin enzyme at S1 and S2 junction • F436Y and 427Asn mutations in "**S"** protein responsible for high stability spike-host receptor complex • Ala348Thr, Arg408Ile, Ala520Ser, His519Gln, Val483Ala, Gly476Ser, and Ala930Val, Asp936Tyr mutations were identified at RBD and HR1 domain respectively • One mutation in ORF9a at position 28,881 causes the change of two amino acids: Arg to Lys and Gly to Arg and

(continued)

Table 1.2 (continued)

	HCoV-NL63, -OC43, -HKU1, -229E	SARS-CoV	MERS-CoV	SARS-CoV-2
				mutation in ORF1ab at position: 1397 in nsp2, 14,408 in RdRp, 17,746 and 17,857 in nsp 143 that causes the amino acid change from Val to Ile, Pro to Leu, Pro to Leu, Cys to Tyr respectively

1.4.2.1 Genetic Diversity Among Human Corona Viruses (HCoVs)

1.4.2.1.1 Genus *AlphaCoVs*

Two of the seven known HcoVs, 229E and NL63, belong to the genus *AlphaCoVs* and have a genome of ~27.5 kb in size. Both viruses have a 70 nucleotides long leader sequence at 5′ terminus. The lengths of the 5′ UTR and 3′ UTR in 229E, is 292 and 462 nucleotides, respectively. In NL63 5′ UTR is 286 and 3' UTR is 287 nucleotides long. The 229E is recombined with the alpha-CoV within the "S" gene, Whereas, NL63 is recombined with the Porcine epidemic diarrhea virus (PEDV) within the "M" gene.. There is a deletion in the "**S"** gene of 229E. However, it is not clear whether this deletion is responsible for the change in tissue tropism or not. Although the host receptor angiotensin-converting enzyme-2 (ACE2) of NL63 and SARS-CoV is similar, there is no sequence similarity between receptor-binding regions of (RBD) "Spike" proteins of NL63 and SARS-CoV (Forni et al. 2016; Liu et al. 2020a, b; Chen et al. 2020a, b).

1.4.2.1.2 Genus *BetaCoVs*

The HcoVs-OC43, HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2 belong to the genus *BetaCoVs.* The genome size of HCoVs-OC43 and HKU1is more than 30,000 bp. The genome size of SARS-CoV, MERS-CoV, and SARS-CoV-2 is 29,727 bp, 30,119 bp, and 29,844 bp, respectively (Song et al. 2019; Lu et al. 2020; Liu et al. 2020a, b). In the genome of OC42 and HKU1, the 5′ UTR is 210 and 205 nucleotides long, while 3′ UTR is 288 and 281 nucleotides long, respectively. The length of SARS-CoV-2 spike protein is by far the longest of 1273 amino acids in comparison to SARS-CoV (1255 amino acids) and MERS-CoV (1270 amino acids) (Song et al. 2019; Lu et al. 2020; Malik et al. 2020a, b). Phylogenetic studies showed that SARS-CoV-2 shares a high sequence identity of about 79.5% to SARS-CoV (Ning et al. 2020), ~96% similarity with a bat corona virus RaTG13. Furthermore,

the spike protein of SARS-CoV-2 has an additional cleavage site for the "furin" enzyme at the junction of S1 and S2 subunits, responsible for its increased infectivity. Whereas this additional site is absent in other related betaCoVs (Zhang and Holmes 2020; Li et al. 2020a, b).

Higher sequence similarity with 12.8% of the difference is present between the spike protein of SARS-CoV and CoV-2 (Lu et al. 2020). The carboxy-terminal domain (CTD) of the S1 sub unit of SARS-CoV and CoV-2 is responsible for binding to the human host receptor (ACE2). Furthermore, most of the aminoacids that contact with ACE2 are identical between them (Sun et al. 2020). But at the same time, several key residues of the spike protein RBD between these two viruses are reported to be different (Lu et al. 2020). The most important variation observed is Val404 (SARS-CoV-RBD) that is substituted with Lys417 (in SARS-CoV-2-RBD), resulting in a strong interaction of SARS-CoV-2 RBD with ACE2. This substitution results in a salt bridge formation between Lys 417 and Asp30 of ACE2 (Yan et al. 2020). A total of 380 amino acid substitutions are present between the genome of SARS-CoV and CoV-2 (Petrosillo et al. 2020). SARS-CoV-2 spike protein has lower free energy and better RBD solubility (more soluble) allowing this virus to survive at higher temperatures and easier binding to ACE2, resulting in higher infection ability (He et al. 2020).

1.4.2.2 Genomic Variability of Corona Viruses (CoVs) in Animals and Humans

1. SARS-CoV variability in animals and humans: The genomes of SARS-CoV like viruses isolated from mammals in China exhibit differences in their genomes compared to the counterparts infecting humans. For example, the GZ0 strain of corona virus has an additional stretch of 29-nucleotides in the 3′ end domain of ORF8a, which fuses ORF8a and ORF8b into single ORF (ORF*), encoding122 amino acid protein. Whereas, this sequence is absent in the human corona viral genomes. Deletion of this region (29ntds) in most human corona viral isolates is a reason for their increased fitness and spread in human hosts (Stadler et al. 2003). Furthermore, some other deletions were also reported in the same ORF, such as deletion of 82 nucleotides, in SARS-CoV strain isolated from farmed Civets in Hubei, China, and from humans during very early cases in Zhongshan, China. These deletions supporting the hypothesis that the early SARS-CoV infections of humans came from wild animals. In some human SARS-CoV strains, deletion of 415 nucleotides (loss of entire ORF8) is also detected (Lau et al. 2015; Chinese SARS molecular epidemiology consortium 2004). In the spike protein, Ser487 of animal CoVs is substituted with Thr487 in human SARS -CoV strains. This substitution increases the affinity of RBD of spike protein for human ACE2 receptors. One more substitution of Lys479 (of S protein) with Asp479, also increases the RBD binding ability for human ACE2 by interacting with its His 34 residue. This residue is absent in the ACE2 receptor of palm Civet (Li et al. 2005; Zhang and Holmes 2020). Apart from these amino acid substitutions, some other transitions and transversions were also detected in human isolates of SARS-CoV. It caused amino acid switching in Spike protein and nonstructural proteins

(Nsp). For instance, one A to G transition, one G to T transversion, and two C to T transitions were discovered at nucleotides 21,721, 17,564, 22,222, and 27,827, respectively, resulting in amino acid switches from Asp77 to Gly (in S protein), Glu 1389 to Asp (in nonstructural polyprotein), Thr244 to Ile (in S protein), and Arg17 to Cys (in ORF 8a) respectively (Chinese SARS Molecular Epidemiology Consortium 2004).

2. MERS-CoV variability in humans and animals: Before the MERS-CoV outbreak in 2012, the MERS-cluster of CoVs was discovered in bats and reported that bats are natural reservoirs for these viruses. Between bat and human MERS-CoVs, the sequence identity of the genome and Spike proteins are around 85% and 45–65% respectively (Cui et al. 2015; Fan et al. 2019). HKU4 and HKU5 are closely related to this virus and both can infect bats. BothHKU4 and MERS-CoV recognize the same host receptor-dipeptidyl–peptidase 4 (DPP4). However, HKU4 is not able to enter into human cells (Rabaan et al. 2017; Luo et al. 2018). MERS-CoV can infect humans because it already has two mutations: Ser746Arg (S746R) and Asn762Ala (N762A) at S1/S2 boundary, which makes it susceptible to cleaved by human proteases. Whereas these mutations are absent in the HUK4 Spike protein (Yang et al. 2015). No evidence is reported concerning the use of human DPP4 receptors by HKU5 (Fan et al. 2019). Similar to HKU4, another strain called NeoCoV can also infect bats and it is 85% identical to MERS. However, NeoCoV binds with different host receptors because the S1 subunit of NeoCoV spike protein is highly different from the MERS-Spike protein S1 subunit (Anthony et al. 2017).

   Apart from HKU4, two MERSr-CoVs known as HKU25 and 422CoV can also recognize the DPP4 host receptor (Fan et al. 2019). However, HpBatCoV HKU25 binds with hDPP4 with a lower affinity compared to MERS. Thus, this evidence indicates that MERS-CoV has evolved its Spike protein and gained the ability to utilize the DPP4 receptor of both camels and humans, resulting in the interspecies transmission of MERS-CoV (Lau et al. 2018). Many positive selected sites are also present in the Heptad repeats 1 and 2 (HR1 and HR2) regions (of S2 subunit) such as Arg^{652} and Val^{1060}. Along with this, one mutation is present in the HR1 region of MERS isolated from camels at position 1020, and three different amino acids: Glutamine/Arginine/Histidine is present in this position. These mutations show the importance of HR1 and HR2 regions in the evolution of the *betaCoVs* Spikegene (Forni et al. 2015). During the MERS-CoV outbreak in South Korea (2015), the Ile529Thr/Asp510Gly (I529T/ D510G) point mutations (in MERS-CoV S protein) reduced the affinity of MERS-CoVRBD for DPP4 (Rabaan et al. 2017).

3. SARS-CoV-2 variability in Humans and Animals: Bat CoV RaTG13 is the most recent ancestor of SARS-CoV-2 with ~96% similarity. A total of 147 amino acid differences are present between the genome of SARS-CoV-2 and RaTG13 (33 amino acid differences in the "S" protein of these two strains). A mutation Tyr436Phe in the RaTG13 strain is responsible for poor stability of the spike-host receptor complex. Tyr436 in human SARS-CoV-2 spike protein forms a hydrogen bond with ACE2 (Asp38) receptor, which is responsible for the increased

stability. Tyr436His mutation is detected in mice SARS-CoV also. Apart from this mutation, a 427Asn mutation (in the spike protein) is present both in humans and civet SARS-CoV-2 while it is absent in batCoVs (Cagliani et al. 2020; Galindo et al. 2020; Li et al. 2020a, b; Paraskevis et al. 2020). In addition, 25 mutations in SARS-CoV-2 S protein alter amino acid sequences including, Ala348Thr, Arg408Ile, Ala520Ser, His519Gln, Val483Ala, Gly476Ser mutations at RBD and Ala930Val, Asp936Tyr at HR1 domain (Lokman et al. 2020).

In Human SARS-CoV-2, mutations in ORF1ab at position: 1397 in Nsp2, 14,408 in RdRp, 17,746, and 17,857 in Nsp143 caused amino acid (s) change from: Val to Ile, Pro to Leu, Cys to Tyr, respectively. Furthermore, one mutation in ORF9a at position 28,881 caused two amino acids switches: Arg to Lys and Gly to Arg (Pachetti et al. 2020). Thus, this high level of viral diversity in the human SARS-CoV-2 genome suggests that this virus has started to adapt to the human environment (Zhao et al. 2020).

The mutation contributing to the modification of SARS-CoV-2 pathogenicity is the mutation at site 11,083 in the ORF1ab region that encodes for Nsp6 (Benvenuto et al. 2020; van Dorp et al. 2020). The SARS-CoV-2 RBD amino acid sequence is 97.4%, similar to Pangolin CoVs S protein RBD, higher than RaTG13 having only 89.2% S protein RBD amino acid similarity. This suggests that Pangolin might be the intermediate host for SARS-CoV-2. However, more studies are needed to confirm this (Zheng 2020; Sen et al. 2020). Some studies state that bat CoV RaTG13 is the common ancestor of SARS-CoV-2 and Pangolin-CoVs (Kasibhatla et al. 2020).

4. CoV-229E and -OC43 variability in humans and animals: Deletions are present in the spike genes of human and camelid corona viral strain 229E. Whereas, no deletions are reported in the bat-associated 229E strain. Recombination can be the reason for these deletions.. Similar to these viruses, in HCoV-OC43 a deletion of 290 nucleotides is reported downstream of the spike gene which is believed to be responsible for host switching or adaptation to humans (Corman et al. 2018; Drexler et al. 2014). Apart from deletion, recombinations are also observed in the genome of OC43, at nsp2/nsp3, nsp12/nsp13, and NS2a/HE junction of ORF1ab. However, the role of these recombinations is not clear yet (Lau et al. 2011).

1.5 Emergence of Corona Viruses as Human Pathogens

1.5.1 Pandemic Human Corona Viruses

The seven HCoVs reported to date show the worldwide distribution in nature. However, the large outbreaks were caused mainly by the three viruses, that is, SARS-CoV, MERS-CoV, and SARS-CoV-2.

The first grievous epidemic caused by the HCoVs was recorded in the year 2002 by SARS-CoV in Guangdong province of China on November 16, 2002. An older man in Foshan was the first one who got infected. Later on, the infection was spread

outside China through a doctor who had been treating SARS-CoV patients in Guangdong, traveled to Hong Kong, and transmitted the infection to 16 other hotel guests. When these infected individuals returned to their destinations: Vietnam, Singapore, Canada, and the United States, they carried the infection to their respective countries. By the end of July 2003, SARS-CoV spread to 32 countries (Yin and Wunderink 2018; Hilgenfeld and Peiris 2013; Stadler et al. 2003; Meo et al. 2020) with a mean reproductive number (R_0) of 2–5 (Li et al. 2020a, b; Guarner 2020).

The second epidemic, caused by MERS-CoVs, was first reported in June 2012, when a 60-year-old male was hospitalized in Saudi Arabia and died 14 days later. In September 2012, a 49-year-old man who had a travel history in Saudi Arabia was infected with the same virus. In April 2012, a group of 13 people was reported to be infected with the same virus (MERS-CoV) in Jordan. It was the first case where a cluster of humans was reported to get infected. The majority of cases have occurred in the Arabian Peninsula and infection has spread to other countries (26 countries across the globe), from index cases with travel history to the Middle East or North Africa (Yin and Wunderink 2018; Omari et al. 2019; Douglas et al. 2018). Usually, HCoVs peak in winter and spring but few cases were also observed in early summer (Woo et al. 2012; Lee et al. 2013).

The third and most recent outbreak of SARS CoV-2 was first reported in Wuhan, China. Fifty-five percent of the patients had a history of exposure to the seafood market of Wuhan city and this was the first stage of epidemic transmission. While in the second stage of epidemic transmission (that was the community dispersion stage), only 8.6% of SARS-CoV-2 infected patients had a history of exposure to the seafood market of Wuhan city. All these patients became infected in January 2020. The SARS-CoV-2 epidemic spread to other parts of China and other countries around the world when migrant workers and people of Wuhan started to travel to their hometowns in other cities of China or other countries to celebrate Spring Festival (Chinese New Year). Later on, it spread to almost all countries and territories of the world (Ge et al. 2020; Wu et al. 2020; Ye et al. 2020a, b).

1.5.2 Endemic Human Corona Viruses

HCoVs 229E, OC43, HKU1, and NL63were also distributed throughout the world. They cause diseases in temperate climates, especially during winter and spring (Poutanen 2020). The HCoV-NL63 epidemic has been observed to peak during spring and summer. It was first isolated in the Netherlands (2004) from a 7-month-old infected child. It has an incubation period of 2–4 days. In 2005, several children were affected by this virus in New Haven, USA. HCoV-HKU1 was first discovered in Hong Kong. Furthermore, cases were also reported in America, Australia, France, and Brazil. HCoV 229E and HCoV OC43 were discovered in 1966 and 1967 respectively (Table 1.3) (Su et al. 2016; Kiyuka et al. 2018; Liu et al. 2020a, b).

Table 1.3 Emergence and epidemiology of all known HCoVs

	SARS-CoV	MERS-CoV	SARS-CoV-2	HCoV-229E	HCoV-OC43	HCoV-NL63	HCoV-HKU1
Genus	Beta-CoV, lineage B	Beta-CoVs, lineage C	Beta-CoV	Alpha-CoV	Beta-CoV, lineageA	Alpha-CoV	Beta-CoV, lineageA
Natural host	Bat	Bat	Bat	Bat	Rodents	Bat	Rodents
Intermediate host	Palm civet	Dromedary camel	Unknown	Camelids	Bovines	Unidentified	Unidentified
Possible ancestor	Recombination between SARSr-CoVs of *Rhinolophus bats*. Shows 95% genome sequence identity with bat SL-CoV: Rs3367 and RsSHC014	Neo-CoVs	Unknown but shows 96.2% nucleotide identity with BatCoVRaTG13	Alpaca (alpha) CoV	BCoV	NL63r-bat CoV strain BtKYNL63-9b X Hipposideros- associated CoVs 229E like viruses	CoVs strain of *Rodentia*
Year of appearance/ isolation	2002	2012	2019	1966	1967	2004	2005
Transmission region	Globally	Regionally	Globally	Globally	Globally	Globally	Globally
Transmission patterns	From animal to human and from human to human.						
Fatality rate	10.87%	34.77%	2.08%	N/A	N/A	N/A	N/A
R_0	2–5	2.7–3.9	3.3–5.5	N/A	N/A	N/A	N/A
Incubation period(days)	2–10(7)	2–10 (5.5)	2–14(5.2)	2–5	2–5	2–4	2–4
Seasonal occurrence	Winter (Dec-Jan)	Summer (May–July)	Winter (Dec-Jan)	Winter	Winter	Summer and autumn	Winter
References	Hu et al. (2015);Su et al. (2016); Zeng et al. (2018); Liu et al. (2020a, b); Ye et al. (2020a, b);Meo et al. (2020); Lu et al. (2020); Decaro and Lorusso (2020); Li et al. (2020a, b)						

1.6 Comparative Pathophysiology of HCoVs

1.6.1 Less Pathogenic Strains (229E, NL63, OC43, and HKU1)

Out of seven known human coronaviruses (HCoVs), HCoV-229E, NL63, HKU1, and OC43 cause mild acute respiratory illness, depending upon the age and comorbidities such as cardiac and lung-related problems of the infected individuals. These are less pathogenic, causing upper respiratory tract infections (Fig. 1.4). SARS-CoV, CoV-2, and HCoV-NL63 employ the same host receptors (ACE-2 for entry into the host cell. Although these HCoVs share the same receptor, their binding affinity varies, because of the lower affinity with ACE2. HCoV-NL63 causes moderate respiratory infection and usually causes mild upper respiratory tract infections. However, few strains of this virus were reported to cause lower respiratory tract infection and pneumonia (Li et al. 2020a, b; Divani et al. 2020). HCoV-OC43 and -229E account for 5–30% of human respiratory tract infections. HCoV-OC43 uses either HLA class I molecules or sialic acids for cell entry (further studies are still required for the confirmation of OC43 host receptor). It can replicate in the surface layer of the upper respiratory tract. Whereas the host receptor for 229E is human aminopeptidase N(hAPN). OC43 and 229E both create superficial infections in nasal tissue after 3–4 days of infection. During winter and spring, these viruses usually cause common colds (Hierholzer and Tannock 1988; Lau et al. 2006; Owczarek et al. 2018; Li et al. 2019; Niu et al. 2020;). In addition, OC43 also has neuroinvasive properties. As a result, it can also be involved in neurological diseases. Whereas the receptor of HCoV-HKU1 is not identified yet. These viruses cause pneumonia (which is described as a monophasic disease) and bronchiolitis (Woo et al. 2005; Owczarek et al. 2018; Niu et al. 2020).

1.6.2 Highly Pathogenic Strains (SARS-CoV, MERS-CoV, and SARS-CoV-2)

Now coming to the more pathogenic strains of HCoVs have caused severe outbreaks and are responsible for several deaths (Fig. 1.4).

1.6.2.1 SARS–CoV

The route of entry of SARS-CoV (2002) in humans is via the respiratory tract, mainly by droplet transmission. The host receptor of this virus is ACE2. It is a dipeptidyl carboxypeptidase (under normal physiological conditions), and a homolog of the ACE protein. Both (ACE2 and ACE) are key enzymes of the Renin-Angiotensin system (RAS). ACE2 plays a protective role in lung failure. Both SARS-CoV and SARS-CoV-2 down-regulate the function of ACE2. In the RAS system, angiotensinogen is converted to Angiotensin I (Ang I) by rennin, and then Ang I is converted into Ang II. The main function of ACE 2 is the downregulation of Ang I and Ang II levels, which can bind to Ang II type I (AT1) receptor and causes a certain type of lung injuries such as pulmonary hypertension, pulmonary fibrosis,

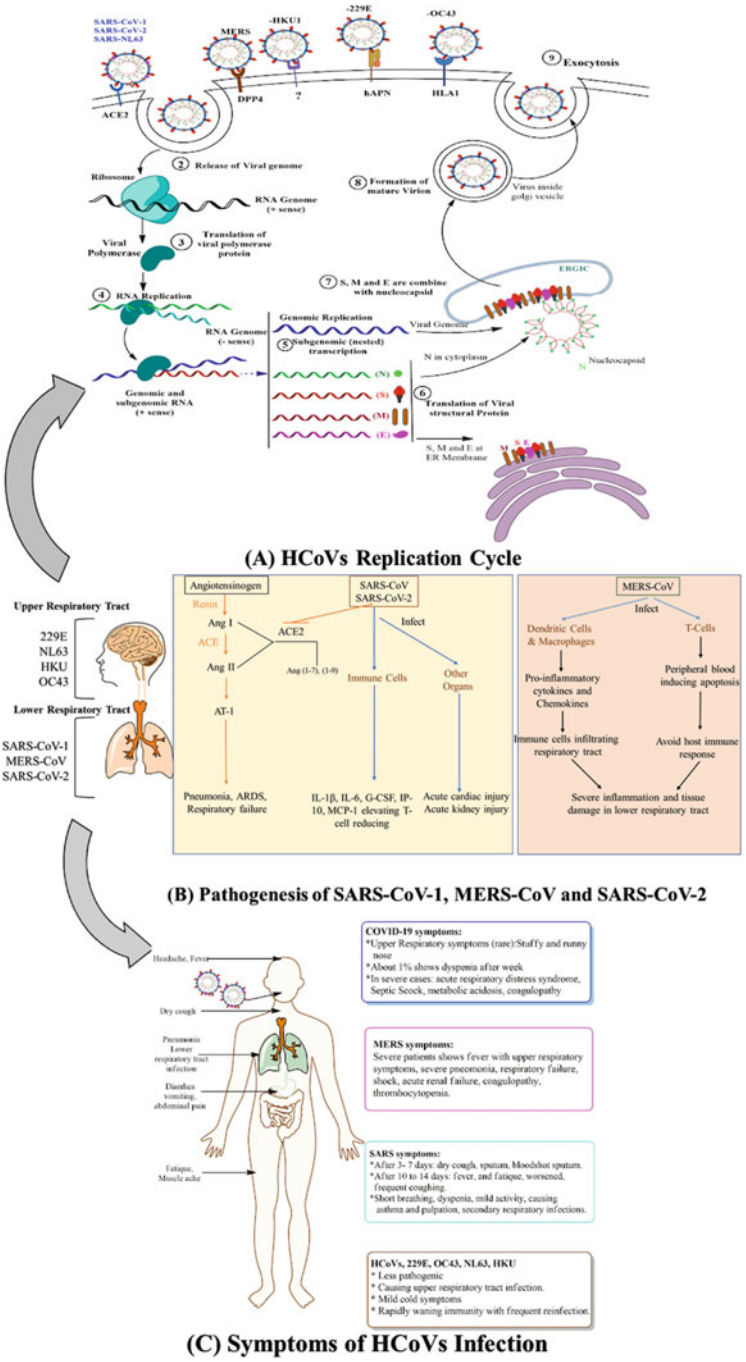

Fig. 1.4 HCoVs Replication, Pathogenesis, and Symptoms: Less pathogenic strains HCoVs-229E, -NL63, -HKU1, and -OC43 cause upper respiratory tract infection. Highly pathogenic strains SARS-CoV-1, SARS-CoV-2, and MERS-CoV, cause lower respiratory tract infection. (**a**) HCoVs Replication cycle. After binding of viral spike protein with host cell receptor, the virus

and acute lung injury. Thus, during SARS-CoV and CoV-2 infections, downregulation of ACE2 contributes to the pathological changes in the lungs (Fig. 1.4b) (Hui et al. 2014; Yin and Wunderink 2018; Damas et al. 2020; Chen et al. 2020a, b).

After SARS-CoV infection, patients can die as early as day 4 and even as late as 108 days. In general, a disease caused by SARS-CoV occurs in three phases. In the first phase, patients show symptoms like sore throat, muscle pain, and dry cough. Shortness of breath and fever are also presented by patients and continued up to the second phase of illness and Oxygen deficiency (hypoxia). During the third phase, patients develop acute respiratory distress syndrome (ARDS) (Weiss et al., 2011). In the infected patients, macrophage, neutrophil, and monocyte infiltration in the lungs are increased. Further serum proinflammatory cytokines and chemokines such as TNF-α, IL-8, IL-6 are also elevated. This leads to the extensive lung damage of infected individuals (Liu et al. 2020a, b). Dysregulation of urokinase is also detected in these infections, leading to more severe lung pathology (Arabi 2017). SARS-CoV infections can also cause heart attack, acute coronary syndrome (ACS), and hepatic damage due to a decrease in albumin and elevated bilirubin levels. Some patients also show symptoms like delirium, a severe disturbance in mental abilities, restlessness, and acute renal impairment that leads to multi-organ failure (Renu et al. 2020). In this viral infection, SARS-like viral particles are found in monocytes and lymphocytes, mostly T cells, some B cells, and NK cells (Gu et al. 2005). Apart from this pathogenesis, the nucleocapsid protein of SARS-CoV is involved in suppressing RNAi in mammalian cells (Cui et al. 2015).

1.6.2.2 MERS-CoV

The receptor for MERS-CoV is (DPP4also known as CD26 (Lu et al. 2013). This receptor is widely expressed on epithelial cells in the kidney, small intestine, liver, prostate, activated leukocytes, and inhuman respiratory tract. It is mainly expressed in alveoli (Yin and Wunderink 2018). MERS-CoV patients show symptoms similar to SARS-CoV patients such as fever, dry cough, sore throat, headache, muscle, and abdominal pain, sometimes renal failure also. However, MERS-CoV has a fatality

Fig. 1.4 (continued) enters into the host cell via membrane fusion or endocytosis. In cytoplasm viral RNA is translated into viral polymerase. During replication, the copies of -Ve sense RNA produced are used as templates to produce full-length + ve sense RNA genomes and subgenomic RNA. viral structural protein spike (S), membrane (M), envelope (E) proteins are translated in the ER and get assembled with viral RNA-N complex into the ERGIC (ER-Golgi intermediate compartment) membrane and then transported via vesicle to be released out from the cell. (Source: Rohanbir Singh, MD). (**b**) Pathogenesis of SARS-CoV-1, MERS-CoV, and SARS-CoV-2. SARS-CoV-1 and SARS-CoV-2 down-regulate the activity of ACE2, which leads to an increase in the amount of AngII and causes lung injury. MERS-CoV infects the dendritic cell, macrophages, and T cells lead to severe inflammation and tissue damage in the lower respiratory tract. (**c**) Symptoms of HCoVs infections. HCoVs- 229E, -NL63, -HKU1, and -OC43 infection causes mild cold symptoms while SARS-CoV-1, SARS-CoV-2, and MERS-CoV cause the symptoms of the common cold to more severe diseases (Chen et al. 2020a, b)

rate (37%) higher than SARS-CoV (10%). MERS-CoV induces more severe dysregulation of the host cellular transcriptome, rapidly after infection, resulting in the apoptosis of surrounding cells, through greater suppression of the antigen presentation pathway (Hui et al. 2014; Banik et al. 2015). Moreover, some patients also showed fever, diarrhea, and abdominal pain without respiratory symptoms (To et al. 2013). It can infect many human immune cells such as dendritic cells (DC), macrophages, and T cells. Its infection of DC cells and macrophages results in the production of pro-inflammatory cytokines and chemokines such as TNF-α, IL-6, IL-8, CCL-2, CCL-3, CCL-5, which are involved in the infiltration of immune cells in infected patients` lower respiratory tracts and also contribute to severe inflammation, tissue, and immune damage (Liu et al. 2020a, b; Chen et al. 2020a, b). Damage of the alveolar barrier in MERS-CoV-infected patients is also reported, which leads to the susceptibility of pathogen entry as well as systemic spread as in the case of SARS-CoV infection (Farahi et al. 2013).

Septic shock, acute respiratory distress syndrome, mild upper respiratory illness, and respiratory failure are found in MERS patients. In some cases of MERS infection, cardiac arrest, acute kidney injury, and white matter disease (also called leukoaraiosis that affects nerves that link various parts of the brain to each other and the spinal cord) are also reported (Renu et al. 2020). In the noninfected host cells, a translation elongation factor 1A (EF-1A) plays a regulatory role in the assembly of the cytoskeleton, microfilaments, and cytokinesis rings. In the case of MERS infection, its N protein interacts with EF-1A in the host. This interaction disrupts the formation of cytokinesis loops and inhibits lymphocyte proliferation, resulting in the reduction in the number of lymphocytes in infected patients (Li et al. 2019).

1.6.2.3 SARS -CoV2

Similar to SARS and MERS-CoVs, SARS-CoV-2 also infects the lower respiratory tract and causes severe pneumonia by downregulating the function of ACE2 as discussed above (Yang et al. 2020; Chen et al. 2020a, b). In the lower respiratory tract, it infects type 2 alveolar epitheliocytes, where it replicates and then releases from cells. As a result, damaged epithelial cells produce alamins, which are sensed by neighboring epithelial and myeloid cells. Moreover, virions being released from cells are infected or being captured by dendritic cells, macrophages, and neutrophils lead to the pathological activation of IL-6, IL-1 TNF, and other proinflammatory cytokines. The level of IL-6 is ~1.7 times higher in COVID-19 non-survivors in comparison to COVID-19 survivors. Therefore, from these observations, it can be suggested that hyperactivation of the immune system and excessive production of IL-6 lead to pneumonia during SARS-CoV-2 infections (Gubernatovora et al. 2020; Divani et al. 2020).

IL-6 weakens papillary muscle contraction, resulting in myocardial dysfunction. Since SARS-CoV-2 uses the ACE2 receptor, which is also highly expressed in the heart apart from the lungs. Thus, there is a high chance of developing cardiac arrest in SARS-CoV-2 patients (Renu et al. 2020; Geng et al. 2020). CoVs are also neurotropic, even cross the blood-brain barrier, and transport to the brain through axonal transport (Divani et al. 2020). Apart from this, in some SARS-CoV-2 patients,

impaired kidney function is also identified. The expression of ACE2 is increases in diabetes patients. Therefore, patients with diabetes are more susceptible to SARS-CoV-2 infections (Renu et al. 2020).

1.7 SARS-CoV-2 Variants and the Second Wave of COVID-19

As discussed above, the viruses have a higher mutation rate. These mutations lead to the development of SARS-CoV-2 variants. These variants can spread more quickly, with higher virulence, and vaccines are less effective against them (Giovanetti et al. 2021). According to the US government interagency group, SARS-CoV-2 variants have been classified into three classes: Variant of interest, a variant of concern, and the variant of high consequence. Out of these three categories, no SARS-CoV-2 variant has been found under the third category of High consequences. The rest of the two categories are discussed subsequently.

1.7.1 Variants of Interest

Variants under this category possess specific genetic marker that leads to the reduction in treatment efficacy, increases transmission ability, increases disease severity, causes changes in receptor binding, and reduces antibody neutralization, that is, reduces the impact of vaccines. There are three variants of SARS-CoV-2 under this category: P.2 is the first variant identified under this category in April 2020. It was first detected in Brazil. The other two variants are B.1.526 and B.1.526.1 first detected in New York in October and November 2020, respectively. Moreover, one variant has been detected in the United Kingdom in December 2020 named B.1.525. These variants are found to have reduced neutralization by some EUA monoclonal antibodies, convalescent, and postvaccination sera (SARS-CoV-2 variant classifications and definitions 2021).

1.7.2 Variants of Concern

Variants fall under this category when there is evidence of a higher transmission rate, higher resistance to the therapy/therapies, and decreased neutralization by antibodies generated either by vaccination or by the previous infection. These variants cause more severe diseases that increase the death rate. There are five variants of SARS-CoV-2 under this category. B.1.1.7 was first detected in the United Kingdom, P.1 was first detected in Japan/Brazil, B.1.351 was first detected in South Africa, B.1.427, and B.1.429 was first detected in the US-California. These variants have reduced neutralization by some EUA monoclonal antibodies. However, a moderate reduction is found in neutralization by convalescent and postvaccination sera (SARS-CoV-2 variant classifications and definitions 2021).

The detail of these variants has been given below:

- B.1.1.7 (U.K. variant): It was initially detected in the UK. Recently, it was reported that this variant is more transmissible, and associated with increased hospitalizations and deaths.
- B.1.351 (South Africa variant): This variant was initially detected in South Africa. This variant appears to spread more easily. It also has a moderate impact on the effectiveness of monoclonal antibody medications and moderately reduces the effectiveness of antibodies generated by a previous COVID-19 infection or COVID-19 vaccine.
- P.1 (Japan/Brazil variant): This variant was initially identified by travelers from Brazil. This variant has a moderate impact on the effectiveness of monoclonal antibody medications. It also reduces the effectiveness of antibodies generated by a previous COVID-19 infection or a COVID-19 vaccine.
- B.1.427 (California variant): This variant appears to spread more easily. It also has a significant impact on the effectiveness of some treatments and moderately reduces the effectiveness of antibodies generated by a previous COVID-19 infection or COVID-19 vaccine.
- B.1.429 (California variant): These two variants were first identified in California. This variant appears to spread more easily. It also has a significant impact on the effectiveness of some treatments and moderately reduces the effectiveness of antibodies generated by a previous COVID-19 infection or COVID-19 vaccine.

Amino acid Substitutions detected in these variants are summarized in Table 1.4.

1.7.3 Surge of COVID-19 in 2021

As expected by many, the human race is facing the second wave of COVID-19 (Xu and Li 2020; Ali 2020). Many countries face a second wave that leads to an increase in the COVID-19 cases and increased death rates. In some countries, an initial SARS-CoV-2 outbreak occurred around March 2020, followed by a decrease in the number of confirmed cases after May 2020, and cases increased again in November 2020. The numbers of confirmed cases and deaths have increased in the new wave, which does not, reflects better diagnostic strategies. This second wave is also found to be different from the first one as this time, older individuals are less affected. Furthermore, during the second wave, younger individuals are found to be symptomatic, whereas in the first wave most of the younger individuals were asymptomatic (Diaz and Vergara 2021). These pieces of evidence suggest that the second wave is more infectious and dangerous compared to the first wave.

1.7.4 Second Wave of COVID-19 in Indian Perspective

India had its first wave of the epidemic in May 2020, followed by a profound decay in the number of cases after Sep 2020, peaking again in March 2021 (New COVID-19 cases worldwide 2021). In India, the UK variant B.1.1.7 was first identified in December 2020 by the Indian Council of Medical Research (ICMR)—National

Table 1.4 Amino acid substitutions in the different variants of SARS-CoV-2

Variant of Interest								
S. No.	Name	Amino acid substitution						
		Spike	ORF1a	ORF1b	ORF3a	ORF8	N	5′UTR
1.	P.2	E484K, D614G, V1176F	L3468V, L3930F	P314L			A119S, R203K, G204R, M234I	R81C
2.	B.1.526	T95I, D253G, D614G	L3201P, T265I, Δ3675/3677	P314L, Q1011H	P42L, Q57H	T11I		R81C
3.	B.1.526.1	D80G, F157S, L452R, D614G, D950H, Δ144						
3.	B.1.525	A67V, E484K, D614G, Q677H, F888L, Δ69/70, Δ144	T2007I	P314F			A12G, T205I	R81C
Variant of concern								
S. No.	Name	Amino acid substitution in spike protein						
1.	B.1.427	L452R, D624G						
2.	B.1.429	S13I, W152C, L452R, D614G						
3.	B.1.351	K417N, E484K, N501Y, D614G						
4.	B.1.1.7	N501Y, A570D, D614G, P681H, Δ69/70, Δ144Y						
5.	P.1	K417N/T, E484K, N501Y, D614G						

Note: According to the World Health Organization, one mutation is shared by all these variants known as D614G. This mutation is found in the spike protein gene, it was first documented in the US in late January or early February 2020. After several months this mutation replaced the initial strain of SARS-CoV-2 identified in China (Groves et al. 2021)

Institute of Virology (NIV) in travelers from the UK to India (Yadav et al. 2021a). Furthermore, ICMR –NIV has also identified the South African variant B.1.351 and Brazil variant B.1.1.28 in the travelers from South Africa and Brazil to India respectively (Yadav et al. 2021b). Moreover, India's COVID-19 vaccine, Covaxin, is effectively neutralize the U.K. variant B.1.1.7 (Sapkal et al. 2021). The National Institute of Virology detected another double variant named B.1.617 in October 2020 (New coronavirus variant: What is B.1.617 and is it more Dangerous 2021). Two mutations E484Q and L452R were found in the genome of this variant that leads to a higher infection rate and increased ability to remove antibodies. However, this variant is found only in one Indian state, Maharashtra (Expert reaction to cases of variant B.1.617 (the 'Indian variant') being investigated in the UK 2021).

1.8 Therapeutic Options, Control Measures, and Challenges

The SARS-CoV and MERS CoV pandemics were mainly controlled by practicing the best hygienic practices and taking measures for viral containment. There were no specific drugs or vaccines were developed/tested against these CoVs. However, there were attempts to use some drugs and prepare the vaccines for these outbreaks. Before the larger clinical trials, these outbreaks were controlled through viral containment measures. The SARS- CoV-2 outbreak in 2019 necessitates the whole world to develop specific drugs and vaccines for Corona viral diseases. Some of the drugs approved for viral diseases like Ebola, HIV were used to treat SARS CoV-2 infections as investigational therapies. We give a brief account of these drugs and their efficacy in controlling Coronaviral diseases, especially the COVID19 pandemic.

1.8.1 Repurposing of Drugs

The CoV outbreaks are managed through the repurposing of the drugs as there are no specific drugs. The treatment is mostly symptomatic and sometimes it is compassionate. The front runner of the drug molecules in treating the SARS CoV2 is Remdesvir, an ebola viral drug followed by anti-HIV drugs such as Lopinavir and Ritonavir. Remdesivir got approval to treat severe COVID-19 patients. It inhibits replication of virus via interfering with RNA-dependent RNA polymerase (RdRp) activity (Abd El-Aziz and Stockand 2020; Sternberg et al. 2020). Lopinavir and Ritonavir are being explored to treat corona viral infections, especially SARS CoV-2. These drugs inhibit the proteases of CoVs and decrease the viral load (Tobaiqy et al. 2020; Totura and Bavari 2019; McKee et al. 2020) as mentioned in Table 1.5. IFN-α in combination with systemic corticosteroid is used for the treatment of SARS-CoV infections. They significantly improve oxygen saturation, decrease the level of creatine kinase, and rapidly return LDH to its normal level (Loutfy et al. 2003). While corticosteroids should not be used routinely in SARS-CoV-2 patients, it can be used in low or moderate doses for a short period of time

Table 1.5 List of drugs used against corona viral infections (SARS-CoV-1 &2 and MERS-CoV)

Drug	Class	Target
Remdesivir	Antiviral drug	RdRp
Chloroquine phosphate	Antimalarial drug	ACE2
Hydroxychloroquine	Antimalarial drug	Elevate the pH in endosomes block endosome-mediated virus cell entry
Camostatmesilate	Serine protease inhibitor	TMPRSS2
Lopinavir/ritonavir	Antiviral drug	Viral protease
Fostamatinib	Tyrosine kinase inhibitor	3Clpro
Ouabain	ATP1A1- binding steroids	Inhibits clathrin-mediated endocytosis
Telbivudine	Antiviral drug	3Clpro
Flvipiravir	Antiviral drug	RdRp

References: Saxena (2020); Sternberg et al. (2020); McKee et al. (2020); Abd El-Aziz and Stockand (2020); Magro (2020)

(Tobaiqy et al. 2020). Convalescent plasma therapy is a promising treatment for many viral infections such as H1N1, West Nile Virus, including the SARS-CoV, MERS-CoV, and SARS-CoV-2 infections (Cheng et al. 2005; Brown and McCullough 2020). The recombinant monoclonal antibodies are also explored to treat corona viral infections. The CR3022 and CR3014 effectively neutralize the SARS-CoV infections in animal models, and are being explored for SARS-CoV-2 infections (Shi et al. 2020a, b).

1.8.2 Control of Corona Viral Diseases Through Vaccines

After the beginning of vaccination history in the laboratory of Louis Pasteur, this approach has become an excellent way to reduce infection and disease wherever applied (Plotkin 2005). Therefore, CoV vaccine development started after the first large outbreak caused by SARS-CoV in 2002, followed by the MERS-CoV outbreak in 2012. However, vaccine development against HCoVs has accelerated as WHO announced the COVID-19 outbreak as a pandemic on 12th March 2020. SARS-CoV-2 vaccine development employs not only the traditional vaccine development platforms such as inactivated and live attenuated platforms but also many latest production platforms such as DNA and RNA vaccines, non-replicating viral vector vaccines, recombinant protein vaccines, and cell-culture based vaccines (Francis 2017; Wallis et al. 2019).

Taking advantage of structural and some genomic similarities between SARS, MERS, and SARS-CoV-2 (Huang et al. 2021) some of the vaccines that have been developed against SARS-CoV-1 and MERS, the same platform used as SARS-CoV-2 vaccine candidates. These are screened for identifying its ability for inducing

Table 1.6 SARS-CoV-2 vaccines that use the same platform as SARS-CoV-1 and MERS vaccine candidate (COVID-19 treatment and vaccine tracker 2020)

Vaccine name	Developer	Same platform as a vaccine candidate for
Platform: DNA based		
No name	Epivax/Pharmajet/Immunomic	SARS-CoV
Platform: Inactivated virus based		
Picovacc	Sinovac	SARS-CoV
Platform: Non-replicating viral vector based		
No name	Prefix	MERS
PIV5 based vaccine	University of Georgia/University of Iowa	MERS
Platform: Protein subunit based		
No name	AZLB/Institute of microbiology, Chinese academy of sciences	MERS
No name	Sanofi Pasteur	SARS-CoV
Platform: Replicating viral vector-based		
TMV-083	Institute Pasteur/Themis/University of Pittsburgh/ MERCK	MERS
Platform: RNA based		
CVNCOV	Curevac	MERS
DS-5670	Daiichi-Sankyo/University of Tokyo's Institute of S medical sciences	MERS

strong cross-neutralizing antibodies to SARS-COV-2 (COVID-19 treatment and vaccine tracker 2020) summarized in Table 1.6. Few SARS-CoV-1 and MERS vaccines have made it to clinical trials (Amanat and Krammer 2020). Different vaccine production platforms for SARS-CoV, MERS-CoV, and SARS-CoV-2 are as follows:

1.8.2.1 Whole Virus Inactivated or Killed

Being the most rapid approach to vaccine production, this platform has been used for many years as a successful vaccination platform. The vaccine preparation involves heat or chemical inactivation of the whole virus. Despite many limitations, such as limited and short vaccination response, the risk of infection to people involved in the inactivation process of viruses (Afrough et al. 2019; Francis 2017; Kotarya et al. 2020). Still, many vaccines have been developed under this platform against all three HCoVs, that is, SARS-CoV, MERS-CoV, and SARS-CoV-2. Some of the vaccines are entered in the clinical phase, such as vaccine ISCV against SARS-CoV-1, EMC/2012. Alum/MF59 vaccine against MERS (List of candidate vaccines developed against SARS-CoV 2020; List of candidate vaccines developed against MERS-CoV 2020). Some of the vaccines against SARS-CoV-2 have been either achieved regulatory authorization or approval for use in several countries, such as Corona Vac developed by China-based biotechnology company Sinovac; BBIBP-CorV developed by Sinopharm in collaboration with Beijing Institute of Biological Products,

and Covaxin developed by Bharat Biotech in partnership with India's National Institute of Virology. Another vaccine is CoviVac, approved for use in Russia only. It is developed by the Chumakov Federal Scientific Center for Research and development of Immune and Biological Products. However, late-stage trials of this vaccine have not begun yet (Craven 2021).

1.8.2.2 Live Attenuated Vaccines (LAV)

Vaccines under this platform are produced by either reducing or removing the virulence of live viruses. Thus, these vaccines are highly immunogenic. In SARS-CoV-2 LAV, either the key virulence factors (E protein) are deleted or the effects of NSP exonuclease are inactivated (Yong et al. 2019; Al-Kassmy et al. 2020; Dhama et al. 2020; Kotarya et al. 2020).

Two LAV vaccines have been developed against SARS-CoV. One is LAV Nsp16 mutant that lacks 2′-OMTase, and another one is the Live attenuated SARS-CoV MA-ΔExon. MERS-ΔE, and MERS-dNSP16 are LAV against MERS-CoV (List of candidate vaccines developed against SARS-CoV 2020; List of candidate vaccines developed against MERS-CoV 2020), COVI-VAC LAV has been produced by using Codon deoptimization technology against SARS-CoV-2.

1.8.2.3 DNA Vaccines

Genetically engineered DNA molecules are used as a vaccine candidate in this platform. It encodes antigen (s) upon entering the body, leading to the induction of both humoral and cellular immune responses (Smith et al. 2020). This platform is used for producing vaccines against SARS-CoV-1, MERS-CoV, and SARS-CoV-2. It has plasmid DNA that contains the sequence of the spike protein of respective viruses.

These DNA-based vaccines can be administered in two ways. Some are delivered orally while some vaccines are administered via creating temporary pores (by delivering electrical pulses) in the cells near the injection site by using a handheld device (Kotarya et al. 2020; King 2020; COVID-19 treatment and vaccine tracker 2020). DNA vaccine VRC-SRSDNA015, pIRES-ISS-S1 have been developed against SARS-CoV-1. Vaccines pVRC8400-S1 and pcDNA3.1(+)- S1 or S (1–725) against MERS-CoV, Vaccines INO- 4800, GX-19, and so on, have been developed against SARS-CoV-2 (List of candidate vaccines developed against SARS-CoV 2020; List of candidate vaccines developed against MERS-CoV 2020).

1.8.2.4 Non-replicating Viral Vector Vaccines

Vaccines under this platform have involved Ankara Viral system, Adenoviral vectors systems like efficient viral vectors and in the recent past, this vaccine production platform was greatly improved (Kotarya et al. 2020). Some of the vaccines under this platform are an rADV-S vaccine that expressed Trunctuated S protein and AdHu5 s AdC7-nS vaccines were produced against SARS-CoV-1. MVA-MERS-S vaccine produced with tissue plasminogen activator (tPA) gene, and ChAdOx1 MERS vaccine with or without leader sequence of human tPA were produced against MERS-CoV (Alharbi et al. 2017; Rauch et al. 2018; Kaur

SP & Gupta V2020; List of candidate vaccines developed against SARS-CoV 2020; List of candidate vaccines developed against MERS-CoV 2020). Authorized/approved SARS-Co-V-2 vaccines under this platform are AZD 1222, also known as Covishield, developed by Astra Zeneca and the Oxford Vaccine group. In India, this vaccine is jointly developed by the Serum Institute of India and AstraZeneca. Other vaccines such as Sputnik V; Ad26.COV2.S and Convidicea (Ad5-nCoV) are also authorized/approved for emergency use in some countries (Craven 2021).

1.8.2.5 Protein Subunit Vaccines

Several vaccines have been developed under this platform against SARS, MERS, and SARS-CoV-2 due to the many advantages, such as the fact that they contain noninfectious recombinant protein, developed using a "reverse genetics" approach, can combine many pathogen epitopes in a single vaccine, do not require cold chain and vaccine production is easy (Strugnell et al. 2011; Kotarya et al. 2020). Vaccine preparation involves either purification of pathogen's protein or using recombinant technology to produce recombinant proteins (Vetter et al. 2018), mostly the "Spike" protein (Shi et al. 2020a, b). Russia has granted regulatory approval to the EpiVacCorona vaccine against SARS-CoV-2, developed under this platform (Craven 2021).

1.8.2.6 Virus-Like Particle (VLP) Based Vaccines

These are similar to subunit vaccines, formed by self-assembly of the capsid protein of the virus that can mimic native virus. Still, it cannot replicate as it doesn't have genetic material, but it can induce a strong immune response even without any adjuvant and can also trigger T cell-mediated responses. (Urakami et al. 2017; Wallis et al. 2019; Yong et al. 2019; Kotarya et al. 2020).

1.8.2.7 RNA Vaccines

Since RNA based vaccines have the potential to overcome drawbacks of viral vector-based and DNA based vaccines such as: during acute infection, it mimics antigen expression better than DNA vaccines. As a result, it induces a better antigen-specific immune response, does not have any risk of integration in the host genome and construction is easy (Ulmer et al. 2012; Kotarya et al. 2020). Therefore, many biotech companies have used mRNA platform-based vaccines against SARS, MERS, and SARS-CoV-2. An mRNA-based vaccine named mRNA1273 was developed by Moderna, the USA in association with the National Institute for Allergy and infectious diseases, Vaccine Research Center. Vaccine 3 LNP-mRNAs BNT162b2 is developed jointly by BioNTech (Germany) and Pfizer. These vaccines are in front-runner positions and have been either approved or authorized for emergency use in many countries (Craven 2021; COVID-19 treatment and vaccine tracker 2020). mRNA1273 is prepared by using mRNA of SARS-CoV-2 spike protein, which has a transmembrane region and contains two subunits of spike protein: S1 and S2 with furin cleavage stabilized as prefusion confirmation as 2 proline substitutions at S1 subunit at positions 986 and 987 (S-2p) in nano lipid

formulation (Kotarya et al. 2020) (Rego et al. 2020; Jackson 2020; Corbett et al. 2020).

Vaccine BNT162 includes two classes: one is BNT162b1 that is modified nucleoside RNAs of RBD antigen and another one is BNT162b2 the whole spike protein of SARS-CoV-2. In vaccine preparation, a single nucleoside modified mRNA was encapsulated in a nano lipid formulation and administered to the individuals (Khuroo et al. 2020).

1.9 Control Measures

According to the CDC (Centres for Disease Control and Prevention), CoVs-infected individuals should be isolated as soon as possible. Alcohol-based hand rubbing or hand washing is believed to be an effective way to prevent the spread of CoVs between people. While caring for confirmed or suspected coronaviral patients, personal protective equipment (PPE) should be used. People are also advised to maintain physical distancing of at least one meter from others or avoid crowded places and avoid unprotected contact with farm or wild animals. (Lai et al. 2020; Chen et al. 2020a, b). Most of the viruses spread through respiratory droplets expelled during coughing, sneezing, talking, or singing (according to the World Health Organization), so wearing face masks is effective. SARS-CoV and CoV-2 both can be detected on surfaces such as on copper, cardboard, plastic, and stainless steel for up to 4 h, 24 h, and 2–3 h respectively (Kampf 2018; Ashour et al. 2020; Hasöksüz et al. 2020; Petrosillo et al. 2020; Ning et al. 2020; Shi et al. 2020a, b; Zheng 2020). Therefore, surfaces, different tools, objects, and so on, should be disinfected regularly with ethanol to limit the spread of the virus (42.6% (w/w). CoVs survive longer on disposable gowns compared to cotton gowns. Thus, for personal protective clothing, cotton is preferred. To reducing the risk of airborne transmission, instead of high-flow masks, the low-flow nasal cannula should be used for oxygen delivery (Cheng et al. 2007). Quarantine is believed to be a very effective way of preventing the spread of this virus. During the pandemic, travelers are advised to self-quarantine for at least 2 weeks (Yang et al. 2020). During the MERS-CoVepidemic, immunocompromised individuals and persons having some medical conditions were advised to avoid close contact with dromedary camels, meat, and urine in virus-circulating areas**.**

1.10 Challenges

The natural reservoirs and the intermediary hosts for the CoVs vary and we still need to explore this area. The natural reservoirs for CoVs infecting humans are identified as bats; however, many species of bats are present with worldwide distribution. For example, the HCoVs, SARS-CoV2, -NL63, and HCoV-HKU1 bats are identified as natural reservoirs. These CoVs acquire the ability to infect humans via intermediary hosts such as dromedary Camels, Pangolins, and others. The intermediary host for

SARS CoV2 is expected to be Pangolin. However, some more studies are required to confirm this. For HCoV-229E and HCoV-OC43 the Camelids, bovines are suspected as intermediate hosts. Although we know the natural reservoirs for these viruses, we do not yet know all the Bat and Pangolin CoVs.

The next challenge is the gain of adaptability to human hosts. These viruses use different receptors for their entry into the cells, such as ACE2 and DDPP4. Furthermore, if the two viruses use the same cellular receptor, the new viral species exhibits more affinity towards the receptor, resulting from mutations. Once they infected the human hosts with higher mutation rates, they adapt themselves to the new host, which means the genome of the human adopted corona viral isolate significantly differs from their natural and intermediary host viral isolates. In such situations, a vaccine candidate or the drugs based on the receptors may not work for longer periods.

Next, the transmission dynamics of these viruses, for example, if we consider SARS CoV-2, is transmitted through respiratory droplets, fecal-oral route as well as airborne transmissions. In such situations, containment of these viruses becomes difficult. Furthermore, reports about the existence of viable viral particles on different surfaces are also different. In the case of SARS CoV-2, the viable virus can be detected after 3-4 days on steel surfaces.

The spread of the virus through asymptomatic individuals also poses a great challenge, as we don't know who is transmitting the virus. Furthermore, it is still unclear which mechanism most people acquired MERS-CoV, whether there is also a third host/hosts speculated in virus transmission.

Although there are many challenges related to Human CoVs, except SARS - CoV-2, other outbreaks were controlled and not present at this point. Furthermore, significant development has been achieved in diagnosing these diseases and the pathophysiology of these diseases. The vaccines entered into phase III clinical trials for SARS -CoV-2 and are expected to be available to the humankind at the beginning of 2021. Repurposing the drugs for investigational therapy gained important conclusions, and Remdesvir was found to be an effective drug in SARS CoV2 infections. This is the first time the DNA/RNA-based vaccines have undergone phase III clinical trials.

1.11 Conclusions and Future Prospects

Pieces of evidence suggest that dispersal of the human population and different CoVs occurred simultaneously worldwide about 15,000–1000,000 years ago and enormously increased in the last 10,000 years ago.

As time passed, the continuous evolution in the viral species caused the interspecies host to jump and make the animal CoVs adopt humans as hosts, which led to the emergence of HCoVs that were first identified in mid1960 and after that, many CoVs have been identified to date that can infect humans. From which the highly pathogenic HCoVs: SARS-CoV-1 (2002) followed by MERS-CoV (2012) and the recent SARS-CoV-2 (2019) have caused three pandemics within less than 20 years, has

alarmed the world that the human race could face further Corona viral pandemics in the future, maybe SARS-CoV-3. As we know, animal CoVs have the potential to serve as a genetic reservoir for human disease; thus, for the future control of a disease caused by another type of CoVs, the identification of genome and genes among CoVs in animals those are in close contact with humans, is extremely important.

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Testing Strategy of Covid-19: A Mechanistic Approach

2

Sikander S. Gill, Rajwant K. Gill, and R. C. Sobti

Abstract

The deadliness associated with the COVID-19 disease caused by the SARS-CoV-2 virus plunged the entire global community into the worst times of this century. It was globally realized that a timely diagnosis, effective treatment, and prevention were the key factors in its management. Responding to the emergent scenario, sequencing of the genome of this virus was performed and shared with the scientific community in the nick of time. Thereafter, diverse sets of test kits to detect the SARS-CoV-2 and to detect the antibodies in the patients of the COVID-19 were developed at the war scale. It was indeed a war but with a microscopic spiky package of 50–200 nanometres in diameter having a genome of about 29.9 kb encoding deadly tools in its arsenal.

For the reason, patients of the COVID-19 exhibit diverse symptoms from mild influenza-like to potentially fatal ones that overlap with other respiratory diseases, only efficient testing was essential during the early stages of infection to identify COVID-19 patients among others. The diverse test kits designed exclusively for rapid and accurate outcomes proved instrumental in identifying individuals among asymptomatics, presymptomatics, and symptomatics. The test kits have also been playing an appreciable role in identifying communities with hot spots to facilitate proper management. To meet the demand of higher throughput and simplification of the testing process, novel ways were devised that did not otherwise allow the testing spree to get hit with pandemic supply bottlenecks.

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Mechanistic models have played an essential role in shaping public health policy. The regulatory agencies, both at the world health and the regional public health levels, shared the knowledge and experience on the test kits that helped in the development and improvement in the testing capability and efficiency of the testing infrastructure. The information about the emergence of variants of the SARS-CoV-2 happening due to intrinsic behavior of the viral genomes drew attention of the test kit developers, regulatory agencies, and end-users to be vigilant over the test outcomes. Offering a mechanistic approach, in this chapter, testing strategies for the detection of SARS-CoV-2 virus and COVID-19 disease are delineated.

Keywords

SARS-CoV-2 · COVID-19 · Test kit · RT-PCR · Serology test · Antigen test · Strategies · Limit of detection · Viral load

2.1 Introduction

It has been a challenging task to control the COVID-19 pandemic since the SARS-CoV-2 virus has a long incubation period. This challenge is further augmented by the occurrence of a high percentage of asymptomatic patients and variants of the virus accumulating selective mutations (Álvarez-Díaz et al. 2020; Matic et al. 2021). Therefore, to contain the spread of COVID-19, it is essential to screen asymptomatic or presymptomatic patients to monitor trends in infection and its transmission in the communities along with detection of variants and infected hot spots in the population and communities (Álvarez-Díaz et al. 2020). These objectives could only be achieved by deploying infrastructure that have robust, cost-effective, sensitive, and accurate testing facilities along with a healthy strategy.

In fact, the testing rate of the COVID-19 and its success when viewed with other measures have a relationship with both the doubling time of the epidemic of SARS-CoV-2 and the reproductive number of the disease (Indrayan and Mishra 2020; Kea et al. 2021). In this context, the reproductive number designates the ability of any disease to generate secondary infections from a person infected with the causative agent of the disease. In other words, this means the disease would spread around since an increasing number of individuals would catch the infection from the infected individuals. Thus, in the case of COVID-19, this number reflects transmissibility and potential of the SARS-CoV-2 in its propagation in the infected population, and then the disease would eventually turn out from an epidemic to a pandemic. Similarly, the doubling time of the epidemic is the number of days required for an epidemic to double. It implies that testing for infected individuals is a key pillar in mitigating and controlling the ongoing pandemic. Therefore, to bring the reproduction number of the virus below 1, it demands deployment of ideal testing systems to be engaged and the testing capabilities to be ramped up globally.

What is an ideal test system: An ideal test method must incorporate the following features that would allow mass testing of a large chunk of the population not only in the developed countries but also in countries with limited resources (deCampos-Stairiker et al. 2021; Zowawi et al. 2021; Razvan et al. 2021; Gill et al. 2021; Guglielmi 2021):

1. Accurate: Accuracy of the test includes two attributes of the test, one is sensitivity that conforms to the ability of the test to offer the test outcome of an individual with high confidence as positive. Moreover, a test that is sensitive is less likely to result false negative. The second attribute is specificity that will ensure an individual is not infected. Accordingly, a highly specific test is less likely to result in false positives. This attribute also includes robustness of the test in offering accuracy despite some variability in the test process.
2. Cost-effective: A cost-effective test termed cheap has the attribute of reaching the masses for being affordable within the limited resources of the country.
3. Scalable: The test system should be able to meet adequate throughput requirements so that a large number of tests can be performed. The scalability basically depends upon the rapidity of the test and the number of samples that can be tested in one single operation. Numerous groups are aiming at improving testing capacity to eventually achieve population scale testing.
4. Portable: Normally, the test systems with high sensitivity and specificity are heavy machines and have a hunger for power and other resources including technical staff. Thus, such systems cannot be taken to remote areas for screening populations in deep pockets. However, there are some reverse transcription-polymerase chain reaction (RT-PCR)-based test systems that are portable being light in weight, with small footprint, and can be operated on battery power (deCampos-Stairiker et al. 2021; Razvan et al. 2021; Gill et al. 2021).
5. Rapid: The rapid tests other than molecular tests normally do not offer reliable information, especially in the early and post-infection stage when viral load is high in the test sample. This is part of the reason that different antigen tests tend to result in comparable sensitivity to high viral loads. However, their sensitivity varies when viral loads are low during the incubation and recovery zones of the course of Covid-19 disease. The definition of rapid test also covers RT-PCR tests that offer reliable outcomes under 30 minutes and contribute to the validity of this attribute.

Testing for SARS-CoV-2 infection: While the wait for the inoculation of the vaccine is over and as expected to achieve wonders, to some extend it has started displaying its outcome in reducing the COVID-19 spread but much below 100% efficacy, testing and screening of the populations are still the need of the time (Hopkins and Toy 2021). Testing and screening is also of an utmost importance in view of the emergence of variants with capability of overcoming the efficacy of the vaccines and immunity (Bernal et al. 2021). There is no doubt screening programs cannot wipe this pandemic, but intelligent implementation and judicious public health strategies have so far provided some element of safety. Since it is

essential to identify infectious persons among asymptomatic and pre-symptomatic persons, only a proper testing can help in this task. This identification in return can encourage people to take appropriate measures for their safety from the COVID-19 disease. Thereby, informed decisions taken by the tested individuals can mend their own behavior in reducing the spread of the disease (Schwartz et al. 2021). Thus, clear guidelines and recommendations from the regional authorities of public health can mitigate the impact of the pandemic.

In view of this, an array of testing options are available for both the symptomatic and the asymptomatic Covid-19 patients. With the rampant spread of this virus, at a similar pace, many tests and test facilities also rose up (Carter et al. 2020; Thakur et al. 2020). As such, the available tests, although work satisfactorily, but differ from each other in terms of their throughput, cost, user-friendliness, and certification levels for example, the tests that have approval from the US-FDA or CE-IVD or validated by country's authorities or laboratory-developed (validated) test by certain labs like CLIA labs in the United States.

Categories of tests: The development of a plethora of accurate and sensitive tests for detection of the SARS-CoV-2 was started by the scientific community after its genome sequence was shared on January 10, 2020. Similar developments in parallel also took off for the detection of antiviral antibodies in SARS-COV-2 patients. For simplicity, these tests can be divided into the following categories:

(a) **Antigen test:** These tests are immunoassays meant to recognize the specific antigen(s) present on the surface of the target or the virus to be detected in the sample. It is often called a "rapid test" because its turnaround time is relatively much shorter than an RT-PCR test. For example, an antigen test, the lateral flow test (LFT), is designed to pick up the antigen of the SARS-CoV-2 infection. A suitable sample like a nasopharyngeal sample is taken from the test individual and placed on the absorbent pad of the test strip. The specific antigen present in the sample reacts with the antibody that is immobilized on the test strip offering an outcome of a colored line indicating infection. The test outcome is offered within a time frame of 5–30 min. The test that gives the outcome in less than 30 min is termed a rapid test (Larremore et al. 2020; Kent 2021; Dinnes et al. 2020). Accordingly, Roche's rapid PCR-based test and Lumex's microchip-based RT-PCR test are also rapid tests (Razvan et al. 2021; deCampos-Stairiker et al. 2021).

Normally, these tests happen to be low priced to produce and thus affordable to screen a large number of people and offering high throughput in testing. This also implies that in the event of the "negative" outcome from an antigen test, the patient needs to undergo a molecular test to rule out a false-negative case. Some antigen tests can be performed as "point-of-care" (POC) tests. These tests have similar specificity, but sensitivity is lesser than most of the nucleic acid Amplification Tests (NAATs).

Such tests do offer rapidity at the cost of the accuracy of the test but have their utility in quick screening tasks (Kent 2021; Schwartz et al. 2021; Mina and Andersen 2021). In symptomatic individuals, the rapid antigen tests have

particularly useful applications in testing, for example, surveillance of communities or the people in hot spots (Schwartz et al. 2021). Because of their rapidity, it can be deployed at the POC sites to make the results available in minutes. Therefore, these tests can be applied in screening programs for quickly identifying infected populations and considered appropriate for population-wide screening in epidemiological situations. The areas or population clusters with high test positivity are the ideal spots to use the rapid tests (https://www.raps.org/news-and-articles/news-articles/2020/12/covid-antigen-testing-framework-laid-out-by-ec). In this way, the rapid antigen tests offer an excellent tool to empower the overall testing capacity of a country. However, the antigen tests must be validated against a laboratory-based NAAT or an alternate rapid antigen test for confirmatory purposes. Additionally, rapid antigen tests must be approved by the regional regulatory agency of the country to ensure its sensitivity and specificity meets minimum requirements for testing.

Such tests, however, at the population level, represent an opportunity and offer substantial benefits in reducing the spread of the virus. So far, it is a fact that the antigen tests are not as perfect as NAATs in their diagnostic ability but quite instrumental in testing large sections of the public and at the airports ensuring travel safety at airports (Chan 2021).

(b) **Antibody test or serology test or blood test:** This is the type of test detects antibodies to the SARS-CoV-2 in the test sample. As RT-PCR is the gold standard method for the detection of active infection, the serology test is the gold standard method for detecting the previous infection of SARS-CoV-2 (Yuen et al. 2020; Schuler et al. 2021). The antibodies detected by the serological test are produced by the immune system in its fight against the invader pathogen—the SARS-CoV-2. For example, the test detects IgG and IgM antibodies that are specific for the virus antigens, including the receptor-binding segments of the spike protein and nucleocapsid protein. Normally, both virus-specific IgG and IgM antibodies are detected by some serological assays, while only IgG is recognized by others (Schuler et al. 2021).
Typically, by the time these antibodies develop in the patient, infection of the SARS-CoV-2 is cleared from the patient. Therefore, antibody tests although specific cannot be deployed as a diagnostic tool for detecting the SARS-CoV-2 infection, as the antibodies become available normally several (Føns and Krogfelt 2021) days after an infection with this virus (Wang 2020; Deeks et al. 2020). Moreover, to assess the presence of antibodies in response to a past infection or in response to vaccination, a specific antibody test is needed for each task. This is because an antibody test recognizing past infection may have different targets (antigens) on the virus than those on the vaccine (Watson 2020; Føns and Krogfelt 2021).

In view of the launch of vaccination programs worldwide, it becomes highly essential to screen people to determine whether the antibodies detected are from the previous infection or in response to the vaccination. Although the current serological assays differ in detecting different antigens on the surface of the virus with test-specific conditions, this becomes important to keep a note that the

current vaccines are produced against spike protein of the virus (Yuen et al. 2020).

Thus, serology tests are a better measure to allow a quarantined person to come out of isolation. Most of the serology tests are not rapid tests that cannot be performed as POC or at home. These tests are performed on the blood sample at a central lab and thus can take more than a day for the outcome. Recently, the first antibody test-based POC Covid-19 test has been accepted by the FDA that offers outcome within 15 min from the drop of blood from the subject (Føns and Krogfelt 2021).

Accordingly, a POC is considered the best approach. Another POC named Finger-stick serological test for COVID-19 has emerged as an effective test for identifying individuals with prior infections (Pickering et al. 2020). Operationally, the following are the major types of test methods deployed in the detection and diagnosis of the SARS-CoV-2 and COVID-19:

1. **Enzyme-Linked Immunosorbent Assay (ELISA):** It is a plate-based test that involves binding of the immobilized antigen in the wells that look for antibodies produced by the patient body in response to the SARS-CoV-2 infection
2. **Lateral Flow Immunoassay:** It involves immobilized antigens on the test kit, and being a qualitative test, it is meant to determine the presence of antibodies as a positive or negative result from a sample. The other way round, it can also be used as an antigen test by applying the sample on immobilized antibodies specific to SARS-CoV-2 antigens.
3. **Neutralization Assay:** To determine the ability of the antibodies to inhibit propagation of the virus in vitro since antibodies are specifically produced against the antigenic determinants on the surface of the SARS-CoV-2.

(c) **Molecular test:** The molecular testing or NAAT detects the genetic material of SARS-CoC-2 in the sample. The advancement in the molecular tests has lead to the development of the following diversity of tests:

1. **RT-PCR:** The majority of the molecular tests involve RT-PCR that detects specific signature sequences in genetic material of the pathogen present in the test sample. For example, the sequences involved in the test are specific for SARS-CoV-2 virus only but not for any other virus-like Ebola virus or Zika virus. The RT-PCR test requires the presence of RNA from the SARS-CoV-2 for its detection that would be recognized by the specific sequences present in the test kit. However, this test is valid only for detecting the virus in the body prior to the emergence of symptoms or antibodies of the disease produced against the pathogen. This implies that the tests can inform if the patient has the virus infection exceedingly early in its course of infection or not (Gueudin et al. 1996; Tsang et al. 2021).

   The levels of virus in a sample are normally quantified by the amplification cycles needed to detect the virus. If it takes less than 25 cycles typically known as cycle threshold (Ct), it represents high levels of virus indicating high infection, suggesting that the patient is likely infectious. The RT-PCR diagnostic tests are termed gold standard and known to be the most sensitive

for detecting the SARS-CoV-2 active infection and hence extremely popular in use (Zhang et al. 2021). However, the accuracy of the test swings within the window of the course of infection for being highly accurate and sensitive in between the false-negative zones. Therefore, to contain the spread of the virus at an early stage of transmission, and to identify infected individuals for isolation, molecular testing is essential. The molecular tests detect one or more genes in the viral genome (RNA) to detect a current or recent infection but do not always offer direct evidence on the presence of a virus or infection being transmitted to other individuals. The duration of outcome from this test is about 15–45 min, whereas some tests can take about 1–3 days.

Since RT-PCR test methodology in most of the cases takes much longer than the antibody-based rapid tests, therefore, the throughput from the RT-PCR test is low. Therefore, with the purpose of increasing throughput of the molecular testing, a pooling of test samples is also strategized. It is, however, not recommended for the areas with >5% positivity rate (Denny et al. 2020). While pooling the samples, attention must be paid toward maintaining the sensitivity of the test to a larger extent. In the pooling process, several test samples are mixed together in a lot that is eventually taken for testing. In this strategy, the following two options are recommended by the FDA (US FDA 2021):

- **Sample or media pooling**: Pooling aliquots of viral transport media (VTM) containing a single patient sample in each tube.
- **Swab pooling:** Putting swabs from a certain number of patients into a single tube of VTM.

2. I**sothermal Nucleic Acid Amplification (INAA)**: Despite its popularity and gold-standard status, the RT-PCR method suffers from another limitation. It requires multiple temperature cycles called thermocycling and an instrument that generates thermocycles for the process to go forward. An alternative development of INAA came up that performs the process of amplification at a constant temperature and thus removes the need of the thermocycler. Further advances driven by the urgency of testing the SARS-CoV-2 resulted in the development of many advanced devices based on this principle of INAA as follows:
   - Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP): This method is an isothermal nucleic acid amplification technique performed in a single tube in combination with reverse transcription (RT) step. It is a low-cost alternative to detect certain diseases including SARS-CoV-2 (Lamb et al. 2018; Coelho et al. 2021). The first FDA's emergency use authorization (EUA) over-the-counter COVID-19 test is Lucira™–PCR At-Home Test Kit that detects nucleic acid from SARS-CoV-2 (Park 2021).
   - **Nucleic acid sequence-based amplification (NASBA)**: The method is also known as primer-dependent technology for amplification of nucleic acid at isothermal temperature. It was developed in 1991 by J. Compton,

and its first application was in the diagnosis and quantification of HIV-1 in patient samples (Kievits et al. 1991).

- **Transcription-Mediated Amplification (TMA)**: It employs retroviral replication to amplify specific genetic sequences of viral RNA using two enzymes—retroviral reverse transcriptase and T7 RNA polymerase that are more efficient than RT-PCR enzymes (Soler et al. 2020).
- **Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Based Assays**: The test involves a group of sequences that are recognized in the test, and specific CRISPR-associated enzymes make specific cuts in viral RNA sequences. A CRISPR-based detector assay offered positive-predictive and negative-predictive agreement at 95% and 100%, respectively, for the detection of SAR-CoV-2 (Broughton et al. 2020).
- **Rolling Circle Amplification (RCA)**: This assay is more sensitive than LAMP and completes in 90 min. A sensitive detection was achieved to detect SARS-CoV RNA and thus offers an economical and sensitive option (Wang et al. 2005).
- **Isothermal NASBA (nucleic acid sequence-based amplification)-based high-throughput test**: This method is a combo of NASBA with sequences in molecular barcode formats deployed to detect the virus causing COVID-19 (Wu et al. 2021a, 2021b). However, its limitation offers 95% limit of detection (LOD <50 copies) of the SARS-CoV-2 virus/per reaction, and outcome duration of 1–2 h is not making these tests attractive and feasible for routine testing of SARS-CoV-2 (Zeinoddini et al. 2017). Among several proposals, two devices have been authorized so far: SAMBA II and Abbott ID NOW, but expensive instruments and reagents are limiting their widespread acceptance (Wu et al. 2021a, 2021b). However, in the view of the need for regular, high-throughput, and rapid testing, several POC diagnostic tests including SAMBA II and Abbott ID NOW have been authorized for use. However, their use in real-world testing and screening is limited due to relatively expensive instrumentation or reagents.

3. **Microarray:** This method involves the labeling of both the viral cDNA generated from the sample and a reference cDNA each labeled with a different fluorescent label. The mixture of the two cDNAs is loaded into the microwells of the array which has immobilized specific DNA probes. Through rapid hybridization followed by a wash, the unbound cDNA is washed away leaving behind the bound cDNA (hybridized) with immobilized DNA probes leading to detection of the target virus (Chen et al. 2010). In the past, this test was used to identify variants in SARS-CoV having mutations in the spike gene with 100% accuracy. Similarly, an array test has been applied in the detection of coronavirus strains with high fidelity (Guo et al. 2018). However, this method suffers from high costs.
4. **Amplicon Metagenomic Sequencing:** This test method is supplementary to metagenomics sequencing. It employs amplicon-based sequencing to rapidly

identify the SARS-CoV-2 as well as other pathogens involved in secondary infections contributing to the severity of COVID-19 (Moore et al. 2020).

5. **Next-generation sequencing (NGS)**: The DNA sequencing technology that involves the sequencing of multiple small fragments of DNA in parallel helps in determining sequences. Such NGS readouts-based centralized testing at a centralized facility is also a potential testing method. Its accuracy encourages scaling of testing, while the data collection is also easy and simple. But its access to the patients and access to the test results delay the early isolation for quarantine measures for asymptomatic or presymptomatic subjects. This technique is instrumental in identifying the variants of SARS-CoV-2. In addition, its cost and cumbersome nature do not make it an attractive test system for routine screening (Yángüez et al. 2020).
6. **Biosensor test**: These tests involve the specific interaction of biological molecules that are transduced normally into an electrical or optical signal. For example, a surface plasmon resonance (SPR)-based test has recently been developed for detection of the SARS-CoV-2 by immobilizing viral surface antigen on a gold substrate (Reed et al. 2019; Soler et al. 2020; Rybicka et al. 2021).

How to choose a test: The three types of the SARS-CoV-2 tests differ in their attributes of an ideal assay and other characteristics. Therefore, the regulatory agencies recommend selecting the test for the SARS CoV-2 detection based on the purpose of use. Picking the test and transcribing the outcome of the test need to be based on the objective or purpose of the test to be used. For example, the occurrence and symptoms of the COVID-19 and to test the contact chain of the individuals being tested need to be considered in view of the following:

- Rapid tests versus slow tests
- Lab tests versus POC tests versus performed at home.
- Sensitive tests versus specific tests versus sensitive and specific tests
- Few false-negative results versus few missed detections versus few false-positive results versus false-positive tests
- Frequently performed affordable tests versus unaffordable tests
- Cost-effective tests versus expensive tests
- User-friendly tests versus cumbersome tests

Types of samples for testing: The application and usability of a test method depend upon the sample type, and accordingly a test is authorized to be used with specific type(s) of samples (Tsang et al. 2021; Razvan et al. 2021; Gill et al. 2021). For example, the swabs for COVID-19 are taken from anterior nares, mid-turbinate, nasopharyngeal, and oropharyngeal parts of the body. Specific guidelines may be available at the local authority for the collection and logistics of samples (US-CDC 2021a). The samples that are mostly used for testing the presence of the SARS-CoV-2 are as follows:

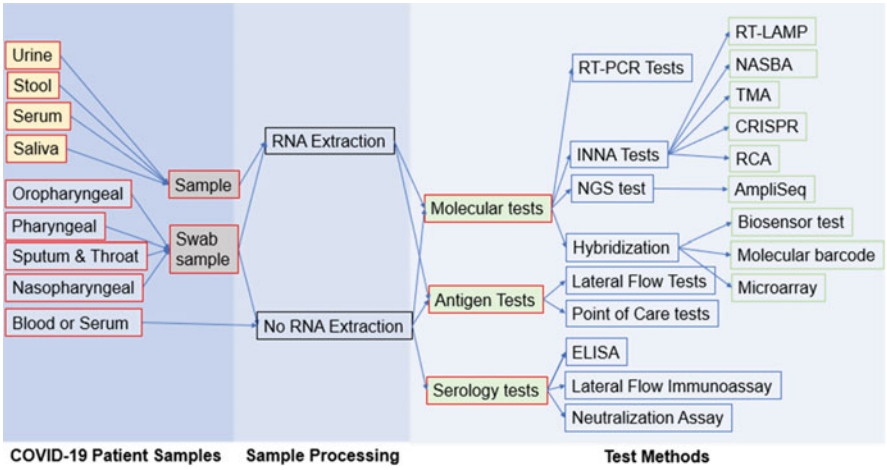

Fig. 2.1 Schematics of workflow from sample to test method for detection of SARS-CoV-2

- Samples for RT-PCR:
- Anterior Nares or Nasals: location of the swab is just inside the nostrils.
- Mid-turbinate: location of the swab is further down inside the nose.
- Nasopharyngeal: location of the swab is deep inside the nose near the throat.
- Oropharyngeal: location of the swab is the back of the mouth at the middle part of the throat (pharynx).
- Bronchoalveolar lavage: location is the lung, but the sample is taken with a medical procedure involving a bronchoscope that is passed through the mouth or nose. Fluid is first squirted before collecting it for testing.
- Saliva: samples are not taken with a swab, but saliva is collected by spitting into a saliva collection device or a tube.
- Sputum: it is a mix of saliva and mucus or phlegm expelled from the upper respiratory tract.
- Extraction-free samples: The majority of the RT-PCR methods require labor-intensive procedures for RNA upstream of running the test with the sample (Fig. 2.1). In addition, these tests require expensive equipment like a thermocycler. In addition, the test complexity, cost, and bottlenecks of supplying the RNA extraction kits limit the application and throughput of these tests. Recently, however, novel RT-PCR tests have been developed that do not need extraction of RNA or require minimized sample processing, miniaturized amount of reagents in test, lyophilized reagents in the microchip, and offer user-friendliness in performing the test (deCampos-Stairiker et al. 2021; Vogels et al. 2021; Razvan et al. 2021; Gill et al. 2021)
- Samples for antigen test:
- Nasal
- Nasopharyngeal

Table 2.1 Glossary of COVID-19 terms

#	Term	Expansion
1	SARS-CoV-2 virus	It is a microscopic spiky package containing RNA as its genetic material enclosed in a molecular envelope
2	POC test	It is point-of-care test also called bedside testing. It is a medical diagnostic test performed at or near clinical infrastructure
3	Home collection test	The test sample is taken at home and tested in a laboratory
4	Direct to consumer (DTC) test	It allows collection of the sample at home without a prescription while the test is performed in a laboratory
5	At-home testing	The consumer performs the test at home by taking a sample using a home test kit
6	Over the counter (OTC) test	The consumer performs the test at home by taking a sample using a home test kit without a prescription
7	Swab	A brush-like tool for collecting the specimen from a patient that must collect cells from the swabbed infected area. The test laboratory lyses the swab to extract intact virus or viral genetic material
8	Bronchoalveolar lavage (BAL)	The lung fluid sample is taken from the lungs with a medical procedure
9	VTM	The virus transport medium solution keeps the swabbed specimen stable during its transit to the testing laboratory. The nature of the VTM decides what methodology the testing laboratory needs to extract the genetic material for testing
10	Diagnostic test	Identifies an active infection of the SARS-CoV-2
11	Antigen test	A diagnostic test that detects specific antigen on the proteins from the virus
12	Antibody (serology) test	A diagnostic test or antibody produced by the immune system in response to an infection happened a few days earlier
13	Molecular test	A diagnostic test that detects a signature part of the genetic material of the virus in the sample
14	NAAT	Nucleic acid amplification test is one type of molecular diagnostic test for example RT-PCR
15	INSIGHT	It is a NASBA-based test known as isothermal NASBA sequencing-based high throughput test combining NASBA with molecular barcode for detection of the SARS-CoV-2
16	Pooled sample testing	To enhance the test spree by testing several samples pooled together where most of the population is negative
17	Negative test results	A negative result means the test did not detect the SARS-CoV-2 virus. When the outcome of the test is negative, it may be because it is too early to test the sample when the infection has not reached its detectability • In the case of asymptomatic patients, a negative test must not rule out infection • Preventive measures must be kept in place to reduce the risk of spreading COVID-19 • Negative result of unvaccinated individual still requires quarantine for the *period established by the public health authorities* • Fully vaccinated people have a low risk of infection

(continued)

Table 2.1 (continued)

#	Term	Expansion
18	Negative test result (serology)	A negative result means the test did not detect antibodies to the COVID-19 virus
19	Positive test result (serology)	A positive result means the test detected antibodies as an adaptive immune response to the COVID-19 virus possibly due to a recent or prior infection of COVID-19
20	Positive test results	Since no test is perfect, therefore, chances are there that a test may turn with a false outcome. A positive result means the test did detect the SARS-CoV-2 virus and the likelihood is the presence of COVID-19. When the outcome of the test is positive, allow for identification and isolation of infected persons, as well as a case interview to identify and notify the case's close contact(s) of exposure and the need to quarantine
21	False-negative result	Since no test is perfect, therefore, chances are there that a test may turn with a false outcome When the test outcome of a positive (infected) sample is negative- this means the test tells you don't have COVID-19, but in reality you are infected. Whether it is true negative or not, can be confirmed by either retesting the sample or by testing replicates of the same sample or comparing it with another test method
22	False-positive result	When the test outcome of a negative (uninfected) sample is positive. This means the test tells you have COVID-19, but in reality, you are not infected When the test outcome of a negative (uninfected) sample is positive- whether it is true positive or not, can be confirmed by either retesting the sample or by testing replicates of the same sample or comparing it with another test method. Following are the possible reasons for false-positive results: 1. Pre-analytical errors: For example, mislabelling of samples 2. Analytical errors: These errors occur during the laboratory testing for example reagent are contaminated 3. Post-analytical errors: This involves interpretation of results for example incorrect interpretation of data
23	Positive predictive value	It is the chance that an individual with a positive screening test really has the disease. A test with poor positive predictive value would have high false-positive rate
24	Negative predictive value	It is the chance that an individual with a negative screening test really does not have the disease
25	Pretest probability	Is the chance estimated before taking the test that the test patient is having the disease
26	Post-test probability	Is the chance estimated after taking the test that the test patient is having the disease

- **Samples for antibody tests:** For this test venous blood or serum samples are normally taken in a clinical setting. For the antibody tests like finger stick, the blood sample is taken from finger-tip. However, other body fluids like saliva and sputum samples are also used.

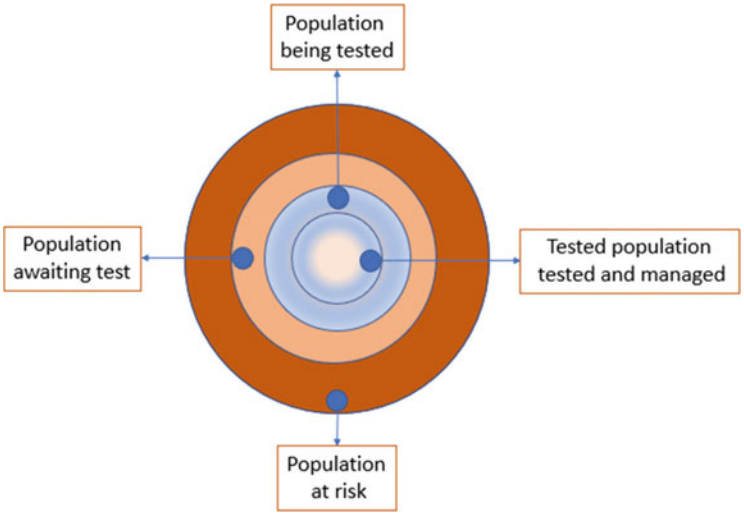

Fig. 2.2 Population testing under limited resources

Test terminology for COVID-19: These terms may clarify the terminology of the testing language to interpret the results (Table 2.1).

The testing framework: Ideally, to design an appropriate testing framework in the pandemic situation, every country needs to set out a goal of achieving zero infectivity rate of the outbreak in the community. In this scenario, two key points are important for the regional public health agency to consider—to rapidly detect all infected persons and to identify the source of infection. The main objective of this operation is to ascertain all the possible chains of infectivity in the pockets of population. An appropriate role by the regional public health agency to perform targeted testing is required for better management of the disease (Pettit et al. 2020, https://www.health.gov.au/resources/publications/coronavirus-covid-19-testing-framework-for-covid-19-in-australia, https://gov.wales/covid-19-community-testing-framework).

The current pandemic has strained the supply of materials, drained resources, and affected labor capacity all over the globe. Moreover, the testing framework demanded by the SARS-CoV-2 testing has further aggravated the testing scenario. It is this scenario that is reflected on the testing infrastructure of the testing community and the health of communities (Fig. 2.2). Therefore, their abilities in monitoring and stopping the transmission of the COVID-19 have experienced hindrance in their task. With the evolution of the pandemic, more new tests came to the market that have offered some solutions to these issues. Some regionally relevant and novel frameworks such as 4Ps that involve actions—prioritize, propagate, partition, and provide—were evolved (Pettit et al. 2020). Such programs were devised to augment testing capacity with the existing limited resources in the event of disease outbreaks. The utilization of the 4Ps-like strategies in the testing framework can prove helpful in rapidly expanding the current testing capacity and for these strategies to be

Table 2.2 Testing in different scenarios

Attribute	Diagnostics testing	Screening testing	Surveillance testing	Genetic variant Testing
Purpose	To identify current infection	To identify infected asymptomatic or unknown cases	To monitor population • Burden of disease • Characterize the incidence of disease	To detect variants (mutants)
Whom	In a symptomatic or asymptomatic person known or suspected exposure to the SARS-CoV-2	Who do not have known, suspected, or reported exposure to the SARS-CoV-2	Population	Positive samples with compromised or negative test outcome
Benefit	For better management of the transmission	For taking measures to prevent further transmission	Gaining information at a population level	• A rapid way of detecting variants • Confirmation by sequencing.
Examples	• People having COVID-19 symptoms • Identified by contact tracing • Suspected contact with a confirmed or suspected case of COVID-19	• Testing followings: – Employees in a workplace – School staff – A person before or after travel	• Testing results are not reported to the individuals • Not for a person's health care • Example: wastewater	• Accula SARS-Cov-2 Test (Hogan et al. 2020)

applicable in future pandemics. As the COVID-19 pandemic evolves along with more tests and testing methods become available, the testing framework includes different testing strategies as categorized in Table 2.2.

During this pandemic, people from all walks of life, dealing with the impacts of this scenario, are passing through painful times (Morris et al. 2020; Kumar et al. 2021). In this situation, the cost of COVID-19 is so high that some sections of the population groups like ethnic minority or remote area populations may experience a disproportion in the burden of the disease. Therefore, strategies should be drawn to take health equity more seriously than before. All such groups deserve to have equal access to affordable and timely testing of the SARS-CoV-2 to reduce transmission among those communities (Paremoer et al. 2021; Jensen et al. 2020). To provide health equity in terms of testing strategy, considering free testing, determining categories of available test, for example, RT-PCR versus antigen test, and removing barriers to testing, for example, offering drive-through testing, need to be considered (US-CDC 2021b).

Testing in different scenarios: While reviewing the current pandemic situation, in early July 2020, the U.S. CDC released the following five workplace testing

scenarios. The design of these scenarios was based on the most current knowledge and experience available for protection again COVID-19 and on minimizing its spread (Gieseke 2020, Paltiel et al. 2020; US-CDC 2020; US-CDC 2021c; Table 2.2).

- Testing individuals who have signs or symptoms of COVID-19.
- Testing asymptomatic individuals who have recent known or suspected exposure to the SARS-CoV-2 virus.
- Testing asymptomatic individuals without known or suspected exposure to the SARS-CoV-2 for early identification in case community transmission is particularly high in an area to test and identify positive cases.
- Testing to resolve cases of isolated individuals, for example, to decide when to come out of isolation.
- Testing for public health surveillance for the SARS-CoV-2 to identify hotspots, to understand disease trends, to assess the disease burden in a community or a workforce, and to assess factors promoting the risk of infection. These measures are meant for evaluating the effectiveness of the control measure (Bracis et al. 2020).
- Testing for variants of the SARS-CoV-2 (WHO 2021a; Álvarez-Díaz et al. 2020; Matic et al. 2021).

How to choose a suitable test: Before picking a test, it is important to know the basic purpose and target of the testing, whether it is for diagnostic or screening or surveillance or for variant detection. The second aspect to consider is the attributes of the analytical performance of the test, for example, how rapid, robust, accurate, and sensitive the chosen test is. The third aspect for consideration is knowing the pretest probability of the test, since the positive- and negative-predictive values of every method depend upon these factors. The pretest probability accounts for the incidence of infection in the population and the testing objective of the person to be tested (Tordjman et al. 2020). To screen a population or community with a high infection rate, an antigen test is considered as a method of choice to screen the population for being rapid. Although it has a low positive-predictive value that may lead to a high number of false positives, it may still prove its worth in meeting the objective of testing. On the other hand, to screen a community with a high infection rate and high test demand, choosing a laboratory-based RT-PCR test may not be a worthy choice. The RT-PCR in such a situation may lead to testing delays due to its intrinsic limitations of turnaround duration to return the results on the tests. Updated information and recommendations from the regional regulatory agencies on what test should be used for a particular purpose, for example, a testing program, can be excellent support (FDA 2021). However, the rapid microchip RT-PCR test system that can provide accurate results in less than 30 min and for being cost-effective and portable test system can be an acceptable choice for certain strategies (deCampos-Stairiker et al. 2021; Razvan et al. 2021; Gill et al. 2021).

In view of the false-negative results emerging with molecular tests from patients with the SARS-CoV-2 genetic variant, the staff of the testing laboratory and health

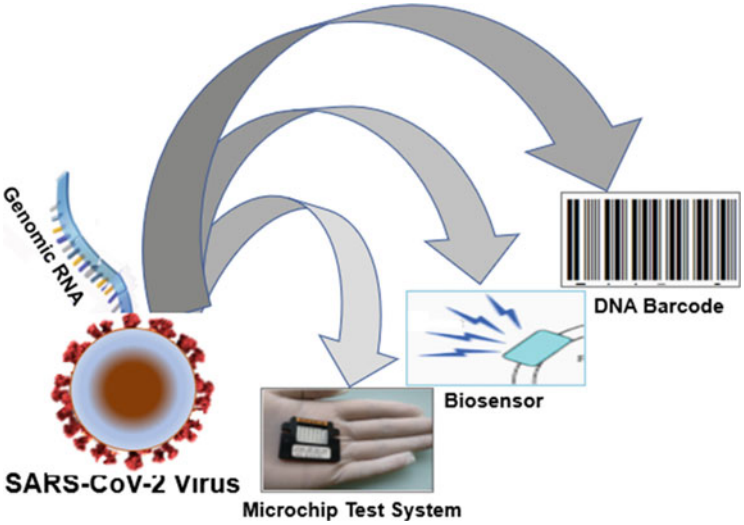

Fig. 2.3 Advanced molecular testing technologies

care need to be vigilant. Similarly, the false-positive test outcomes potentially emanating from antigen tests due to the changes in antigen determinants on the surface of the viral coat also warrant attention to the notification from the regulatory agencies of the country or the WHO or the FDA (WHO 2021b; FDA 2021).

COVID-19 and testing in 2021 and onward: Ever since the start of the pandemic, the COVID-19 is no longer a respiratory illness only. It has been affecting multiple organs in the body thus throwing a challenge to the treatments. Its causative virus has been mutating to attain stringent capabilities and thus impairing the testing capabilities of some test kits. However, in view of relentless research and developments carried out globally, the currently horrifying COVID-19 is no longer going to enjoy the same status in 2021 and onward. In offering a respite from this devil bug, the following developments are expected to change the scenario (Nania 2020; Scudellari 2020). Seems like intermission has dawned through testing strategies and vaccination programs.

1. Vaccination programs on two major vaccines with FDA authorization—one from Pfizer-BioNTech and another from Moderna—are being administered that started reducing the R number of the SARS-CoV-2.
2. An antiviral drug called remdesivir has been approved by the U.S. FDA.
3. A few monoclonal antibodies that imitate the immune response to COVID-19 have been granted EUA by the U.S. FDA. The clinical trials have demonstrated progress in delaying hospitalization of the covid patient.
4. Improved therapies are expected to be administered more effectively.
5. COVID-19 testing will become faster and more accessible to be performed at home without requiring a prescription [Fig. 2.3].

6. It remains to be seen how long the immunity conferred by vaccine stays (Hopkins and Toy 2021). But the know-how gained on the RNA vaccine production is expected to produce improved vaccines in the future.

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3 The Broad Impact of Infectious Disease Epidemics on Human Civilization: A Public Health Perspective

Amarjeet Singh

Abstract

Slow acceptance and delayed response of the state/public have been a common feature in the history of infectious disease pandemics. Globalization affects both the genesis and the control strategies of the pandemics. The general public, health professionals, political leaders as well as administrative authorities, all have shown some or other changes in their routine working or living style while handling the COVID-19 situation. Gradually, the new ways of living 'the new normal' have transformed into new behaviour. The response of different sections of society has also been different. The bulk of the changes was linked to the fear and panic about the chances of spread/catching of infection which brought the world to its knees.

The second wave of the Corona pandemic in 2021 saw shortage and black marketeering of medical supply essentials. Vaccination has emerged as the dominant strategy this time. In the atmosphere of too many rumours, fake news, disinformation and so on, a third wave has now been predicted.

There is a significant role of public health discipline in controlling any pandemic like Corona. Generic, age-old preventive measures are the only way out for the infections which spread through the respiratory route, with a fast person-to-person spread.

Corona pandemic has provided a lesson for us that for communicable disease control, there is a need to inculcate a nature-friendly responsible behaviour to ensure peaceful co-existence between people and microbes.

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Keywords

Pandemics response · Public health · Infectious disease · Global health governance · New normal · Health behaviour

3.1 Introduction

Since antiquity, human beings have been exposed to a variety of catastrophes. Our continued existence is a testimony to the fact that we, as a human race, have successfully survived different kinds of threats to our lives. Be it nature's onslaught like an earthquake, floods, a storm, tsunami and an avalanche or manmade disasters like accidents involving aeroplanes/trains/ships/ road vehicles or collapse of building/bridge and so on, we have learned to negotiate a variety of emergencies.

Our response to such situations has been varied, per the nature of the challenges faced. These problems are tackled to deal with the impact by limiting the damage caused. Avalanche, tsunami, earthquakes and accidents are usually spaced apart in time and place. Their immediate effect is localized. Their repetition is also not that frequent, and the impact is not that lingering. Every time, man faces a catastrophe, he develops strategies to tide over the prevailing crisis and prepares for preventing any similar next onslaught. Yet, the subsequent repetitions do not spare the human race. Some damage is always there. For example, man has built quake-proof buildings and has evolved tsunami warning systems. Still, these disasters do take their toll.

However, the morbidity and mortality-related impacts of infectious disease epidemics and pandemics are in a different league altogether. Quite often, we are not even sure of the nature of the causative organisms, mode of spread or the remedies. Quite often, the infections keep spreading and lingering on for years, as witnessed in the 2021 Corona pandemic second wave, that is propagated epidemics.

3.2 Historical Aspects

Middle Ages onwards, without even knowing the causative organisms, quarantine mechanisms were instituted in many countries. After the repeated infectious disease outbreaks troubled different countries in the eighteenth to nineteenth centuries, there were widespread discussions across the society. There were efforts to improve their civic hygiene status, even without clear-cut elucidation of aetiology (Singh et al. 2013).

In the modern era, despite significant medical progress focusing upon microbiology, pharmaceutical research, genetics, immunology and vaccinology. over the last centuries, infectious diseases still represent a considerable threat to society. Microbes are steps ahead of various human endeavours to control them. Every few years, some or other epidemic starts.

Man has always underestimated nature, in particular, the potential of microbes to cause diseases. After specific disease control programs were launched in the 1950s

onwards, a rapid decline in the incidence of smallpox, malaria and filaria led to fostering of a complacent attitude among us in the late 1960s. Many public health experts predicted that causative organisms of these diseases would be eliminated by the end of the twentieth century!

Like revolutions, economic crises and wars, infectious diseases pandemics have long-term social, political and cultural consequences. Every such disaster has led to changes in the way we live. Human civilization has kept on progressing through the centuries. With survival at its root, we have changed our values, norms, attitudes, beliefs and behaviours, often in the wake of pandemics. Our governance systems have also changed accordingly.

The panic and fear associated with mortality/morbidity due to pandemics have important economic implications through impact on trade with other countries, health, transportation, agricultural and tourism sectors. The interconnectedness of modern economies in a globalized world disrupts international supply chains also. There are micro- and macro-level changes at individual, family, community/society, country and international scales. From time immemorial, throughout our history till today, from the Black Death to the present COVID-19, there have been disruptive global implications of such disasters on human health, culture and demography. Pandemics have often led to the weakening of institutions and prevailing power structures. Even the collapse of empires has been attributed to epidemics.

The synergy between wars and epidemics in shaping history has also long been recognized. Quite often, the diseases ended the wars. Napoleon Bonaparte's nineteenth-century imperial expansion was halted by yellow fever. His eastern ambitions were thwarted by dysentery and typhus. Spanish Flu, a pandemic beginning towards the end of World War I, killed up to 50 million people. Eventually, Germany and Austria had to slow down their offensives (History.com 2020).

The public's understanding of how a disease is transmitted affects the course of an epidemic. In the twenty-first century, the only thing that has changed is the prevailing understanding of disease aetiology and pathogenesis (this is dependent upon the technology level of the era). Even before the advent of microscopy, bacteriology, information technology, artificial intelligence and so on, man tried to control the situation of epidemics per his understanding of the disease. Even in the twenty-first century, despite all high-end research, the initial individual and community containment measures employed were almost similar to the non-pharmaceutical interventions that were used almost a century ago for any infectious disease with pandemic potential. The information, education and communication strategies adopted and advice given were also similar. These measures form part of a much broader, layered approach to behavioural intervention. This extends from individual actions (hand hygiene, cough etiquette) to global efforts (containment at the source, advisories and screening for travellers), isolation of ill persons; voluntary home isolation and quarantine of their suspected contacts from the 'well'; providing prophylaxis to their household contacts; social distancing; simple sanitary/personal hygienic measures; wearing facemasks universally; following lockdown guidelines; reduced travelling; avoiding stigmatization; avoiding blame games; school closures;

keeping children and teens at home; avoiding hoarding of essential commodities and so on.

3.2.1 Cholera Forcing

Even up to the nineteenth century, public health infrastructure was inadequate in most countries, resulting in filthy and foul conditions, laying the basis for an outbreak. This way, epidemics such as cholera became the key stimulus for the improvement in safe water and sanitation. For centuries, diseases have forced cities to make changes that benefitted future generations, for example the 'cholera forcing' phenomenon (Hamlin 2009). This implies that cholera 'forced' beneficial changes in public health. Concepts of cleanliness and sanitation were promoted in Europe and the United States with strict sanitary enforcement in cities**.** Local governments rigorously controlled street cleaning, public baths, water bodies' maintenance, disposal of dead bodies and so on. A sort of sanitary revolution ensued. It was realized that addressing the environmental causes of epidemics was a public responsibility. Waterworks, water closets, drains, sewers and sewage disposal works were seen as a moral good as part of a single integrated sanitary system.

A series of central themes have recurred about our social responses to epidemics ever since the Black Death (Plague), the deadliest pandemic recorded in human history killing millions of people in Europe and Asia in the fourteenth century. It had lasting economic and social consequences. There were changes in the structure and development pattern of urban populations. Although its cause was unknown, plague spread was linked to shipping movements. This led to the establishment of quarantine stations to hold and disinfect humans and cargo in port cities through legislation for international disease control. However, sanitary efforts were implemented by municipalities with little coordination between city and states (Birn et al. 2018; Boire et al. 2013).

This led to urban planning reforms. Cities invested heavily to create sanitation departments for garbage removal. Cities started using the environmental engineering approach to prevent epidemics, for example improved ventilation, drainage of stagnant water, street cleaning, cleaner wells, fumigation and burial of garbage. Urban space improved with the development of a citywide zoning code for effective civic work monitoring. This improved human and environmental health.

Initially, there was no official disease notification system. The establishment of plague boards in many Italian towns and cities by the fifteenth century was a precursor to the formation of an international health authority. Thus, epidemic crises do make us do what we cannot do otherwise, for example after the SARS outbreak in 2003, China strengthened its national and local surveillance systems to prevent and control diseases and also expanded its laboratory capacity (Qiu et al. 2018).

3.2.2 Technology Forcing

Similarly, the 'Technology forcing' concept implies that epidemics catalyze technological changes that were not possible earlier. This way, the threats of disease have scared us into accepting the deficiencies in basic systems and services that needed to be corrected. Diseases like cholera, thus, proved to be a net benefit to humanity (Hamlin 2009).

3.2.3 Scapegoating

There is also a long history of scapegoating, violence and mass hysteria accompanying epidemics, for example massive persecution of Jews who were blamed for spreading plague. Undesirable social groups are often blamed for an epidemic and are unfairly treated in the name of preventing disease transmission. White scapegoating of black South Africans in 1918 precipitated the first legislative steps towards apartheid (Snowden 2019). Widespread stigma was also seen during the COVID-19 crisis against the people belonging to some ethnic groups and some professional groups (Pandey 2020a). The root cause for the same was linked to the fear and panic among people about the chances of spread/catching of infection.

3.2.4 Cholera Denial

Slow acceptance and delayed response of the state/public have been a common feature in the history of human epidemics. Leaders are either lauded for their bold decisions or criticized for incompetent crisis handling. It is usually difficult to convince people about the seriousness of the problem. After the official declaration of an epidemic, people often demand government action to control it. Acknowledging an epidemic threatens various economic, institutional and personal interests.

The 'Cholera denial' concept implies that governments' response to epidemics like cholera has usually been its denial for simple reasons (Hamlin 2009). If governments admit the existence of an outbreak of any infectious disease like cholera, it will adversely affect trade and tourism. The 'illusion of normality' favours political stability. Also, there are dreading prospects of costly sanitary reforms. Even plague triggered the decline of the economies across the globe. Wages tripled during those times, as labour demand rose. Hence, official mortality data usually hide the real situation. Rather, they attribute deaths to other causes. Even in the twenty-first-century information age, the WHO estimates indicated underreporting to the extent of 90–99% in the formal system, even for corona-related morbidity/mortality. Governments often attempt to conceal outbreaks from the world to protect economic assets and trade. This is because of the economic and political costs associated with reporting disease outbreaks.

3.2.5 Role of Globalization

It affects both the genesis and the control strategies of the pandemics. The extent, as well as the speed of travel of people and goods, affects the spread of pandemic disease locally and globally. In a globalized marketplace, with plenty of international travel and tourism trade, whenever a pandemic strikes, it becomes the focus of international attention. This forces various countries to hide the data to protect the economy. Resultant infectious disease denials have the potential to undermine the control activity efforts compromising the protection of the global community. Historically also, quarantine-linked closures of a port or a city to foreign traders or goods did cost communities a great deal of money. This has often created great hardships for people. Most of the time, the merchants oppose any disease control efforts involving impediments to commercial activities and capital flow.

Even in the state health care delivery system, health professionals dread the declaration of an epidemic, as it might lead to enforcement of the 'Epidemic Disease Act' with related restrictions. Also, once this Act comes into force, the medical technocrats (medical officers/civil surgeons) have to report to bureaucrats (District Magistrates/Collectors/Commissioners)! (Singh et al. 2013).

3.3 Behavioural Changes in the Wake of COVID-19

Change is an inescapable part of our lives. Nothing remains static forever. In human civilization history, social changes have been evolutionary (gradual/slow) or revolutionary (fast/rapid). Mostly, these changes take years to take shape. Sometimes, however, changes occur overnight or in a matter of few days. This is often seen during pandemics when we are confronted with questions of our survival. There is no option but to alter our routines to mitigate the adverse impact of outbreaks. Adaptation to the changed scenario becomes the key issue concerning our survival.

With every disease epidemic, there is a secondary behavioural epidemic that affects our social lives.

For example, strange things happened as an aftermath of the contemporary Corona crisis. The general public, health professionals, political leaders as well as administrative authorities showed some or other changes in their routine working or living style while handling the COVID-19 situation. Sometimes, it takes a crisis to force us to adopt changes in our habits. The response of different sections of society has also been different.

The behaviour implications of epidemics are many. Corona is special in itself, in that there are many seemingly 'first-time' changes. Almost every aspect of our lives has drastically changed due to corona fear – the way we live and work. There have been restrictions on socialization, organizing weddings/parties shopping/entertainment/eating out and so on. Now, we cannot move out of our homes without masks. We are washing or sanitizing our hands after touching any surface. These changes are difficult to incorporate into our routines. But gradually, 'the new normal' gets transformed into new behaviour.

There is regular news about corona-related complications and deaths. This has instilled fear in the general public. Threat or fear of infection has led to the stigmatization of suspected or declared positive cases. This has further worsened the situation. There was discrimination against patients, high-risk groups, doctors / nurses/health care workers, migrant labours and domestic help and so on. (Pandey 2020a). Overnight, there was a sense of being lost/confusion/disillusion.

Panic buying (and hoarding) was seen on a large scale. People had to compromise on their brand choice due to the nonavailability of the products. There was mass mask panic. Everyone was trying to procure masks that overnight went out of stock. Even routine health services like immunization got seriously disrupted.

Loneliness was experienced by many people. Elderly (above 65 years) and children (below 10 years) were prohibited from venturing out of their homes. The fear and stigma affected different groups differently.

It is often said that behavioural changes are difficult to bring about. But people changed their behaviours significantly since January 2020 in the wake of the corona scare, and they did it very fast. Various domains of our lives were affected due to the Corona crisis, with significant changes, for example professional and office work, family life, eating habits, social life, shopping, markets, industrial sector, entertainment, reading habits, street vending and so on. Various established 'norms' were transcended. Many 'deviations from the normal' were witnessed for the first time. Strange things are happening as an aftermath of the contemporary Corona crisis.

The bulk of the changes was linked to the fear and panic about the chances of spread/catching of infection which brought the world to its knees. It is too early to say how permanent are the COVID-19-related changes seen across the world. Almost all the countries have followed some level of lockdown to curtail its spread, affecting not just the routine life but also their economies**.** The following text tries to analyse these to understand the intricacies involved concerning human behaviour as well as governance (Singh et al. 2020).

3.3.1 International Scenario

- We realized that there are global consequences of local problems. The need to prioritize global public health over national sovereignty became clear. A sort of global interconnectedness has been highlighted by COVID-19. Countries discovered their helplessness due to their dependence on the rest of the world. They felt a sense of isolation and loneliness while responding to the crisis.
- Long, extensive/extended lockdowns/shutdowns (factories/offices), were enforced in almost all the countries with drastic restrictions on people's movements.
- Western, well-to-do countries were affected more (many sunk into deep crisis). America reacted strongly and criticized China vehemently. It also withdrew support to WHO, as it failed to discipline China. There was widespread criticism of the agency, even as WHO kept changing its statements about the Corona crisis (Kannan 2021).

- Some countries/ethnic/professional groups were stigmatized as the epicentre for the genesis and the spread of infection. Even for the second wave, mutant strains from the United Kingdom, Brazil and India were blamed.
- With this crisis, the North-South paradigm ended. It increased inequities. It further concentrated power among the elite in the global north. The crisis deepened the divide between North and South, challenging the multilateral system and global solidarity on an unprecedented scale. The monopoly of traditional 'development actors' of the last 40 years eroded.
- Also witnessed were isolationism, anti-immigration policies and institutionalized racism.
- Focus shifted to research on issues like laboratory diagnostics, vaccines, therapeutics and non-medical methods of prevention.
- It brought developing countries closer to the self-determination of their destiny.
- Rich countries, due to huge economic losses, used COVID-19 as the excuse to cut development assistance to others.
- China and South Korea used to be aid recipients. Now, they helped the WHO, Italy and other European countries to cope with the crisis. China distributed masks to European countries that had been doing it in developing countries for years. In 2021, it offered help to India also. But it was politely refused.
- The COVID-19 pandemic became the new excuse for nationalism with a commitment to producing pharmaceuticals, medical supplies and equipment domestically. There was an outcry in virtually every country about the lack of equipment and supplies to test for and protect against COVID-19. This led countries to re-examine their supply chains for critical health and livelihood-related products.

3.3.2 India/Country-Level Scenario

- Prime Minister of India addressed the nation 6–7 times during the initial 6 months of the crisis. He also visited three vaccine manufacturing units in November 2020. He also led from the front by allaying the fear and apprehension among the general public about the vaccine (The Hindu 2021a).
- A '*Janta* Curfew' was successfully enforced across the nation in 2020.
- Extensive communication of the sets of prevention/disease management-related guidelines/SOPs from ICMR/MOHFW was done. These were regularly reviewed and revised.
- Extensive behavioural restrictions were enforced on people, who complied with these, for example almost universal use of the masks (there was a penalty for violation, which was enhanced to Rs 2000 in Nov. 2020). Social distancing and hand hygiene (sanitizers use)-related behaviour changes were also seen.
- There was extensive use of power by the government, by the police and even by resident welfare associations (Maids were not allowed in homes; restrictions were imposed on inmates).

- Extensive use of statistics/data/graphs/models/predictions was witnessed in media reports. Mostly, it was (wrongly) used by different states to prove themselves better than others.
- The use of IT applications/*Aarogya Setu* by people was also widespread, voluntary as well as enforced.
- Online money transactions rose. Consumer shopping habits changed.
- Massive interstate migrant labour exodus ensued (on foot or otherwise).
- Media reports focused on a public health crisis for such a long time.
- Yet, the second wave of the Corona pandemic saw shortage and black-marketeering of oxygen cylinders, remdesivir, steroids, vaccines and hospital beds. The problem has emerged as a simple administrative/management issue. The court is also exercising its discretion in cautioning the centre as well as the state governments to deal with the shortages/mismanagement. The caseload and mortality are very high (much more than that in 2020). The mutual blame game is playing a dominant role in 2021.
- Health remained on the political agenda for quite a long time.

Prolonged, extensive shutdowns for months was observed in -

- Banks.
- Markets, malls, cinemas, eating establishments.
- The entertainment industry (films/TV/Theatre).
- Hospitality sector.
- Air/road/rail/water-based transport/travel; both international and domestic.
- Teaching institutions (schools/colleges/universities).
- Tuition classes (a ubiquitous feature of most cities).
- Religious places.
- Conferences, picnics, outings.
- Exams/viva (now held through Skype/Google Meet, etc.).

3.3.3 Community-Level Scenario

- Widespread stigma and discrimination were witnessed against the international travellers, the quarantined/suspect/confirmed/cured cases as well as against the health care staff (Pandey 2020a). Concealment of cases resulted because of this stigma.
- Home quarantine was seen by this generation of people for the first time (after a long time since smallpox).
- Widespread interruptions in the provision of essential services and supplies (daily needs) were commonplace. There were extensive/intensive efforts by the civic administration to tackle the same.
- People could not avail of the services of plumbers, electricians, carpenters, tailors, teashops, *paan, bidi*, liquor shops, barbers, haircuts and beauty salons.

- People realized what was essential and the bare minimum necessary things in life. Many brooded upon the futility of worldly possessions, money and so on. Sudden drastic changes in lifestyle made people realize the transience of life. Life philosophy took a new meaning.
- Panic buying (and hoarding) of essentials was seen on a large scale.
- Fear and phobia gripped people about the spread of the disease.
- Loneliness was experienced by many people.
- Elderly (above 65 years) and children (below 10 years) were prohibited from venturing out of their homes.
- From April 2021 onwards, mutual support is slowly dominating the scene.

3.3.4 Home-Level scenario

- Kitchens' ambiance changed. New, as well as old, recipes were tried.
- The kitchen became the new office/home/recreational hub for urbanites.
- All the family members stayed at home with more time for each other.
- People realized the importance of the kitchen garden.
- Many routines were suspended, for example home delivery from eateries, which had picked up very fast in the last decade; social interaction (visits), formal as well as informal, were reduced and leisure walks were restricted.
- Drawing rooms remained unused for months (no guests).
- 'Work from home' (which had been started long back in the private sector) was widely practised, even in govt. offices.
- The burden on housewives increased since maidservants were not available.

3.3.5 Professional Life scenario

- Public health professionals demanded their due place in the plan of the things; there were discussions on their being ignored.
- A flurry of webinars and video uploads were seen in all fields (professional as well as other domains of education).

3.3.6 Health Care Sector scenario

- The use of PPE kits was quite widespread by health professionals.
- Routine OPDs were suspended.
- The concept of screening OPDs was implemented in hospitals.
- Closure of bulk of the private clinics/hospitals was seen.
- Reluctance/apathy of doctors for teleconsultation relaxed (many startups ventured into telemedicine service provision).

3.3.7 Media scenario

- Widespread TV debates/YouTube uploads on CORONA (individual as well as institutional).
- Newspapers were discontinued by many people.
- Fake news also floated.

All said and done, one thing that has dominantly emerged in this crisis, that is the self-reliance/self-sufficiency concept and capacity of villages have been realized by many while exposing the ever-increasing dependence of urbanites on maidservants, essential service providers, departmental stores and street vendors. The importance of neighbourhood provision stores also dawned upon people.

The COVID-19 pandemic has transformed the global health community's acceptance and use of digital health technologies.

- Health care systems across the globe adopted digital health technologies and telemedicine (+remote learning and support), which were scoffed at by the majority earlier.
- Doctors, patients and home care providers discovered the effectiveness of virtual consultations.
- Artificial Intelligence (AI)-based technologies are being increasingly employed to provide insights into complex questions of individual behaviours.

Prolonged confinement due to the recommendations about not to gather in large groups and keep our distance from fellow humans to limit the spread of COVID-19 led to

- People spending more time in front of a screen, computer/smartphone.
- An opportunity to come up with imaginative alternatives to face-to-face contact and to consider the importance of virtual reality.
- Reduced travel forced hoteliers to fast-track technology adoption and revisit their distribution and marketing strategies.
- Rethinking in our daily lives (work, school and entertainment).
- Organizations found it imperative to digitally transform the places of work and education to be able to operate effectively.
- Shift to Work from Home – Companies that were resistant to the concept of a distributed workforce were forced to allow working from home so that work could still be done while taking precautions to halt the spread of the virus. Companies built remote working policies in the event they needed to shift work home in the future when it may be required again due to another outbreak.
- Commercial modelling changed to meet the personalization expectations of the customers, for example hotels now looked more at domestic tourism and travellers and that calls for micro-targeting.
- Increasing willingness to do meetings and discussions digitally or telephonically. These were previously done face-to-face.

- New ideas and technologies emerged that may persist after the disaster subsides. Development of adaptive learning technologies with mechanisms for immediate feedback with more personalization on the part of the technology.
- Billions of children were impacted by school closures. Students had to study from home. The poorest and most marginalized girls may never return to school. Teachers left unpaid during the closures may be lost to the profession. Schools in poor and rural areas, closed during the pandemic or repurposed as health facilities, may not reopen early.
- We have been forced to learn quickly about what works and what does not.
- We learnt which parts of our bureaucracy respond most effectively so we can lean on them in the future.
- We learnt how to better use data, evidence and technology to improve our ability to react to crises.
- We also learnt how to respond to the uncertain environments that are likely to be an inevitable part of our future, for example resources constraints.
- We also realized what matters most in our lives and to prioritize things accordingly.
- We also realized the immense value of data, public health research and epidemiological surveillance for effective pandemic response.
- We also realized the need to manufacture health commodities and strengthen supply chains closer to where materials are needed domestically and globally.
- Inefficient and wasteful spending became more noticeable.
- Governments requested communities to support the needy.
- We were forced to re-examine the emergency health preparedness and reprioritization of development assistance due to the global recession.

We got opportunities to transform traditional approaches to development, by involving the private sector, academia and local partners to promote sustainable investments in community-level preparedness. Public sector service delivery was emphasized to build the resilience needed to respond to and prevent such crises in the future.

- Diseases like HIV/AIDS, tuberculosis and malaria received less attention.
- China got its place at the global policymaking table.
- We also realized that under-investment in the public health of one country is a threat to global health security everywhere.
- A paradigm shift was there in global health governance and multilateral cooperation with international organizations announcing newly expanded mandates to match the needs of a pandemic.
- We generated many innovations in medical science, pandemic response and economic recovery.
- There has been an acceleration in some policy changes that were evolving for several years in global health, for example task-shifting to allow more basic services by community health workers who were assigned distribution of some prescription drugs over the counter.

3.3.8 Behavioural Changes in Routine Life in Various Sections of Society

Responses of different sections of society have also been different as elucidated below:

3.3.8.1 Patients

- With widespread outbreaks burdening the health care agencies, people could not find hospital beds despite having money. COVID-19 patients feared quarantine, isolation, losing livelihood, spreading disease and not being able to protect family members. The stigmatization forced people to conceal symptoms. Many patients absconded from hospitals/quarantines. The COVID label discouraged them from adopting healthy behaviours.

3.3.8.2 High-Risk Groups

- Mortality rates were higher in senior citizens, pregnant women, young children and in people with comorbidities. So, the fear of getting infected was high in them. This resulted in behaviours like 'not availing health care services' or 'not seeking help'. The elderly, anticipating death, started registering/changing 'the will' of their property.

3.3.8.3 Doctors/Nurses/Health Care Workers (Corona Warriors)

- They feared violence; they were also afraid of getting infected due to their exposure status. This impacted the quality of care and adversely affected other health care services like routine immunization. Hospital and civic administration indulged in a mutual blame game. Many doctors and frontline workers across India were attacked and killed due to corona-related stigma (Pisharody 2020). They were even asked to vacate their rented accommodations.

3.3.8.4 Migrant Labour

- Total lockdown led to a loss of jobs due to the closure of industries, factories, shopping centres and even agriculture-related work. They suffered from a higher risk of hunger, the spread of disease, poor health status, maternal and child mortality. The migrant workers were left with no money. They hardly had any liquid assets or savings to pay for their rent and food. This forced them to go back to their native villages due to their wish to be with their people. With no transportation facility available, they were forced to walk on foot for days to reach their homes. Many died due to hunger, exertion, accidents and comorbidities during the transit (Pandey 2020b; Anonymous 2020).

3.3.8.5 Domestic Help

- They were labelled as the new untouchables. Many Residents Welfare Associations (RWAs) did not allow the entry of maids and other workers to enter their societies. They lost jobs as people were too scared to allow them entry

into their homes. Financial constraints due to the stigma attached to them adversely affected their lives.

3.3.8.6 Children and Adolescents

- With the closure of schools and colleges, the children and adolescent were forced to stay back in their homes. They could not go out to play or socialize. They had to adjust to the new online system of education. The virtual world of online classes on mobiles, tablets and computers/PCs became a 'new normal' for them. The idle time due to lockdown led to changes in their lifestyle-related behaviours, sleep cycle, exercise, physical activity and attitude.

3.3.8.7 Adults

- Men and women were also affected in both positive and negative ways. They worked from home. They had more time for each other and their families. But the negative side was that in some homes, females had to do all the household work, due to their reluctance to hire domestic help in the wake of the COVID-19 crisis. Males had more idle time with hardly any work to do. Few of them helped their wives and family. Many inculcated new hobbies and engaged in other recreational means of keeping themselves busy. But on the flip side, few were frustrated, angry and stressed due to financial issues. Many men, who were used to consuming alcohol regularly, suffered withdrawal symptoms. All this resulted in the surge of domestic violence cases against women.

3.3.8.8 Elderly and People with Disabilities

- The anxiety in this group was highest as they were the most vulnerable age group. The fear of death from disease affected their lives greatly. They faced many health-related issues. Restricted home supplies affected their quality of life. The need for caretakers was acutely felt by them. Their loneliness levels also increased.

3.3.8.9 Scenario in Different Income Groups

3.3.8.9.1 Below Poverty Line (Poor People)

- They were the worst-hit group. Their survival was at the mercy of the donations, free ration and food subsidy provided by the local governments, volunteers and NGOs. Many working-class jobs like security guards, taxi drivers, hair/make-up artists and retail assistants were adversely affected. This was because people were reluctant to use their services during the lockdown. They feared getting COVID infected through a high probability of physical contact.

3.3.8.9.2 Middle-Class people

- They did not face the basic survival problems during the lockdown as they had enough savings. But they did have concerns regarding losses in business due to COVID. They were affected most with the closure of gyms, entertainment,

international travel and trips and so on. They were seen to be busy making videos to showcase their workout sessions and culinary skills.

3.3.8.9.3 Upper Middle- and Upper-Class People

- Affluent people generally have sufficient stocks of savings. They have already amassed huge wealth, which can meet such kind of contingent situations. Even when money is not an issue for them, they stockpiled and hoarded the items of necessities. This reduced the availability of groceries to other economically vulnerable people, especially during the time of lockdown when the food supply was limited.

3.3.9 Impact on Different Aspects of the Life of the General Public

3.3.9.1 Home

Social interactions (visits), formal as well as informal, were suspended.

3.3.9.2 Food

Avoidance of non-vegetarian food was observed. Addiction to junk food forced people to experiment with cooking at home. Restricted availability of vegetables and fruits was there.

3.3.9.3 Shopping for Grocery

Shops were shut down. Only the essential commodity shops operated. Due to social distancing norms, only a few customers could enter the shops. People preferred to make cashless payments. The customers and shop owners were scared to handle cash due to a fear of infection. Even after the opening of shops after the lockdown phase, people were afraid to go for marketing. Window shopping was no longer visible. The markets and malls wore a deserted look. People shifted to online shopping or doorstep delivery of items.

3.3.9.4 Entertainment

People explored new entertainment options. The use of the internet increased. People made tik-tok videos (now banned) of cooking, singing, dancing and exercising and so on. No new shows are telecast. With no new theatre release, movies were released on over-the-top (OTT) platforms like Netflix and Amazon Prime. Old popular serials telecast by Doordarshan (*Ramayana, Mahabharat, Buniyad, Yeh Jo Hai Zindagi, Idhar-Udhar, Shriman-Shrimati*) were eagerly lapped up by the people. Games and sports events/tournaments were cancelled.

3.3.9.5 Work/Offices/Occupation

3.3.9.5.1 Permanent Employees

The government employees were at ease, as they enjoyed free time without the tension of salary deduction.

3.3.9.5.2 Loss of Job for Temporary Workers, Future Gloomy

Temporary and private employees faced salary deduction and job loss. Companies reduced the number of employees (Load shedding). No new vacancies or advertisements were there.

3.3.9.5.3 Hospitality Industry

Employees of hotels, restaurants and entertainment industry suffered a huge loss of jobs.

3.3.9.5.4 Conferences, Meeting, Interviews/Exams and Outings were Suspended

These were conducted online (Zoom, Google meet, etc. platforms have become commonplace).

3.3.9.5.5 Work from Home

'Work from home' was widely practised, even in govt. offices.

3.3.9.5.6 School/Colleges

Online system of education increased mental stress in children as well as in their parents.

3.3.9.5.7 Outing/Socializing

These were completely stopped. People feared meeting and greeting people. Guests were no longer welcome – '*Atithis* were no longer *Devo Bhava*'.

3.3.9.5.8 Marriages/Rituals/Functions/Ceremonies

Only a few close relatives attended family functions. Even at cremations, attendance was low. People attended weddings/cremations in 'virtual mode'.

3.3.9.5.9 Sleep

People slept till late as they had nowhere to go. They took afternoon naps. Few even had disturbed sleep due to financial problems.

3.3.9.5.10 Mental Health Issues

The rising financial, interpersonal and other routine issues inflicted a great impact on the mental health of people during corona times. Many health problems increased including depression, anxiety, stress, panic attacks and so on. There was also an increase in the incidence of violence (domestic and community) and abuse (elderly, maids, children and women). The new norms, like hand hygiene practices, also resulted in increased obsessive-compulsive disorders among the public. Tensions and conflicts within homes, departments, agencies (ICMR, WHO, etc.) and countries (India/USA vs China) also escalated.

3.3.9.5.11 Exercise

For fitness enthusiasts, indoor exercises became the norm.

3.3.9.5.12 Transport and Travel

The local and interstate public transport system was disrupted. No cabs/autos/rickshaws were allowed to run on roads. They were gradually resumed, but with many restrictions. Trains and air travel restrictions (domestic/international travel) were imposed. Tourism was greatly affected. People were unable to travel for work or leisure.

3.3.9.5.13 Rent/Accommodation

People were unable to pay rents for homes, offices, shops and so on. Many poor people were rendered homeless.

3.3.9.5.14 Health care sector

People facing dental, body aches and other minor health ailments were unable to access health care facilities. The use of PPE kits and so on was quite widespread by health professionals.

3.3.9.5.15 Pets

People disowned their pets with a fear that animals might spread infection. PETA worked to safeguard the animals on roads.

3.3.9.5.16 Religious Places

Gurudwaras/temples/mosques were closed. People refrained from paying daily obeisance at religious places. Many religious festivals like *Eid, Ram Navami, Gurpurab* and Good Friday were not celebrated. Pilgrimage and religious processions were prohibited. Even *Kumbha Mela* was suspended in between during the second wave of corona in April 2021.

3.3.10 Behavioural Economics and COVID-19

It is pertinent to gain insight into how people across various sections have behaved during the lockdown period where restrictions were imposed on their movements. By and large, initially, people cooperated with the local authorities for the lockdown in the hope that the threat of the coronavirus shall fade away shortly. They obeyed the instructions issued. They even accepted the restrictions imposed on their movements.

The general public utilized this opportunity to their advantage by interacting with their close ones via mobile phones.

It proved to be a boon that during the lockdown period, the environment became free from hazardous pollutants. Roads were free from traffic jams. Water in the canals and rivers became clean. Wildlife could get access to the surroundings of the civilized world.

Interestingly, the companies started advising their employees to work from home which came as a bonus as they could spend more time with their families. On the other hand, the companies saved on their expenditure on providing basic amenities

to the workforce and the rent expenses. Thus, such an arrangement proved advantageous to both parties.

However, as further extensions in the lockdown period became a new normal, the patience in the public started disappearing.

Behaviour economics implies that the rationality of people's decision-making is influenced by certain emotional, psychological, circumstantial, cognitive and social factors that may or may not follow the predictions of standard economic models. People, in general, seldom behave in a rational or unbiased manner in making cost–benefit decisions. At times, they take casual or short-natured actions depending upon their convenience. So, people may not be expected to follow a disciplined path based on economic models during the COVID-19 crisis. They do not have the patience to wait for a long duration (Eichenbaum et al. 2020).

People have learnt to live with bare minimum resources. They have understood the difference between need, desire and luxury. The lockdown seems to have reduced wasteful and extravagant behaviour. People have learnt the importance of savings. Production and spending inevitably decline for a time due to lockdowns. People's decisions to cut back on consumption and work might have reduced the severity of the epidemic. But this also exacerbated the recession caused by the epidemic.

3.3.11 COVID-19 Resulted in Unprecedented Job Loss

Although the private sector has resilience plans to protect itself from catastrophic events like hurricane, earthquake, cyber, terror and flood, but no such thing was there for epidemics. In future, there will be a need to have special officers make epidemic-preparedness plans for risk assessment-based mitigation.

There is a need to give opportunity for the emergence/growth of small- and medium-sized businesses in many countries and to support 'social enterprises' as part of the recovery in terms of both local jobs and service/product development.

3.3.12 Social Impacts: Rumours and social chaos

During the early period of the outbreak, tension surged in the community. Due to a lack of trustworthy official information, folk tales about the epidemic situation spread through word of mouth, mobile phone SMS, social media transmission and other ways. The spread of all kinds of rumours exacerbated the spread of social panic and escalation of panic buying of drugs. The lack of understanding of the Corona crisis by authorities or the media caused several experts to become dissatisfied. People questioned the information provided by the government; they wondered about the status of control of the epidemic; they asked whether it was under control.

3.3.13 The Pandemic Brought us Many Gains Also-

1. A reduction in CO_2 and other polluting emissions.
2. There was increased spending on prevention and health care, which negatively impacted the family budget.
3. A reduction in crime (e.g. homicide rate) and road accidents;
4. Less use of paper. Use of PDF documents increased by 50% during the pandemic.

It is too early to determine the pandemic's lasting effects. But it allowed us to make and implement decisions that are difficult to do normally.

3.4 Role of Various Stakeholders in Corona Pandemic with a Specific Focus upon Public Health Discipline

The bulk of activities about Corona pandemic control comes under the domain of Public Health. There has been gross confusion about how the control measures were instituted. Multiple agencies gave their recommendations. However, there was seemingly a general disregard for public health experts' advice. The broad trend is that the media seemed to be calling the shots in such debates while undermining the role of experts. For example, on 15 November 2020, a prominent TV news channel telecast a debate on the corona situation after Diwali. As usual, despite an AIIMS, New Delhi faculty (Community Medicine) being on the panel, a cardiologist was asked about the preventive measures, masks, social distancing and so on. Thus, everywhere, clinicians were seen to behave like public health experts.

In the wake of the Corona Pandemic, Community Medicine departments and Schools of Public Health in medical colleges would play their crucial roles in this crisis. However, it was seen that public health professionals were constrained to submit to the terms of the prevailing power structure. They felt restricted. They were unable to realize the full potential of their discipline/speciality.

In the setting of a medical college, during any infectious disease outbreak, at the very outset, whenever authorities initiate any action like contact tracing, the clinical experts (usually the Internal Medicine people) take the center stage. This is often supported by the administration because nobody seems to seriously bother about prevention. Winslow also said that the utility or value of Public Health/prevention is intangible. Public health professionals, unlike surgeons (who may boast of removing a bladders tone or a tumour), can never claim that they prevented an outbreak since it never happened! (Bhalwar 2008).

The main focus was on the clinical management of the COVID cases. Discussions usually centred on finding out appropriate medicines or developing a vaccine for the emergent disease. Laboratory people also flexed their muscles. Debates about false positive/negative and confirmed/suspect cases gained the limelight. When, eventually, state/district or country-wise data about the outbreak was projected by the media, political issues dominated everything else.

Epidemiology usually becomes subservient to the power structure in the given circumstances. Despite the importance, public health professionals/perspectives are usually ignored. Ironically and rather paradoxically, all the while, public health measures-related discourses are owned up, dictated and dominated by others. There is never any opportunity for Community Medicine departments and Schools of Public Health to translate their academic principles/conceptual frameworks into practice, for controlling the disease outbreaks.

In the Corona case also, in a particular tertiary medical care institute, initial contact tracing efforts suggested by public health experts was nipped in the bud by Internal Medicine people. Their logic was that it would create panic. So, all prevention was thrown out of the window. The advantage of early pre-emptive action was lost. In many medical colleges, public health experts were, by and large, not involved in decision-making in the initial phases. It was quite surprising yet humiliating. Ideally, in any medical college, it should be a team effort for the control and management of any disease outbreak. Every concerned discipline should have its well-defined role, with a clear accountability mechanism. But in practice, it does not happen. For example, during the Corona crisis, everyone had an axe to grind. Everyone wanted to try for one-up-man-ship, intent on our scoring over others.

Another thing that became clear in 2020 that in a crisis like Corona, generic, age-old preventive measures (which have been a part of the routines established in our culture) is the only way out, for example generic social distancing measures like the use of '*Namaste*' instead of westernized 'hand shake', hand hygiene, elaborate SOPs on kitchen hygiene, washing of feet on entering the home, keeping shoes out, use of masks, asceticism (avoiding hedonistic pleasure-seeking activities like eating out/parties/picnics/tourism) and advocacy for using squatting type latrines (Singh 2007). On August 5, 2020, Jug Suraiya also wrote in an article, 'Wash vs. Wipe' in The Times of India, against the use of toilet papers (Suraiya 2020).

Back to the basics appears to be the clarion call. This is because no specific measures against Corona are available. Vaccines came into the picture when the incidence of new cases is already on the decline. However, during the second wave, the equation seems to have changed. Vaccination is the centre of focus now.

Despite the focus on steroids and remdesivir, effective medications are still a far cry. This is likely to be true for any virus-based pandemic, even in the future. Again, this is particularly true for the infections that spread through the respiratory route, where person-to-person spread is very fast.

Here, most humbly, we mention that many of these interventions were initiated by our department well before the Corona crisis set in, for example '*Salaam–Namaste-Sat Sri Akal*' campaign (Feb. 2019), PGI website YouTube upload of patient education materials (2018), The concept of mobile phone-based medical consultation is listed in HSPROD database in the name of Dr. Surya Bali and me (2005), WhatsApp-based teleconsultations and 2010, App-based clinical care coordination, patients/caregiver counselling as well as referrals through vendors like Jiyyo Innovations (2018).

3.4.1 Role of National-Level Policy-Makers/Executives/Experts

Eradication is most likely to work when doctors, politicians, pharmaceuticals, the media and citizens work together. It is teamwork (with an active role of people). At the national level, this involved The Prime Minister, Ministry of Health & Family Welfare, Bureaucrats (Secretary. Health), Politicians (ruling/opposition), Director health services, ICMR, Public Health Professionals, Disaster management team, IT professionals, Economists, Media, Law & legal services and many others. Role of Central Govt. involves identifying pandemic crisis and declare it, liaising with WHO and other agencies, initiating control measures, promulgating Epidemic Disease/ Disaster Management Act, interacting with states, issuing guidelines/SOPs/ Directives and monitoring the situation.

3.4.2 Role of Apex-Level Medical Institutions

The bulk of the action is at the medical colleges' level, involving officials/services like Director/Principal and Hospital Administration people. Clinicians, emergency OPD/wards, nurses and laboratory services also play their roles. Public health professionals, statisticians, immunologists, virologists, microbiologists, immunologists, IT professionals, sanitation department and ministerial staff also contribute actively in mitigation and control of the situation. Various activities seen in the pandemic situation are – Screening OPDs, Tele-consultation, Contact tracing, Isolation/Quarantine and Surveys and so on. Conflict resolution is also needed in most hospitals because of the regular inter-departmental clashes.

3.4.3 Role of Public Health Professionals in Control of Coronavirus Crisis

They are involved in providing services like epidemiological data analysis and interpretation, modelling/trend prediction, formulating strategies, developing SOPs, monitoring and evaluation, research, manpower planning and deployment, organizing quarantine centre duties, educating the public (developing IEC material, posters presentations, SOPs, training/capacity building, webinars, videos), training manpower, media handling, coordination and communication. They also contribute as members of expert committees and as TV channel experts. They also provide outreach services and help other states besides advising local administration (Centre, States and International outreach). Apart from that, they delve into the domains of health economics, environment health and behavioural sciences pertinent to the prevailing epidemics.

In any epidemic, public health strategies must be given the highest importance, for example forcing people to stay home from shopping and work, especially if they are sick or at risk.

3.4.4 Application of General Principles of Public Health

As described hereafter, the public health conceptual framework operates through five levels and three types of prevention. It gives a wider horizon of thinking about how society can cope with pandemics (Singh 2004). The first two levels come under primary prevention, which is applied before the disease onset.

3.4.4.1 Level 1: Health Promotion

This means preparedness, provision of general civic amenities, infrastructure and enforcement of laws/regulations. It involves a balance between health education and legislation. Health promotion includes a set of activities – not directed at any particular disease. These intend to improve the general health and well-being of the population as a whole rather than focusing on people at risk for specific diseases, for example safe water supply, safe disposal of excreta/wastes, clean air (free of pollution), arranging adequate health services, adequate nutrition, healthful housing, control of insects and rodents, safe working conditions, recreation facilities, health education, marriage counselling, genetic counselling, sex education, physical education, periodic health screening, improvement of the standard of living and so on.

Thus, compliance with the principles of health promotion leads to a reduction in the chances of disease occurrence. It is prevention in a true sense, when the individual may be well but is already exposed to various risk factors (Patro and Singh 2011; Singh et al. 2013).

Here, it must be noted that as per Health Field theory and even otherwise, we know that solutions to health problems also lie in non-health sectors.

Basically, the epidemiological triad-related factors need to be addressed, that is agent/host/environment. For example, several mutants have emerged which have been said to be responsible for the second wave.

For Corona-like situation, health promotion includes health education, social distancing, hand hygiene, use of masks; legislation, penalty, quarantine/isolation, curfew, lockdown and so on. This highlights the need for a comprehensive, global plan for addressing infectious disease pandemics.

Given the globalization of commerce, travel and economics and the worldwide migration of people, goods and ideas, there is a need for a coordinated, international approach to control any pandemic. This will include the following features:

- Universal implementation of core disease surveillance and control capacities with international cooperation and coordination.
- Protection of international trade from unnecessary embargoes on disease reporting; maintaining vital global supply chains.
- Compensation of citizens and governments of low-resource countries at risk for emerging infectious diseases for the sacrifices made (e.g., economic consequences of disease reporting, culling of infected animals, quarantine and isolation) for the benefit of the global community. This includes countermeasures and economic support.

- Enhance economic and environmental resilience, create jobs and improve well-being in both rural and urban communities.
  The modalities and suggestions that are given hereafter form a part of the health promotion strategy that will be effective to prevent/mitigate any pandemic situation.

3.4.4.1.1 Ways to Improve our Societies After the Pandemic

1. *Financial Inclusion*. Cash transfer programs need to be expanded; access to the financial system needs to be broadened.
2. *Greater investment in hospitals*. After the pandemic is over, hospital beds will need to be increased.
3. *Greater use of information and communication technology (ICT) and digitalization*. More investments in ICTs are needed to revive the economy. This will reduce transportation needs.
4. *More awareness about the effects of crises*, e.g. *climate change*. The pandemic has increased our concern for climate change.
5. *Aversion to unnecessary travel and meetings*. Video conferencing technology is now a much more acceptable, efficient, cost-effective and friendly substitute. The pandemic has highlighted the time and money we waste in attending meetings and conferences.
6. This crisis has also been a vindication of the public sector. Earlier, government inefficiencies generated distrust and negative perceptions about the public sector. Now, it is seen as a supportive organization ensuring essential supplies and services to the people at their doorsteps. The public sector responded efficiently and effectively on several fronts, from public health to the preservation of incomes for households; it also offered a lifeline of support to businesses. The public sector is indispensable when it comes to externalities and other market failures. It has a longer horizon to plan than do private agents. In the context of solutions to problems as diverse as climate change and inequality, the state is needed.
   To address the health, social and economic impacts of COVID-19, interventions by the state have become central again. In financial recovery for development after the Corona crisis-related damage, stimulus and recovery packages are needed. The private sector is key, yet the state is back!
7. Health crises like COVID-19 have raised questions about urban living. Metropolitan cities have to transform by developing plans to reduce density. This will contribute to greater human-to-human contact and illness. There are discussions on the reversal of urbanization trends. Suburbs and rural areas fared worse during and after airborne pandemics. Low-density areas also suffered more during pandemics because they had fewer, smaller and less well-equipped hospitals. And because they are not as economically resilient as large cities, post-crisis economic recovery takes longer.
8. Lockdowns prevent travel-related disease spread but are difficult to maintain. Policy-makers need to search for longer term solutions.

9. Rather than expecting to find an absolute 'right' answer to a problem, the more viable approach is to find a solution that is both understood and accepted by the public who ultimately must implement and abide by the decision. The public's trust must be attained by ensuring that the decision-making process, as well as the evidence used in it, is transparent and open to public debate. Also, the resulting decisions must be clearly articulated and justified.

Some states fail to engage the public in pandemic planning. They may exclude them from the process. They keep their plans secret, even from hospital workers and other healthcare providers. Engaging with the public in this process will build community resilience. It is generally necessary to develop a strong consensus among the constituents of a community. This should consider varying culture, social class, economy and political scenarios. The government needs to maintain its credibility through adequate preparedness planning by developing 'living documents' that are subjected to constant review, testing and revision based on evidence and experience. It should keep the public alert yet not alarmed.

There is a need for a transparent, ethical decision-making process that incorporates public debate and deliberation. Its goal is the selection of interventions that are both understood by, and acceptable to, most people. In public health emergencies, citizens are more willing to sacrifice self-interest in favour of the common good, if they believe that everyone else is doing so; conversely, if they perceive that others are receiving preferential treatment, they may not act selflessly.

3.4.4.1.2 Common-Sense Steps cities Can Take to Fight the Coronavirus

- Closing some streets to cars. This will allow citizens to get outside and walk maintaining social distancing for physical and mental health promotion.
- 'un-pave the way', creating urban greenbelts for walking and biking at a safe distance in even the densest of places
- Easy access to nature has additional benefits
- Keeping productive farmland and a fresh food supply nearby.
- Protecting the most exposed city residents.
- Anti-poverty centres, anti-eviction legislation and rent control measures to prevent homelessness during the pandemic.
- Keeping people safely inside their homes will help to stop the spread of the virus. It is likely to reap public health dividends beyond the pandemic.

The lack of a holistic ecosystem-based approach is disastrous. A 'green deal' agenda needs to be brought back. When Egypt faced H1N1 swine flu in 2009, the problem was misdiagnosed. The focus was on slum clearance and culling pigs rather than breaking human-to-human transmission. With the disappearance of pigs, the streets were filled with garbage. Rat populations boomed. Typhoid, cholera and other diseases resurged.

3.4.4.1.3 Ways to Tackle Ethical and Legal Issues in Pandemic Planning (Lemon et al. 2007)

Public engagement in decision-making is needed on issues affecting personal freedom, for example quarantine, school closings, allocation of limited medical resources and use of evidence-based non-pharmacological interventions (NPIs). Public communication is needed, before, as well as during a pandemic, to explain the rationale for disease control measures. Interventions of proven efficacy need to be implemented under conditions in which their benefits to society outweigh their harms (including justifiable harms). The establishment of a clear authority for public health decision-making is essential during a pandemic along with adherence to ethical principles. The focus should be on the least restrictive means of achieving public good. As a long-term measure, capacity building should be envisaged to address potential public health emergencies in future.

Foundation for legal decision-making during a pandemic situation involves transparency, sound science, global involvement and procedural justice. The focus of ethics can be on the content of policies (about pandemic preparations/response). Alternatively, the focus can be on the process by which policies are established. There is a need to incorporate both duty-oriented and outcome-oriented considerations (e.g. utility and justice) into the principles. Ethics may coincide with prudential considerations.

Substantive principles include *Principle of utility*: We must act to produce the greatest good; *Principle of efficiency*: Produce an objective or maximize the total benefit from a given level of resources; *Principle of fairness*: treat all cases alike and avoid unfair discrimination; *Principle of liberty*: impose the least burden on personal freedom/self-determination necessary to achieve legitimate goals.

A basic ethical challenge involves the potential conflict between preserving society as a whole and protecting the greatest. The moral challenge we face is how to respect commitments to social justice in the face of the overwhelming, systematic inequalities that form the backdrop for the harsh realities of pandemic fling upon its weakest members, for example limited access to technology and barriers to communication and community engagement. These aspects represent particularly feasible targets for policy, intervention or development endeavours. Governments should identify opportunities to mitigate the burdens imposed by such inequalities under pandemic circumstances, in the broader context of public health.

3.4.4.1.4 Application of the Other Lessons Learnt

The *global hot spots* where epidemics historically have emerged overlap with some of the world's *least developed countries*. Serious financial limitations can make it hard for leaders in such countries to respond to outbreaks in time. It has, now, been realized that early cases of an outbreak should trigger the immediate release of funds to control it, by way of sovereign or regional-level epidemic insurance policies. Dedicated funds will automatically flow into pre-programmed rapid-response efforts.

Universally, there is a need to eliminate the wild-animal trade. It will stop many epidemics from occurring in the first place. Most viral epidemics spillover from wild

animals, particularly animals closely related to us, like mammals. This will also break the link between wild animals and dense cities with vast human populations. Such a ban won't eliminate contact with wildlife viruses. But chances of spread will reduce.

As far as epidemic control measures are concerned, various governments and international agencies have framed SOPs, laws and regulations. The main issue, not effectively tackled so far, is the resolution of the *chain of command* as to who is in charge of the operations. *Confusion and chaos* seem to be the dominant themes that have emerged in the context of the current Corona crisis.

3.4.4.1.5 Battling Airborne Pathogens

Viral (particularly respiratory diseases) pathogens make up eight of the ten most recent pandemics, which are proving difficult to combat. The essential common features of the resultant catastrophes are rapid spread, high case fatalities, lack of a medicinal cure, non-availability of an effective vaccine (which usually gets developed when the outbreak is already on the decline; though, this time, vaccination has emerged as the dominant strategy, with a variety of vaccines available), link with China as the source of origin and link with non-vegetarian exotic food habits. Breaking airborne disease transmission requires reducing human-to-human contact through physical distancing and business closures, for example wearing masks to impede infectious droplets.

3.4.4.1.6 Vaccines and Pharmaceuticals

In the last 20 years or so, substantial efforts have been made to develop and stockpile vaccines and pharmaceuticals in anticipation of threatened viral pandemics, for example H5N1 avian influenza. It is proved every time that it was unlikely that enough of these measures would be available at the beginning of such a crisis, and their effectiveness usually remains far from assured. For example, even for vaccines, a wide variety, developed by many countries, individually, is being pushed. It is not clear which one will be effective for which country; if any worthwhile benefits will be there?

3.4.4.2 Level 2: Specific Protection

This applies to the sections of populations who have been exposed to the infection, for example health care workers and contacts of positive cases. For such people, specific protective measures need to be taken. For example, the vaccine roll-out program has been offered first to health care workers in January 2021 (Dey 2021). Moreover, the elderly, immune-compromised and diabetic/hypertensive people have been prioritized for special care during the Corona pandemic. This group also gets preferential attention for secondary prevention enlisted hereafter.

3.4.4.3 Secondary Prevention (Level 3: Early Diagnosis and Treatment)

It is applicable when the disease begins. This is the domain of clinical specialities. Hospitals are involved in this level of prevention, for example OPDs/wards, ventilators, Remdesivir, ICU, emergency/isolation wards or special wards (like

COVID hospitals) and so on here, specialists in respiratory medicine, emergency medicine and anaesthesia are at the forefront.

3.4.4.4 Tertiary Prevention

It has two parts.

- Level 4 – Disability limitation. It means limiting the damages, (e.g. corona complications by ensuring oxygen supply; ICU management, Plasma therapy (?) and facilitating recovery for those who survive corona.
- Level 5 – Rehabilitation. It implies bringing back the routine lives of the survived patients to the extent possible. This will include medical, social, vocational, occupational as well as psychological rehabilitation.

3.4.5 Public Health Professionals' Perspectives on COVID-19

A Joint Task Force of eminent public health experts of India was constituted by the Indian Public Health Association (IPHA), Indian Association of Preventive and Social Medicine (IAPSM) and Indian Association of Epidemiologists (IAE) in April 2020 to advise the Government of India for containment of COVID-19 pandemic in the country. Their second Joint Statement severely criticized the government by labelling the measures taken by it as 'draconian.' They alleged that public health experts were ignored and side-lined by the administration/government. They lamented that there was limited engagement with expert technocrats in the areas of epidemiology, public health, preventive medicine or social sciences. They alleged that the policy-makers relied overwhelmingly on general administrative bureaucrats. They asserted that the historic and systematic neglect of public health as a discipline and non-involvement of related experts in policy making and strategy formulation has cost the nation enormously especially in the current pandemic. They advised the government to constitute a panel of these experts and social scientists at central, state and district levels to address both public health and humanitarian aspect of the crisis (IPHA, IAPSM, and IEA 2020).

The role of biomedical experts vis-a-vis political/administrative authorities was also highlighted in these discussions. A question posed was – 'Are biomedical experts only to generate evidence and questions posed by politicians/civil administrators to take pandemic control-related operational decisions?'

Public health professionals need to realize that political compulsions are an integral part of the decision-making for such a crisis. They have to understand that once the Epidemic Disease Act and Disaster Management Act come into force effectively, the bureaucracy has to be proactive. They should focus on evolving a long-term mechanism so that, in future, due consideration is given to them for tackling the disease burden, in routine, as well as during pandemic situations, particularly after the invocation of the Epidemic Disease Act.

Such crises are more of a management and administration issue, rather than just exclusively being an epidemiological conundrum to be solved. This has been amply

proved by the instances of widespread mismanagement of hospital supply and services (oxygen cylinders, medicines, hospital beds, manpower etc.). Lacklustre handling of lockdowns and civic life also points towards such a conclusion.

It needs to be realized that there will always be scepticism, criticism and differences of opinion, whenever some policy making and strategy formulation is attempted by the administration/government for pandemic situations. Often, quick actions are warranted. Health authorities will often need to act rapidly and authoritatively even based on incomplete knowledge. This is called 'the public health paradox'. There is no way to avoid the dilemmas posed by acting without full scientific knowledge. Public health professionals must expect most people to be entirely unprepared when the next pandemic strikes.

3.5 Conclusions and Recommendations

The corona pandemic is a kind of wake-up call for us. The lesson learnt is that, for communicable disease control, there is a need to inculcate a nature-friendly responsible behaviour to ensure peaceful co-existence between people and microbes. On this planet, all issues are interconnected. Our survival demands collective action. Many crises will trouble us in the future if we fail to live sustainably. We need to stop exceeding the planet's limits. Issues like deforestation, biodiversity loss, climate change and so on make the chances of future pandemics more likely.

There is a need to phase out fossil fuels and deploy renewable energy technologies. We need to move towards green infrastructure, reforestation and invest in a more circular, shared regenerative, low-carbon economy. We need to use science to prevent pandemics by designing economies that mitigate the threats of climate change and biodiversity loss.

The biggest mistake would be to not take advantage of this situation to resolve issues that will impact our future. The COVID-19 crisis forms a strong case to usher in global systemic change. This pandemic demanded a forceful, immediate response, which was duly provided. This proved that it is possible to make transformational changes overnight.

But, the second wave in 2021 caught all of us napping. Now, in hindsight, some experts have pointed out flaws in the government's approach to modeling the pandemic (The Hindu 2021b). In fact, a model is as good as its input variables. Under-reporting does not help (Mukherjee et al. 2021). Humanity was a witness to a kind of fatigue and complacency in the general public, bureaucracy as well as in health care providers. A new mutant virus variety also emerged. All this resulted in a sharp spike in cases and deaths. The situation became out of control. Hospital services were overwhelmed everywhere, including the national capital, New Delhi (The Hindu 2021c; Babu et al. 2021). This indicated that the guard should not have been let down. Even in the Spanish Flu outbreak in 2018–2019, a similar trend was observed.

We have suddenly entered a different world with a different economy. Governments are rushing to protect their citizens medically and economically in

the short term. But in managing the crisis, governments also must look at the long-term implications. The goal should be to shift from industrial to regenerative agriculture. We need to live within our planetary boundaries while ensuring the welfare of marginalized communities. Disaster is a laboratory for innovation. Such a crisis should not go to waste. COVID-19 reflects that more such global crises may come.

3.5.1 Behavioural Strategies for Disease Containment and Mitigation

These are critical to pandemic planning. Change is the law of nature. Behavioural change requires efforts. People should be ready to accept changes and learn to face any future corona-like crisis. Some people accept changes easily, some take time and while few others never change. Much of behaviour change has been 'forced upon the public, in the present crisis.' It is mainly due to anxiety/apprehension of contracting the disease or due to the fear of enforcement of the law of the land about social distancing, masks use and so on.

As human beings, we are quite resilient and entrepreneurial. We are perfectly capable of beginning again. If we learn from our failings, we can build a brighter future. We need to embrace this moment of upheaval as an opportunity to start investing in resilience, shared prosperity, well-being and planetary health. The short-term implications of the pandemic are evident everywhere. But the long-term consequences, their impact on reshaping health and development institutions, occupations and priorities are still debatable. The COVID-19 crisis has the potential to radically change development for the better. It will not be a drastic, but rather an evolving, change. We need to adapt to the 'new normal'.

About behavioural change efforts, we can say that 'You can tell people to change; they may or may not change. You force people to change, they may concede and comply'. But, it remains to be seen as to when will the 'reverting to original lifestyle' may happen. We have to wait to witness, 'Till what time these changes will be sustained?' We will come to analyze (and realize) what are the temporary changes and what are relatively permanent ones.

3.5.2 The Problem of Authority Needs to Be Resolved

Disaster response can be slowed and sometimes even brought to a halt by confusion among national, state and local jurisdictions about who has authority and responsibility for various decisions and actions. There has been a great deal of ambiguity. Among the legal and ethical barriers to public health intervention during a pandemic, the first and most critical is the establishment of an authority for decision-making.

3.5.3 Role of the Government and the Administrators

Continued faith of people is important. The government must be seen to be working for the welfare of the public at large. Bringing people's act together is also important. Boosting the morale of essential service providers is another area that needs government support. Over the centuries, after the nation-state concept emerged, government-level control activities are, usually, at the centre stage of local action in an epidemic situation. This time, the state-led restrictions, the exercise of power by the police and bureaucracy were much more than that in the Swine flu pandemic. Interstate differences in the skills and attitude of administrators in handling the Corona crisis also became apparent. Some states handled it well, while others committed mistakes.

Strict action is required against anyone who indulges in overpricing of essential medicines or in the trading of fake drugs. Penalty should be imposed on people who spread rumours about the virus and cause a state of panic among the general population.

Enforcement of quarantine for International Travelers has been patchy. There is a need to emulate the examples of countries like Australia or Canada where any incoming migrant is compulsorily asked for 2-week quarantine in hotels. They are not allowed to move out of their rooms.

There are examples of successful control within India also. For example, in army units, as early as Feb. 2021 (after the first warning of the second wave), precautionary measures were instituted on a war footing, very strictly. They were successful in warding off the spike in corona cases. Even some localized areas (villages/tribal areas) were similarly successful. The secret was ensuring very basic social distancing and personal hygiene measures.

3.5.3.1 Widespread Blame Games

These were also witnessed during the Corona crisis. Frequent intra- and interdepartmental fights/debates within and outside the hospital/medical colleges were there. Ruling and opposition political parties also blamed each other. Such a blame game is counterproductive. This should be consciously avoided.

3.5.3.2 Impact of Shutdowns

This was also hotly debated. Contrary opinions were expressed. On one hand, some countries were quoted to have realized that delay in (or lack of) imposition of shutdowns was a blunder; on the other hand, many experts criticized their own country for 'unnecessary' and 'draconian' lockdowns.

3.5.3.3 Pandemic as an Opportunity for 'Technology Forcing'

The pandemic presents a huge opportunity for digital transformation. This is also the time for the redesign of many things. The pandemic will be the catalyst for the mass adoption of a technology whose time has come. Future solutions may take advantage of technologies that don't even exist, for example digital immunity passports. Here, apps will certify individuals' immunity to viruses they have been vaccinated against.

They will also be linked to diagnostic test results so that individuals, when recovered and immune, can re-enter the workforce. These systems will give people the confidence to return to normal more rapidly. Such data will give health officials real-time susceptibility maps showing what regions need to be quarantined and where to focus vaccination efforts. Tele-consultation is being used to reduce health workers' exposure to corona; this was initiated by the lead author in PGIMER, Chandigarh, India, even before the pandemic broke out (Singh et al. 2020; WHO 2020).

3.5.3.4 One Health Concept

COVID-19 crisis shows how interconnected and interdependent our world has become. It illustrates our common vulnerability, across borders. There is no way of tackling it at the national level only. It has revealed the limitations of a segmented approach to development. International cooperation and multilateralism are back! A more holistic, comprehensive and coordinated approach is back. One health concept is being advocated by one and all (Chaturvedi 2021).

3.5.3.5 Pandemics as Collateral Damage of our Behaviour

Historically, many of the currently prevalent communicable diseases (TB, Influenza, Swine Flu, Rabies, etc.) have resulted from the domestication of animals. Many zoonosis/vector-borne diseases can be linked to our uninvited forays into the forests (Singh 2019). Although the exact cause for its origin is not known, COVID-19 is believed to have its origin from one Huanan Seafood Wholesale Market, Wuhan, China. Many Asian countries like China, Japan and Korea consume raw, live exotic animals. The desire to consume such animal food for taste, nutrients or exploring new flavours has resulted in the entry of zoonotic viruses in humans and thereby facilitating ecological niche building for the virus. These exotic or wild foods (e.g. Bats, snakes, etc.) are readily available at 'wet markets' or 'wildlife markets' (Maron 2020). So, the focus on our dietary preferences needs to be changed.

3.5.3.6 Pandemics Viewed as a By-Product of International Power Game

Many countries compete to prove their supremacy and wish to become super-power nations. The urge to control and dominate other countries through means like fear, war and so on is also common. Biological warfare like developing deadly viruses and spreading them to different nations is one such extreme behaviour for establishing supremacy.

War or civil strife in any part of the world compromises all gains of special health campaigns, including pandemic mitigation efforts.

Now it is a trade war between the United States and China. The erstwhile American President Mr. Trump, many times, in his press meetings called the coronavirus a 'Chinese virus', as the virus was alleged to have been developed in their laboratories as a means of bio-warfare. The Chinese knew that it spread from their labs; it had the means to confine its spread within their mainland. To top it, China hid the disease extent and impact from the whole world. So, global equilibrium, peace and cooperation are essential to ward off any pandemic threat.

The whole world was caught unaware by Corona. Except for China, no one knew the impact, spread and the ways to control it, leading to worldwide panic. Western, well-to-do countries were affected more. The Western world was hit badly with high case fatality rates. Countries panicked and took extreme knee-jerk measures of shutdown/lockdown which hit their economies badly. The intentions and complicity of China (in generating and inflicting this malady) have remained a mystery. Reports indicate gross manipulation of data by China (under-reported number of Corona cases and deaths). Real 'truth' seems to be still elusive! It is only in January 2021 that, now, China has, rather reluctantly, 'allowed' WHO experts to visit its labs in Wuhan! Even now, in May 2021, truth eludes us! Meanwhile, there has been a query in Quora – 'Is the second COVID wave a planned biological war against India when the entire Indian subcontinent, Pakistan, Bangladesh, Nepal, Bhutan have no second wave? Are China and the United States working in collusion as from vaccine to economy Modi amazed the world?' (Sharma 2021).

3.5.3.7 Final Comments

There has been a pervasive sense of helplessness during the Corona crisis. Movement restrictions and suspension of commercial air transport were severe constraints for any form of humanitarian action to facilitate access of the affected people to medical facilities or necessities.

The same situation, rather worse, is likely to be there in any such pandemic, in the future. Uncertainty is the watchword. Already, second wave onslaught has devastated the world with the involvement of a group of mutant Coronavirus strains infections from the United Kingdom, Brazil and India (Canton 2021). Predictions of yet another third wave are already there. This has made people jittery (Bhaskar 2021). It appears that the availability of the latest scientific knowledge has not helped us in managing the situation. After all these lockdowns, people, more or less, stopped heeding to any government directives. Now, again, in May 2021, fresh restrictions have been enforced through strict fines/punishments. The good news is that now experts say that the second wave is declining and that the third one can be prevented (The Hindu 2021d).

All said and done, we, as human beings, need to be in control of the situation.

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Human Impacts on Natural Habitats Leading to Covid-19 Pandemic

4

Priya Mishra and Naveen Kumar Arora

Abstract

In the history of mankind, coronavirus disease 2019 (COVID-19) has emerged as one of the most alarming pandemics. The causative organism of COVID-19 is severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2) that has affected humans with high infection and mortality rates. SARS-CoV-2 is proposed to be a zoonotic virus with reservoir animals being bats or pangolins. The continuous emergence of zoonotic diseases in the last 100 years has indicated the linkages between anthropogenic activities and the onset of novel pathogenic microorganisms in the human population. Effect of the devastation of the environment and natural habitats are reasons for an increasing number of zoonotic diseases impacting mankind in the last few decades. Several of the zoonotic microbes are known to have jumped from wild animals or birds to humans causing severe outbreaks. Deforestation, unplanned urbanization, air pollution, climate change, bushmeat trading, and consumption are some of the important factors that are correlated with each other and influence the emergence of pandemics such as COVID-19. COVID-19 has also proved to be a learning for the future suggesting the importance of environmental sustainability and achieving the targets of United Nation Sustainable Development Goals (SDGs 2030).

Keywords

Covid-19 · Zoonoses · Pandemic · Unplanned urbanization · Anthropogenic activities · Environmental sustainability

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4.1 Introduction

The world has witnessed several perilous pandemics since the beginning of human civilization. The emergence of zoonotic diseases is directly linked with the human invasion of natural habitats which has caused devastating effects on natural ecosystems leading to ecological imbalance. Recently, another zoonotic disease emerged in Wuhan city in the Hubei region of China in December 2019. In the very beginning, the disease was reported as "pneumonia of unknown etiology," including symptoms such as fever, cough, sore throat, fatigue, difficulty in breathing, vomiting, nausea, and diarrhea, while in severe cases acute respiratory distress syndrome and cardiac issues leading even to the death of the infected individual (Rume and Islam 2020; Zhu et al. 2020a, b). Later, the Chinese Center for Disease Control and Prevention reported that the novel virus belongs to the coronavirus family. Subsequently, the International Committee on Taxonomy of Viruses (ICTV) termed the virus as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which has similarities with previously known Severe Acute Respiratory Syndrome coronaviruses (SARS-CoVs) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV). The World Health Organization (WHO) named the disease Coronavirus Disease-2019 (COVID-19; Cascella et al. 2021; Gorbalenya et al. 2020). According to WHO, globally, there have been 1,29,902,402 confirmed cases of COVID-19 with 2,831,815 deaths (as of 7:01 pm CEST, April 03, 2021).

When the outbreak of COVID-19 occurred, there were some assumptions that the virus could have laboratory origin, but the genetic information related to the presence of altered receptor binding site (RBD) in spike protein with high affinity to angiotensin-converting enzyme 2 (ACE2) from humans and some other species suggest that the virus may have evolved from the related SARS-CoV virus by natural selection process (Andersen et al. 2020; Rabi et al. 2020). Several workers claimed different possible sources of origin of novel COVID-19 virus such as snakes, pangolins, or bats (Lu et al. 2020; Cyranoski 2020). However, SARS-CoV-2 related β-CoVs belonging to pangolins share the highest similarities with the SARS-CoV-2 RBD (all six RBD residues) suggesting them as the potential intermediate host of SARS-CoV-2 (do Vale et al. 2021).

Ecological pressure created by human activities such as unplanned urbanization and habitat destruction are largely involved in the continuous emergence and spread of zoonotic diseases (Mishra et al. 2021; Gibb et al. 2020) (Fig. 4.1). As humans invade natural habitats, they disturb the habitats of wild animals, resulting in contact with the pathogens that can jump over from wild animals. Climate change and unplanned urbanization have emerged as the main culprits as they create a huge impact on the environment. World history has past pandemics or epidemics as proof, where anthropogenic activities have proven to be the major causes of the emergence of novel zoonotic pathogens or diseases. For instance, in the mid-fourteenth century, the "black death" occurred due to a simultaneous increase in rodent population which was likely to be due to climate change along with complex interaction with other factors related to human activities. This disease affected 75 million people in Europe and Asia (Meyer, 2019). The emergence and frequent reemergence of Ebola

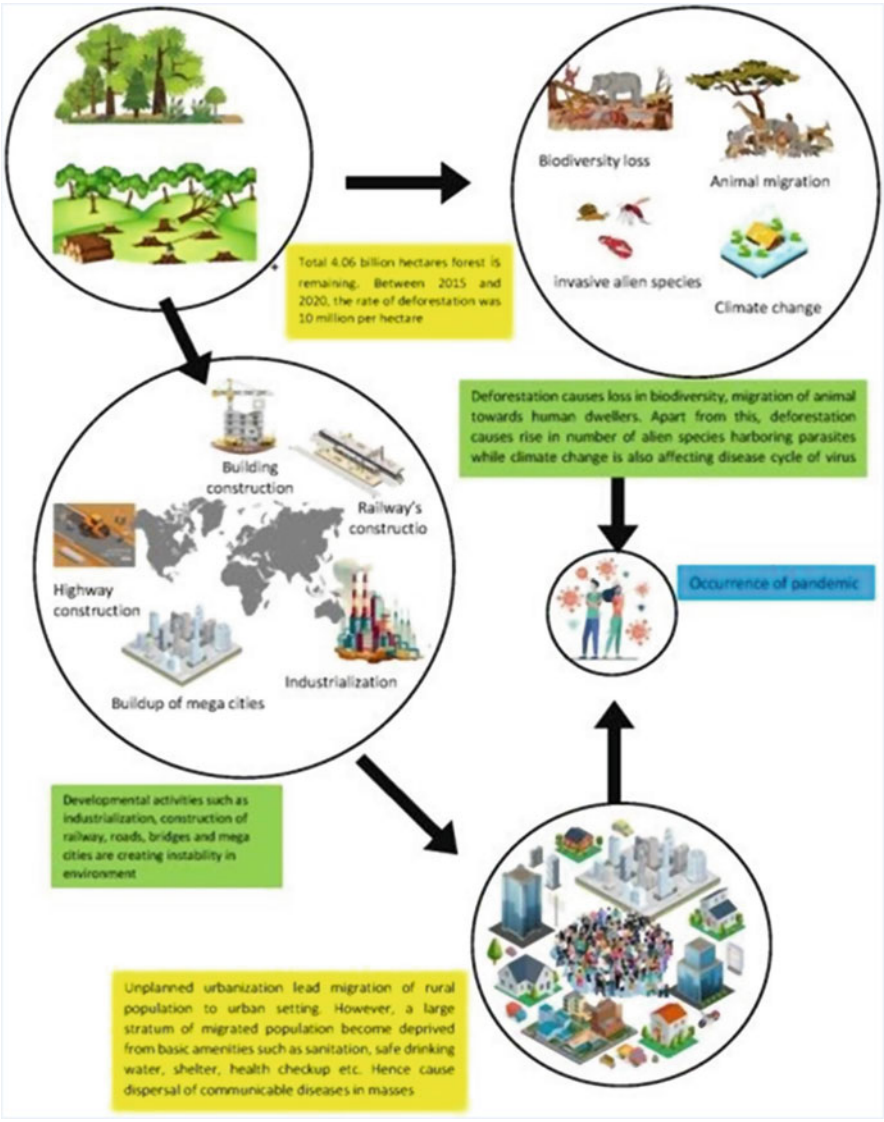

Fig. 4.1 Various factors responsible for Covid−19 emergence

virus disease (EVD) in Africa is another example of a zoonotic disease related to climate change and deforestation (Kamorudeen et al. 2020).

This chapter focuses on the impact of anthropogenic activities on natural habitats and their correlation with the emergence of a pandemic such as COVID-19 which has already taken millions of lives worldwide and is still raging around the globe. There are also some key points to be learned from this pandemic on how humans can

protect nature and take care of the environment with a new perspective to prevent the emergence of future pandemics.

4.2 Human Impacts on Natural Habitats

Unfortunately, human invasion in natural habitats has created a threat to a large number of plants and animal species on earth. Several species of plants, as well as animals, have been lost or have become endangered (Arora 2018a). In IUCN red list, 44,838 mammal species are listed of which 16,928 species are threatened and 16,928 species (38%) have become extinct. Human activities have a massive impact on global warming, climate change, and environmental pollution resulting in biodiversity loss (Arora et al. 2018). These factors combinedly disturb ecological balance which apart from other consequences also result in the emergence of zoonotic diseases. Unplanned urbanization and climate change are among the most important factors resulting in the spread of diseases and diminishing the health of environment, resulting in the arrival of novel pathogens in contact with humans.

4.3 Unplanned Urbanization

In the twenty-first century, the world has moved towards the rapid expansion of the urban area without following proper regulations. According to an estimate, 6.7 billion people will be living in urbanized areas, while only 3.1 billion will be living in rural settings by 2050 (Fig. 4.2). Over the past few decades, unplanned urbanization has increased the pressure on the environment and intensified issues, such as global warming, pollution of soil, air, and water, and deforestation. According to Dr. Samlee Plianbangchang, WHO's Regional Director for South-East Asia, "Urbanization is one of the major threats to health in the twenty-first century. Closing the urban equity gap and promoting healthy cities requires urgent action, including the efforts of both rich and poor urban dwellers. To reap the potential benefits from urbanization, we must act collectively." The dynamic growth of cities is, although, increasing the economic growth, but, on the other hand, this scenario can worsen the chances of spread of communicable diseases that could lead the world toward severe health consequences.

Humans are diminishing the forests for building more infrastructure which increases the habitat loss of the wild animals and in turn affects the biodiversity. Several ecologists believe that the loss of habitat as well as biodiversity due to unplanned urbanization is linked with the emergence of zoonotic diseases (Mishra et al. 2021). Due to habitat loss, some species become extinct while those species that survive (such as rodents, bats, etc.) can enter the human dwellings. Also, these animal species can be the reservoir of some dangerous pathogens that may jump into humans (Tollefson 2020). The extinction of the host of some pathogenic microbes could lead to the shift of the host and increase the transmission of the pathogenic microbes toward humans (LoGiudice et al. 2003).

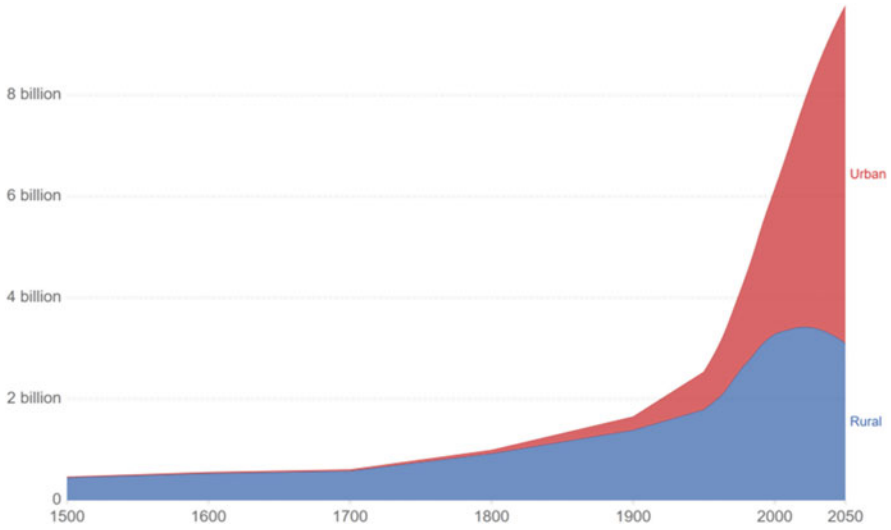

Fig. 4.2 Projection of urban and rural population from 1500 to 2050 (Source: OWID based on UN world urbanization prospects 2018 and historical sources)

The outbreak and uncontrollable spread of the COVID-19 pandemic could be linked with unplanned urbanization. Interurban connectivity has increased, and urban centers have become the hot spots for human pathogens such as SARS-CoV-2 (Sumeet et al. 2017; Connolly et al. 2020). The impact of unplanned urbanization has also been mentioned in Sustainable Development Goal (SDGs) 11. Rapidly increasing urban areas are exerting pressure on the water supplies, sewage treatment, public health, and the living environment. Due to high population density and migration of people to big cities, 17% of the urban population is living unhygienically in slums of the megacities. As per estimates, 43 megacities with more than 10 million inhabitants will develop by 2030. The huge population densities in megacities, especially the unhygienic slum areas, are red zones for transmission of zoonotic diseases. WHO has emphasized to maintain proper hygiene, sanitation, and waste management to prevent human-to-human transmission of COVID-19. Apart from high population density and unhygienic conditions, people living in slums suffer from malnutrition, deficiency diseases, and other microbial infections, resulting in a highly vulnerable population. In several studies, the correlation of regression analysis has shown that high population density is positively correlated with the infection, prevalence, and mortality rates due to COVID-19 (Kadi and Khelfaoui 2020; Bhadra et al. 2021).

Heavily populated urbanized megacities have fast life with competition for survival resulting in different types of stresses and related ailments. In such situations, the occurrence of ailments like diabetes, hypertension, obesity, cardio-vascular, and respiratory diseases affect the immune system and make people vulnerable to zoonotic infections such as COVID-19 (Holder and Reddy 2021; de

Almeida-Pititto et al. 2020). Regarding the COVID-19 pandemic, several reports have shown that the mortality rates are higher among the individuals already suffering from these problems because of defective immune response due to prior existing comorbidities (Tay et al. 2020). Along with increasing unplanned urbanization, the problem of pollution is also accelerating resulting in the evolution of pathogenic microbes (both bacteria and viruses) and rampant development of mutants. Viruses containing RNA as genetic material are more prone to have mutation due to the lack of proofreading capacity of the RNA polymerase enzyme (Ghatak et al. 2020). Continents like Europe, Asia, and Africa where the percentage of urbanization is 75%, 51%, and 43%, respectively, have witnessed a higher number of zoonotic outbreaks (Raj et al. 2014; Richards et al. 2015; Yee et al. 2020) (Table 4.1). The occurrence of air pollution in urbanized megacities due to high industrialization and vehicle emission affects the health of human beings and causes respiratory illnesses that make them more susceptible to diseases such as COVID-19 (Sciaraffa et al. 2017). There have been evidences that prove the effect of air pollution on COVID-19 infections and mortality rate. Short-term and long-term exposure to polluted air, especially with particulate matter 2.5 ($PM_{2.5}$) and nitrogen dioxide (NO_2), increase the rate of COVID-19 infections and mortalities (Ali and Islam 2020). According to a study, the increase of 1 microgram/cubic meter in PM 2.5 along with some other small pollutants in air is linked with 8% increase in COVID-19-related deaths (Travaglio et al. 2021). Studies on heavily polluted Metropolitan areas in Italy as Milan, Lombardy, Florence, and Tuscany demonstrated the positive correlation between virus transmission and air pollution (PM 2.5 and NO2; Lolli et al. 2020). In another study, air pollution concentration and meteorological variables such as PM2.5, PM10, CO, NO_2, and O_3 were found to be positively linked with COVID-19 cases in 120 thickly populated cities of China (Zhu et al. 2020a, b). The Indian Medical Association (IMA) has reported that 13% of severe COVID-19 cases may be linked with air pollution. COVID-19 cases are likely to increase unless the air quality of megacities is not brought under control. Apart from this, medical facilities in thickly populated urban centers are getting easily overwhelmed. COVID-19 has exposed even the most developed megacities on the globe in relation to medical facilities, and major rethinking is required regarding the future planning of urban centers throughout the globe. Unplanned urbanization has to be restricted in a planned manner, and better conditions need to be generated keeping in mind human health by reducing pollution, checking high population density, and creating sustainable ecosystems in and around the urban centers.

4.4 Climate Change

Human activities have a massive impact on climate change which is creating environmental instability. Climatic factors, such as temperature, precipitation, humidity, and so on, are responsible to maintain the integrity of ecosystems (Arora 2019). Changes in any of these climatic factors may affect the migration pattern,

Table 4.1 Major zoonotic diseases reported from different continents

S. No.	Continent	Major Zoonotic diseases	Pathogen	Intermediate host	Year	References
1.	Europe	Black Death	*Yersinia pestis*	Rodent flea	1347–1352	Prentice and Rahalison (2007)
2.	Europe	Spanish flu	H1N1 influenza virus	Pig	1918–1919	Trilla et al. (2008)
3.	North America	Swine flu	Swine influenza virus (S-OIV) A/H1N1	Pig	2009–2010	Peiris et al. (2009)
4.	Africa	Zika virus disease	Zika virus	Rhesus macaque	1947–Present	Petersen et al. (2016)
5.	Africa	Ebola virus disease	Ebola virus	Bat, chimpanzee, gorilla	2013–2016	Holmes et al. (2016)
6.	Asia	Asian flu	H2N2 influenza virus	Wild duck	1957–1958	Webby and Webster (2001)
7.	Asia	Hongkong flu	H3N2 influenza virus	Pig	1968–1970	Tang et al. (2008)
8.	Asia	Severe acute respiratory syndrome	Severe acute respiratory syndrome coronavirus (SARS-CoV)	Bat, civet	2002–2003	Bolles et al. (2011)
9.	Asia	Bird flu	Avian influenza (HPAI) (H5N1)	Poultry	2019–Present	Bui et al. (2017)
10.	Asia	Middle East respiratory syndrome (MERS)	Middle East respiratory syndrome coronavirus (MERS-CoV)	Camel	2015–Present	Al-Ahmadi et al. (2019)
11.	Asia	Coronavirus disease (COVID-19)	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV 2)	Pangolin (possible host)	2019–Present	Yee et al. (2020)

disrupt breading, and increase disease transmission among wild animals (Masson-Delmotte et al. 2018). These changes also modify the disease cycle of various pathogens and make them more protuberant in the region where historically they were unsuitable. For instance, West Nile Virus (WNV) is a vector-borne pathogen that is transmitted during a warmer season. There are several environmental factors that influence their transmission and distribution. Change in weather conditions such as increased temperature affects their disease cycle in North America (Brault 2009; Paz 2015). There has been evidence that the dengue and yellow fever viruses are also dependent on temperature for completion of their cycle. Warmer ambient temperature is directly proportional to shorter incubation period of these pathogenic viruses. The rise in temperature in the United States not only accelerated the metamorphosis of *Aedes aegypti* mosquitoes but also increased the infection rate of dengue and yellow fever viruses among the population due to a shorter incubation period (Shope, 1991). Additionally, COVID-19 cases are also found to be linked with temperature and humidity fluctuations. In a study, COVID-19 incidences were reported to increase with the decrease in temperature, and also the lag nonlinear models verify the essential correlation between cases of COVID-19 and humidity (Shi et al. 2020). Although no concrete evidence has emerged regarding the correlation of high and low temperatures in the spread of SARS-CoV-2 virus, but one thing is certain that increase in global temperature is allowing the human pathogens to spread more and acquire new habitats as warmer days (per year) are increasing with global warming. Every year, rise in temperature is resulting in favorable conditions for pathogens to acquire more range and habitat. There is speculation that with the melting of glaciers and permafrost, novel pathogens (which got preserved in the ice several centuries ago) may emerge and can play havoc because the immune system of the current human population will not be able to recognize them (Fox-Skelly 2017; Yarzábal et al. 2021).

Deforestation is also a major anthropogenic activity that causes global warming, sudden weather fluctuations causing floods, droughts, forest fires, extinction of species, and destruction of habitats resulting in increased human–animal conflicts. Deforestation not only affects climate but also disturbs the unique balance of the ecosystems involving a plethora of plant, animal, and microbial species. With deforestation, this balance is breached, and the human population gets exposed to novel pathogens that were otherwise restricted by their original hosts and reservoirs. The extinction of wildlife also results in shifts as pathogenic viruses and bacteria evolve to move toward human population for their survival. Deforestation forces wildlife to stray and even come in contact with humans or livestock increasing the chances of zoonotic diseases to spread easily. In the Amazon region, approximately 17% of the forest has been lost in the last 50 years, and most of the forest land is now used for cattle ranching and agriculture, thus increasing the probability of animal–human conflicts and transmission of novel zoonotic pathogens.

It is expected that the world population will reach 9.7 billion by 2050 and could reach nearly 11 billion by 2100. Intensive agricultural practices for increased demand of food for continuously increasing population intensified the use of agrochemicals, livestock farming, and also resulted in extensive pollution of water

and soil (Arora 2019). In the last 50 years or so, our dependence on livestock has multiplied several times. In developing and poor countries, livestock cultivation is largely unorganized. Livestock is allowed to graze in the wild habitats and come in contact with wildlife. Unorganized livestock cultivation centers can be the suitable medium for the transmission of pathogens from wilds to humans and vice versa via direct contact or through domestic animals. Land-use change for agriculture also forces the epidemiological interactions between wildlife and farming animals, and such close contact allows zoonotic diseases to spread more vigorously (Jones et al. 2013; Carrasco-Garcia et al. 2016). For instance, MERS-CoV emerged in Saudi Arabia where the probable intermediate hosts were camels. However, the exact mode of transmission of the virus from camels to humans remained unsolved. Most probably the route of transmission could be the consumption of raw camel milk in the Arabian Peninsula (Sharif-Yakan and Kanj 2014).

Trade and consumption of bushmeat to fulfill the demand for food because of low agricultural production or satisfy the taste and greed are other important drivers for the arrival of novel pathogens in the human population. The illegal trade of bushmeat is rampant in several countries on the globe. Exotic species are hunted, and their meat is exported to different parts of the globe. These species may be the reservoirs of exotic viruses and other microbial pathogens. As per The United Nations' Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), an estimated 1.7 million different viruses exist in mammals and birds, and of them, around 10% can be pathogens (IPBES 2020). As per the most accepted theory, COVID-19 has also emerged from the wet market of Wuhan city of China. Intensive farming involving agrochemicals increases the risk of mutations, amplifications, and spread of zoonotic pathogens with the possible evolution of novel strains (Espinosa et al. 2020).

Climate change has also resulted in an increased frequency of natural disasters, such as droughts floods and bushfires. The rise in temperature and increase in events of drought has resulted in desertification as is also pointed out in the SDGs (Arora and Mishra, 2019). Intensive agricultural practices, use of agrochemicals, and inappropriate use of water resources have added to the process of desertification, and it is increasing by the year. This has resulted in the displacement of population and over-reliance on livestock and bushmeat (Arora et al., 2018; Arora 2018b). A cocktail of anthropogenic activities is a perfect recipe for the emergence of novel viruses and other microbial pathogens.

4.5 Environmental Sustainability for the Prevention of Pandemics in Future

COVID-19 would not be the last health emergency faced by mankind; hence, the preparedness for the future and maintenance of resilience in the ecosystems are very important. The past pandemics have shown that the government and health sectors only show their concern toward the problem till it persists and after that their attention shifts toward other social issues, largely related to unplanned development

and upliftment of the economy. Such social behavior can be called as "panic and forget" cycle. According to the WHO report, annually, only US$5 per person will be required as an investment to be prepared and maintain the environment, while the cost inflicted by COVID-19 as per recent estimates is US$ 11 trillion which is increasing by the day. It has left a huge number of people unemployed and makes their survival difficult even if they have not suffered from the disease. The environmental changes, the emergence of zoonotic disease, and social issues are directly linked. That is why we need to shift our focus towards the factors affecting environmental sustainability. Megacities tend to attract rural populations to move from their original habitats for employment and better life, but a major part of this displaced population is trapped in unhygienic and poor conditions. The huge population density in such cities increases pollution as well as chances of transmission of diseases. COVID-19 has proven that the big urban centers are the most affected regions on the planet. Governments have to plan to provide employment to the youth in their resident areas which can prevent the migration from rural to urban areas. Equal distribution of population will help in the reduction of disease transmission and reduce the rush to megacities, where slum areas under poor hygienic conditions are increasing day by day. The concept of green belt development has to be followed in the urban centers. The major aim should be to reduce the emission of GHGs and develop the idea of green cities.

Currently, in relation to COVID-19, a major part of the research is being conducted for the development of vaccines and drugs. However, the research should also be focused on the prevention of outbreaks of future pandemics by creating resilience in the ecosystems through their preservation (Zinsstag et al. 2020). There is an urgent need to intensely comprehend the transmission of pathogens among the environment, wildlife, and humans as a complex socio-ecological system (Zinsstag et al. 2011). Such an integrated approach toward the environment, animals, and humans is termed as "One Health". For the prevention of future zoonotic diseases and attainment of environmental sustainability, there is a necessity to prioritizing "One Health" as a guiding principle (Zhou 2012).

Moreover, to maintain environmental sustainability, the health care policymakers and governments should respond to the drivers that are accelerating climate change and affecting environmental health (Ossebaard and Lachman 2021). Currently, we are in the midst of an emergency for the prevention of COVID-19 and also the massive environmental catastrophe happening simultaneously. However, learning from this can help in achieving prevention for the future; otherwise, there will be more such pandemics knocking at the door of mankind.

4.6 Conclusion

COVID-19 emerged as a highly contagious zoonotic disease that has affected the whole world. Unplanned urbanization correlated with environmental devastation is linked with the spread of COVID-19 and other zoonotic diseases. Globally, several vaccines are being developed to protect the population from this havoc, and

currently, vaccination seems to be the most important solution to this pandemic. The development of vaccines and vaccinating the whole human population will take time and till then the damages will be humongous. The cost of tackling the pandemic is huge, and it may take several years before socially or economically we are at par as the pre-COVID era. There are so many learnings from this pandemic that should be utilized for the future. Mankind has to move to realize environmental sustainability, and this can be done by achieving the targets of SDGs. The cost of saving our environment is far less than that inflicted by a pandemic such as COVID-19. The common belief and slogan at present are "nobody is safe until everyone is safe." However, we need to extend it for every living creature on the planet and make it "no life is safe until every life is safe on the planet." Conservation of biodiversity and our environment can go a long way in protecting us from such pandemics in the future.

Conflict of Interest Statement Authors declare that there is no conflict of interest involved in the work presented in this chapter.

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Transmission of SARS-CoV2 and Strategies for Control of Infection: Lessons Learnt

5

Kirtan Rana, Ritin Mohindra, and P. V. M. Lakshmi

Abstract

Coronavirus has affected all spheres of human life; physical, mental, and social aspects to the limit which has never been experienced before. The major symptoms of COVID-19 infection are fever, cough, respiratory distress, loss of taste, loss of smell, body aches diarrhea, vomiting, and so on. Those having symptoms are tested for their COVID-19 infection status either by Rapid Antigen test or real-time polymerase chain reaction (RTPCR)/Gene Xpert method. Those found COVID-19 positive are shifted to COVID care centers or home isolation for 17 days. The epidemiological triad includes an agent (strains of SARS-CoV-2), host (immunocompromised person), environment (overcrowding, temperature, humidity, contaminated surfaces). Various strategies have been implemented from time to time to break the chain of transmission to contain the spread of infection. Various strategies at an individual level and the community level are implemented. Strategies such as wearing mask, frequent handwashing, maintaining a distance of minimum 2 m between two people, screening for risk factors, quarantine, isolation, surveillance, and contact tracing, defining high-risk areas into hotspots/containment zones or micro containment zones, issuing heating, ventilation, and air-conditioning guidelines, work from home and introduction of vaccine as prophylaxis for prevention against the infection were introduced by India as well as globally. The introduction of infection control

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measures has some good affects such as lowering air pollution level and controlling the unnecessary plight of the vehicle on roads but the people have faced some serious effects also such as, it pushed people more into poverty and more down in nutritional graph raising country rank in hunger index. Whatever the strategy be proposed it should be implemented keeping to view the pros and cons of each strategy.

Keywords

COVID-19 · Modes of transmission · Symptomatic and asymptomatic infections · Phases of pandemic · Strategies to break the transmission · Impact of various strategies · Role of vaccines · Success stories

5.1 Introduction

Coronavirus has affected each and every sphere of human life. The physical, mental, and social aspects have been affected to a larger extent as never before in the near past. The first case of Coronavirus appeared in Wuhan city of China in December 2019 as a case of atypical pneumonia. India got its first case of Coronavirus in January 2020 in Kerala. The World Health Organization declared the coronavirus infection as pandemic on 11th March 2020 (The WHO just declared coronavirus COVID-19 a pandemic|time n.d.). It was postulated that coronavirus (SARS-CoV-2) originated in bats and just like the Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) infections (Coronavirus history: how did coronavirus start? n.d.). The disease due to the 2019 novel coronavirus was named as coronavirus disease 2019 abbreviated as COVID-19 on 11th February 2020 and the virus was named as Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) (About COVID-19|CDC n.d.).

The COVID-19 infection usually presents as fever, cough, sore throat, shortness of breath, diarrhea, vomiting, body aches, loss of smell, loss of taste, and so on (Coronavirus disease (COVID-19) n.d.). In its most severe form, the patient can have acute respiratory distress syndrome or respiratory failure. Mostly the people above 60 years of age or those suffering from any chronic illness such as diabetes mellitus, hypertension, or any kind of malignancy are considered to be at higher risk of contracting the COVID-19 infection.

The person presenting with complaints of fever, cough, breathlessness, running nose, diarrhea, vomiting, body aches, and so on is suspected to be the case of COVID-19. Since the COVID-19 symptoms mimic the seasonal influenza symptoms, it is important to differentiate COVID-19 from other flu-like illnesses. The diagnosis of COVID-19 disease is laboratory-based and is made by real-time polymerase chain reaction (RT-PCR) which is considered as the gold standard test for diagnosing the diseases. Rapid Antigen Testing (RAT) is another test done for diagnosing COVD 19 infection but is less sensitive and specific as compared to the RT-PCR as RAT diagnosis depends upon the type of kit used and skills of the person

performing the test. In India, the Indian Council of Medical Research has released an advisory on the testing strategy for COVID-19 from time to time (Indian Council of Medical Research 2020) Besides symptomatic patients, a large percentage of asymptomatic patients are also tested positive for COVID-19. A study concluded that asymptomatic patients accounted for 40–45% of COVID-19 infections and can shed the virus for more than 14 days (Oran and Topol 2021) Asymptomatic individuals with high-risk exposure to COVID-19 positive people are being tested between days 5 and 14 from the day of last exposure initially, later on, the testing days were revised and were kept between days 5 and 10 to identify asymptomatic COVID-19 infections.

5.2 Modes of Transmission

5.2.1 Epidemiological Triad of COVID-19

The epidemiological triad of COVID-19 infection comprises of agent, host, and environment. The *agent* is the organism, which causes the infection, that is, the SARS-CoV-2 virus. The strain of SARS-CoV-2, pathogenicity and virulence of the organisms, plays an important role in causing infection. The first strain was the L strain that appeared in Wuhan in December 2019, followed by the S strain in early 2020. Other strains were the V and G strain of coronavirus which appeared in mid-January 2020. The G strain further mutated to GR and GH by the end of February 2020 (The six strains of SARS-CoV-2—ScienceDaily n.d.). The *host* characteristics are the age, immunity of person, preexisting diseases such as diabetes mellitus, hypertension, cancers, or any other immunosuppressed state. With an increase in age and with preexisting comorbidities, the immunity of the body falls low and the body gets susceptible to infections. The *environment* includes components which are in immediate surroundings to the human body such as overcrowding, wind velocity, temperature, contaminated inanimate surfaces, humidity, and so on. The factors such as temperature and humidity favor the survival of SARS-CoV-2 outside the human body and overcrowding and wind velocity favors traveling/transfer of droplets effectively among humans (Eslami and Jalili 2020). The surface which is frequently touched such as television and air conditioner remotes, tables surfaces, desktops keyboards, and so on are the common sources for transmission of SARS-CoV-2 among humans through indirect contact. The stability of the virus on the surface is also an important factor for the transmission of the virus. It is found that SARS-CoV 2 virus survives for maximum days (3–7 days) on plastic and stainless steel, followed by paper and glass for 4 days, for 2 days on wood, and for 4 h on copper surfaces (Chin et al. 2020).

5.2.2 Chain of Transmission

The *chain of transmission* is several interconnected steps, which show how COVID-19 pathogen flows from the reservoir to host as illustrated in Fig. 5.1. This infectious

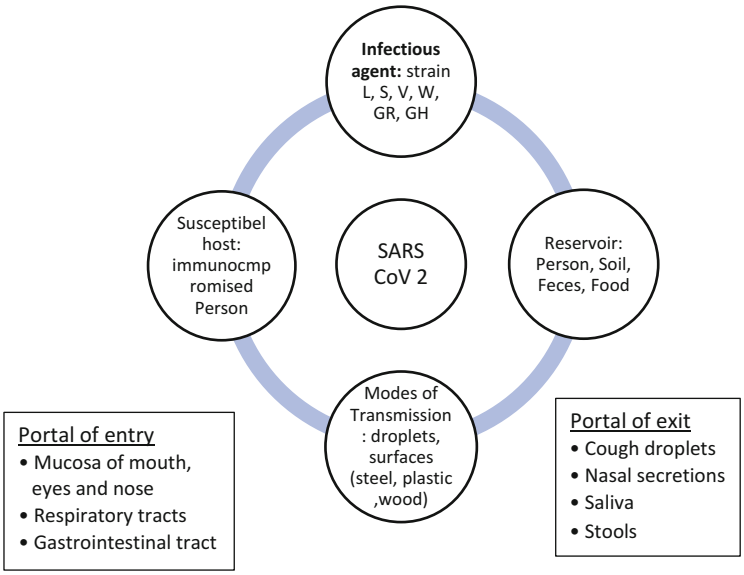

Fig. 5.1 Chain of transmission of SARS CoV2 infection

agent, which is SARS-CoV2, resides in the *reservoir,* which in this case are human beings or nonliving things such as soil, feces, food, equipment, and so on. For further propulsion of infectious agent, it needs some *portal of exit* from the reservoir body. The COVID-19 disease is an airborne disease, which mainly spreads due to the shedding of the virus in the host saliva in the form of droplets. The droplets are generated while coughing, sneezing, or spitting. Other sources are nasal secretions, saliva, and stools. The *modes of transmission* are either direct or indirect. The direct modes are transmission through inhalation of respiratory droplets (>5–10μm in size) or droplet nuclei (<5μm in size), aerosols formed during various surgical and dental procedures, body fluid secretions (feces, saliva, urine semen, and tears, etc.), and from mother to child. The indirect modes include touching contaminated surfaces, sharing common objects such as remote controls, tap knobs, door handle knobs, linen, and so on, and then touching the face (Modes of transmission of the virus causing COVID-19: implications for IPC precaution recommendations n.d.). The *portal of entry* is the route by which the infectious organism enters the human body. Touching one's mouth, nose, eyes with infected hands or objects can pave the entry of the organism into the human body. Not all people who contact the pathogen becomes ill. Just like other bacterial, viral, or fungal infections the one who are immunocompromised owing to long-standing illness, or on immune-compromised drugs or the people who are handling the infected patients are at greater risk of getting infected. A study found that the health care workers are at greater risk of contracting COVID-19 infection as compared to the non-essential workers followed by social and education workers (Mutambudzi et al. 2020). The infection keeps on

spreading if the chain of transmission remains uninterrupted. To control the infection, a break in this chain of transmission is needed.

5.2.3 Incubation Period

The incubation period is the time between exposure to the virus and the onset of symptoms. The incubation period of SARS-CoV 2 on average is 5–6 days, but it can be as long as 14 days from the date of last exposure. The incubation period is used in deciding the quarantine/isolation period.

5.3 Phases of Pandemic

Pandemics are known to affect the large number of populations spanning across various nations at the same period of time. With the advent of globalization and rapid mass transportation systems such as air travel, the disease from one corner of the country can reach another corner or even to other countries in no time and can cause widespread outbreaks. WHO has defined various phases of infection with pandemic potential in 1999 for correlating with the mitigation measures to be adopted, which were revised in 2005. Any infection with pandemic potential has six phases as represented in Fig. 5.2 (World Health Organization 2010). It is very important to understand the phase of the pandemic in a country in order to mitigate the spread of the disease-causing agent.

In phases 1–3 of the pandemic, the transmission is either from organism to humans or there is a limited transmission from human to human. The probability of an established pandemic is uncertain in the first three stages of the pandemic cycle. The community-level outbreaks happen when the pandemic enters phase 4 which is characterized by sustained human to human transmission of an animal or human–animal virus. The probability of pandemic is medium to high in this stage.

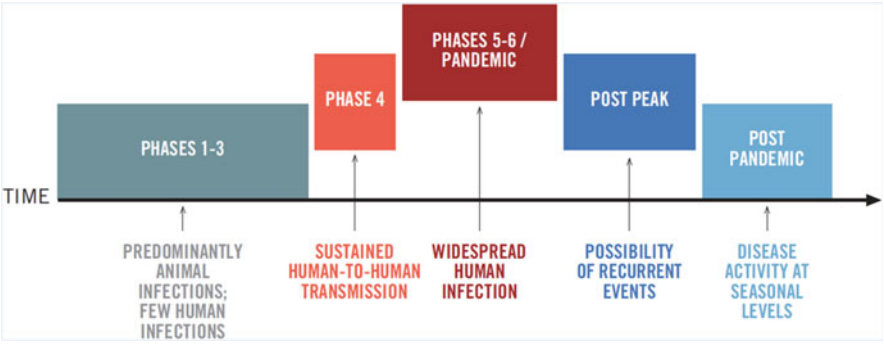

Fig. 5.2 Phases of pandemic

Any country that suspects or has verified such an event should urgently report to WHO so that the situation can be jointly assessed and a rapid pandemic containment operation can be implemented if warranted. In stages 5 and 6, the probability of pandemic shifts from high to the pandemic in progress. Phase 6 is followed by the post-peak period in which there is the possibility of recurrence of events. If there happens recurrence of events after phase 6, there is the possibility that a new wave of infection has occurred. Following phase 6 the period is known as post-pandemic period. During post-pandemic periods many affected countries evaluate their response, revise their plans and look into the recovery rates. In India, Kerala declared the community transmission of COVID-19 infection on July 17th, 2020 in its two areas, Poonthura and Pulluvila and it was the first state in India to declare Community transmission of COVID-19 (In a first, Kerala CM confirms community transmission of COVID-19—India News—Hindustan Times n.d.).

5.4 Strategies to Break the Transmission of SARS-CoV-2

Control of pandemic requires breaking the chain of transmission at multiple stages in the chain of transmission, to mitigate the spread of the infection. Some transmission break strategies are effective between reservior and modes of transmission and others are effective to break the chain between modes of transmission and uptake of organism by the susceptible host as presented in Fig. 5.3.

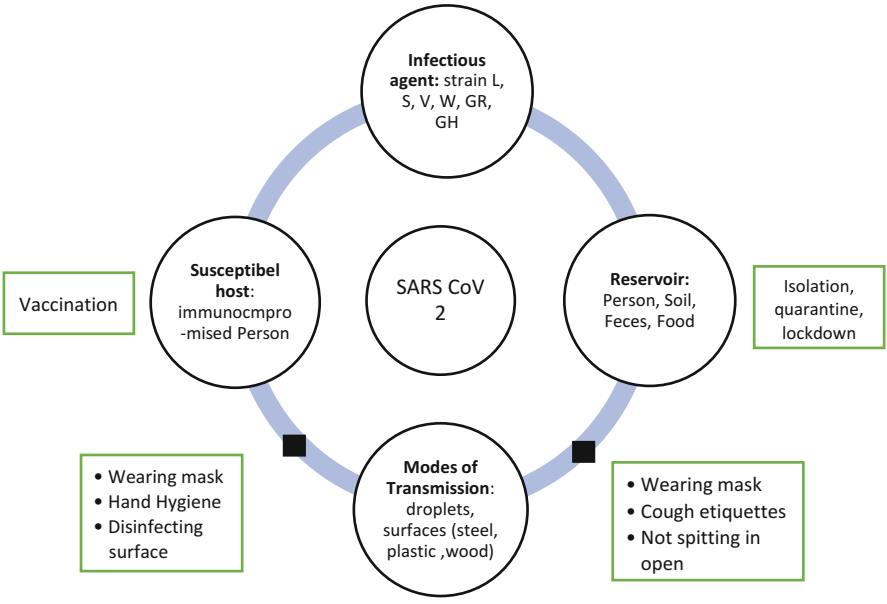

Fig. 5.3 Strategies for breaking chain of transmission

With the declaration of SARS-CoV2 as a pandemic, globally the strategies were being made to cut down the transmission of the virus. Some strategies were common to all the nations as issued by World Health Organization such as *wearing masks at public places, washing hands regularly, maintaining social distancing, not spitting in public places, home quarantine, isolation in COVID care centers or at home*. Some strategies were framed by countries as per their own burden of disease and ways of mitigating the spread. In view of the sudden spike in the number of COIVD 19 cases globally, some interventions were introduced to break this chain of transmission. At the individual level interventions such as *wearing the mask at public places, washing hands regularly, maintaining social distancing, not spitting in public places, home quarantine* for the people having a history of travel were implemented. At community level, the interventions include screening for symptoms of COVID-19 among travelers especially from affected countries and quarantining of the individuals, closure of markets, worship places, schools and colleges, capping on the number of people attending social gatherings, closure of bars and restaurants, curfews, border closure, stoppage of all "non-essential" work and promoting work from home, stoppage of domestic and international travel, and so on (Social Distancing n.d.; Strategies to reduce COVID-19 spread|Coronavirus| COVID-19|CDC n.d.)

1. *Wearing masks* in public places was advised as the strategy to prevent the spread of the COVID-19 infection. It also prevents one from contracting the infection from others. Different types of the mask such as N95, triple-layer, or cloth masks can be used depending upon the type of exposure. The health care workers usually wear N95 masks or triple layer masks depending upon whether they are involved in aerosol-generating procedures or not. N95 mask is recommended for aerosol generation procedures. For people who don't deal directly with the COVID-19 patients, it was recommended that they wear cloth masks. *Personal Protective Equipment* (PPE) is also used by health care workers who are involved in the care of COVID-19 positive patients in dedicated COVID care centers.
2. *Frequent handwashing* with soap and water or with alcohol-based rub is yet another measure that can be adopted at an individual level. It was recommended that whenever a person touches any surface he/she should wash their hands to kill the COVID-19 pathogen in his/her hands.
3. Maintaining a *social distance* of at least 2 m between two people can prevent spreading infection. When a person coughs or sneezes the droplets/aerosols generated can travel up to 2 m distance. In order to be sure that the other person doesn't come in contact with the droplets/aerosols it is advised to have a gap of at least 2 m between two people.
4. *Screening* for symptoms is the first strategy to mitigate the spread of COVID-19 infection. The people are screened for the risk factors of COVID-19 infection. Screening of symptoms starts at the entry point of any country or state. Screening involves measuring body temperature using thermal scanners to rule out fever, asking symptoms such as cough, sore throat, breathlessness, fatigue, and

so on. Those who were found to have any of the symptoms suggestive of COVID-19 infection are referred for testing. Those who do not have symptoms but have a history of travel to country/state/district with the high level of infection are quarantined for 14 days and are tested if he/she develops symptoms suggestive of COVID-19.

5. *Quarantine* was the first strategy adopted after the pandemic was declared. A 14-day quarantine period for all those who had a travel history from the affected countries or a history of high-risk contact with a positive patient was mandatory during in initial phases. The aim of quarantine is to restrict the movement of people suspected to have come in contact with the infected people. The institutional/home quarantine was applicable to all people who have the history of international traveling in the last 14 days (Quarantine period for COVID-19 mohfw—Google Search n.d.).
6. The *surveillance* and *contact tracing* of the people in community settings to find out the hidden cases proved to be the backbone of COVID-19 containment. The government of India issued guidelines to identify and classify contacts as early as possible for preventing the spread of transmission. A risk assessment was done to decide whom to test and whom to quarantine based on factors such as duration of contact (>15 min), proximity (<1 m), nature of exposure (droplets, body fluids, etc.) (Surveillance and Control 2020). During the COVID-19 pandemic unprecedented large-scale use of technology has been promoted in digital contact tracing and surveillance (Berman et al. 2020). To strengthen the surveillance and contact tracing the Government of India launched a mobile-based Application, *ArogySetuApp*, which is used for contact tracing and syndromic mapping, and self-assessment for COVID-19 infections. The application was also used to spread awareness of COVID-19 and to connect essential COVID-19-related health services to the people of India.
7. A *three-tier classification system* of hospitals was developed to manage COVID-19 patients COVID care centers to treat very mild, mild, and suspected cases; COVID health centers for clinically moderate level serious patients; and dedicated COVID hospitals for comprehensive care of the severe and critical patients (Covid-19 India: 3-tier classification of hospitals for diverse levels of infection|India News—Times of India n.d.). People were isolated for 17 days (10 days institutional isolation +7 days home isolation) once they come positive for COVID-19 infection. As the pandemic increased there was an increase in the number of positive cases, shortage of COVID care centers, and increased financial burden on the government, the government then issued guidelines for home isolation. Home isolation is another strategy where all suspects (waiting their test results) and confirmed cases of COVID-19 are separated from others for a similar period of 17 days as earlier in order to break the chain of transmission. A 24*7 caregiver should be available to provide care at home. While providing care the caregiver should wear the mask, avoid touching their own face, nose, or mouth, practice hand hygiene, avoid direct contact with the patient or contact with body fluids, use triple-layer mask and disposable gloves, and above all the caregiver should self-monitor for symptoms (Ministry of

Health and Family Welfare, Government of India 2020). Initially, the area administration used to put quarantine sicker outside the home of those diagnosed as COVID-19 positive but due to the stigma associated with the disease and poster outside their houses, people stop coming for testing. Later on, the practice was stopped as the government wants to encourage more and more people to come for testing (Ministry of Health and Family Welfare, Government of India 2020).

8. Another strategy for breaking the chain of transmission of COVID-19 was to cordon the area from where the maximum cases are being reported. The government declared those areas as '*hotspot*' areas. Initially, the districts where more than six positive cases were found were declared as hotspot districts. There was a restriction in movements of people in hotspot areas and no one was allowed to come out or move in that area. The government ensures doorstep supply of food, medicine, and essential items (What is a hotspot area? What can and can't be done in a hotspot?|India News—Times of India n.d.). Later on, the hotspot areas were changed to *containment zones* which have smaller geographic area coverage, and further the containment zones were changed to *microcontinent zones*. The 3 km area from the containment zone is called buffer zone (Coronavirus: what is a COVID-19 containment zone and why is it created?|Hindustan Times n.d.). The basic idea of changing the hotspot districts to containment and microcontinent zones were to decrease the financial loss, people outside the containment zone can work their routine duties and psychological relief to the people regarding the magnitude of the disease burden
9. The Government of India issued guidelines for managing dead bodies. The staff handling dead bodies in the isolation areas, mortuary, ambulances, and crematorium/burial ground were well trained in the infection control practices. The health workers attending the dead body has to perform hand hygiene and ensure proper use of PPE and place the dead body in a leak-proof plastic body bag. Embalming of dead bodies was not allowed. Autopsies were avoided and to be done only in case of emergency. Viewing dead bodies for the last time by unzipping the face end of the bag only without kissing hugging or touching was allowed (Ministry of Health and Family Welfare 2020).
10. An incident was reported where around 26,000 people from 24 villages in Punjab were home quarantined after they attend religious congregation by the preacher came out to be COVID-19 positive (India's "super spreader" home-quarantines 26,000 people in 24 Punjab villages—The New Indian Express n.d.). A similar 'superspreader' event was reported in Chandigarh, A north Indian Union territory where the primary case infected as many as 236 people (Mohindra et al. 2021). Avoiding/capping down the *social gatherings* was the major strategy to cut down the transmission by enabling more space for people (by capping the people) to move around and maintaining the social distance. The marriage ceremony has a capping of 50 people who can attend a marriage at one point of time (Not more than 50 people can attend wedding functions during the lockdown in Delhi: Arvind Kejriwal, India News Newslwionews.com n.d.). The various sports events which were witnessed earlier by thousands of spectators

have been made virtual just to avoid gathering in stadiums. (Italy going back to games with no fans in stadiums due to rising coronavirus cases—Sports News, Firstpost n.d., Spectating Spectator Sports: COVID-19 is dismantling stadium culture, one screen at a time—The Economic Times n.d.). The Tokyo Olympics which was supposed to happen in 2020, was postponed till 2021 in view of avoiding social gatherings (Tokyo Olympics postponed to 2021 due to coronavirus pandemic|Tokyo Olympic Games 2020|The Guardian n.d.). The Indian Premier League (IPL) was moved out of India and was held in the United Arab Emirates with no spectators in the stadium, due to rising cases of COVID-19 in India (IPL 2020 UAE Schedule: IPL Full Schedule, Time Table, Teams, Players List, Match Dates, Start Date, Latest News n.d.). The workplaces (multinational companies, information technologies, etc.) and schools were allowed to *work from home* (WFH). Some places where work from home could not be possible, Ministry of Home Affairs in coronavirus lockdown 2.0 guidelines allowed to work with 50% employees strength to reduce the number of people gathering at one place and maintaining the social distancing (Coronavirus lockdown 2.0 guidelines|IT companies can now work with 50 percent strength n.d.). The WHO released a document for making workplace COVID-19 free suggesting frequent cleaning of surface and objects, regular hand washing by the employees, contractors, and costumers ensuing facemask and displaying posters promoting respiratory hygiene avoiding face-to-face meetings unless it is an emergency (Occupational Safety and Health Administration 2020; WHO 2020a)

11. In hospitals routine Out Patient Departments (OPDs) were suspended to avoid large gatherings and only the emergency services were continued. In operation theaters, the routine working atmosphere was changed and health care workers were made to work under negative pressure to make the area less contaminated. Further, the fans, as well as the air conditioners, were also stopped in hospitals in order to control the spread of COVID-19.
12. The Indian Society of Heating, Refrigerating, and Air Conditioning Engineers (ISHRAE) issued *Heating Ventilation and Air conditioning (HVAC) guidelines* for the operation of air condition/ventilation system during COVID-19 pandemic (Agarwal et al. 2020)The guideline recommends using room air conditioners between 24 and 30 °C and relative humidity between 40% and 70%. For evaporative coolers, they must draw air from outside to ensure good ventilation and fans to be used with windows partly open. In the case of an exhaust fan facility, it must be kept running to exhaust air for better ventilation. The guideline further suggested using high-efficiency air filter (HEPA) filters for treating exhaust air from COVID-19 patient's area. The quarantine area needs to be well ventilated or a negative or neutral differential pressure needs to be maintained in those areas. The CDC issued guidelines for heating ventilation and Air conditioning (HVAC) systems during COVID-19. As per CDC guidelines, the HVAC system needs to run at maximum outside airflow for 2 h before and after occupied times in accordance with the industry standards. It was emphasized to increase the percentage of outdoor air, potentially as high as

100% (COVID-19 employer information for office buildingslCDC n.d.) The WHO has also issued guidelines for an HVAC system which promoted using natural ventilation, opening windows if possible and being safe. The guidelines discouraged the recirculation of air and in case, it is needed, regular cleaning of the filters is recommended. Further, it was emphasized to generate clean to less clean air movements by revaluating the position of supply and exhaust air diffusers (WHO 2020a, b).

13. *Biomedical waste management* has proven to be the biggest challenge during this pandemic period. More biomedical waste has been generated in the form of one-time use masks as a preventive strategy to mitigate the spread of the COVID-19. The additional personal protective equipment (PPE) kits, kits for sample collection which include the Viral Transport Medium (VTM) and swabs sticks syringes and vial/vacutainers for collection blood samples, and so on have also added to huge amounts of biomedical waste. The biomedical waste generated needs to be disposed off properly in order to avoid indirect transmission of COVID-19 infection to other people. Appropriately using the PPE and other logistics can help in reducing biomedical waste generation. The WHO recommended the rational and appropriate use of PPE based on the risk of exposure and transmission dynamics of the pathogen (WHO 2020b). The Ministry of Environment, Forest, and Climate change published guidelines for managing waste generated during treatment, diagnosis, or quarantine of COVID-19 patients (NCDC 2020). These guidelines recommended the use of double-layered bags with mandatory "COVID 19 waste" labeling, regular disinfection of trolleys, stethoscopes, blood pressure measurement apparatus, walls, lockers, cupboards, refrigerators, railings, and so on, with soap and water, alcohol-based rub, or 1% hypochlorite solution whichever is available. Being an enveloped virus, the surface of the virus can be easily disrupted by using 60–70% alcohol-based antiseptic solutions rather than 100% alcohol-based antiseptic solutions. Waste masks and gloves in the general households should be kept in the paper bag for a minimum of 72 h prior to disposal as dry general waste after cutting to prevent reuse (Central Pollution Control Board 2020) (Fig. 5.4).

5.5 Impact of Various Strategies on Different Spheres of People

No single mitigation strategy has impacted the lives of people during COVID-19 pandemic. Many times unplanned and newly introduced strategies lead to more harm/loss than benefit. The overall impact has been seen because of multiple strategies working together in order to completely cut down the ways of SARS-CoV-2 virus transmission in the community.

Due to the restriction in movements of the people during "lockdown," there has been less plight of the vehicles on roads, rails, and in air, which has raised the air quality index of many cities which were having the worst quality of air index. Imposing lockdowns has disrupted the normal life of people also as it took away

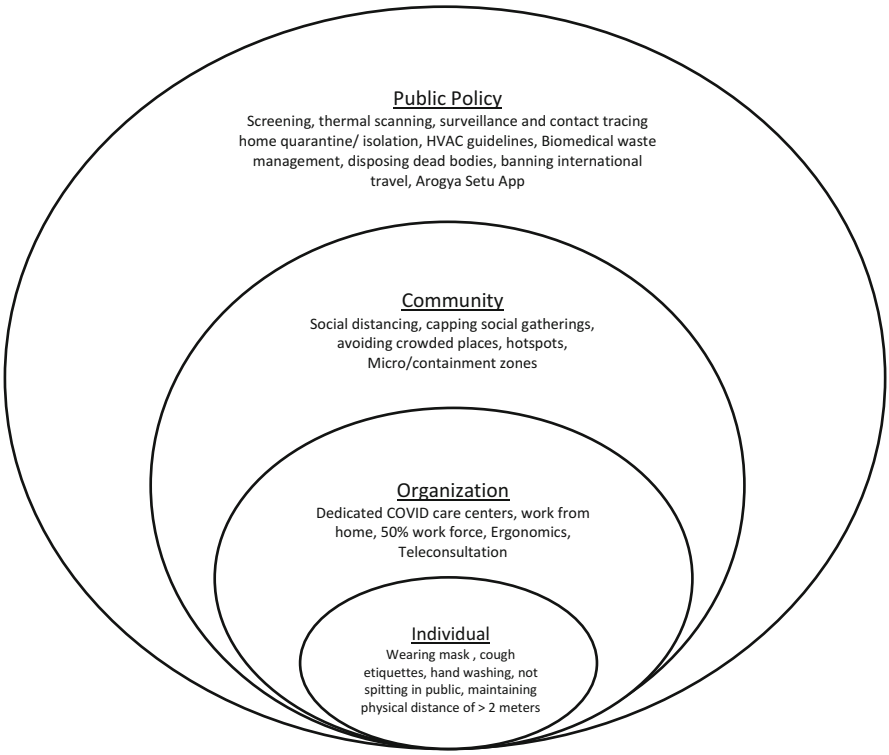

Fig. 5.4 Socio-ecological model for controlling the spread of COVID-19 infection

many jobs pushing them more into poverty. Lack of food and water supplies have pushed them more down in the nutrition graph. Hunger, lack of provision of medicines to the people of chronic disease, migration of people from cities to their home town leading to mishaps have contributed a lot to the total morbidity and mortality index which can be contributed to the ongoing COVID-19 pandemic. The curfews have disrupted the social life of the people. The screen time of the people has increased as their movements were restricted and were made home-bound due to lockdown. Lockdown has also resulted in an increase in psychological problems such as depression and anxiety disorders leading to suicide attempts/suicides.

Work from home (WFH) was started in many sectors to avoid gathering at workplaces. It was considered as a good way of protecting the office workers by cutting down the risk of getting COVID-19 infection. It has been seen that WFH has benefits of flexibility and agility in working arrangements, increased productivity, improved staff health, and wellbeing, attracted new talents, financial benefits, the convenience of doing work but at the same time, it has seen some consequences such as isolation, difficulty in monitoring performance, home distractions, potential burnouts, negative impact on mental health, and so on (Advantages and disadvantages of employees working at homelnibusinessinfo.co.uk n.d.).

Virtual classes have been seen as the future of the current education system. The children need not visit schools for education instead they can be educated at their respective homes. Mobile phones, computers, and a good internet connection are just needed for attending the virtual classes. Though it seems very fancy, but it has increased the screen time of the children to a large extent make them vulnerable to the harmful effects of non-ionizing radiations and leading to many cancerous growths such as gliomas, acoustic neuromas, meningiomas, and parotid gland tumors (Ahlbom et al. 2004). Further, the screen time has severely impacted the refraction of the users. Many places in our country are still where there is no good quality internet connection which has compromised the studies of children. In an incident in Kerala in June 2020, a girl committed suicide as she was unable to join the online class to carry on her studies in absence of logistics required to attend online classes ("I'm going": Kerala girl commits suicide after missing online class—India News n.d.) Further, it has been found that children reported more distraction while attending virtual class than they used to have in the real class.

The healthcare system has been affected more because of the COVID-19 pandemic. It has changed the health care seeking behaviors of people. The direct contact between doctor and patient has been reduced very much. The technology has come into play for seeking medical consultation in the form of "teleconsultation" which can prove to be an advanced method of providing care to the patients. But the consequences such as lack of bonding between doctor–patient and scarcity of time for consultation cannot be denied. The service provided to the number of patients per day is recorded to be less than those when the OPDs were run physically.

5.6 Mortality and Comorbid Conditions Associated with COVID-19

The diseases which were responsible for the major proportion of total morbidity and mortality and were considered to be of prime importance and emergencies are totally ignored during this COVID-19 pandemic due to the shutting down of normal OPD's. The focus of the health care system has shifted more towards COVID-19.

In India, the overall mortality due to COVID-19 among COVID-19 positive patients was found to be 1.43% (MoHFW|Home n.d.). More than half of the deaths that have happened in India were above 60 years of age and 70% of the total deaths were among males (Ssentongo et al. 2020). COVID-19 has been found to be associated with many chronic diseases. A study found 2.25 times more risk of deaths among patients having cardiovascular diseases, 80% higher risk of deaths among patients suffering from hypertension, 1.5 times more in those having diabetes, 2 times among patients with congestive heart failure, and three times those having chronic kidney diseases among those who were COVID-19 positive (Analysis of existing comorbidities and COVID-19 mortality n.d.).

5.7 Role of Vaccine

Vaccines have proven to be game-changer in managing infections. With the development of the first smallpox vaccine by Edward Jenner, the vaccines have moved a long way in managing different types of infections. Never in the history of vaccination, a vaccine has been made so quickly. Though WHO and different national health agencies have published infection prevention control guidelines yet people are waiting for COVID-19 vaccine. The various pharmaceutic companies have been trying hard to achieve the feast. Though certain pharmaceutical companies have claimed to develop the vaccine but the long-term profile of these vaccines is yet to be discovered (NCDC 2020). Another important aspect of a vaccine is its acceptability among the masses. Though the vaccine will be launched over a period of time but the psychological effect of vaccine such as the insecurity about long-term complications of vaccine, vaccine hesitancy, and acceptance in the community is the biggest challenge for the health care providers and the health agencies (MacDonald et al. 2015). For successful rollout of COVID-19 vaccine, the health care providers need to understand the vaccines hesitancy determinant matrix which includes contextual influences (arising due to historic, sociocultural, environmental, health system, economic, political), individual and group influences (personal perception, influence of the social/peer environment) and vaccine-specific issues directly related to the vaccine or vaccination such as vaccination schedule, cost reliability (WHO 2014).

5.8 Success Stories

Implementing the containment strategies stringently has helped in reducing the number of COVID-19 positive cases the many countries. An emergency like a pandemic can be controlled when both government and people participate responsibly. Countries such as Taiwan, South Korea, New Zealand, and Australia have been able to successfully manage the spread of COVID 19 to the maximum extent. Taiwan tested and quarantined travelers from Wuhan, China, banned the export of surgical masks, and traced the mobile sim cards to ensure those who were given quarantine were actually abiding by the rules. Singapore adopted an aggressive approach to contact tracing by scanning people's Identity cards at the supermarket, built temporary bed spaces at breakneck speed to house COVID-19 patients. South Korea did excessive testing and employed real-time tracking of COVID-19 patients, offering cash payments to the citizens to ride out the economic turbulence. New Zealand moved swiftly to shut down the country in less than 3 weeks of its first case instituting "level 4 lockdown" which meant that people could interact with people within their home in an attempt to eliminate the virus all together with text messages to the people to explain what is expected of them (The best global responses to COVID-19 pandemic|Time n.d.).

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Pathogenesis of COVID-19 Infection

6

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Abstract

Coronavirus disease 2019 (Covid-19) has produced a grave global pandemic and resulted in a serious public health crisis accompanied by loss of life and economy. The causative virus is known, but the exact pathogenesis and cascade of events that ensue are still poorly understood. It is now known that the virus enters through the respiratory route by attaching to ACE-2 receptors in the ciliated nasal mucosa. Infection of the lower respiratory system causes a surge in the release of a variety of cytokines that trigger a "cytokine storm" resulting in collateral damage to lungs and vascular endothelium. The damage to vascular endothelium results in multisystem involvement.

Keywords

Pathogenesis · Covid-19 · Cytokine storm · ARDS

6.1 Introduction

COVID-19 is an infectious disease that is caused by a positive-sense single-stranded RNA (+ssRNA) virus known as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). This virus belongs to an already known broad family of viruses called coronaviruses. The covid-19 pandemic has afflicted the entire world in terms of both loss of life and economic crisis. The pathogenesis of COVID-19 has not been completely elucidated, but it includes the interplay between inflammation and coagulation in broader terms. There is an enormous release of proinflammatory

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cytokines and chemokines from the immune cells of the body which results in the so-called "cytokine storm." The cytokine storm leads to plasma leakage, vascular hyperpermeability, and disseminated vascular coagulation resulting in multiorgan failure (Zafer et al. 2021). The scientific community of the world and health care workers have worked tirelessly to understand the specific cellular and molecular immune mechanisms to prevent disease transmission in the community and to develop a Covid vaccine for the prevention of disease (Saad and Moussa 2021). Fortunately, now the vaccine has been rolled out, and the vaccination program is intensively going on all over the world. To complicate the pandemic further, a new variant of SARS-CoV-2 has been recently reported from the United Kingdom which has the potential to spread rapidly; however, so far, there is no evidence to suggest that the variant has the potential to cause severe disease or mortality. This variant has resulted from multiple mutations in the spike protein as well as mutations in other genomic regions of the RNA virus. Research is underway to understand the properties of this variant in greater detail. The current surge in India has brought out the emergence of double and even triple mutations in the covid-19 virus. Such viral mutations have rendered the virus highly infectious even with a short exposure and the mutated viruses are potentially fatal within few days. Children, younger individuals, and persons without any comorbidity are getting affected by these new strains. A brief outline about the pathogenesis of Covid-19 is given subsequently.

6.2 Virus Entry and Spread

The transmission of Covid-19 virus occurs primarily through the respiratory route, that is, aerosols and large droplets. Coughing and sneezing can throw viruses in the form of microparticles which remain suspended in the air for few hours. Infected surfaces and fomites have also been considered as the route of infection in some cases. Close and prolonged contact for more than 20 min is said to be significant for contracting the infection.

The coronovirus-19 is a spherical envelope with multiple spike-like projections on its surface. When viewed under transmission electron microscopy, it gives a crown-like appearance and thus the name "coronavirus". The average size of viral particles is reported to be 70–80 nm (Prasad et al. 2020); however, it may range from 60 to 140 nm. The virus contains membrane glycoprotein (M), envelope protein (E), nucleocapsid protein (N), and spike protein (S). The S-glycoprotein enables the virus to attach to host cells through a receptor called angiotensin-converting enzyme 2 (ACE-2). This binding results in cleavage of the S-protein of the virus by host cell proteases leading to fusion of viral and cellular membrane which facilitates the entry of the virus into the host cell. The upper respiratory tract, especially the posterior nasal mucosa, highly expresses ACE-2 receptors and acts as the crucial factor for viral entry into respiratory epithelial cells (Wan et al. 2020; Hoffmann et al. 2020). However, many other cells in the human body express ACE-2 receptors which include endothelial cells, pericytes, epithelial cells of GIT, muscle cells, T-cells, cardiomyocytes, and distal renal tubular cells (Hamming et al. 2004; Chen

et al. 2020; Ziegler et al. 2020). All these cells can be directly affected by this virus. Upon entry into ciliated cells of the upper respiratory tract, the virus undergoes local replication and propagation (Sims et al. 2005), and an immune response is mounted in the next 2–3 days. The asymptomatic affected individual may test positive for covid-19 during this period and can transmit the infection even when there is a low viral load.

Due to infection of the upper respiratory tract, symptoms such as fever, malaise, and dry cough are observed. At this stage, C-X-C motif chemokine ligand 10 (CXCL-10) and interferons (IFN-β and IFN-λ) are released from the virus-infected cells (Tang et al. 2005). Most patients do not progress further as the mounted immune response is ample and enough to restrict the spread of infection.

6.3 Cytokine Storm

In some cases, the virus infection may progress to invade the lower respiratory tract and the virus enters into the type 2 alveolar epithelial cells. The virus replication leads to the generation of more viral nucleocapsids. Consequently, the heavily affected alveolar cells synthesize and release a variety of cytokines and inflammatory markers. These include tumor necrosis factor-α (TNF-α), IFN-λ and IFN-β, CXCL-10, monocyte chemoattractant protein-1 (MCP-1), and multiple interleukins (IL-1, IL-6, IL-8, IL-120, and IL-12); (Yang 2020). The massive production of pro-inflammatory cytokines and chemokines leads to the so-called "cytokine storm" in which many defense cells such as neutrophils, CD4 helper T cells, and CD8 cytotoxic T cells are sequestrated in the pulmonary parenchyma. The body defense cells try to fight off the virus and may succeed to a larger extent, but in doing, so they also cause pulmonary damage. The reasons for the consequent lung damage include rapid viral replication and covid-19-induced downregulation of ACE-2 receptor resulting in shedding of its active ectodomain (Fu et al. 2020). The reduction of ACE-2 receptors is known to increase vascular permeability resulting in pulmonary edema. Another implicated mechanism involves the presence of anti-S protein neutralizing antibodies in the infected lungs which promotes pro-inflammatory response by binding to Fc receptors (FcR) expressed by macrophages and monocytes. Additionally, this FcR and anti-S-IgG complex may facilitate the continual viral replication in the lungs of patients (Fu et al. 2020; Takada and Kawaoka 2003). Due to the persistent infection, the infected host cells undergo apoptosis resulting in the release of viral particles. The viral particles then infect the remaining viable pneumocytes in the same manner. The constant injury triggered by both inflammatory cells and viral replication results in the loss of both type 1 and type 2 pneumocytes. The result is diffuse alveolar damage and hyaline membrane formation which eventually culminates in an acute respiratory distress syndrome (Cascella et al. 2020; Xu et al. 2020).

6.4 Acute Respiratory Distress Syndrome (ARDS)

ARDS is defined as respiratory failure induced by a known clinical insult that is graded based on the severity of arterial blood oxygenation. The severe stage is characterized by life-threatening respiratory insufficiency, cyanosis, and severe arterial hypoxemia that is non-responsive to oxygen therapy. In fatal human SARS-CoV-2 infections, about one-fifth of cases experience marked respiratory insufficiency requiring mechanical ventilation. The histopathology findings in such cases at autopsy show evidence of ARDS such as PAS-positive thick hyaline membrane formation (Xu et al. 2020; Ding et al. 2003; Ng et al. 2016). There is evidence that genetic predisposition and synthesis of inflammatory cytokines are closely associated with the occurrence of ARDS. The levels of the above mentioned cytokines and/or chemokine correlate directly with disease severity. Raised plasma IL-6 and IL-8 levels also contribute to the adverse outcomes of ARDS (Thompson et al. 2017). Such biomarkers of inflammation operating in the pathogenesis of SARS-CoV-2 infection indicate that a possible treatment is achievable for ARDS associated with covid-19 infection.

6.5 Immune Dysfunction

Zhu et al. (2020) and Huang et al. (2020) have documented that lymphopenia, a common finding in Covid-19 infection, is associated with the severity and mortality of this disease. High intensities of pro-inflammatory CD4+ T cells and cytotoxic CD8+ T cells have been found. These findings suggest that there is an overactivation of T cells as well as antiviral immune responses (Xu et al. 2020). Higher concentrations of highly proinflammatory CCR6+ and Th17+ CD4 cells have been found in Covid patients. In addition, CD8+ T-cells have been shown to contain high concentrations of cytotoxic granules with perforin and granulolysin positivity.

With this brief background of the pathogenesis of Covid-19 infection, it is apparent that the covid-19 virus can enter any cell that contains ACE-2 receptors. Therefore, multiple body organs are likely to be affected because many human cells possess ACE-2 receptors. A review by Tabary et al. (2020) has described that the lung is the primary target organ for Covid-19 infection, but based on autopsy data, it has been found that other organs such as brain, heart, kidney, skin, gastrointestinal tract, spleen, and blood can all be involved. Thus, a diverse range of pathological changes is observed in this infection. The pulmonary pathology comprises of diffuse alveolar damage, pulmonary microangiopathy, fibrin thrombi, and interstitial inflammation. Currently, pulmonary changes have been divided into early (exudative), intermediate (fibro-cellular), and late (fibrotic) stages based on histological and imaging findings during the course of the disease. Since endothelial cells have ACE-2 receptors, the viral entry in endothelial cells can produce endotheliitis and microthrombi formation in any of the organs, for example, brain, and therefore in some patients Covid-19 infection may manifest as stroke. The infection of epithelial

cells of GIT with new strains has manifested in many patients with gastric bleeding, small gut perforations, and gangrene.

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Pathology of COVID-19 Infection

7

B. D. Radotra

Abstract

COVID-19 pandemic which started in 2019 in Wuhan, China, later spread to other parts of the world as a major disaster. Two years back, it was considered predominantly a pulmonary disease, however, it is now thought to be a multi-system disorder. With the emergence of new mutants, the spectrum of disease has changed and widened. The interplay of cytokines and vasculopathy with the development of vascular thrombosis is chiefly responsible for diverse manifestations. The secondary infections in many patients, during as well as in the post-COVID period have resulted in higher morbidity and morbidity. A brief description of pathology in various organs is given below.

Keywords

COVID-19 · SARS-CoV-2 · Pathology · ARDS

7.1 Introduction

COVID-19, which is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is now very much known to be a systemic disease. Although it can involve any system in the body, particularly with the emergence of new mutants of the virus; it is the respiratory system that is the primary organ of involvement. This is the reason why most COVID-19 patients report to the hospital with symptoms referable to upper and lower respiratory tracts. The other body organs which can be

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affected are the brain, heart, kidney, GIT, liver, hemopoietic, and musculoskeletal system.

7.2 The Pulmonary System

It is well known that the virus enters primarily through the respiratory route, that is, aerosols and droplets, therefore, the respiratory system bears the brunt of the disease. Lung damage is the leading cause of death in the majority of the patients afflicted with COVID-19 infection. The pulmonary pathological features of COVID-19 have been studied mainly from autopsies conducted on the COVID-dead patients. The pathological findings closely resemble to those seen in SARS and MERS (Liu et al. 2020). The affected lungs appear heavy, edematous, and diffusely congested. The pleura may show dullness, but pleural effusion is not a common feature until complicated by secondary infection. The cut surface of the lungs exhibits irregularly distributed foci of consolidation. Areas of hemorrhage or infarction with apparent thrombosis in feeder vessels may be noted (Lax et al. 2020). The infarcts are not typically wedge-shaped because these are due to thrombotic occlusion of multiple smaller vessels rather than a single large vessel. If there are superadded infections, there may be abscesses or lobar pneumonia.

Histologically, four main morphological stages of pulmonary involvement are described:

1. an early stage (day 0–1): There is edema, initial epithelial damage, and evidence of capillaritis. At this stage, the interstitial inflammation may be minimal.
2. The stage of exudative diffuse alveolar damage (DAD) (days 1–7) is characterized by fibrin-rich edematous intra-alveolar fluid, macrophage exudation, and type II pneumocyte proliferation. Occasional multinucleated syncytial giant cells, as well as enlarged pneumocytes containing large nuclei, amphophilic cytoplasm, and prominent nucleoli, are noted. These cells may show cytopathic changes but generally do not contain apparent viral inclusions. Hyaline membrane formation (Fig. 7.1) occurs during this phase (Menter et al. 2020; Polak et al. 2020; Borczuk et al. 2020). Microthrombosis may be frequently observed with associated intra-alveolar hemorrhages. Areas of dilated alveolar ducts and collapsed alveoli can occur side by side.
   The presence of occasional intravascular megakaryocytes (Valdivia-Mazeyra et al. 2021) is not an uncommon finding. It may be remembered that neither presence of intrapulmonary megakaryocytes nor microthrombi are specific for COVID-19 as it is known to occur in DAD of other causes (Valdivia-Mazeyra et al. 2021; Hariri et al. 2021). The inflammatory cells in the exudative phase consist of CD3 positive lymphocytes and rare plasma cells located in alveolar walls. In addition, the alveolar spaces are filled with macrophages which are CD68+, CD163+, and CD206+ (Pandolfi et al. 2020; Bradley et al. 2020).
3. The organizing stage (1 to several weeks): This stage is characterized by interstitial and intramural fibrosis (Fig. 7.2). The alveoli are filled with loose connective

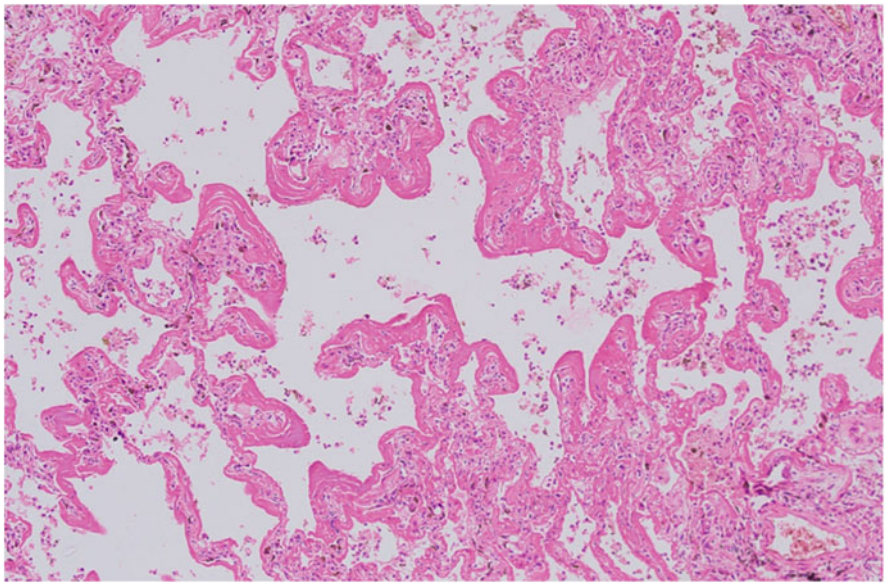

Fig. 7.1 Extensive hyaline membrane formation in lungs during the exudative phase of COVID-19 infection

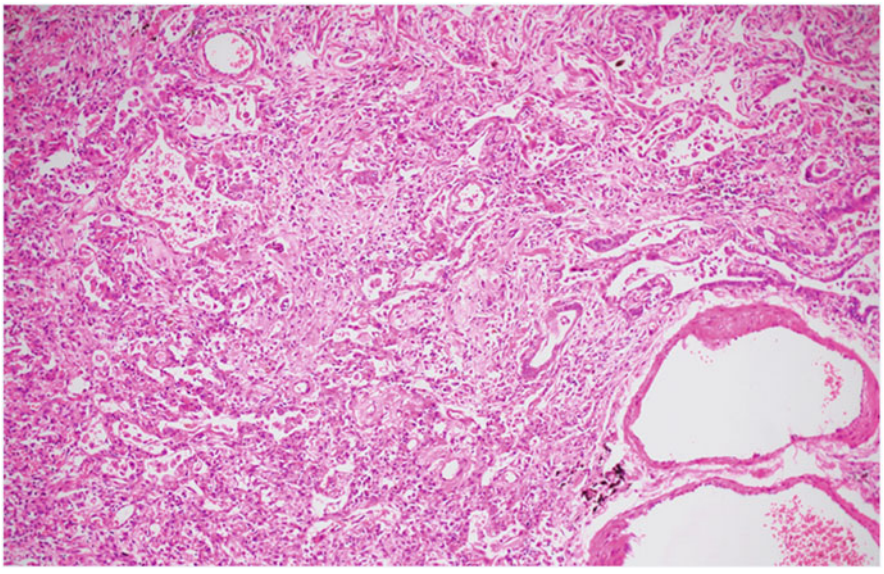

Fig. 7.2 Interstitial and intra-alveolar fibrosis of the lungs in organizing stage of alveolar damage

tissue. Interstitial myofibroblastic proliferation follows and bronchopulmonary squamous metaplasia may set in (Lax et al. 2020; Carsana et al. 2020).

4. The fibrotic stage of DAD (weeks to months): there is the deposition of septal collagen. The lungs show diffuse fibrosis and the alveolar architecture is lost. At this stage, the lungs fail to expand.

   It is pertinent to mention that different stages of COVID-19 pathology may show some overlap. Such overlap is known to happen with other diffuse interstitial lung diseases as well, at autopsy examination. Actually, the stages represent a continuum of the disease process which is very much evident from postmortem tissues. The excised antemortem biopsy samples where only limited material is examined may not show the spectrum of pathology of the entire lung, based on which staging is done. This reason explains the overlap of different stages in pulmonary findings at autopsy. Therefore, the DAD manifestations frequently coexist side by side with the organizing stage, a reflection of the temporal heterogeneity of COVID-19 (Lax et al. 2020; Calabrese et al. 2020; Ye et al. 2020).

   Fungal or bacterial superadded infections usually take over in patients in ICUs and lead to bronchopneumonia. The incidence of superadded infections in autopsy series ranges from 32 to 57% (Skok et al. 2020). The incidence of pulmonary embolism and microthrombi are evident in 20% (Hariri et al. 2021) and 57% (Edler et al. 2020) of COVID-19 patients, respectively. Pulmonary thromboembolism may be the direct cause of death in many patients.

7.3 Extra Pulmonary Involvement

7.3.1 The Cardiovascular System

It has been documented that COVID-19 contributes to cardiovascular complications, including acute myocardial injury, acute coronary syndrome, myocarditis, stress-cardiomyopathy, arrhythmias, and cardiogenic shock (Kang et al. 2020). Zou et al. (2020) published a comprehensive review and meta-analysis on the incidence, comorbidities, outcomes, and possible mechanisms of acute cardiac injury in COVID-19 patients. They concluded that the risk of cardiac injury in COVID-19 hospitalized patients is alarmingly high, particularly in old age; furthermore, that the incidence is similar in the Chinese and Western populations. Myocarditis in COVID-19 positive patients with raised troponin and ECG changes has been reported by Doyen et al. (2020). The myocarditis may be multifocal and mainly lymphocytic in nature (Fig. 7.3). Other documented histological findings in COVID-19 patients are hypertrophied cardiomyocytes along with inflammatory infiltrate, focal edema, interstitial hyperplasia, fibrosis, degeneration, fibrin thrombi, and necrosis (Fig. 7.4). The coronary artery, endocardium, and pericardium may also be affected (Yao et al. 2020a, b; Tavazzi et al. 2020).

Since the vascular endothelial cells express ACE-2 receptors, endothelial cells are an easy target for the SARS-CoV-2 virus (Hamming et al. 2004) and thus the presence of viral inclusions along with inflammatory cells and apoptotic bodies in

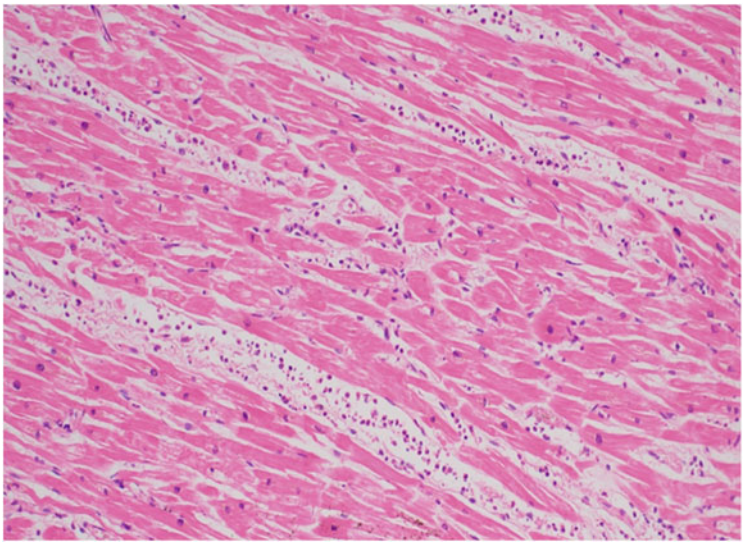

Fig. 7.3 Myocarditis showing myonecrosis, infiltration by lymphocytes and plasma cells along with interstitial edema

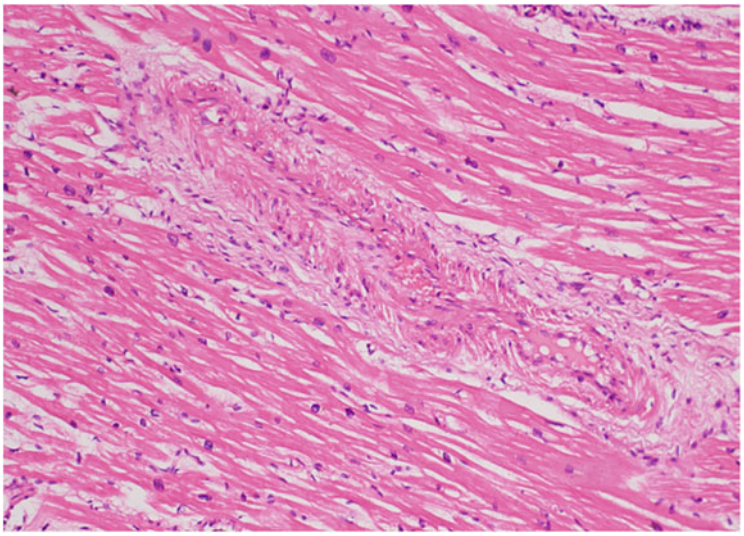

Fig. 7.4 Heart: Interstitial vessel showing organizing fibrin thrombus with luminal occlusion

these cells can be easily explained (Xiao et al. 2020). An increased risk of venous thromboembolism in COVID patients is largely due to prolonged immobilization, hypercoagulable status, active inflammation, and propensity for DIC.

7.3.2 The Nervous System

CNS involvement is being increasingly reported in COVID-19 patients and symptoms may vary from mild to severe in nature. Headache, anosmia, dizziness, dysgeusia, confusion, and impaired consciousness have all been reported in COVID-19 patients. Studies from China, France, and other European countries have reported varying percentages of neurological signs and symptoms (Mao et al. 2020; Helms et al. 2020; Paterson et al. 2020). The pathological disorders described in COVID-19 patients include stroke, Guillain-Barre syndrome (GBS), meningoencephalitis, acute hemorrhagic necrotizing encephalopathy, and cerebral venous thrombosis (Lou et al. 2021).

Meningitis and meningoencephalitis are infrequent manifestations of COVID-19 infection. Except for mild edema, the external examination of such a brain may not show any abnormality. The meningitis is usually mild and lymphocytic in nature. In meningoencephalitis cases, microglial activation, microglial proliferation with microglial nodule formation, perivascular, and parenchymal lymphocytic infiltration are noticed like any other encephalitides. In routinely stained slides of brain tissue, viral inclusions are not found, however, the SARS-CoV-2 has been detected in the brain by RT-PCR, immunohistochemistry, and electron microscopy (EM). The regions of the nervous system where the virus has been detected by these techniques include olfactory epithelium, olfactory bulbs, olfactory tubercle, frontal lobe, cerebellum, medulla, cranial nerves, and trigeminal ganglia. The virus may be present in other areas of the brain but there is no information about it due to the paucity of sampling from other areas at autopsy. The virus has also been demonstrated in cerebral endothelial cells. The combination of endotheliopathy and COVID-19 associated coagulopathy leads to thrombi formation resulting in either hemorrhage or infarcts which are more devastating and fatal manifestations of COVID-19 infection. The size of the hemorrhage may be small or large. Similarly, the size of infarcts will also vary from small to large depending upon the size of the vessel involved by coagulopathy. Pre-existing comorbidities such as hypertension, diabetes, and atherosclerosis will influence the outcome of the stroke. The brain gross and microscopic pathology will depend on the type of stroke in these patients, whether it is ischemic or hemorrhagic.

Peripheral neuropathy, demyelinating polyneuropathy, ascending paralysis, facial paresis, ophthalmoplegia have all been reported in COVID-19.

7.3.3 Musculoskeletal System

As with other viral infections, myalgia is a common manifestation of COVID-19 infection. It may be mild but at times it is very severe. Myositis, rhabdomyolysis, necrotizing autoimmune myositis, hematoma, gangrene, and COVID toes have all been described (Fig. 7.5). Elevated creatine kinase levels are noted, and acute kidney failure may ensue when rhabdomyolysis occurs. The myopathic process and myonecrosis can be diagnosed by imaging techniques (Ramani et al. 2021). The

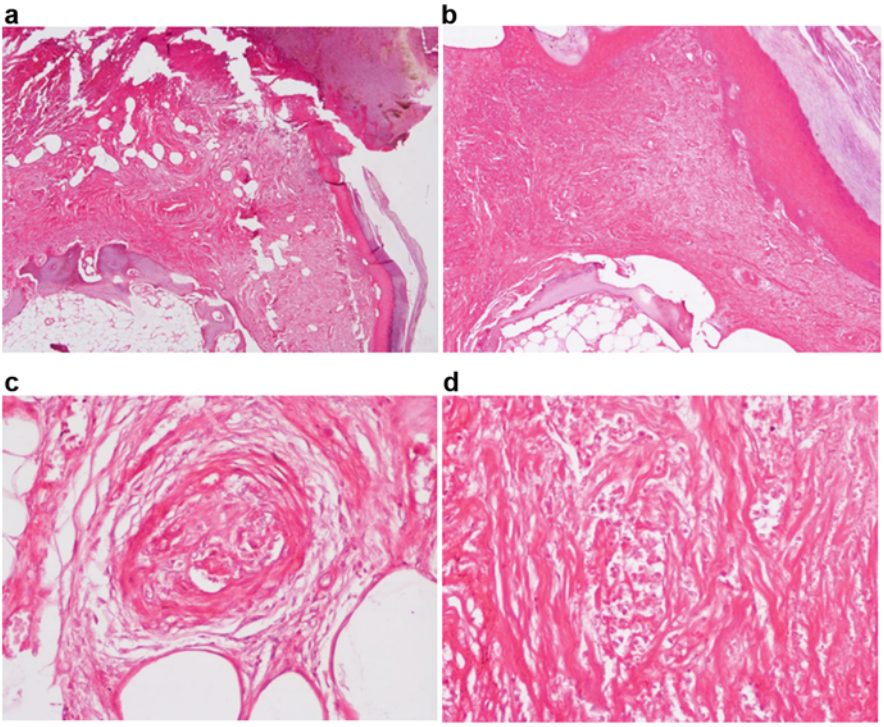

Fig. 7.5 COVID toe: (**a**) Gangrene of the toe showing necrotic skin and underlying tissue. (**b**) higher magnification of **a**. (**c**) Vascular thrombosis in underlying soft tissue. (**d**) Necrosis and neutrophilic infiltration of soft tissue

long-term survivors may demonstrate muscle loss and atrophy. Such patients may manifest with diaphragm dysfunction, respiratory inefficiency, and weaning off from ventilation may be difficult in them. The mechanism of muscle involvement may be due to the direct attachment of SARS-CoV-2 through ACE-2 receptor on a myocyte; however, an immune-mediated mechanism may be an alternate pathway. A small number of patients have shown arthralgia and arthritis as a symptom in COVID-19 infection. Serology needs to be done to exclude other causes of arthritis including other infections. The thrombotic events in COVID-19 infection and vasopressor medications given for hemodynamic support may lead to gangrene formation particularly in patients who have prior peripheral vascular disease, atherosclerosis, and diabetes.

7.3.4 The Liver

There is a dearth of literature about liver pathology and liver parenchymal changes induced or related to SARS-CoV-2. It appears that liver failure is not a main concern

and the liver is not the target of significant inflammatory damage by the virus. The pathological findings observed in liver tissue are highly suggestive of marked derangement of the intrahepatic blood vessel network, secondary to systemic changes induced by the virus (Sonzogni et al. 2020). The gross examination of the liver does not reveal any significant findings; however, it shows varying degrees of steatosis, congestion, ischemia, and fibrosis in the subset of cases. The other histological findings include lobular necro-inflammation and minimal-to-mild portal inflammation. Lobular cholestasis, sinusoidal dilatation, venous flow obstruction, newly organized thrombi, and granulomatous inflammation may be present in some cases (Lagana et al. 2020).

7.3.5 The Kidney

Acute kidney injury (AKI) is a common symptom in COVID-19 infection which occurs in 0.5–80% of patients (Sharma et al. 2021). Among kidney lesions, acute tubular injury is the most common pathology. Microscopic findings include diffuse proximal tubule injury with loss of brush border, non-isometric vacuolar degeneration to even frank necrosis (Su et al. 2020a, b). The Collapsing glomerulopathy and thrombotic microangiopathy are other notable lesions in both antemortem and postmortem tissues. The glomeruli show swollen endothelial cells with the presence of fibrin in glomerular capillaries. There is an edematous expansion of the interstitial spaces in distal collecting tubules and collecting ducts (Yao et al. 2020a, b). Non-specific fibrosis along with lymphocytic infiltrates may be found beneath the renal capsule.

Other rare findings such as anti-neutrophil cytoplasmic antibody vasculitis and anti-glomerular basement membrane disease are described. Occasional findings include segmental fibrin thrombus, podocyte vacuolation, focal segmental glomerulosclerosis, and shrinkage of capillary loops with the accumulation of plasma in Bowman's space (Yao et al. 2020a, b; Santoriello et al. 2020). Although direct viral infection of the kidney is possible and the virus has been demonstrated in some studies, it is certainly not a commonly reported finding.

7.3.6 The Gastrointestinal System

Although it is well known that most COVID-19 infected patients present with respiratory symptoms, some patients particularly those harboring new mutant strains manifest primarily with gastrointestinal (GI) symptoms like diarrhea, loss of appetite, nausea/vomiting, and abdominal pain. This is attributable to the high expression of ACE-2 receptors on GIT epithelial cells (Su et al. 2020a, b). Pathologically, the gastric mucosa shows congestion with a few bleeding points. There may be epithelial degeneration, necrosis, and shedding of the mucosa. The lamina propria and submucosa reveal infiltration by lymphocytes, monocytes, and plasma cells in the esophagus and stomach. Recently, we have seen many cases of intestinal

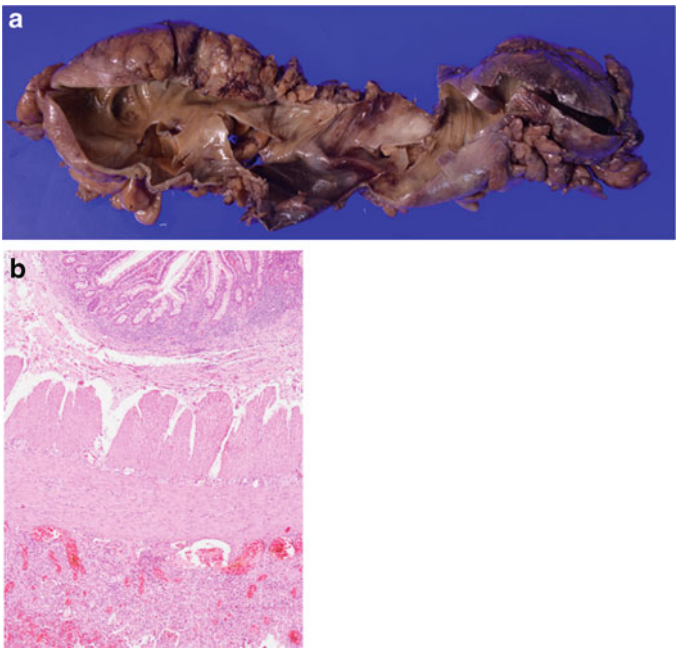

Fig. 7.6 (**a**) Resected segment of the intestine showing dull serosa. (**b**) Acute serositis of intestine due to perforation

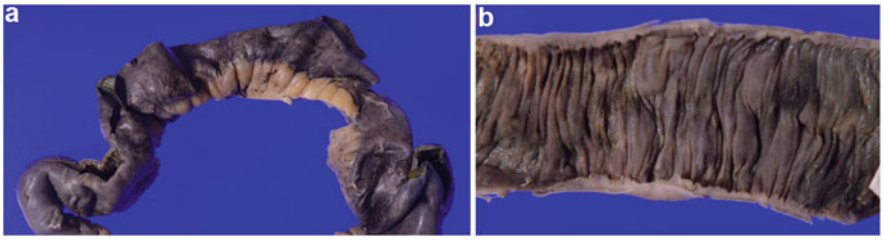

Fig. 7.7 (**a**) resected gangrenous ileum showing a blackish discoloration of the outer aspect. (**b**) On opening, there is mucosal edema and patchy ulceration

perforations presenting with acute abdomen and peritonitis (Fig. 7.6). The intestine also manifests with gangrene which develops as a result of microthrombi known to occur in this disease. (Figs. 7.7 and 7.8). It is also reported that mucosal epithelial cells of the gastrointestinal tract may be apparently normal with occasional inflammatory infiltrates (Yao et al. 2020a, b). The endocrine pancreas may show evidence of tissue degradation.

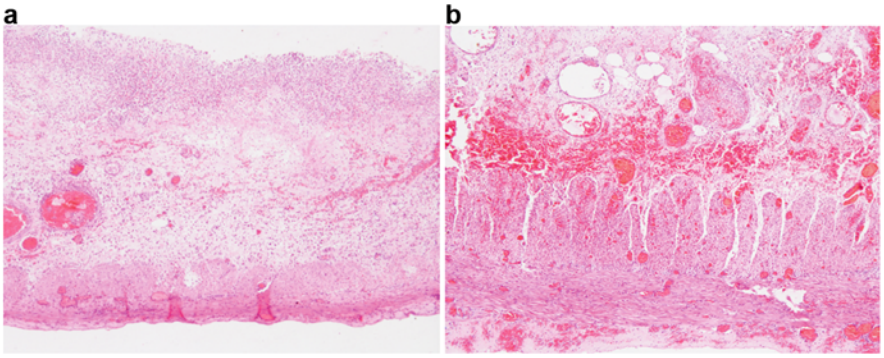

Fig. 7.8 (**a**) Section of ileum showing loss of mucosa, submucosal edema, and muscle necrosis. (**b**) Myonecrosis, submucosal hemorrhages, and vascular thrombi are seen

7.3.7 The Skin

The skin biopsies show a wide spectrum of histopathological patterns in COVID-19 infected patients. Prominent dilated blood vessels with a swollen endothelial layer and congested vessels are common findings. Perivascular infiltration by cytotoxic CD8+ lymphocytes and eosinophils is noted. In a subset of cases, diffuse coagulopathy affecting small vessels is evident. In the early phases of the disease, numerous collections of Langerhans cells in the epidermis can be seen after being activated by the virus (Gianotti et al. 2020). As a result of endothelial damage and microthrombi formation, the skin biopsies demonstrate ulceration, apoptotic keratinocytes, small vessel vasculitis, RBC extravasation, and lobular panniculitis (Fig. 7.9).

7.3.8 The Genital System (Testis)

All SARS-infected testes demonstrate histological findings with extensive germ cell destruction and decreased spermatogenesis in the seminiferous tubules. The basement membrane shows thickening and peritubular fibrosis may set in. Leucocytic infiltration and vascular congestion in the interstitial tissue are other histological findings. The Sertoli cells show swelling, vacuolation, and cytoplasmic rarefaction (Shen et al. 2020).

7.3.9 The Hematopoietic System

Recently, Elsoukkarya et al. (2021) have described pathological findings of 32 patients at autopsy from a single center. They found variable degrees of autolysis in tissue from spleen and lymph nodes. However, in preserved areas intact lymphoid follicles with centrally located germinal centers were seen. The subcapsular and

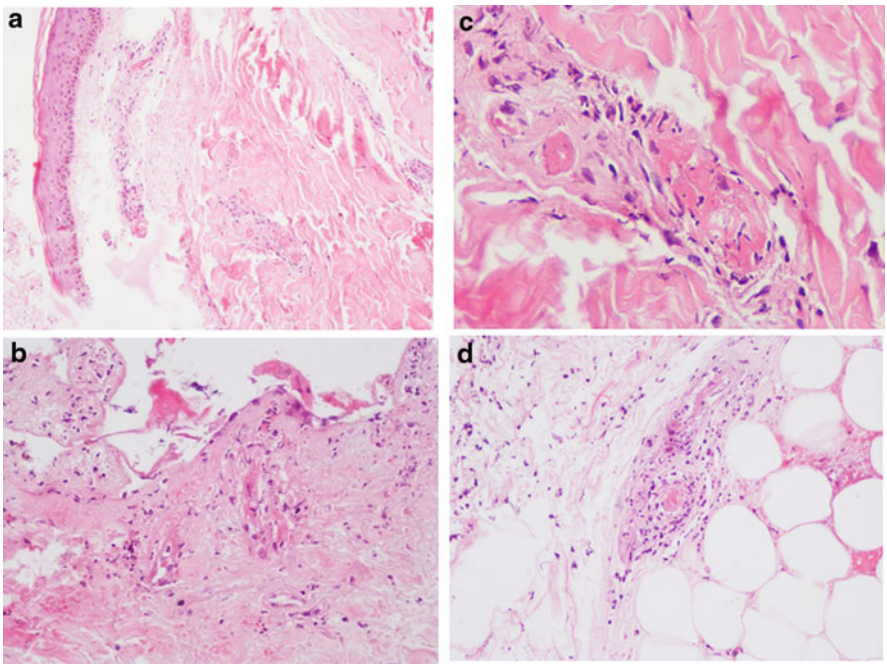

Fig. 7.9 (**a**) Skin biopsy from a COVID positive patient showing preserved epidermis and mild upper dermal infiltrate. (**b**) Complete ulcerated epidermis with only a few keratinocytes attached to basal lamina. (**c**) Small vessel vasculitis and microthrombi and (**d**) panniculitis and vascular occlusion with fibrin

intraparenchymal sinuses were frequently expanded and often contained a variable number of larger transformed cells with prominent nucleoli and amphophilic cytoplasm. The paracortical areas contained largely small lymphocytes or plasma cells. In other studies, lymphocyte depletion involving specific compartments with increased phagocytosis and sinus histiocytosis were prominent findings. Medullary areas of lymph nodes show the prominence of plasma cells and histiocytes.

Examination of the spleen has shown the reduction of cell composition, atrophy of white pulp, and infiltration by neutrophil and plasma cells. Red pulp congestion and an increase in red pulp to white pulp proportion are noted. The depletion of T and B cells occurs due to necrosis, apoptosis, and atrophy of corpuscles in the spleen of infected cases. Bone marrow samples showed reactive changes with tri-lineage hyperplasia, prominence of plasma cells, and histiocytes (Tabary et al. 2020; Hanley et al. 2020).

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COVID-19: Clinical Spectrum—It's Multiorgan Syndrome

8

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Abstract

COVID-19 outbreak caused by SARS-CoV-2 has emerged as a global challenge for the entire health care system worldwide. It has affected the developed as well as developing countries markedly. What began as pneumonia-like illness later evolved into a multiorgan disease leading to severe morbidity and even death. The clinical data and global literature explicitly suggest that in addition to respiratory symptoms, the COVID-19 patients may present with hematological, cardiovascular, renal, gastrointestinal, neurological, ocular, and skin manifestations. The underlying mechanism for multisystem involvement is the expression of angiotensin-converting enzyme 2 (ACE2) receptors at multiple extrapulmonary tissues. Injury to various organs may be attributed to cytokine storms or to disturbances of coagulation and vascular endothelium. The aim of this review is to emphasize the impact of SARS CoV-2 infection on not only the lungs but other organ systems too.

Keywords

COVID-19 · SARS-CoV-2 extrapulmonary manifestations · Multisystem disorder · Symptoms

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8.1 Introduction

The COVID-19 pandemic caused by novel Coronavirus (SARS-CoV-2) began as viral pneumonia initially reported from Wuhan city of China in December 2019. Within a period of few weeks, it engulfed the whole world and this global health crisis is still ongoing. The transmission of the virus primarily takes place through the aerosols via the respiratory route. As per the report of WHO on COVID-19, the severity of COVID-19 in patients ranges from completely asymptomatic to severe pneumonia and death (WHO report 2020). Initially, it was considered as a lung disease but later as the understanding of the disease evolved, it has been found to involve various other organs. The plausible mechanism for multisystem involvement is the expression of ACE2 at multiple extrapulmonary tissues. Apart from lung involvement, COVID-19 patients may present with hematological, cardiovascular, renal, gastrointestinal and hepatobiliary, endocrinological, neurological, ocular, and skin manifestations making it a multisystem disorder (Hoffmann et al. 2020; Guan et al. 2020).

Case definitions of COVID-19 as per the criteria defined by WHO are as follows:

1. Confirmed case: A person with positive RT PCR or SARS CoV rapid antigen test with or without clinical signs and symptoms.
2. Probable case: A person who tests inconclusive for COVID-19 virus but fits the clinical criteria and is a contact of a confirmed case.
3. Suspect case: A person who has an acute respiratory illness and has a history of contact with a confirmed or probable COVID-19 case.

8.2 Clinical Presentation

Initially, animal to human transmission was presumed to be the main mode of transmission linked to the seafood market of Wuhan. However, this could not be established in the absence of insufficient evidence and human-to-human transmission was believed to be the reason for the spread of this deadly virus. Based on the data available, the incubation period is said to vary from 3 to 7 days (median 5.1 days) and up to 2 weeks as the longest time from infection. The majority of the cases occur in about 4–5 days after getting exposed to the virus. The clinical presentation of COVID-19 patients is the variable and a large number of the cases may be asymptomatic (Midha et al. 2020). There is no clear definition of the term "asymptomatic" in regard to COVID-19 infection. Some of the patients who do not present with usual signs and symptoms of Coronavirus infection may or may not show COVID-19 changes on radiological imaging. The classic example of asymptomatic infection due to COVID-19 has been observed on the outbreak on a cruise ship. Out of the total 712 patients who tested positive for SARS CoV, 58% did not have any symptoms of COVID-19 (Batista et al. 2020). Additionally, a substantial number of all pregnant women screened at the time of delivery were found to be

asymptomatic, though confirmed positive for COVID-19 (Lopes de Sousa et al. 2020).

The clinical spectrum of symptomatic COVID-19 patients is quite variable. Most of the patients with SARS CoV infection experience mild to moderate disease without any need for admission to the hospital. The symptoms most commonly seen in these patients include fever, cough, sore throat, malaise, and so on. Other clinical findings are myalgia, headache, body ache, ageusia (loss of taste), anosmia (loss of smell), skin rash, or bluish discoloration of fingers or toes (Giacomelli et al. 2020). The severe form of the disease will include symptoms like chest pain and dyspnea and some severe cases present with atypical symptoms without fever such as loss of appetite, reduced alertness, delirium, fatigue, and so on, especially in immune-compromised and geriatric patients (García 2020). Pediatric patients are either asymptomatic or are mildly symptomatic. Based upon the clinical presentation of COVID-19, the manifestations can be divided into pulmonary and extrapulmonary. The disease presentation of COVID-19 on various organs of the human body has been illustrated in Fig. 8.1.

8.2.1 Pulmonary Manifestations

The patients infected with SARS-CoV-2 most commonly present with fever, cough, sore throat, dyspnea, and fatigue. In severe cases, the disease progresses to acute respiratory distress syndrome (ARDS) and respiratory failure. It is well known that initial manifestations of COVID-19 are similar to Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) but the impact of COVID-19 is much more as it affects multiple organs of the body (Johnson et al. 2020). Nevertheless, a subtle indicator of COVID-19 infection is the development of dyspnea after experiencing mild flu-like symptoms. SARS CoV-2 infection leads to lung fibrosis in severely ill patients. Although fibrosis is a result of a normal repair mechanism yet the mechanism of pulmonary fibrosis in COVID-19 patients is not clearly understood. It may be the result of viral and immune-mediated mechanisms. Several other factors like advanced age, the severity of illness, smoking, and so on can predispose these patients to severe lung damage and pulmonary fibrosis in COVID-19 survivors (Ojo et al. 2020). A rare finding observed in critically ill COVID-19 patients is mediastinal lymphadenopathy. This is described as a reactive phenomenon to viral disease and inflammation (Valette et al. 2020). The clinical presentation of COVID-19 has been further classified into mild, moderate, severe, and critical illness (Acute respiratory distress syndrome, ARDS) (Guan et al. 2020; García 2020).

Mild/uncomplicated illness: This would include symptoms like mild fever, sore throat, dry cough, nasal congestion, headache, malaise, loss of taste and or smell, and gastrointestinal disturbance.

Moderate disease: Such patients usually present with respiratory symptoms like cough, dyspnea, and tachypnea.

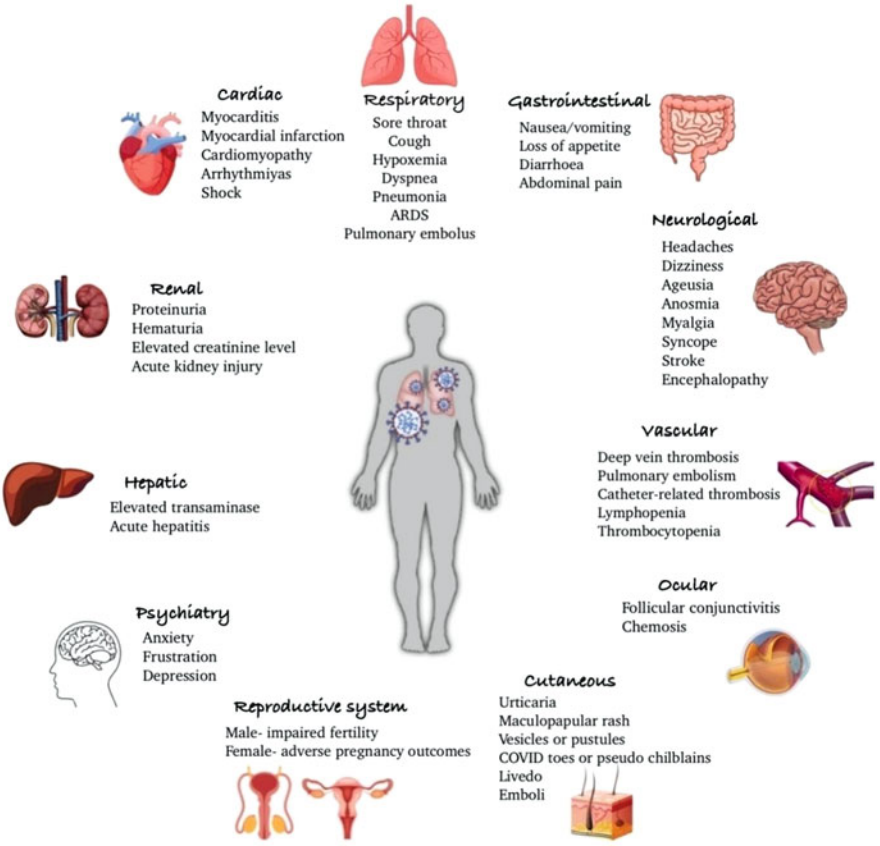

Fig. 8.1 Effect of SARS-CoV-2 infection on various organs of the human body leading to multi-organ failure in severe cases

Severe disease: These patients usually present with severe pneumonia, acute respiratory distress syndrome (ARDS), sepsis, or septic shock. Most common clinical presentations include the presence of severe dyspnea, tachypnea, respiratory distress ($SpO_2 \leq 93\%$, $PaO_2/FiO_2 < 300$), and/or more than 50% lung infiltrates within 24–48 h of onset of symptoms.

ARDS: This is a clear sign of onset or worsening of respiratory failure. According to one study, CT scan of 86% of these patients shows ground-glass opacity, 29% show consolidation and 19% have crazy paving. Bilateral involvement is seen in 76% of patients and peripheral disease distribution in 33% (Li and Ma 2020).

8.2.2 Extrapulmonary Manifestations

8.2.2.1 Gastrointestinal and Liver

The manifestation of gastrointestinal (GI) symptoms and liver involvement in COVID-19 is variable (3–70%) and is associated with the severity of the disease (Tian et al. 2020). This disease can cause acute gastritis and enteritis, leading to nausea, vomiting, diarrhea, pain abdomen, abdominal distention, and constipation. Some patients present with gastrointestinal symptoms at the onset of disease without any respiratory symptoms and some develop GI symptoms as the disease progresses (Mao et al. 2020). Mesenteric ischemia with severe hypotension has also been reported as one of the gastrointestinal manifestations of COVID-19 in patients with other comorbidities like type 2 diabetes mellitus, obesity, hypertension, and hepatic cirrhosis in the absence of any pulmonary manifestation (Norsa et al. 2020). Another rare gastrointestinal manifestation of COVID-19 is hemorrhagic colitis (lower GI bleed) reported in patients with comorbid conditions. Patients may experience flaring up of inflammatory bowel disease, that is, ulcerative colitis and Crohn's disease during COVID-19 infection (Carvalho et al. 2020; Gulen and Satar 2020). Hepatic involvement in COVID-19 has also been reported in the substantial number of patients and is presented as increased transaminase levels and acute hepatitis. One-third of critically ill COVID-19 patients suffer from hepatocellular injury and cholestatic liver dysfunction. Patients with underlying chronic diseases such as cirrhosis of the liver and autoimmune hepatitis need to be watchful of acute flare and decompensation (Fan et al. 2020).

The presence of angiotensin-converting enzyme 2 (ACE 2) receptor for viral transmembrane S-protein on the surface of certain intestinal epithelial cells in the ileum and colon is responsible for direct invasion and multiplication of the virus in the gut epithelium, leading to degeneration and necrosis of the gastrointestinal mucosa. The presence of ACE 2 receptors on oral mucosa could also explain the basic pathogenesis behind loss of taste in SARS-CoV-2 infection. Further, these receptors are also expressed on the cholangiocytes, which might indirectly dysregulate the liver function. However active multiplication of the virus has not been proven inside the hepatocytes. Cytokine storm and ischemia/ hypoxia due to respiratory complications of COVID-19 can also damage the liver. However, the possibility of liver damage due to the use of certain hepatotoxic drugs like acetaminophen, antivirals, antibiotics, steroids, and certain herbal medications during the management of COVID-19 cannot be ruled out (Chai et al. 2020; Zhao et al. 2020; Gupta and Kaushal 2021).

8.2.3 Renal Manifestations

The renal abnormalities associated with COVID-19 range from mild proteinuria, hematuria to acute renal injury (AKI). These renal manifestations can also be a marker of multi-organ dysfunction (MOD) and severe renal disease can occur often requiring renal replacement therapy (RRT). It is reported that up to 20% of ICU

patients with COVID-19 require RRT. The mortality is reported to be higher in patients of COVID-19 with acute kidney injury. According to a recent data analysis, coexpression of ACE2 and transmembrane protease serene 2 (TMPRSS) genes in renal tubular cells and kidney podocytes is necessary for the entry of SARS CoV-2 into host renal tissue.

8.2.4 Cardiac Manifestations

Cardiovascular manifestations in COVID-19 patients are more substantial than other extrapulmonary clinical manifestations. Approximately 12% of COVID-19 patients present with elevated levels of troponin T (TnT), C-reactive protein (CRP), NT-proBNP, or have myocardial infarction though they do not have any history of cardiovascular disease. The levels of these cardiac markers positively correlate with the severity of COVID-19 infection and adverse outcomes of the COVID-19 patients. Complications such as ARDS, ventricular arrhythmias, acute coagulopathy, and AKI are seen more frequently in patients with elevated TnT levels. COVID-19 may manifest itself as a cardiac event even in absence of lower respiratory tract symptoms.

Clinical manifestations like myocarditis, myocardial infarction, heart failure, shock, and increased incidence of cardiac arrhythmias are seen in COVID-19 patients. Cardiac arrhythmias sometimes go unnoticed in asymptomatic patients or maybe overshadowed by pulmonary manifestations (Gao et al. 2020; Zheng et al. 2020).

Multiple factors associated with the underlying pathophysiology of cardiovascular manifestations observed in COVID-19 patients have been reported. The presence of ACE2 receptors on cardiac myocytes, endothelial cells, smooth muscle cells, and fibroblasts explain the direct entry of virus-mediated injury in COVID-19 patients (Gallagher et al. 2008). Myocarditis in COVID-19 is presumed to be positively correlated with the viral load. Additionally, few autopsy studies have reported the isolation of the virus from the myocardial tissue thus lending credence to direct invasion and subsequent damage to cardiac tissue. Cytokine storm due to systemic inflammatory response syndrome is another presumed mechanism of myocarditis in these patients. Furthermore, higher levels of ACE2, in patients with prior cardiovascular disease may be one of the predisposing factors leading to more severe COVID-19. Moreover, ARDS, pulmonary thromboembolism, or vascular endothelial/smooth muscle tissue injury may lead to isolated right ventricular dysfunction (Gupta et al. 2020; Epelman et al. 2008; Walters et al. 2017) Disproportionately increased hypercoagulability in COVID-19 may further exaggerate the risk of myocardial infarction which otherwise also increases due to viral infections in general. In addition, many drugs used in the treatment of COVID-19 may result in prolonged ventricular repolarization and hence arrhythmias (Gupta et al. 2020; Ullah et al. 2020; Fried et al. 2020).

8.2.5 Vascular System

Elderly and immobile COVID-19 patients may frequently present with venous thromboembolism (VTE), a cardiovascular or respiratory complication due to increased coagulopathy. The occurrence of VTE was more commonly seen in lobar and segmental pulmonary arteries as compared to smaller subsegmental arteries. Some of the patients including few pediatric patients presented with Kawasaki-like disease/Kawasaki shock-like syndrome with coronary artery aneurysms. Luminal dilation/engorgement or mural thickening, subsegmental vessel enlargement microvascular dilation has been a frequent finding on the CT chest of the patients with confirmed COVID-19. The possible multifactorial mechanism responsible for vascular manifestations in COVID-19 patients includes expression of ACE 2 receptors on endothelial lining the vascular beds of different organs recruitment of immune cells to a site of viral infection, inducing apoptosis of endothelial cells, the release of cytokines and chemokines, excessive activation of the complement system leading to elevated plasma levels of lactic dehydrogenase, dimerized plasma fragment D (D-dimer), and decreased platelets (Tal et al. 2020; Porfidia and Pola 2020; Verdoni et al. 2020; Jones et al. 2020).

8.2.6 Neurologic Manifestations

Various neurological manifestations have been reported in COVID-19 patients. SARS CoV-2 can affect both the central nervous system (CNS) and peripheral nervous system (PNS). CNS related manifestations include headache, confusion, hallucinations, impaired consciousness, acute cerebrovascular disease and epileptic seizures, ataxia, acute disseminated encephalomyelitis (ADEM), and encephalopathy, whereas PNS-related manifestations are chemosensory disorders like hyposmia/anosmia, hypogeusia/ageusia, hypoplasia, neuralgia, myalgia, and Guillain-Barre syndrome (acute inflammatory demyelinating polyneuritis) (Wu et al. 2020a, b; Azhideh 2020; Sedaghat and Karimi 2020). The affinity of the virus for nervous tissue is again explained by the expression and distribution of the ACE2 receptor in neurons, astroglial cells, microglial cells, endothelial cells, and skeletal muscle tissue. According to reports, some COVID-19 patients directly present with neurologic symptoms whereas others with severe COVID-19 infection develop respiratory failure which might lead to cerebral hypoxia. Older individuals with comorbidities have a higher risk of impaired consciousness, delirium, and encephalopathy as a result of intracranial hemorrhages. COVID-19-associated epileptic seizures might be occurring due to the release of pro-inflammatory cytokines, tumor necrotizing factor α, and granulocyte colony-stimulating factors. Epilepsy/seizures may either be due to direct invasion of the virus to the brain or adverse drug reaction of antiviral drugs. Cytokine storm may also be responsible for acute necrotizing encephalopathy (ANE) observed in few patients (Sharifi-Razavi et al. 2020; Poyiadji et al. 2020; Kansagra and Gallentine 2011).

8.2.7 Hematologic Abnormalities

The hematological manifestations of COVID-19 include lymphopenia and thrombocytopenia. Lymphopenia has been found to be an important immunological marker and predictor of severe disease and mortality in critically ill COVID-19 patients (Arentz et al. 2020). Some studies revealed low counts of CD4+ T cells and CD8+ T cells in patients with severe COVID-19 (Bhatraju et al. 2020). Leukocytosis though rarely seen, is sometimes observed in older patients with underlying chronic conditions. The incidence of thrombocytopenia is variable (mild to moderate) but is often associated with poor outcomes (Terpos et al. 2020; Lippi et al. 2020; Connors and Levy 2020).

Studies have revealed that there is an increased risk of vascular thrombotic events due to COVID-19. Pathologic processes though not clearly understood, might involve either direct vascular and endothelial injury thereby producing microvascular clots or apoptosis of endothelial and mononuclear cells due to inflammation. COVID-19 induced coagulopathy leads to increased prothrombin time, low platelet counts, and elevated levels of D-dimer which has been associated with poor prognosis (Yuki et al. 2020). It has been seen that the patients with severe COVID-19 infection and multiorgan failure rarely develop overt disseminated intravascular coagulation (DIC) unlike classic DIC induced by bacterial sepsis or trauma.

8.2.8 Cutaneous Manifestations

Skin lesions in COVID-19 patients could be the result of post-viral hyperimmune activation or use of disinfectants and other drugs. Following dermatologic findings have been reported in patients with COVID-19 (Genovese et al. 2021):

- Urticarial rash with intermittent to severe itching mainly involving the trunk and limbs. Histopathological findings of the lesions include vacuolar interface dermatitis associated with superficial perivascular lymphocytic infiltrate.
- Confluent erythematous or maculopapular rash with symmetrical lesions predominantly localized on the trunk and showing centrifugal progression. Sometimes the eruptions and pruritis coexist. Superficial perivascular lymphocytic and neutrophilic infiltrates are observed on histological examination.
- Chilblain-like acral pattern mainly involving the feet or sometimes hands have become a famous COVID-19 cutaneous manifestation in otherwise asymptomatic individuals. The common symptoms seen are pain, burning sensation, and pruritus. On histology, these lesions display focal vascular necrosis.
- Papulovesicular lesions, papules, vesicles, and pustules are not very common and these involve the chest, abdominal region, and back. Histopathological examination reveals acantholysis and dyskeratosis.
- Livedo reticularis/racemosa lesions may be observed in COVID-19 patients with severe coagulopathy.

- Generalized purpuric severe vasculitic lesions evolving into hemorrhagic blisters localized in the intertriginous regions are sometimes seen Histopathological examination reveals vasculitis, cellular infiltrates, fibrin, and endothelial swelling.

8.2.9 Reproductive System Involvement

Many genitourinary complications have been reported due to COVID-19 disease progression mostly as a result of hyper coagulopathy. Decrease in total testosterone and increase in serum luteinizing hormone have been reported. In addition, an interesting case of bilateral orchitis resenting with testicular pain has been reported. COVID-19 infection impacts the male reproductive system more than the female reproductive system. The reason may be a high expression of ACE2 receptors on spermatogonia and supporting testicular cells which makes the male reproductive system a good target for SARS-CoV-2 infection (Wang and Xu 2020; Zhang et al. 2020a, b). A study has highlighted the presence of SARS CoV 2 in the semen of male patients infected with COVID-19. However, no viral RNA could be demonstrated in a biopsy taken from testes of the COVID-19 patients thereby indicating that SARS-CoV-2 does not directly infect testes or the male genital tract.

Another study supports the presence of SARS-CoV 2 in the vaginal fluid although low expression of ACE2 receptors has been demonstrated in fallopian tubes, ovaries, vagina, and endothelium. SARS CoV-2 RNA has been detected in the breast milk of infected patients (Ma et al. 2020; Song et al. 2020; Bridwell et al. 2021).

8.2.10 Ocular Manifestations

Ocular involvement in COVID-19 is uncommon with prevalence varying between 0.7 and 3%. Conjunctivitis and conjunctival congestion has been reported as one of the initial presenting symptom of COVID-19 in some studies (Zhang et al. 2020a, b; Chen et al. 2020). Additional ocular symptoms include watery eyes, ocular irritation, chemosis, folliculitis, conjunctivitis, and foreign body sensation. On examination, unilateral or bilateral eye involvement is observed. Conjunctival congestion, epiphora, and mild eyelid edema are seen. The reports of COVID-19 patients presenting with keratoconjunctivitis have also been published. The route of entry of the virus into the eyes could be from aerosols and respiratory droplets or transfer of virus from respiratory tissue to ocular tissue through the nasolacrimal system and vice versa (Wu et al. 2020a, b; Belser et al. 2013).

8.2.11 Psychiatric Manifestations

COVID-19 patients present with a wide range of psychological manifestations during the acute phase of the illness as well as following recovery from

COVID-19. Acute psychological reactions to COVID-19 infection include fear, anxiety, irritability, stress, confusion, and low mood (Sahoo et al. 2020). Psychiatric manifestations that have been noted during the illness and hospital stay are anxiety, insomnia, aggression, irritability, confusion, and varying degrees of impairment of consciousness. COVID-19 patients may also exhibit attention deficits, memory impairments and are at risk of developing post-traumatic stress disorder (Xiao et al. 2020). Negative psychological consequences such as anxiety, anger, irritability, fear, boredom, aggression, stress depression, suicidal ideation, apathy, and burnout may be noted especially during long periods of self-quarantine (Philip and Cherian 2020).

8.3 Special Considerations

8.3.1 COVID-19 and Pregnancy

Although pregnant females are more susceptible to COVID-19 infection, but the disease presentation is the same as in nonpregnant women (Wang et al. 2020; Bunyavanich et al. 2020). The changes in the maternal immune system that occur during pregnancy make pregnant females more susceptible to COVID-19 illness There is a strong concern regarding the vertical transmission of COVID-19 from mother to fetus.

Histopathological examination of placental and fetal membrane samples from a few patients with SARS-CoV-2 infection have revealed the presence of viral RNA but no virus has been demonstrated in vaginal swabs and amniotic fluid COVID-19 positive pregnant women (Akhtar et al. 2020).

8.3.2 COVID-19 and Children

Few cases of COVID-19 have been reported in children. Infected children either remain asymptomatic or experience a mild illness as compared to adults. Some children develop a serious condition called Multisystem Inflammatory Syndrome in Children (MIS-C). The most probable reason for less severe manifestations of COVID-19 in children is that ACE2 receptors are still evolving as are the T cells and associated cytokines (Williams et al. 2020). According to a review report of 72,314 COVID-19 patients from the Chinese Center for Disease Control and Prevention, less than 1% of the patients were younger than 10 years of age (Wu and McGoogan 2020).

8.4 Conclusion

COVID-19 is a disease that has put the entire world into a halt. The presentation of COVID-19 is highly variable. This article summarizes the data available on almost all pulmonary and extrapulmonary manifestations of this enigmatic multisystem viral infection that continues to evolve as the pandemic is still ongoing. Multicentre collaborative studies on this disease are further required at the national and international levels in order to improve patient care. High-quality transparent and ethical studies by researchers and clinicians will help in decreasing the morbidity and mortality associated with COVID-19 and the global community will inch closer to success against this pandemic.

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Organ Involvement in COVID 19: Lung and Beyond

9

Ashish Bhalla and Vikas Suri

Abstract

Coronavirus Disease 19 (COVID-19) evolved into a pandemic to torment the world since the last 15 months and has resulted in millions of individuals getting affected. It has caused significant strain on the health care systems in developed and developing countries alike. It has killed more than two million patients globally. The potential of this virus to cause multi-organ dysfunction with associated significant mortality and morbidity has made it the most formidable enemy we have faced since the great plague. COVID-19 is becoming a mystery with its plethora of typical and atypical clinical presentations. Its ability to get attached to widely distributed human angiotensin-converting enzyme-2 (hACE2) receptors, has enabled it to cause multi-organ dysfunction and extensive disease. In this chapter, we review the pulmonary and extra-pulmonary manifestations of SARS-CoV-2 and try to elucidate organ-specific patho-physiology. Organ dysfunction leading to a myriad of cardiac dysfunction, symptoms related to gut and liver, nervous system involvement, renal and ocular injury is being discussed in this chapter. An effort to raise awareness of the potential to cause long covid syndrome is being made to identify the possible burden of morbidity we might have to experience post covid 19 pandemic.

Keywords

SARS CoV-2 · Corona virus · COVID-19 · Organ involvement

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9.1 Introduction

SARS-CoV-2 virus pandemic has had a major impact on health care systems around the world. Patients infected with coronavirus present with a variety of symptoms varying in severity. Majority of the patients remain asymptomatic or develop mild and moderate symptoms at the most. Only some develop severe symptoms (dyspnoea, hypoxia, or $> 50\%$ lung involvement on imaging) and critical illness (acute respiratory distress syndrome, respiratory failure, shock, or multiorgan system dysfunction. The number of pre-existing/coexisting conditions is strongly associated with SARS-CoV-2 severity and mortality. The highest levels of the SARS-CoV-2 virus are detected in the respiratory tract. The other organs may also show some level. The important organs involved are the kidneys, liver, heart, brain and blood. Possible organ tropism of the virus might influence the course of the disease and may aggravate potential underlying conditions. In this chapter, we aim to discuss the possible pathogenesis of the organs involved, the acute effects and the long-term effects.

9.2 Lung Involvement

It is the primary organ involved and leads to major clinical manifestations in the form of disease or disability. The severity associated with SARS-CoV-2 infection ranges from no symptoms or mild pneumonia (in around 80%) to severe disease (characterized by hypoxia in 15%), a critical disease associated with shock, respiratory failure is only in around 5% of patients. Patients usually present with cough (initially dry), fever, expectoration, fatigue and dyspnea. The frequency of these reported symptoms varies in different studies from different geographic areas. 20–41% of all hospitalized patients go on to develop acute respiratory distress syndrome (ARDS).

SARS-CoV-2 infection causes alveolar and interstitial inflammation. This results in fever, cough and dyspnoea. Levels of pro-inflammatory cytokines and chemokines are significantly increased in patients with COVID-19 infection. The dysregulated cytokine response 'cytokine storm' plays a central role in the pathology of COVID-19 lung. What triggers the 'cytokine storm', the exact mechanisms behind it have not been identified as yet. There is evidence of extensive hemophagocytosis but it is very distinct from the classic macrophage activation syndrome (MAS). In addition to this, an immune suppression stage characterized by lymphopenia, low CD4 and CD8 T cell counts is also noted in many patients, increasing the risk of bacterial super infection.

Autopsy studies have demonstrated acute interstitial pneumonia and diffuse alveolar damage (DAD). There is also significant macrophage infiltration, formation of hyaline membranes, alveolar wall oedema/thickening. These pathological changes manifest as severe hypoxemia. The microvasculature involvement with hyaline thrombosis is also noted in many studies on autopsy. Pulmonary haemorrhage, vessel wall oedema, intravascular neutrophil trapping and immune

cell infiltration are other important pathological changes observed. The pulmonary vascular changes can at best be described as 'diffuse pulmonary intravascular coagulopathy' (PIC). These vascular pathological changes are mostly limited to the lungs. Pulmonary vasculopathy is driven by the proximity of type II pneumocytes and the pulmonary vasculature. Extensive microthrombi may lead to pulmonary infarction, haemorrhage, pulmonary hypertension and secondary right ventricular stress. Hypoxemia, high flow oxygen and alveolar trauma due to mechanical ventilation also seem to contribute to the development of PIC.

Hypoxia is one of the most important presenting features of COVID-19 lung pathology, but interestingly, it is often very well tolerated by patients. Therefore, the term 'happy hypoxia'.

Lung compliance in covid 19 related ARDS is usually well preserved. Hypoxia-driven tachypnea allows high volumes and hypocapnia which fails to stimulate the sensation of dyspnea, a pathophysiological mechanism akin to hypobaric hypoxia observed at high altitudes.

Management is usually based on the duration of symptoms, type of lung injury and extent of lung injury. A high flow oxygen for hypoxia at early stages, simple and effective use of gravitational forces (using prone/lateral positioning), helps in effective diaphragmatic excursions, perfusion redistribution and better oxygenation. Awake proning has been incorporated in many hospital protocols and is very effective in preventing/delaying intubation and reversing hypoxemia. Patients with severe ARDS need higher PEEP volumes, prone positioning and may also need extracorporeal support in select settings.

The excessive increase in pro-inflammatory cytokines (IL-1 and, IL-6), interferon, TNF-α is an inflammatory over-reaction to SARS-CoV-2 infection. This dysregulated response ultimately leads to endothelial cell dysfunction, vascular endothelial damage, capillary leak and diffuse alveolar injury [35]. In this context, anti-IL-6 inhibitors (tocilizumab), inhibitors of JAK kinases (baricitinib), and corticosteroids have shown promising results in the management.

Not all symptoms resolve and may persist for a varying period depending on the severity of the pathology leading to Persistent or Long COVID (Fig. 9.1).

9.3 Heart and Blood Vessels

Underlying cardiovascular comorbidities (hypertension, diabetes, cardiovascular disease) are known to worsen outcomes in COVID 19 patients. Cardiovascular inflammation also complicates myocardial injury, ventricular dysfunction causing heart failure and arrhythmias responsible for sudden death in a certain group of individuals. Obesity has independently emerged as an independent risk factor for adverse cardiovascular outcomes.

Evidence of myocardial injury in patients has been a remarkable finding which persists even after clinical recovery. ACE2 expression in cardiac and vascular tissue potentiates direct damage due to viral infection. Presence of these receptors in multiple organs and their interaction with the SARS CoV2 virus could be

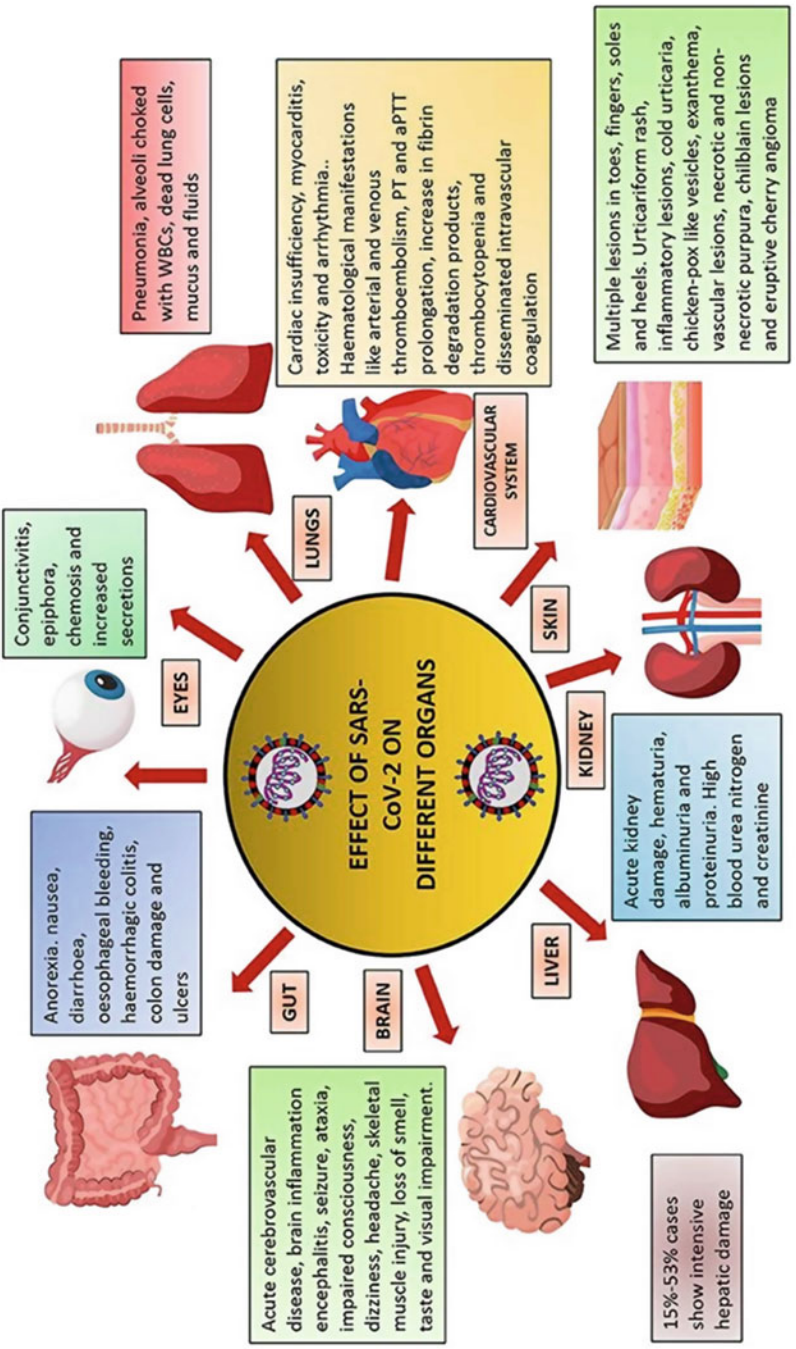

Fig. 9.1 Organ specific symptoms in COVID 19

responsible for multi-organ dysfunction characteristically seen in severe infections (Fig. 9.2). Down regulation of these receptors and resultant decrease in angiotensin 1–7 levels also compromises ventricular function leading to severe myocardial dysfunction.

Increase in inflammatory cytokines stimulates leucocyte adhesion molecule expression on the endothelial cells thereby making underlying atherosclerotic lesions vulnerable to disruption resulting in clinically pronounced acute coronary syndrome. Systemic cytokines induce dysfunction of the coronary microvasculature with resultant myocardial ischaemia/injury further compromising cardiac function (Fig. 9.3).

Mismatched myocardial oxygen demand and supply coupled with hypotension, during the cytokine storm syndrome, can reduce organ perfusion and may result in cascading effects on organ perfusion leading to multi-organ dysfunction.

Fulminant myocarditis with resultant severe hypotension/arrhythmias COVID-19 may result in severe left ventricular systolic dysfunction/cardiogenic shock.

A syndrome not unlike 'Kawasaki-like syndrome' has been reported too. These patients suffer due to a combination of circulatory dysfunction with macrophage activation syndrome. The manifestations are due to vascular and skin involvement. Persistent cardiovascular inflammation and residual vascular endothelial dysfunction could contribute to sudden cardiac deaths or persistent dyspnoea on exertion on follow-up.

9.4 Gastrointestinal Tract and Liver

The virus's avid affinity for ACE2 receptors located in the ileum and colon is responsible for luminal symptoms. ACE2 receptor involvement is partially responsible for luminal inflammation, which manifests as diarrhoea in infected patients. Heavy load of virus in intestinal epithelial cells, development of diarrhea and shedding of enterocytes are possible mechanisms resulting in the virus transmission through the faecal–oral route. Only a small fraction of patients manifest gastrointestinal symptoms alone. The most common reported symptom was the loss of appetite. Nausea and vomiting are seen in the majority, nearly 70% of the patients, while diarrhoea and abdominal pain are seen in less than one-third of symptomatic patients (Fig. 9.4a).

Transient elevation of liver enzymes is commonly seen in most viral infections. Similarly in COVID-19 infected patients mild elevation of liver enzymes is described but this rise is usually less than 5 times the normal. Some authors have reported liver enzyme abnormalities in more than 50% of infected patients. Liver dysfunction is usually present in patients having the severe disease at the time of presentation to the hospital. Acute liver failure in the absence of underlying chronic liver abnormality has not been reported with Covid 19 infection. During the course of management, it becomes difficult to differentiate the individual contribution of either the infection or the other treatment modalities, such as antiviral agents or other drugs administered. The liver function abnormalities can be attributed to direct

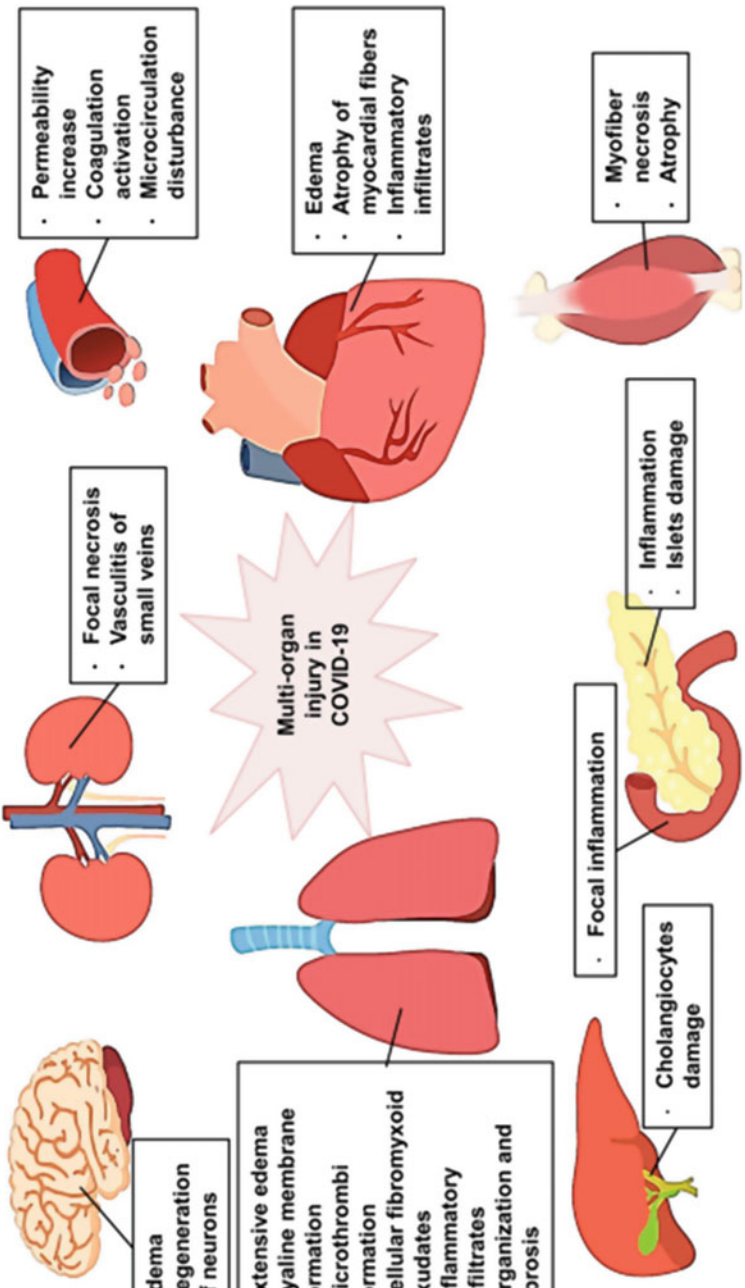

Fig. 9.2 Multi-organ dysfunction in COVID 19

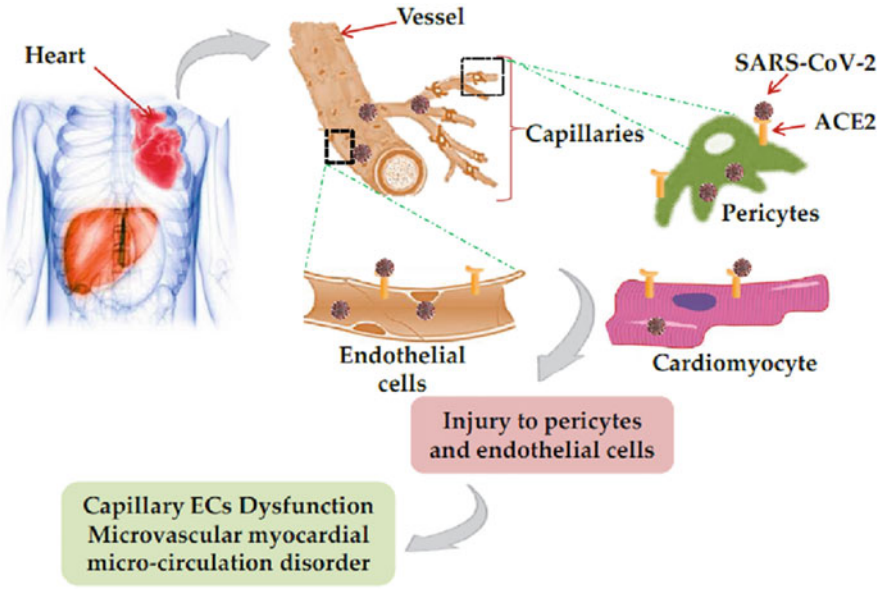

Fig. 9.3 Cardiac and vascular damage

involvement of hepatocytes with the virus and resultant cytopathic effect or a result of a combination of cytokine storm, sepsis, hypoxia and/or hypotension.

In covid 19 survivors, the persistence of loss of appetite and symptoms suggestive of reflux have been reported even after 6 months of recovery. This is an important component of long COVID syndrome.

9.5 Haematological Manifestations

COVID-19 infection has a significant impact on the haematopoietic system. The most important haematological finding is lymphopenia, which has important prognostic potential. Cytokine storm occurs approximately 7–14 days from the onset of the initial symptoms with, significant lymphopenia becoming more evident. Neutrophil to lymphocyte ratio (NLR) has prognostic value in determining the severity of the illness. Cytokine activation has the potential to impair lymphocyte turnover. More than 95% of patients with severe COVID 19 patients have significant lymphocytopenia as compared to non-severe cases. Nearly two-third of severe cases have significant thrombocytopenia and leukopenia. Thrombocytopenia has been independently noted to have a significant association with the severity of the COVID-19 disease. Other important biomarkers of acute severe inflammation and infection, noted to be associated with poor prognosis, are high serum procalcitonin, high serum cortisol, high CRP and serum ferritin.

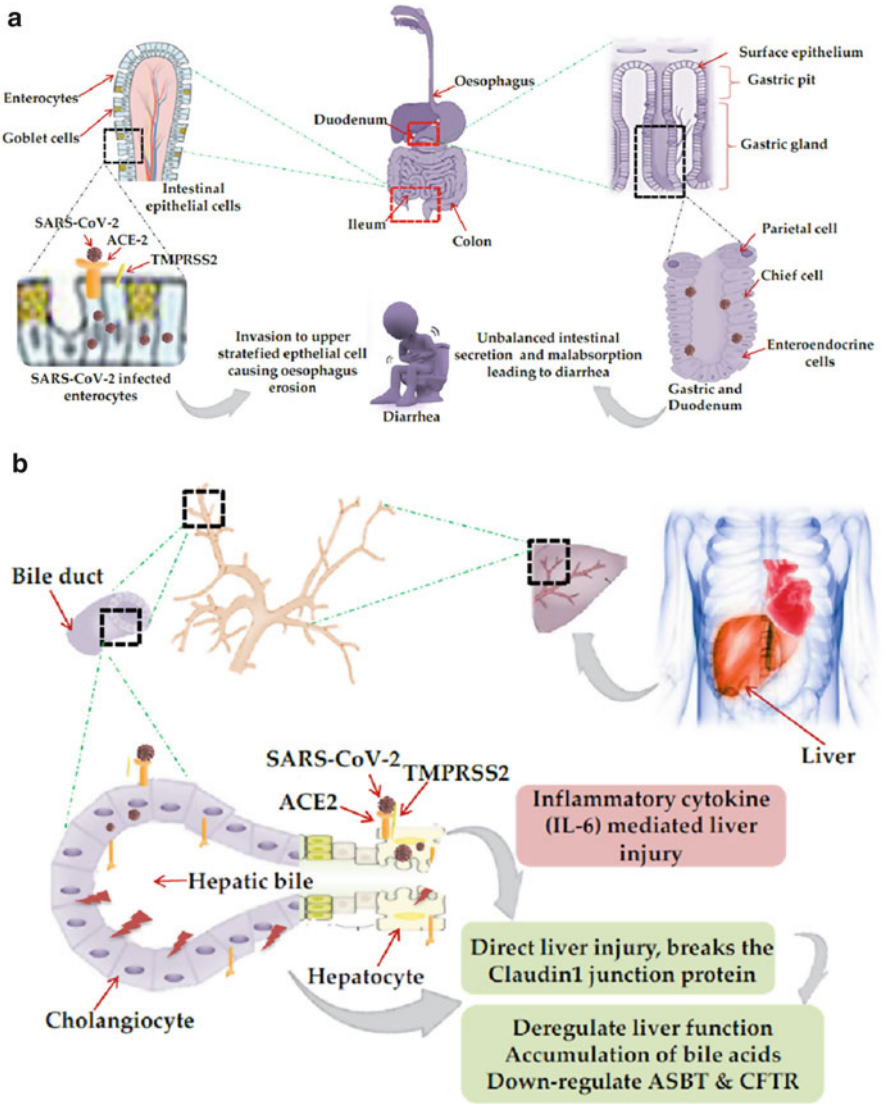

Fig. 9.4 (**a**) Gastrointestinal pathology. (**b**) Hepatic damage is caused by 1. Direct cytopathic effect of the virus 2. Virus induced inflammatory cytokine mediated damage

Hypercoagulability of blood, indicated by elevated D-dimer levels is common among hospitalized COVID-19 patients having severe disease. Gradual increase of D-dimer levels during the course of the disease is associated with deterioration and poor survival. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) prolongation, rising fibrin degradation products, and life-threatening disseminated intravascular coagulation (DIC) are important parameters to be looked

at for in severe cases. Significant endothelial dysfunction coupled with deregulated immune response can be implicated for these abnormalities. Up to 10% of COVID-19 infected patients develop clinically evident venous thromboembolism (VTE). Presence of significant underlying comorbidities like active malignancy/chronic liver disease/bedridden state/active trauma or surgical interventions, along with endothelial cell damage increases the risk of VTE. Pharmacological thrombo-prophylaxis, as in any other critically ill patient, with low molecular weight heparin, is recommended to prevent VTE. It has been noted that if during the acute episodes, therapeutic anticoagulation is administered, the risk of life-threatening bleeding increases significantly. Therefore unless indicated only thrombo-prophylaxsis, as indicated for the management of any bedridden critically ill patient, an appropriate dose should be administered.

9.6 Neurological Manifestations

Coronaviruses are neurotropic viruses and have been involved in direct CNS infection leading to neurological complications. Post-infectious, as well as para-infectious complications are increasingly being reported now with COVID 19 infection.

As many as one-third of patients have been reported to present with neurological manifestations. The commonest CNS symptoms reported are dizziness and headache. The most commonly hyped symptoms are taste impairment and anosmia but they have more psychological effects than actual association with severity. Other less frequent symptoms reported are altered consciousness, acute cerebrovascular disease, neuralgia, visual disturbances, rarely gait disturbances and seizure. Severely infected patients report a higher incidence of neurological symptoms.

Clinical manifestations might be due to direct viral infiltration or a result of hyper-inflammatory status (Fig. 9.5). Dysregulated immune response and resultant metabolic dysfunction might contribute to neurological manifestations in COVID-19 infection. Neurological manifestations of COVID-19 infection may also be a manifestation of severe systemic illness or related to it. Critically ill patients are more likely to develop encephalopathy, myopathy, autonomic neuropathy and polyneuromyopathy. SARS-COV-2 virus is postulated to enter the CNS through the retrograde neuronal route. Infection of olfactory neurons enables the virus to spread directly from the respiratory tract to the CNS. The extensive distribution of ACE2 receptors in the brain and endothelial cells may be responsible for direct neurological and skeletal muscle damage by the virus.

Severe systemic inflammatory response triggers dysregulation of the coagulation and diffuse intravascular coagulation resulting in an increased incidence of thrombotic complications in patients with COVID-19 infections. Imbalance between procoagulant and anticoagulant mechanisms may lead to cardiac dysfunction making a patient more vulnerable to cardio-embolism and subsequent complications. Hypotension or hypertension may also contribute by causing impaired cerebral perfusion or raised intracranial pressure causing cerebrovascular events.

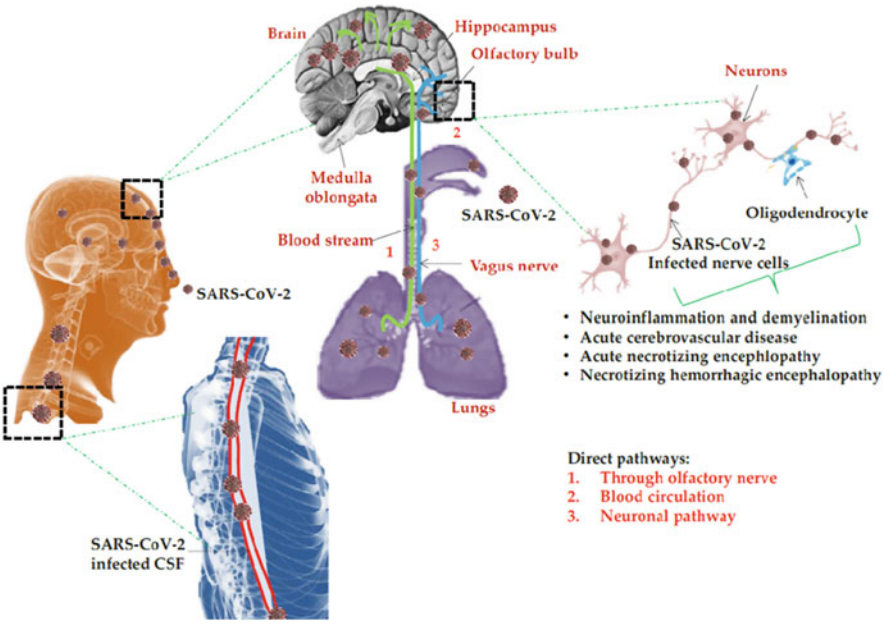

Fig. 9.5 Neurological damage by SARS CoV 2

Cerebrovascular disease related to hyper-coagulability may be associated with the COVID-19 pandemic but ischaemic stroke in covid 19 hospitalized patients have been reported denovo in less than 1% of patients. Whenever coexistent, patients with stroke and COVID-19 infection are known to have an increased risk of poor outcome and severe disability.

9.7 Anosmia and Ageusia

Olfactory (OD) and gustatory (GD) sense dysfunctions have been hyped as common symptoms of COVID-19 but the exact prevalence is not known as there could be under or over-reporting. Prevalence of smell and taste disturbances varies considerably. In a group of patients, nearly 85% were reported to have OD (anosmia or hyposmia) and GD (hypogeusia or ageusia). Women are more likely to suffer. Early olfactory recovery is a norm in the majority, while in some patients, symptoms could last even 14 days. OD and GD are not usually associated with milder disease but are present in patients with moderate to severe COVID19 infection.

Over the course of the pandemic, OD and GD have emerged as important screening tools despite the lack of a clear pathogenic mechanism explaining them. Specific viral neuro-invasivity and neuro-tropism, especially for olfactory nerves and the possibility of the temporal lobe, the amygdala, insula, limbic lobe (psycho sensorial syndrome) involvement may be a pathogenic mechanism.

9.8 Renal Involvement

Since ACE2 receptors are present in several cells in the kidney like podocytes, mesangial cells, epithelium of the Bowman's capsule/tubules and ducts, the theoretical possibility of kidney damage is definitely there in COVID 19 infection. The most frequent abnormality is mild-to-moderate proteinuria without any pre-existing kidney damage. Several mechanisms have been postulated for this abnormality. High levels of inflammatory cytokines like IL-1β, IL-8, IFN-γ and TNF-α in these patients suggest a possibility of cytokine release syndrome (CRS). This is somewhat similar to sepsis-associated AKI, wherein uncontrolled inflammation leads to acute kidney injury. Altered haemodynamics can further add to acute renal dysfunction. Acute kidney injury (AKI) is more common in critically ill patients which may be seen in nearly one-third of patients in the intensive care unit. Angiotensin-converting enzyme 2 (ACE2), transmembrane serine protease 2 (TMPRSS2) and cathepsin L (CTSL) expressed in many kidney cells, have been implicated for inducing SARS-CoV-2 associated kidney injury. Affinity of SARS-CoV-2 to attach to receptors on renal cells leading to viral overload in the cells could be another reason for acute kidney injury. It is also being increasingly recognized now that kidney involvement is associated with worse outcomes. Most of these acute changes are reversible.

9.9 Skin Manifestations

Skin abnormalities can be observed in as many as 20% of COVID-19 infected patients. The presentation of clinical abnormalities is heterogeneous and varies from urticarial to vesicular and from purpuric to papulosquamous lesions. Clinical involvement in skin, in the form of a morbilliform rash, urticarial lesions, livedo reticularis, and vesicular, varicella-like eruptions have been commonly reported (Fig. 9.6). A severe multisystem inflammatory syndrome having significant mucocutaneous findings akin to severe Kawasaki disease has also been reported with COVID19.

It is not very clear if these skin manifestations are due to direct virus inoculation/invasion or secondary to dysregulated host immune response. Some of these lesions could be a result of vasculopathy associated with SARS COV2 infection. The nature of the association between COVID-19 and skin lesions and the systemic implications of their presence remains to be determined.

In addition, skin involvement due to mechanical/friction dermatitis, and contact dermatitis due to personal protective equipment (PPE)/sanitizers have been reported in health care workers. Although not directly related to the covid 19 virus, these skin manifestations need to be taken into account as pandemic related skin manifestations and add to the burden of clinical cases during COVID-19 infection management.

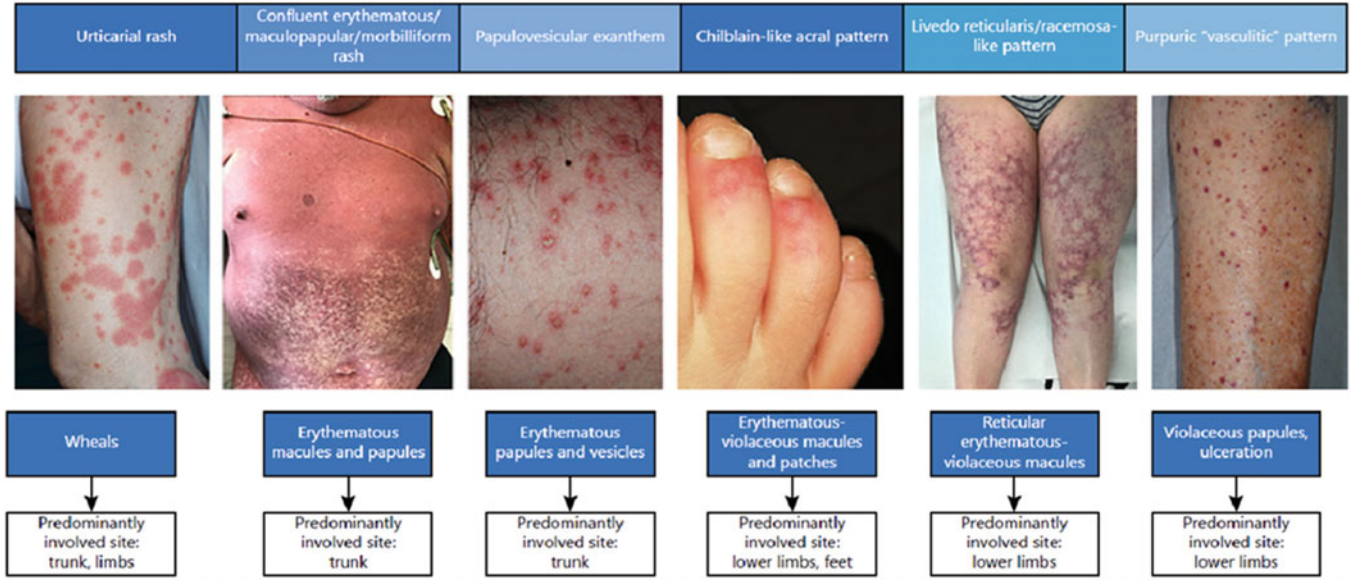

Fig. 9.6 Skin manifestations

9.10 Endocrine Abnormalities

Stress of infection and dysregulated immune response leads to variety of endocrine abnormalities and may unmask pre-existing subclinical diseases. SARS-CoV2 expresses amino acids mimicking host adrenocorticotropic hormone (ACTH) and antibodies to ACTH mimics may lead to a relative cortisol insufficiency. In one study among patients, high cortisol levels were observed at presentation. This may indicate hormonal stress due to severe systemic. Very high cortisol at presentation in severely ill patients has been found to be associated with poor outcomes. Autopsy studies have shown necrosis of the adrenal gland. High load of virus in the glands may point towards damage and resultant altered cortisol dynamics in patients. The hypothalamic–pituitary–adrenal (HPA) axis may also be involved by the virus directly, leading to hypophysitis or direct hypothalamic damage. High level of ACE2 expression in these tissues may explain possible viral tropism and resultant damage.

Unmasking of Type 2 diabetes mellitus, severe illness in established poorly controlled diabetics and predisposition to infections/vascular adverse events complete the conundrum of virus related damage in diabetics. A number of patients develop 'sick euthyroid syndrome' especially when the critical phase of illness sets in.

9.11 Ocular Manifestations

The human conjunctiva is considered to be a potential site for SARS-CoV-2 infection and possible transmission. Currently there is no evidence that viral replicates and can be shed especially in tears. Direct viral inoculation may lead to injury and inflammation of the conjunctiva or cornea. Therefore the current recommendations necessitates the use of protective eye gear for all healthcare workers involved in the care of COVID 19 infected patients. In some initial studies, as many as 30% of patients have been noted to have ocular manifestations. The commonest manifestation is dry eye and foreign body like sensation whereas keratitis and conjunctival follicular reactions have also been observed by some. (Ocular Manifestations of COVID-19; Nasiri et al) Other common manifestations include conjunctival congestion, chemosis or epiphora. Some studies have suggested possible prolonged shedding of virus in the tears and significant retinal involvement. The exact extent and patho-physiology responsible for the ophthalmic manifestations associated with SARS-CoV-2 are not fully understood.

9.11.1 Persistent/Long COVID

Large number of investigators have shown persistent residual respiratory and cardiac dysfunction in patients recovering from severe COVID19 infections, especially the ones requiring high flow oxygen and ventilation. These lung and cardiac changes

take a long time to recover. Another important complication reported is serious psychological morbidity and chronic fatigue in survivors. Nearly one-third of survivors report these chronic symptoms. Older patients and patients with multiple pre-existing comorbidities are more likely to have these symptoms. Persisting symptoms in covid survivors are likely to adversely affect the quality of life and result in significant morbidity. Persistence of these symptoms points towards the potential of COVID 19 infection to leave the world crippled.

Take Home Message

COVID-19 probably presents with a broad spectrum of clinical signs and symptoms. There may be significant involvement of deep-seated vital organs (heart, lung, CNS & Kidney) or milder involvement of superficially located organs (eyes and skin). Multisystem involvement associated with deregulated immune response leading to organ failure might lead to adverse clinical outcomes and increased morbidity/mortality. The high binding affinity to omnipresent ACE2 receptors is postulation for multi-organ involvement. The exact role of the dysregulation of ACE2 receptors expression, presence of pre-existing comorbidities, dysregulated immune response and/or direct viral damage are some questions that need to be addressed. The unpredictability of this pandemic needs more careful surveillance, customized control measure implementation, and specifically focused medical research looking at various treatment strategies to guide our management. There is a need to address the long-term effects and morbidity in COVID survivors to improve quality of life in post COVID era.

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COVID-19 and Mucormycosis

10

Varsha Gupta and Anku Goel

Abstract

In early December 2019, several cases of pneumonia of unknown etiology were reported from Wuhan, Hubei province, China. The disease resembles severe acute respiratory syndrome coronavirus (SARS CoV) of 2012 and was subsequently named SARS CoV-2 causing the 2019-novel coronavirus disease (COVID-19) by the World Health Organization (WHO). The first case of COVID-19 in India was reported on January 30, 2020. In the first wave, daily cases peaked in mid-September 2020 and began to drop by January 2021. However, a second wave beginning in March 2021 was experienced which was much larger than the first, with extreme shortages of hospital beds, oxygen cylinders, and other medicines including vaccines in parts of the country. In the second wave, an association of COVID-19 patients with mucormycosis further complicated the situation. COVID-19 associated mucormycosis (CAM) has been increasingly reported particularly among patients with uncontrolled diabetes. An increase in CAM cases could be probably due to immunosuppression caused by the use of steroids, other immunomodulators like tocilizumab. Rhino-orbital-cerebral mucormycosis is the most common presentation. The most common causative agent isolated is *Rhizopus arrhizus.* Simple KOH examination with broad, ribbon-like, aseptate hyphae that branch at right angles is diagnostic of mucormycosis. This can be further confirmed by culture examination. However, newer tests like MALDI-TOF for species identification are also being explored. The main treatment modality is surgical debridement, removing all the infected, dead, and necrotic tissue followed by simultaneous administration of antifungal antibiotics in the form of Amphotericin B. Liposomal amphotericin B is the drug of choice, however, if not available, amphotericin B deoxycholate, posaconazole,

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and isavuconazole can be given for the treatment of CAM. Prognosis and clinical improvement depend upon the stage of disease, the surgical management as well as the availability and administration of antifungal drugs. In media, mucormycosis is being projected as black fungus throughout this pandemic, though it is a misnomer and should not be used in the medical literature.

Keywords

COVID-19 · Mucormycosis · SARS CoV · COVID-19 associated mucormycosis (CAM) · Amphotericin B

10.1 Introduction

Towards the end of 2019, an outbreak of a pneumonia-like infection occurred in Wuhan, China, and rapidly spread across the globe. With the utilization of genome sequencing technology, the disease was identified as coronavirus disease or more commonly known as coronavirus disease 2019 (COVID-19), and the etiological agent was identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Khan et al. 2020a). This respiratory illness has significantly impacted all sectors of life, primarily healthcare and the economy, and as a result, on March 11, 2020, the WHO officially recognized COVID-19 disease as a global pandemic. The coronaviruses are enveloped, positive-sense single-stranded viruses that utilize the human angiotensin-converting enzyme 2 (ACE2) receptors located on cells in many organs/tissues, including the lung, heart, kidney, bladder, eyes, nasal and oral cavities, brain, thyroid, liver, gallbladder, stomach, pancreas, intestine, reproductive system of males and females, and skin, to gain entry, and trigger a range of clinical manifestations (Jin et al. 2020). Evaluating the data available from various parts of the world has demonstrated that the SARS-CoV-2 virus follows an exponential growth, and the mean basic reproduction number, which indicates the number of new infections that can arise from a single case of COVID-19 infection, is estimated to be 1.4–4 (Khan et al. 2020a).

Severe COVID-19 disease is associated with an increase in pro-inflammatory markers, such as IL-1, IL-6, and tumor necrosis factor-alpha, less CD4 interferon-gamma expression, and fewer CD4 and CD8 cells; this, therefore, increases the susceptibility to bacterial and fungal infections (Pemán et al. 2020). One such opportunistic fungal infection involved is mucormycosis that are ubiquitous in the environment. Infection with SARS-CoV-2 drastically impacts the immune system via induction of an inflammatory storm, an increase in neutrophil count as well a decrease in lymphocyte count, specifically CD4+, CD8+, and T cells. Consequently, these patients are at increased susceptibility in developing opportunistic infections such as mucormycosis, due to decreasing lymphocyte cells (Pemán et al. 2020). CD4+, CD8+, and T cells serve a prominent role against infection with mucormycosis via recruiting cytokines, such as IL-4, IL-10, IL-17, and IFN-γ (Pasero et al. 2020).

10.2 Mucormycosis

Mucormycosis is an infection caused by a group of filamentous molds mucormycetes. Mucormycetes belong to phylum Glomerulomycota (former zygomycota), subphylums Mucoromycotina and Entomophthromycotina, and the orders Mucorales and Entomophthorales (Chakrabarti et al. 2006; Prabhu and Patel 2004; Petrikkos et al. 2012; Riley et al. 2016; Danion et al. 2015; Kwon-Chung 2012). The order Mucorales comprise numerous genera and species, for example, *Rhizopus arrhizus, Rhizopus microsporus var. rhizopodiformis, Mucor species, Lichtheimia* (formerly *Absidia corymbifera*), *Rhizomucor pusilus, Cunninghamella species, Mortierella species, Apophysomyces elegans*, *A.*variabilis, *A.*trapeziformis, *A.ossiformis*, *A.mexicanus*, *Saksenaea vasiformis, S. erythrospora, S.oblongispora, and Cokeromyces recurvatus* and other species (Riley et al. 2016; Danion et al. 2015; Kwon-Chung 2012; Ribes et al. 2000). *Rhizopus* is the most common genus associated with mucormycosis, followed by *Mucor* and *Lichtheimia* (Petrikkos et al. 2012; Park et al. 2011). Other genera (e.g., *Rhizomucor, Cunninghamella, and Saksenaea*) are less common. *Lichtheimia* spp. were identified as the major cause of mucormycosis in a single hospital in Spain, indicating geographical variation and the need to know local epidemiology of various mucor species (Guinea et al. 2017). Molds from the subphylum Entomophthromycotina, order Entomophthorales are substantially different from Mucorales. Entomophthorales produce chronic subcutaneous infections in immunocompetent hosts, usually in tropical or subtropical climates (Prabhu and Patel 2004). Unlike Mucorales, Entomophthorales are not angioinvasive and rarely disseminate (Vilela and Mendoza 2018). In this chapter, we shall be discussing molds within order Mucorales only.

There are three ways a human can contract mucormycosis—by inhaling spores, by swallowing spores in food or medicines, or when spores contaminate wounds. The major route is via inhalations of spores, which then spread to the paranasal sinuses and lungs. Clinical and experimental data clearly demonstrate that individuals who lack phagocytes or have impaired phagocytic function are at higher risk of mucormycosis. For example, severely neutropenic patients are at increased risk for developing mucormycosis (Ibrahim et al. 2012). In the presence of hyperglycemia and low pH, which is found in patients with diabetic ketoacidosis (DKA), phagocytes are dysfunctional and have impaired chemotaxis and defective intracellular killing by both oxidative and nonoxidative mechanisms (Chinn and Diamond 1982). In addition to host factors that predispose patients to mucormycosis, Mucorales possess virulence factors that enable the organism to cause disease. One such trait is the ability to acquire iron from the host (Vilela and Mendoza 2018). The clinical observation depicts that patients with DKA are uniquely susceptible to mucormycosis and lends support to the role of iron uptake in the pathogenesis of the disease. Patients with DKA have elevated levels of free iron in their serum, and such serum supports the growth of *R. arrhizus* at acidic pH (7.3–6.88) but not at alkaline pH (7.78–8.38) (Artis et al. 1982).

Another clinical observation highlights that patients receiving dialysis who are treated with the iron chelator deferoxamine, predispose them to *Rhizopus* infection

as deferoxamine acts as a xenosiderophore (Boelaert et al. 1993). Deferoxamine strips ferric iron from transferrin and attaches itself to the mold through an inducible receptor, and the iron is transported intracellularly by an active reduction of the ferric form into the more soluble ferrous form (de Locht et al. 1994). In addition, in vitro studies of radiolabeled iron uptake from deferoxamine in serum show that *Rhizopus* is able to incorporate eight-fold and 40-fold more iron than can *Aspergillus fumigatus* and *Candida albicans*, respectively (Boelaert et al. 1993).

Mucormycosis infections are characterized by extensive angioinvasion that results in vessel thrombosis and subsequent tissue necrosis (Spellberg et al. 2005; Ibrahim et al. 2003; Ben-Ami et al. 2009). Ischemic necrosis of infected tissues can prevent the delivery of leukocytes and antifungal agents to the foci of infection. Glucose-regulated protein (GRP78) was identified to act as a receptor that mediates penetration through and damage of endothelial cells by Mucorales (Liu et al. 2010). Elevated concentrations of glucose and iron are consistent with those noted during DKA-enhanced surface GRP78 expression and resulting penetration through and damage of endothelial cells by Mucorales in a receptor-dependent manner (Ibrahim et al. 2012). This angioinvasion likely contributes to the capacity of the organism to hematogenously disseminate to other target organs (Ibrahim et al. 2012). Mucormycosis is non-pathologic in immunocompetent individuals as a result of the presence of an intact immunity via neutrophils which hence permit the elimination of these spores (Pasero et al. 2020). The common symptoms linked to mucormycosis are swelling in one side of the face, fever, headache, nasal or sinus congestion, and black lesions on the nasal bridge or upper inside of the mouth (Coronavirus n.d.).

However, in immunocompromised patients such as those with uncontrolled diabetes mellitus, diabetic ketoacidosis, presence of an open wound, HIV/AIDS, cancer, COVID-19, and organ transplant, mucormycosis can result in a severe invasive fungal infection (Hernández and Buckley 2020). Invasive mucormycosis is a life-threatening fungal infection that most frequently occurs in patients with underlying comorbidities impacting immune system function (Gomes et al. 2011; Ribes et al. 2000; Roden et al. 2005; Prabhu and Patel 2004). Based on its clinical presentation and anatomic site, invasive mucormycosis is classified as one of the following six major clinical forms: (1) rhinocerebral, (2) pulmonary, (3) cutaneous, (4) gastrointestinal, (5) disseminated, and (6) uncommon rare forms, such as endocarditis, osteomyelitis, peritonitis, and renal infection (Goodman and Rinaldi 1991; Lopes et al. 1995; Stas et al. 1996). The most common reported sites of invasive mucormycosis have been the sinuses (39%), lungs (24%), and skin (19%) (Torres-Narbona et al. 2007). Dissemination developed in 23% of these cases. The overall mortality rate for the disease is 44% in diabetics, 35% in patients with no underlying conditions, and 66% in patients with malignancies (Petrikkos et al. 2012). The mortality rate varied with the site of infection and host: 96% of patients with disseminated infections, 85% with gastrointestinal infections, and 76% with pulmonary infections died (Petrikkos et al. 2012). In children, mucormycosis manifested as cutaneous, gastrointestinal, rhinocerebral, and pulmonary infections in 27%, 21%,

18%, and 16% of cases, respectively, in one study (Zaoutis et al. 2007). The skin and gut are affected more frequently in children than in adults (Petrikkos et al. 2012).

Rhino-orbital-cerebral mucormycosis (ROCM) is most frequently seen in those with poorly controlled diabetes mellitus, whereas immunocompromised patients (including those with hematological malignancies and transplant recipients) frequently present with pulmonary involvement and disseminated infection (Roden et al. 2005; Lanternier et al. 2012; Chakrabarti et al. 2006). In a case report, the authors presented a case of rhino-orbital mucormycosis in a 57-year-old female with poorly controlled diabetes mellitus (Xess et al. 2012). The only mold cultured at 25 °C, 37 °C, and 40 °C from a specimen of the nasal crust was identified phenotypically and independently using nuclear ribosomal DNA sequence data as *Thamnostylum lucknowense*, a species that was originally isolated from soil in Lucknow, India, and designated *Helicostylum lucknowense* (Rai et al. 1961), implicating it as an etiological agent of rhino-orbital mucormycosis. A multifaceted approach, including the elimination of predisposing factors, aggressive surgical debridement, and effective antifungal therapy, is critical to improve patient survival. However, despite these interventions, the outcome of invasive mucormycosis remains ominous (Ribes et al. 2000; Roden et al. 2005).

In media mucormycosis is being projected as black fungus throughout this pandemic, though it is a misnomer and should not be used in the medical literature (https://www.thehindu.com/news/cities/Madurai/calling-mucormycosis-black-fungus-is-a-misnomer/article34740553.ece). The Government of India on May 20, 2021 wrote a letter to all the states and union territories asking to make mucormycosis or black fungus a notifiable disease under the Epidemic Diseases Act 1897. A notifiable disease is any disease that is required by law to be reported to government authorities. This allows the authorities to monitor the disease and provides early signaling of possible outbreaks. Telangana was the first state to declare mucormycosis a notifiable disease followed by Odisha, Karnataka, Tamil Nadu, and other states (https://www.hindustantimes.com/india-news/these-states-have-declared-black-fungus-a-notifiable-disease-101621565107184.html).

10.3 COVID-19 and Immunosuppression

Initially, it was debated whether a person taking immunosuppressant be at higher risk of getting COVID-19 or whether the immunosuppressive state would cause more severe COVID-19 infection (Thng et al. 2020). As understood so far, COVID-19 infection may induce significant and persistent lymphopenia and deranged neutrophil lymphocyte ratio (NLR) which in turn increases the risk of opportunistic infections (Pasero et al. 2020; Saha et al. 2020; Yang et al. 2020). The optimal threshold at 3.3 for NLR showed a superior prognostic possibility of clinical symptoms to change from mild to severe thus indicating that elevated NLR can be used as an independent prognostic biomarker for COVID-19 patients (Yang et al. 2020). And since the lymphocytes play a major role in maintaining immune

homeostasis (Salehi et al. 2020), the patients with COVID-19 are highly susceptible to fungal co-infections (Monte Junior et al. 2020).

10.4 Mucormycosis Coinfection in COVID-19

Mucormycosis is a fungal infection that is often life-threatening (Monte Junior et al. 2020). It is characterized by vascular invasion by the fungal hyphae which leads to thrombosis and necrosis (Monte Junior et al. 2020). There has been a surge of mucormycosis coinfection in COVID-19 patients, many cases being reported worldwide.

Although systemic corticosteroid treatment can reduce mortality in those with the most severe courses of COVID-19 disease, but together with immunological and other clinical factors may also predispose patients to secondary fungal disease. COVID-19-associated mucormycosis (CAM) has recently been increasingly reported, particularly among patients with uncontrolled diabetes. CAM may be a relevant complication of severe COVID-19 in those with uncontrolled diabetes (https://www.researchgate.net/publication/351558444_The_emergence_of_COVID-19_associated_mucormycosis_Analysis_of_cases_from_18_countries/link/609d25e192851cca598aab02/download).

Symptoms of some fungal diseases can be similar to those of COVID-19, including fever, cough, and shortness of breath. Laboratory testing is necessary to determine if a person has a fungal infection or COVID-19. Some patients can have COVID-19 and fungal infection at the same time (Bhatt et al. 2021). There are cases reported in which fungal infection arises as a post-COVID-19 sequelae. Cases of acute invasive fungal rhino-orbital mucormycosis (Mekonnen et al. 2021) and cavitary pulmonary mucormycosis (Pasero et al. 2020; Khan et al. 2020b) in patients with COVID-19 have been reported. Patients hospitalized for COVID-19 are at risk for healthcare-associated infections (Hossain et al. 2020), including candidemia, or bloodstream infections caused by *Candida* and other fungal diseases (Bhatt et al. 2021).

10.5 Time of Presentation

Variable but usually around the third week of onset of symptoms of COVID-19 (https://dghs.gov.in/WriteReadData/News/2021051711193015559 88MucormycosismanagementinCovid-19.pdf).

10.6 Reasons for Increase in Mucormycosis in COVID-19 patients (https://dghs.gov.in/WriteReadData/News/20210517111930155598 8MucormycosismanagementinCovid-19.pdf)

1. Hyperglycemia due to uncontrolled pre-existing diabetes and high prevalence rates of mucormycosis in India per se.
2. Rampant overuse and irrational use of steroids in the management of COVID-19.
3. New-onset diabetes due to steroid overuse or severe cases of COVID-19 per se. Since it is known that ACE 2 receptors are also seen in the pancreas so their effect is insulin resistance and hyperglycemia (Jin et al. 2020).
4. Prolonged ICU stay and irrational use of broad-spectrum antibiotics leading to immunosuppression and also some effect of poor neutrophil response.
5. Pre-existing co-morbidities such as hematological malignancies, use of immunosuppressant, solid organ transplant, and so on.
6. Breakthrough infections in patients on Voriconazole (antifungal drug) prophylaxis.

10.7 Diagnosis

Mucormycosis is difficult to diagnose rare disease with high morbidity and mortality. Diagnosis is often delayed, and the disease tends to progress rapidly (Cornely et al. 2019a). Urgent surgical and medical intervention is lifesaving. Guidance on complex multidisciplinary management has the potential to improve prognosis, but approaches differ between health-care settings (Cornely et al. 2019a).

Diagnosis in cases of CAM includes potassium hydroxide (KOH) wet mount staining and microscopy, histopathology of debrided tissue, and culture (https://dghs.gov.in/WriteReadData/News/20210517111930155598 8MucormycosismanagementinCovid-19.pdf).
Mucormycosis diagnosis mainly requires demonstration of characteristic broad, ribbon -like, aseptate (nonseptate) hyphae 5–15 μm that branch at right angles invading tissues on KOH wet mount preparation (Fig. 10.1) and histopathology, accompanied by culture growth from specimens of involved sites (https://dghs.gov.in/WriteReadData/News/20210517111930155598 8MucormycosismanagementinCovid-19.pdf; Walsh et al. 2012; Donnelly et al. 2020). The mucormycetes can be easily grown on conventional media-like sabouraud dextrose agar (SDA) with antibiotics at temperatures, that is, 25 and 37 °C (Fig. 10.2). However, expertise is required to confirm the diagnosis. Pathogen identification and antifungal susceptibilities are critical to determine appropriate antifungal therapy (Steinbrink and Miceli 2021). Investigations include non-contrast computerized tomography (NCCT) of paranasal sinuses (to see bony erosion), high-resolution computerized tomography scan (HRCT) of the chest ($\geq$10 nodules, reverse halo sign, CT bronchus sign, etc.), and CT Angiography. Magnetic resonance imaging (MRI) brain to be done for better delineation of central nervous

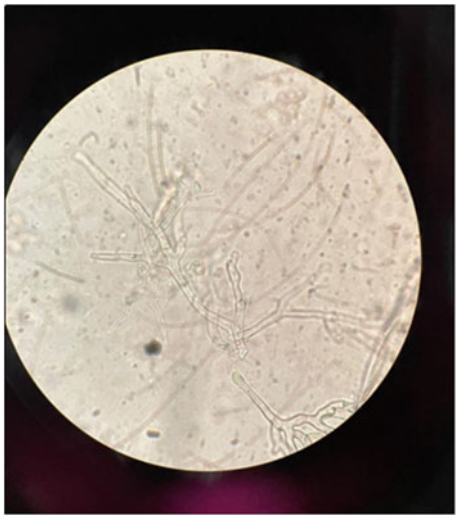

Fig. 10.1 Microscopic appearance of broad, ribbon-like, aseptate hyphae with wide-angle branching seen in wet mount (KOH X 400)

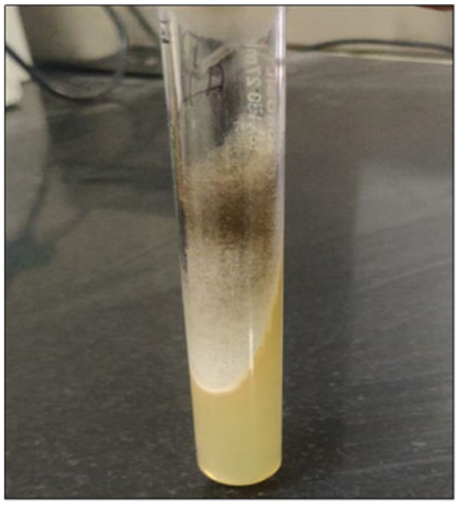

Fig. 10.2 Macroscopic appearance of colonies of *Rhizopus* species on SDA culture tube

system (CNS) involvement (https://dghs.gov.in/WriteReadData/News/202105171119301555988MucormycosismanagementinCovid-19.pdf). Radiographic findings alone are nonspecific and are usually insufficient for complete and accurate diagnosis of mucormycosis. Several CT findings like pleural effusion and multiple pulmonary nodules, along with clinical evidence of sinusitis, the "reverse halo" sign, point toward mucormycosis as opposed to other fungi (Chamilos et al. 2005; Jung et al. 2015; Hammer et al. 2018).

Non-culture-based serologic tests for the diagnosis of invasive fungal infections are currently available. However, such serum markers, including 1,3-beta-D-glucan (BDG) and *Aspergillus* galactomannan, are derived from fungal cell wall components not present in Mucorales (Ostrosky-Zeichner et al. 2005; Miceli and Maertens 2015). Thus, although a positive BDG or galactomannan can be suggestive of fungal infection with alternative pathogens to mucormycosis (i.e., to "rule out" mucormycosis), these tests will not be able to identify a specific pathogen (Steinbrink and Miceli 2021). Currently, there are no serum assays specific to mucormycosis (Steinbrink and Miceli 2021).

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) may be resorted for identification of mucor species after culture growth (https://dghs.gov.in/WriteReadData/News/2021051711193015559 88MucormycosismanagementinCovid-19.pdf). MALDI-TOF can be used for better identification of culture specimens and also help in identification of some species which are resistant to antifungals, but further development of available databases is necessary for more widespread use (Cornely et al. 2019b). Molecular methods, including polymerase chain reaction–based approaches, are increasingly used because of their ability to improve detection in tissues, and often aid in identification to the level of the species, through targets such as the internal transcribed spacer or 18s ribosomal RNA (Cornely et al. 2019b; Walsh et al. 2012; Bialek et al. 2005; Hammond et al. 2011; Soare et al. 2020; Skiada et al. 2020). Additional noninvasive approaches of fungal identification continue to be investigated as well, including gene expression profiling, next-generation sequencing, and breath-based metabolomics (Steinbrink et al. 2020a, b; Koshy et al. 2017).

10.8 Current Management and Treatment Options for CAM

CAM can be prevented by following few simple steps. Use masks if you are visiting dusty construction sites. Wear shoes, long trousers, long sleeve shirts, and gloves while handling soil (gardening), moss, or manure. Maintain personal hygiene including a thorough scrub bath (https://www.icmr.gov.in/pdf/covid/techdoc/Mucormycosis_ADVISORY_FROM_ICMR_In_COVID19_time.pdf).

The ability to treat cases of COVID-19 associated mucormycosis effectively depends on the availability of surgical techniques and antifungal drugs. Antifungal prophylaxis for CAM treatment is not recommended. The calculated dose of amphotericin B to be started from the first day and avoid dose escalation. Fluconazole, voriconazole, echinocandins (caspofungin, anidulafungin, micafungin), or 5-flurocytosine is not active against mucormycosis. A combination of antifungal therapy is generally not recommended, as there is little evidence in support of combination therapy (http://www.fisftrust.org/covid-19/Asscoiated-Mucormycosis/images/Treatment-CAM-20-5-21.pdf). Treatment of COVID-19-associated mucormycosis includes diabetes control, reduction of steroids, and discontinuing immunomodulators. Along with these measures, if eye or lung (localized or one

lobe) is involved, extensive surgical debridement should be done. Medical therapy includes maintenance of adequate hydration; install peripherally inserted central catheter (PICC) or central venous catheter line (CVC). Antifungal therapy should be started for 3–6 weeks (http://www.fisftrust.org/covid-19/Asscoiated-Mucormycosis/images/Treatment-CAM-20-5-21.pdf). Three lipid formulations of amphotericin B (AMB) are available: AmBisome (AmBi, liposomal amphotericin B; Gilead Sciences Inc., Foster City, CA, USA), Abelcet (ABLC, amphotericin B lipid complex; Enzon Pharmaceuticals Inc., Bridgewater, NJ, USA; Cephalon Limited, Welwyn Garden City, UK), and Amphocil /Amphotec (ABCD, amphotericin B colloidal dispersion; Three River Pharmaceuticals Inc., Cranberry Township, PA, USA). Although all these formulations contain AMB, they differ with regard to their lipid composition, shape, size, stability, pharmacokinetics, and toxicity. Lipid formulations of AMB have been shown to reduce the parent drug nephrotoxicity while retaining the drug's activity, providing a better therapeutic index for the drug (Adler-Moore and Proffitt 2008). Specialty biopharmaceutical manufacturer, Celon Laboratories, has launched an alternative drug—Amphotericin B Emulsion, for the treatment of Mucormycosis (https://www.thehindubusinessline.com/news/national/celon-laboratories-launches-amphotericin-b-emulsion-for-mucormycosis/article34699948.ece). Liposomal/lipid amphotericin B 5 mg/kg/day is administered in cases of CAM for 3–6 weeks. The patient's renal function tests, potassium and magnesium levels should be regularly monitored during this time (http://www.fisftrust.org/covid-19/Asscoiated-Mucormycosis/images/Treatment-CAM-20-5-21.pdf). In case lipid amphotericin B is not available, Amphotericin B deoxycholate—1 to 1.5 mg/kg/day is administered for the same duration (http://www.fisftrust.org/covid-19/Asscoiated-Mucormycosis/images/Treatment-CAM-20-5-21.pdf). If polyenes (amphotericin B) is not available or if the patient is intolerant to polyene, then Isavuconazole inj—200 mg tid on day 1–2 and 200 mg/day from day 3 for 3–6 weeks or Posaconazole inj—300 mg bid on day 1 & then 300 mg/day administered for 3–6 weeks (http://www.fisftrust.org/covid-19/Asscoiated-Mucormycosis/images/Treatment-CAM-20-5-21.pdf). Isavuconazole tab (200 mg) and Posaconazole tab (300 mg) are also available and are alternative/step-down therapeutic options. In a scenario, where neither polyene nor isavuconazole/posaconazole is available, then itraconazole (200 mg tid) should be given, for the same duration (http://www.fisftrust.org/covid-19/Asscoiated-Mucormycosis/images/Treatment-CAM-20-5-21.pdf). During this time, the patient's liver function tests should be monitored every week. Further, follow-up disease status to know whether it is stable or with progressive (clinically/radiologically) disease. If the disease is progressive and the patient is on azoles, consider adding polyene, consider therapeutic drug monitoring (TDM), dose adjustment, and any possible drug-drug interaction with azoles (http://www.fisftrust.org/covid-19/Asscoiated-Mucormycosis/images/Treatment-CAM-20-5-21.pdf). If the patient is on polyene and any signs of toxicity are observed, shift to azoles. Shift the patient to isavuconazole, if any drug interaction with posaconazole is observed (http://www.fisftrust.org/covid-19/Asscoiated-Mucormycosis/images/Treatment-CAM-20-5-21.pdf). Posaconazole is a CYP3A4 inhibitor and has been shown to interact with a

number of CYP3A4 substrates or inhibitors including cimetidine, phenytoin, midazolam, cyclosporine, tacrolimus, and rifabutin. Therefore, avoidance of posaconazole with these and other agents that share the same metabolic pathways is recommended. Other agents include calcium channel blockers, calcineurin inhibitors, ergot alkaloids, HIV-1 protease inhibitors, HMG-CoA reductase inhibitors, proton pump inhibitors, and vinca alkaloids. Otherwise, frequent monitoring of drug levels and for adverse drug events as well as dose adjustments may be warranted (https://nsuworks.nova.edu/cgi/viewcontent.cgi?article=1196&context=ijahsp&httpsredir=1&referer=/).

COVID-19 pandemic has resulted in widespread mortality, morbidity, social and economic upheavals of an unprecedented magnitude. Global scientific collaboration and reporting of new information related to this are of paramount importance to increase the knowledge with regard to the novel viral infection. The incidence of rhino-orbito-cerebral mucormycosis is likely to rise, both as a co-infection and as sequelae of COVID-19. Invasive mucormycosis is an aggressive fungal infection with high morbidity and mortality, particularly in patients with underlying medical comorbidities or immunosuppression. Clinical and radiographical presentations can vary between patients based on the immune status of the host and mode of infection. Early diagnosis and management with appropriate and aggressive antifungals and surgical debridement can improve survival.

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Rhino-Orbito-Cerebral Mucormycosis—The Bane of the 'Black Fungus'

11

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Abstract

Rhino-orbito-cerebral mucormycosis (ROCM) is an infection of the nasal passages and orbit caused by ubiquitous fungi of the order Mucorales. These fungi are known to affect patients with phagocyte and neutrophil dysfunction. Patients with uncontrolled diabetes, solid-organ, haematological malignancies and organ transplant recipients on immunosuppressive therapy are especially susceptible. The disease is being seen with alarming frequency in patients with COVID-19 infection or those who have recently recovered from it. Poor glycaemic control due to the indiscriminate use of steroids has been strongly implicated. Patients present with periocular pain, oedema, numbness or skin discoloration along with symptoms of the nasal blockade. Direct microscopy of a deep nasal swab taken from the involved mucosa reveals broad aseptate or pauci-septate fungal hyphae, clinching the diagnosis. CT scan of the paranasal sinuses and orbit would reveal a hyperdense lesion involving the nasal turbinates and sinuses with extension into the orbit. Lack of contrast enhancement indicates necrosis of the tissues. The treatment involves administration of systemic antifungals (Amphotericin B, Posaconazole and isavuconazole) and aggressive surgical debridement of involved tissues. In spite of all measures, the mortality rate is about 46% in these patients. Strict diabetic control and judicious prescription and monitoring of systemic steroids in the setting of COVID-19 infection, keeping a high index of suspicion with early detection of the disease can go a long way in improving the prognosis.

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Keywords

Rhino-orbital-cerebral mucormycosis · Mucormycosis · Black fungus · Diabetes mellitus · COVID-19 · Anti-fungal

11.1 Introduction

Mucormycosis is one of the most fulminant and devastating infections affecting the nasal cavity, sinuses and orbit. Colloquially called the 'Black fungus', this name aptly hints at the nature of the disease. It is a fungal infection caused by several species belonging to the order Mucorales. It is known to affect the nasal mucosa, paranasal sinuses, orbit, lungs and skin. In rare cases, involvement of the gastrointestinal tract, intracranial structures or disseminated infection may be seen. The recent steep rise in the number of patients affected has mirrored the rise of the second wave of COVID-19 infections in India (Sharma et al. 2021). This chapter aims at giving a comprehensive overview of rhino-orbito-cerebral mucormycosis (ROCM).

11.2 Cause and Pathogenesis

Fungi of the order Mucorales are the causative organisms. Species belonging to several genera including *Mucor, Rhizopus, Absidia* and *Cunninghamella* have been isolated. The most frequently reported pathogens in mucormycosis are Rhizopus spp., Mucor spp. and Lichtheimia spp. (formerly of the genera Absidia and Mycocladus), followed by Rhizomucor spp., Cunninghamella spp., Apophysomyces spp. and Saksenaea spp. (Roden et al. 2005; Skiada et al. 2011). *Rhizopus oryzae* is the most common organism and responsible for nearly 60% of mucormycosis cases in humans and also accounts for 90% of the Rhino-orbital-cerebral (ROCM) form (Sugar 2000). These fungi are ubiquitous and are very commonly found in moist, decaying matter. They release spores in the environment and are commonly found colonising the oral cavity, throat, nasal cavity and paranasal sinuses. However, infection in immunocompetent individuals is rare owing to the robust phagocytic elimination of these organisms.

The spores of these organisms are free to grow in immunocompromised individuals and develop into aseptate hyphae. These hyphae have a tendency to progressively invade surrounding tissues including the blood vessels and nerves, along with the destruction of the surrounding bones. This appears to be mediated through extracellular matrix laminin, type IV collagen (Bouchara et al. 1996) and glucose-regulated protein (GRP78) (Liu et al. 2010). This facilitates the spread into adjacent compartments. The angio-invasive property of the fungus leads to thrombosis of the involved blood vessels. Thrombosis leads to tissue infarction and resultant necrosis. The perineural invasion of the fungus results in loss of sensation,

pain, decreased vision and paraesthesias. The angio-invasiveness of the organism explains the haematogenous dissemination to other distant organs.

11.3 Risk Factors

1. Diabetes and mucor: Uncontrolled diabetes and ketoacidosis are the most important predisposing factors contributing to mucormycosis (Roden et al. 2005; Corzo-Leon et al. 2018). In the presence of hyperglycaemia and low pH, which is found in patients with diabetic ketoacidosis (DKA), phagocytes are dysfunctional and have impaired chemotaxis and defective intracellular killing by both oxidative and nonoxidative mechanisms (Chinn and Diamond 1982). The acidic serum helps to promote the growth of the mucor, decreases the iron-binding capacity of serum transferrin, thus increasing the free iron, promoting the reproduction of the mucor. Mucor has ketoreductase that helps it utilise the host's ketones to promote its growth (Ma et al. 2015; Mondal et al. 2012).
2. Neutropenia: Neutrophil and phagocyte dysfunction have been strongly implicated in the pathogenesis of mucormycosis (Ibrahim et al. 2012). Due to this reason, patients with haematological and solid organ malignancy patients receiving chemotherapy, solid organ transplant patients on immunosuppression and hematopoietic stem cell transplant patients are particularly susceptible to mucormycosis. Interestingly, patients with AIDS (lymphocyte dysfunction) did not seem to have an increased risk of mucormycosis (Sugar 2005).
3. Iron overload states like hemochromatosis, deferoxamine therapy, multiple blood transfusions provide an excellent setting for mucormycosis as it allows for the growth of the fungus and increases susceptibility (Ibrahim et al. 2012).
4. Mucormycosis has also been reported in chronic kidney disease, liver cirrhosis and malnutrition patients (Prakash et al. 2019).
5. Long-term systemic voriconazole (especially given in ICUs for prophylaxis) has also been implicated (Gupta et al. 2017).

ROCM is frequently observed in association with uncontrolled diabetes and DKA. Pulmonary involvement is commoner in patients having neutropenia, bone marrow and organ transplant and haematological malignancies. Gastrointestinal involvement is seen in malnourished individuals (Singh et al. 2021).

11.4 COVID-19 and Mucormycosis

The surge in COVID-19 cases in the second wave has brought with it aftermath of increased mucormycosis cases. Globally, the prevalence of mucormycosis varied from 0.005 to 1.7 per million population, while its prevalence is nearly 80 times higher (0.14 per 1000) in India compared to developed countries, in a recent estimate of the year 2019–2020 (Singh et al. 2021). As of May 2021, 101 cases of mucormycosis have been reported in patients with COVID-19, 82 of which are

from India. Most cases presented with concomitant COVID-19 infection or within 2–3 weeks of recovery.

Singh et al. (Singh et al. 2021) in their review discussed the reasons for the increased susceptibility of these patients to mucormycosis:

1. Diabetes Mellitus (DM) is often associated with increased severity of COVID-19 infection, as well as increased risk of contracting mucormycosis.
2. Uncontrolled hyperglycaemia and precipitation of DKA are often seen secondary to corticosteroid intake. Widespread indiscriminate use of steroids has been seen in several COVID-19 patients.
3. Like all viral infections, COVID-19 infection affects both innate and adaptive immunity and predisposes to secondary infections. COVID-19 is known to cause endotheliitis with damage, thrombosis, lymphopenia and reduction in CD4+ and CD8+ levels, predisposing to secondary fungal infections.
4. Transferrin and ferritin undergo glycosylation in hyperglycaemic states, releasing and allowing for increased free iron. An increase in cytokines (especially IL-6) and concomitant acidosis in these patients increases ferritin levels thus increasing free iron availability.
5. High glucose, low pH, free iron and ketones enhance the expression of fungal ligand spore coating homolog (CotH) protein and glucose-regulator protein 78 (GRP-78) of endothelial cells, facilitating angioinvasion, tissue necrosis and haematogenous dissemination.

Diabetes was present in 80% of cases, while corticosteroid treatment was given for COVID-19 in 76.3% of cases according to this study (Singh et al. 2021). Widespread indiscriminate use of steroids has led to systemic hyperglycaemia, which is especially worse in diabetics, but is not restricted to known diabetics. Other factors implicated are the use of Tocilizumab and prolonged mechanical ventilation through unclean oxygen humidifiers.

11.5 Clinical Features of Rhino-Orbito-Cerebral-Mucormycosis

Clinical features correlate with the structures involved. The nasal mucosa and turbinates are the first to be colonised by the pathogens. The patients will first experience nasal symptoms such as nasal discharge and stuffiness. At later stages, dark eschar may be visible in the vestibule of the nose with blood-tinged or dark coloured nasal discharge (Honavar 2021; Deutsch et al. 2019). As the sinuses are progressively involved, sinus headaches may be experienced accompanied by cheek oedema and erythema. Fungal invasion into the surrounding vessels results in vascular occlusion and resultant necrosis of the tissues. Externally it may manifest as reddish or dark discoloration of the skin, which eventually breaks down to form a dark eschar. Palatal involvement as an ulcer or eschar may also be seen.

Orbital involvement is secondary to invasion from the sinuses through intact bone, or with bone destruction. Medial and inferior orbit are commonly involved

Table 11.1 Clinical features of ROCM

Nasal involvement	Sinus involvement	Orbital involvement	Cerebral involvement
• Nasal stuffiness • Discharge • Foul smell • Epistaxis • Nasal eschar	• Facial edema • Facial pain • Dental pain • Sinus tenderness • Sinus headache • Sinus discharge • Malaise • Fever	• Eye pain, decreased vision • Proptosis • Ptosis • Diplopia • Conjunctival congestion and chemosis • Infraorbital nerve anaesthesia • Central retinal artery occlusion • Superior ophthalmic vein thrombosis • V1, V2, 3rd, 4th, 6th cranial nerve palsy	• Altered consciousness • Seizures • Hemi-cranial headache • Bilateral signs and symptoms • Hemiparesis

first, followed by spread to the rest of the orbit (Honavar 2021). Patients may complain of periocular pain, swelling with vision loss or diplopia. Lid oedema, erythema, limitation of ocular movements, proptosis, pupillary involvement (afferent pupillary defect), conjunctival congestion and chemosis may be seen to varying degrees. Extreme proptosis with corneal exposure may lead to epithelial defects or eventually corneal ulceration. Posterior segment involvement in the form of retinal vascular occlusions (commonly central retinal artery occlusion) due to direct invasion into the retinal vessels and embolization, may be seen, especially with apical involvement. Optic disc oedema or pallor resulting from optic nerve compression due to orbital inflammation may be observed. Isolated involvement of the orbital apex by invasion through the adjacent sinus is also seen. In such cases, severe vision loss, pupillary involvement, III, IV and VI cranial nerve palsies are much more marked as compared to proptosis and signs of ocular inflammation.

Intracranial extension of the disease may occur through the cribriform plate, direct extension from the sphenoid and frontal sinuses, throughout the orbital apex, cavernous sinus or through haematological routes (Ma et al. 2015). The manifestation of bilateral vision loss, III, IV and VI cranial nerve palsies or orbital congestion may point towards the intracranial spread. Patients usually present with severe headaches, seizures, hemiparesis, focal neurological disorders or altered sensorium (Ma et al. 2015). The common clinical features of ROCM are summarised in Table 11.1.

11.6 Imaging

Computed tomography (CT) and Magnetic resonance imaging (MRI) are both useful modalities to characterise the extent of involvement of the disease. Nasal cavity involvement is seen as nonspecific inflammatory turbinate hypertrophy and inflammatory fluid in the nasal cavity. CT scan of the paranasal sinuses (PNS) accurately

shows the bony details of the sinuses. Hence it is the preferred choice of imaging by the ENT surgeons, Involvement of the sinuses is seen as a heterogeneous hyperdense opacity within the sinus cavity. In more advanced cases, bone destruction with invasion into the orbit and intracranial cavity may be seen. One study found the ethmoid sinus was the most common paranasal sinus involved (86%) (Therakathu et al. 2018). In the majority of patients (79%) multiple sinuses were involved. The combination of maxillary, ethmoid and sphenoid (49%) was most frequently seen. Unilateral sinus involvement was more common (79.1%) than bilateral (20.9%) (Therakathu et al. 2018). Bone involvement in the form of bone rarefaction, erosions and permeative destruction was seen in 40% cases.

Orbital involvement begins adjacent to the involved sinus in the form of periosteal thickening and adjacent fat stranding. This progresses to involve the medial rectus, inferior rectus, optic nerve and adjacent fat (Fig. 11.1) (Honavar 2021). Further spread of the infection is indicated by diffuse heterogeneous lesion within the orbit and in the area of the orbital apex. Resultant proptosis with optic nerve stretch and tenting of the globe may be seen in extreme cases. Oculoplastic surgeons consider MRI as a better modality to judge the orbital inflammation and invasion and Intra-cranial spread (Mnif et al. 2005). On MRI the lesions are mildly hypointense on T1 weighted images and have variable intensity on T2 weighted images. Early cases may show low intensity on T2 weighted images. An abscess can be identified as lesions with peripheral enhancement and central non-enhancing portion. Intracranial involvement is seen on brain CT as irregular low-density areas within the cerebral parenchyma (Ma et al. 2015). MRI shows low T1-weighted imaging signals and high T2-weighted imaging signals. Contrast-enhanced MRI may show annularly enhancing lesions. Although CT and MRI can both diagnose intracranial inflammation and brain abscess, MRI shows better tissue details and is therefore preferred (Ma et al. 2015).

Although MRI has a greater advantage in delineating the disease extent, often the patients are too unstable to undergo this lengthy scan. In such cases, a CT scan with contrast may be obtained, as it is often quicker and more readily available.

The use of contrast greatly increases the information gained from the scan. Patchy contrast enhancement is seen in the initial stages in the nasal mucosa, paranasal sinus mucosa and orbit. In areas with complete occlusion of the feeder arteries, no contrast uptake is seen in the involved mucosae, turbinates (called the 'black turbinate sign') (Safder et al. 2010), sinuses and orbital tissues. Non-contrast-enhancing areas indicate portions with necrotic tissues and a high fungal load (Choi et al. 2018). This helps in planning the management of the patient. All the devitalised tissues seen as the portions not enhancing with contrast, have to be debrided during surgery. The presence of contrast uptake indicates that the tissues can possibly be salvaged. Therefore, more conservative approaches may be tried. MR angiography is especially being explored for assessing tissue perfusion and identifying vascular occlusions and viable tissue.

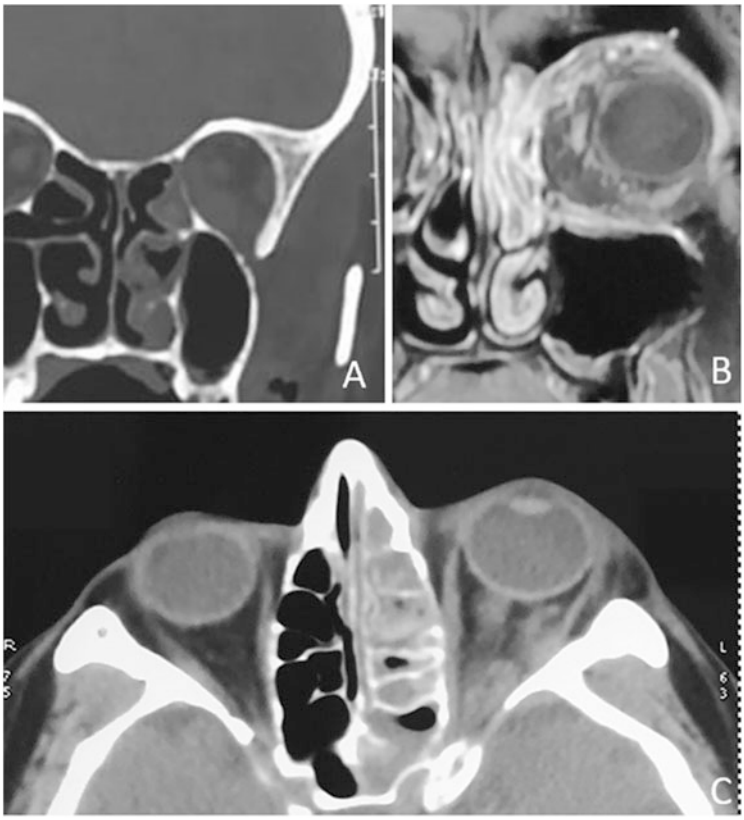

Fig. 11.1 Radiological features of rhino-orbital mucormycosis: (**A**) shows the CT scan of a patient showing a patchy hyperdensity involving the left ethmoid sinus and nasal cavity (turbinates), with adjacent edema of the left medial and inferior recti. (**B**) shows the CEMRI orbit and paranasal sinuses of another patient. This T1 weighted image shows patchy enhancement of the ethmoid sinus and adjacent infero-medial orbital involvement. The enlargement of inflamed nasal turbinates is also noted. (**C**) shows a heterogenous opacity involving the entire left ethmoid sinus. There is predominant left orbital apex involvement seen as a heterogenous hyderdensity at the apex, involving the medial rectus, the optic nerve sheath and surrounding fat

11.7 Microbiological Investigations

The most convenient and fastest modality of confirming the diagnosis is through direct microscopy as a KOH or calcofluor white mount. The sample is obtained from the nasal cavity or paranasal sinus preferably under endoscopic guidance, from the orbital tissue, or from the edge of the eschar to increase the yield. Another sample is also simultaneously taken for histopathology. Direct microscopy with fluorescent brighteners shows aseptate or pauci-septate hyphae, with a wide branching angle ($\geq$45–90°) and greater hyphal width (6–16μm). Histopathological sections may be

stained with haematoxylin-eosin (HE), periodic acid-Schiff stain (PAS), or Grocott-Gomori's methenamine-silver or both. Lesions show infiltration of the tissues by fungal hyphae, haemorrhagic infarction, coagulation necrosis, angioinvasion, infiltration by neutrophils (in non-neutropenic hosts) and perineural invasion (Frater et al. 2001). Often confusion arises while differentiating mucor from aspergillosis. They can be differentiated by molecular diagnostic techniques or PCR. The application of immunohistochemistry with commercially available monoclonal antibodies can also help (Jensen et al. 1997; Jung et al. 2015).

Culture of specimens in brain heart infusion agar, potato dextrose agar or Sabouraud dextrose agar with gentamicin or chloramphenicol and polymyxin-B, without cycloheximide, is strongly recommended for genus and species identification, and for antifungal susceptibility testing. Incubation at 30 °C and 37 °C separately will show rapid growth of fluffy white, grey or brown cotton candy-like colonies with coarse hyphae and interspersed brown or black sporangia (Honavar 2021).

Serological tests for antigen and antibody,1,3-β-D-Glucan and Galactomannan can aid diagnosis in suspected cases (Song et al. 2020).

Polymerase chain reaction (PCR) based molecular identification is also available. DNA sequencing based on bar codes 18s, ITS, 28s, rDNA, MALDI-TOF have limited availability, but can detect fungal DNA in paraffin-embedded tissues and serum. PCR is also a rapid and accurate test (Song et al. 2020).

11.8 Management

Patients presenting with classical signs and symptoms suggestive of mucormycosis with a history of uncontrolled diabetes, immunocompromised status, history or concurrent COVID-19 infection especially with steroid administration need further investigations. They may be classified for practical purposes into the following categories probable, possible and proven cases of mucormycosis (Honavar 2021) (Fig. 11.2).

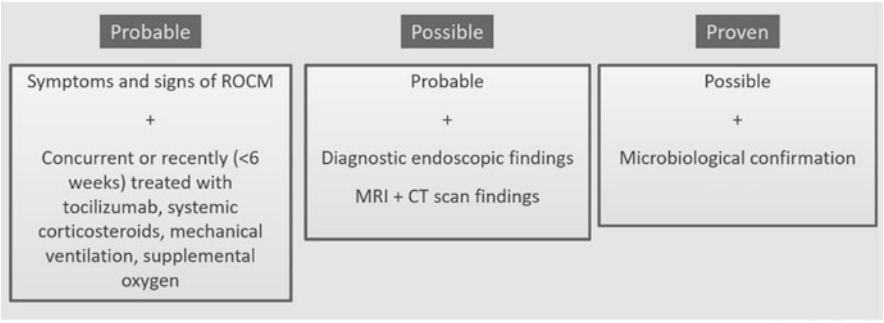

Fig. 11.2 Clinical categorisation of patients

All probable mucormycosis patient's nasal swab samples must be taken for microbiology and histopathology. A contrast-enhanced CT scan of the paranasal sinuses must be done. In cases where the orbital or intracranial spread is suspected, a CEMRI orbit with MR angiography may be preferred. A detailed and comprehensive evaluation of the nose and PNS is a must. Ocular evaluation with documentation of vision, intraocular pressure, pupillary reactions, extraocular movements, anterior segment evaluation, evaluation of retinal perfusion and the disc is essential.

Possible and confirmed cases of mucormycosis must undergo haematological investigations such as random blood sugar, serum electrolytes, renal function tests complete blood count and arterial blood gas (to rule out acidosis) (Craig 2019). Detailed iron studies may be required if iron overload is suspected. Testing for COVID-19 by RT-PCR is preferred. Once confirmed, the patient must be admitted for further management.

11.8.1 Medical Management

The global guidelines for diagnosis and management of mucormycosis were given in 2019 by the European Confederation of Medical Mycology ECMM and Mycoses Study Group Education and Research Consortium (MSGERC). Antifungal treatment with a thorough surgical debridement of involved tissues form the mainstay of the management (Cornely et al. 2019). Systemic antifungals have to be instituted at the earliest.

Amphotericin B is the drug of choice. It acts by binding with ergosterol, a component of fungal cell membranes, forming pores that cause rapid leakage of monovalent ions (K+, Na+, H+ and Cl−) and subsequent fungal cell death. It is available in the liposomal (LAmB), lipid complex (ABLC) and in deoxycholate (C-AmB) forms. The former two are preferred due to their established efficacy and lower renal toxicity. The commonly available forms are:

1. Liposomal amphotericin B: It is administered at the dose of 5 mg/kg/day (7.5–10 mg/kg/day in cerebral mucormycosis) over 4–6 h.
2. Amphotericin B Deoxycholate: 1–1.5 mg/kg/day is administered over 4–6 h. It is generally not preferred due to higher nephrotoxicity.

Dose adjustments are required in case of renal impairment. This treatment is continued for 2–4 weeks. This is followed by step-down treatment with azole antifungals. These act by interfering with the synthesis and permeability of fungal cell membranes, by inhibiting cytochrome P450-dependent 14-alpha-sterol demethylase. Isavuconazole and Posaconazole are approved for use either in combination with Amphotericin B (first line- in severe infections) or as a step-down treatment after initial treatment with Amphotericin B or as an alternative first-line treatment, in case of toxicity or non-availability of Amphotericin B (Rawson et al. 2020). The high cost of these medications and limited availability may be a deterrent.

1. Isavuconazole is administered at the dose of 200 mg thrice a day for the first 2 days, followed by 200 mg daily.
2. Posaconazole is given as 300 mg twice a day for 1 day followed by 300 mg daily.

Treatment is continued for 3–6 months or until 6 weeks after the radiological and clinical resolution or stabilisation (Honavar 2021). In case of renal toxicity or drug resistance, a combination with caspofungins and echinocandins has been explored due to the observed synergistic effect.

Retrobulbar amphotericin B is being explored as an option to increase globe salvage rates in cases of limited retrobulbar disease with viable tissues and preserved vision (Hirabayashi et al. 2017; Safi et al. 2020). Three to five 1 mL injections (3.5 mg/mL) given every consecutive or every alternate day has shown promising results.

11.8.2 Surgical Management

Any necrotic and infiltrated tissue is not viable and cannot be effectively treated with systemic antifungals alone due to lack of penetration. Therefore, thorough debridement is the only option. In case of limited sinus disease, FESS with clearance of the necrotic mucosa might give beneficial results (Nithyanandam et al. 2003). In case of extensive bony involvement and orbital involvement, a maxillectomy (total or partial), zygoma debridement or orbital exenteration may be necessary. During surgery, all visible necrotic tissues must be removed. The extent of debridement needed may also be determined by the non-perfused portion as seen on a CEMRI scan. Post-operative serial dressings and sinus irrigation with amphotericin B may be performed.

Orbital exenteration may be required in case of the presence of extensive necrotic tissue (Lee et al. 2020). Orbital exenteration when needed, maybe preferably performed through a lid sparing anterior approach. There have however been several reports of patients surviving despite withholding orbital exenteration. There are debatable indications for this procedure in mucormycosis management (Hargrove et al. 2006). Some reports have also found exenteration to worsen the outcomes of the patients. Post-operative socket related complications are also known to occur.

Intracranial involvement is mainly managed medically. If there is abscess formation, it may need the expertise of a skull base surgeon or a neurosurgeon for removal of the devitalised tissue (Ma et al. 2015).

11.8.3 Systemic Management

Reversal of the underlying predisposing factor is equally important. Control of blood sugars with the help of insulin injections and constant monitoring is essential (Corzo-Leon et al. 2018). Diabetic ketoacidosis has to be reversed along with adequate hydration and serum electrolyte correction. In patients on

immunosuppressive drugs, the doses may have to be decreased. Neutropenia in hematologic malignancies should be reversed, if possible, with the use of colony-stimulating factors and the withdrawal of cytotoxic chemotherapy. Deferoxamine therapy should be stopped and hydroxypyridine chelating agents should be used as a substitute (Yohai et al. 1994).

11.9 Disease Burden and Prognosis

Mucormycosis is known to have a high mortality rate. The rates are highest with disseminated, gastrointestinal and pulmonary disease (up to 90%) (Deutsch et al. 2019). ROCM form has a mortality rate of about 46% among people with sinus infections. The rate jumps to 76% for pulmonary infections and 96% for disseminated mucormycosis (Roden et al. 2005). The cutaneous disease carries the lowest mortality rate (15%). The factors related to a lower survival rate include delayed diagnosis and treatment, bilateral sinus involvement, hemiparesis or hemiplegia, leukemia, renal disease and treatment with deferoxamine (Yohai et al. 1994). A more recent meta-analysis (Vaughan et al. 2018) revealed that the survival rates in patients with chronic renal disease had improved from 19% to 52% and in patients with leukaemia from 13% to 50% compared to the analysis by Yohai et al. (Yohai et al. 1994) Facial necrosis and hemiplegia are poor prognostic indicators (33% and 39% survival rates, respectively). Early commencement of medical treatment led to better survival outcomes (61% if commenced within the first 12 days of presentation, compared to 33% if after 13 days) (Vaughan et al. 2018).

Even after recovery, the possible sequelae include the partial loss of neurological function, blindness and clotting of intracranial, orbital or pulmonary vessels. Additional poor healing in the surgical field predisposes to post-exenteration socket complications.

11.10 Prevention

Knowing the lengthy treatment process and the dismal prognosis, prevention and early recognition of the disease gives the best chance to the patient. In the COVID-19 era, judicious and supervised use of systemic steroids and tocilizumab must be stressed upon. Aggressive monitoring and control of diabetes mellitus is a must. In patients requiring supplemental oxygen, strict aseptic precautions must be followed (Honavar 2021).

Personal and environmental hygiene must be reiterated to patients. Betadine mouth gargles are advocated by some. Masks used for covering the nose and mouth should not be reused. Some centres are leaning towards the use of Posaconazole prophylactically in high-risk patients.

11.11 Conclusion

ROCM is a disease with high mortality despite all treatment. A high index of suspicion is necessary, especially in the setting of uncontrolled diabetes and recent COVID-19 infection. These patients present with orbital cellulitis like the picture, but with considerably fewer inflammatory signs. Early diagnosis, through appropriate diagnostic techniques and aggressive treatment is the key. Prevention, by limiting and controlling the use of immune-compromising drugs (especially steroids) goes a long way in the management of these patients. Awareness of this disease entity among the physicians and ophthalmologists, specifically in patients with predisposing factors, early diagnosis and treatment, monitoring of therapeutic agents would go a long way in reducing morbidity and mortality.

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Strokes, Neurological, and Neuropsychiatric Disorders in COVID-19

12

Sunil K. Nooti, Vikrant Rai, Harbinder Singh, Veena Potluri, and Devendra K. Agrawal

Abstract

Although Coronavirus disease (COVID-19) is predominantly a respiratory illness, it can affect all organ systems directly or indirectly. The plethora of published information regarding SARS-CoV-2 infection focused initially on general clinical presentations with less attention on specific organ systems involved. However, the extent and specific involvement of the nervous system are now being appreciated with newer studies confirming the sparse earlier findings. Short-term and long-term nervous system complications of recovered and recovering COVID-19 patients can be broadly classified into neurological, neuropsychiatric, and stroke-related manifestations. Here, we critically reviewed the emerging literature on these aspects, where the direct invasion of the virus into the nervous system and/or an aberrant host immune response have been implicated as the predominant mechanisms. However, recognition and further understanding of the extent and severity of nervous system involvement may lead to improved clinical outcomes in the near future.

Keywords

Angiotensin-converting enzyme-2 · Cerebral venous sinus thrombosis · Coagulopathy · COVID-19 · Intracerebral hemorrhage · Ischemic stroke ·

Sunil K. Nooti and Vikrant Rai contributed equally with all other contributors.

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Orthostatic hypotension · Rhabdomyolysis · Severe acute respiratory syndrome · Venous thromboembolism

12.1 Introduction

The short-term neurological complications of the ongoing COVID-19 pandemic have emerged to be a significant cause of morbidity and to certain extent mortality as well. Even though the pandemic is only a year old, long-term sequela of neurological, neuropsychiatric, and stroke-related manifestations are expected to predominate for years and decades to come once the pandemic ends or becomes endemic. A study conducted as early as April of 2020 identified, among patients who had been discharged, that at least 33% had a "dysexecutive syndrome" consisting of inattention, disorientation, or poorly organized movements in response to the command (Helms et al. 2020a). These and many persisting neurological manifestations of COVID-19 have become so common and disabling that online groups for "Long Haul COVID Fighters" have emerged (Rubin 2020). A number of collaborative networks to systematically document neurological manifestations (e.g., CoroNerveStudies Group) have sprung globally in longitudinally tracking and managing the illness prospectively, as proposed by Helms et al. (2020a, b) (Rubin 2020) and more recently by O'Conner et al (2020). The neurological manifestations associated with COVID-19 may vary from mild symptoms including loss of taste and smell, dizziness, and headache and severe manifestations including ischemic stroke, meningitis, encephalitis, and encephalopathy. The neurological manifestations arise both from the central nervous system (CNS) as well as the peripheral nervous system (PNS) (Table 12.1) and can broadly be categorized as neurological, neuropsychiatric, or stroke related.

The literature on the neurological features of COVID-19 initially comprised a large number of case reports and only a few case series and retrospective observational studies. However, a quick PubMed search of keywords related to "Covid" and "neurological" yields more than 2000 articles as of March 2021. A huge number of neurological abnormalities have been described in COVID-19 patients. These involve both the central and peripheral nervous system and range from mild to life-threatening and are also based on subjective descriptions provided by the patient or clinically assessed by neurologists and by advanced investigations. Even though it is not practical to go through each and every publication for a thorough review, we present here a broad and general perspective on the various aspects of neurological manifestations seen in COVID with emphasis on mechanisms.

Table 12.1 Neurological manifestations of COVID-19

CNS		
Neurological	Neuropsychiatric	PNS
Muscle weakness	Brain fog	Anosmia
Dizziness	Inability to concentrate	Ageusia
Headache	Memory lapses	Vision impairment
Impaired consciousness	Mood changes	Nerve pain
Acute cerebrovascular disease including strokes, seizures, and slurred speech	Limb force reduction	Muscle pain
Sore throat and difficulties to swallow	Acute psychosis	Guillain–Barre syndrome
Diplopia	Manic disorders	Peripheral limb weakness
Delirium	Cognitive and behavioral abnormalities	
Polyneuritis cranialis	Psychosocial stress	
Ataxia, Cerebellar ataxia	Catatonia	
Epilepsy		
Encephalitis		
Encephalopathies		
Transverse myelitis		
Altered mental status		
Focal central neurologic symptoms		
Cranial neuropathy		
Movement disorders		
Pyramidal syndrome		
Central oculomotor syndrome		
Motor or sensory deficits		

12.2 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2) Infection

Like SARS-CoV-1, SARS-CoV-2 uses ACE2 as the main docking receptor and needs proteolytic processing of its spike protein with the absolute requirement of a transmembrane serine protease TMPRSS2 (Fig. 12.1). Spike protein is synthesized as a large membrane protein that is cleaved into two components, S1 and S2, which remain noncovalently associated. Cleavage is required for infection and can occur during virus particle production or virus entry into the target cell. The S1 protein forms the "head" of the molecule and mediates binding to ACE2. The S2 protein is anchored in the virus membrane and mediates membrane fusion. The so-called priming involves S protein cleavage allowing fusion of viral and cellular membranes, a process driven by the S2 subunit (Hoffmann et al. 2020). The viral spike protein is crucial in mediating the fusion of the membrane after binding. The

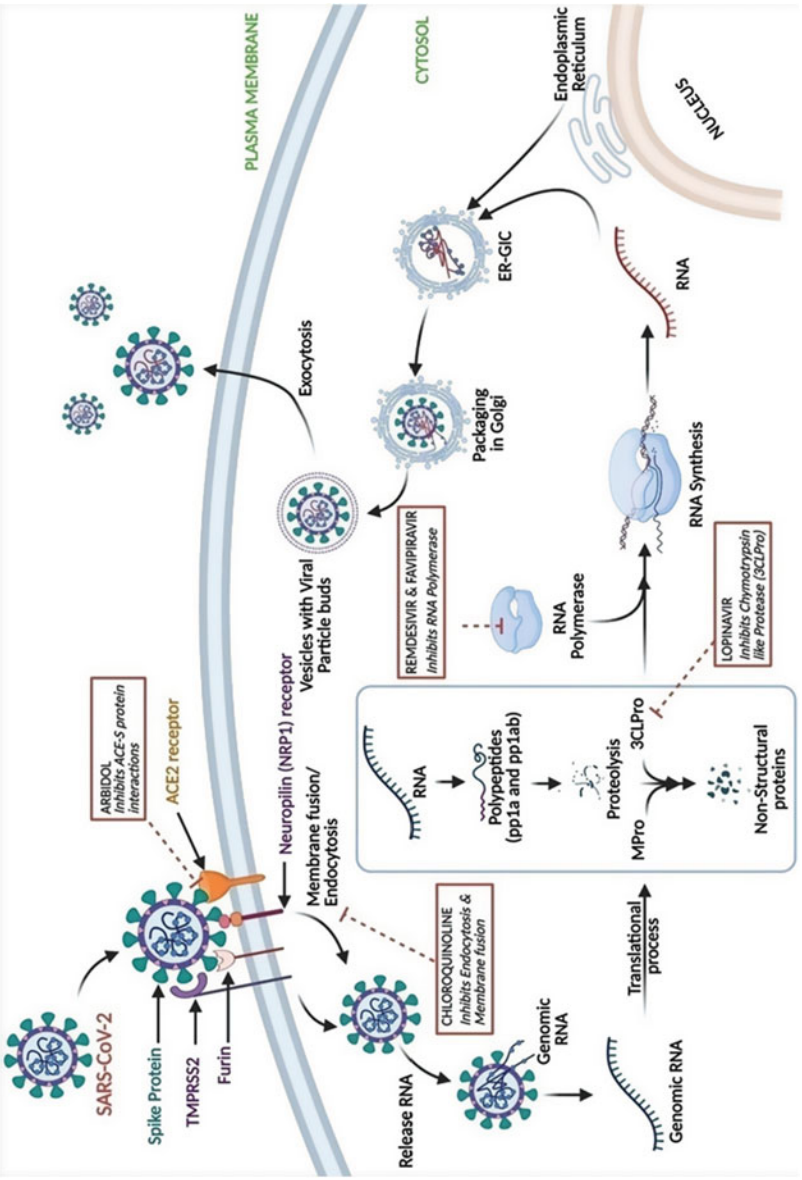

Fig. 12.1 Graphical description of the pathogenesis of direct invasion of SARS CoV-2: Graphical description of the pathogenesis of direct invasion of SARS CoV-2 and various potential therapeutics available in the pathogenesis. Spike protein subunits are responsible for the interactions with the receptors like ACE2,

Neuropilin, and so on, and proteases like TMPRSS2, Furin, and so on, leading to the fusion of SARS CoV-2 and host cell membranes. Viral RNA is released into the cytosol after the membrane fusion. During the translational process, viral RNA is used to synthesize non-structural proteins. Following the synthesis, RNA polymerase also helps to replicate the viral RNA to synthesize a battery of RNA encoding the structural and accessory proteins. With the help of host ER and Golgi machinery, viral genome and proteins assemble to form new vesicles that fuse with the cell membrane to release the virus. *ACE* Angiotensin-Converting Enzyme, *MPro* Main protease, *3CLPro* Chymotrypsin like protease, *TMPRSS* Transmembrane protease, *ER-GIC* Endoplasmic Reticulum-Golgi intermediate compartment

S1 subunit forms the "head" of the molecule and mediates binding to ACE2 while the S2 subunit is anchored in the virus membrane and mediates membrane fusion. Hence S protein priming by cellular proteases is essentially a cleavage of S protein at the S1/S2 interface (by Furin) and a second S2′ site (by TMPRSS2). The cleavage at the S1/S2 interface (which contains the polybasic furin-type cleavage site) is unique to SARS-CoV-2 and is not seen in SARS-CoV-1. It is interesting to note that similar furin-type cleavage sites are found in S proteins of other dangerous viruses such as HIV-1, Ebola, and some highly virulent strains of avian influenza. More recently SARS-CoV-2 has been shown to also utilize basigin (BSG/CD147) (Wang et al. 2020c) and/or neuropilin-1 (NRP-1) (Cantuti-Castelvetri et al. 2020; Daly et al. 2020) as its receptors. A range of proteases including TMPRSS11A/B, cathepsin B, cathepsin L, and furin have also been shown to participate in the protease activity.

12.3 The Burden of Neurological Manifestations

A majority of the population affected with COVID-19 is asymptomatic (45% according to CDC), while those who are symptomatic; a majority have only mild nonspecific symptomatology. COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS CoV-2), which is the seventh known human coronavirus. Beta-coronaviruses SARS-CoV-1, SARS-CoV-2, and Middle Eastern respiratory virus (MERS CoV) are associated with severe disease in humans and have also been implicated in causing neurological complications. SARS-CoV-1 has been reliably detected in the brains of autopsy specimens, specifically in the cytoplasm of the neurons in the cortex and hypothalamus (Gu et al. 2005). Autopsy tissue from a patient with encephalitis revealed neuronal necrosis, glial cell hyperplasia, and infiltration of monocytes and T cells with visualization by electron microscopy, and isolation of SARS-CoV-1 RNA from the brain tissue (Additionally, virions were visualized in neurons on electron microscopy, and SARS-CoV-1 RNA was isolated from the specimen (Xu et al. 2005). Unlike SARS-CoV-1 and 2, MERS-CoV does not bind to the Angiotensin-converting enzyme 2 (ACE2) but binds to dipeptidyl peptidase 4 (DPP-4), which is widely expressed on epithelia, endothelia, and brain (PMID: 26486634 (Li et al. 2016; Lambeir et al. 2003). However, SARS and MERS infected a smaller number of cases (~8000 and 2500, respectively) and had high mortality rates (~10% and 30%, respectively) (according to WHO), due to which the burden of neurological complications in survivors was low compared to COVID-19 which has so far infected more than 130 million confirmed cases and more than 2.5 million deaths as of March 2021.

SARS COV2 invasion of neurovascular compartment can lead to acute cerebrovascular events like ischemic strokes, cerebral venous sinus thrombosis, intracerebral hemorrhage, Rhabdomyolysis, and Orthostatic hypotension. Many of the long-term manifestations in COVID survivors have been found to be similar to myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) whose etiology still remains unknown. Possible mechanisms in COVID-19 for these long haulers that are suggested include unmasking of a previously unrecognized underlying comorbidity,

residual brain damage, or persistent viral replication, or host immune activity (Nath 2020). However, it needs to be seen if COVID-19 studies bring hope in deciphering the true causal factors of these seemingly simple yet complex phenomena. In contrast, with respect to short-term neurological manifestations at the time of presentation or during the course of COVID-19 disease, a large body of clinical data has been collected. Though a significant portion of these data is covered by a number of case reports, large retrospective observational studies as well as case series from tertiary referral centers are rapidly accumulating. Here we provide a broad perspective of the evolving literature with a review on the mechanisms of neurological involvement in COVID-19.

Unavoidable methodological differences have hampered estimations of true incidence and prevalence of neurological manifestations in COVID. In the very first case series from Mao et al. (2020) neurological symptoms were detected in 36.4% (a total of 214 patients), while analysis of the ALBACOVID registry in Spain detected 57% (of 841 patients) as reported by Romero-Sanchez et al. (2020). However, Xiong et al. (2020) in a case series from 56 dedicated COVID-19 treatment centers in China detected new-onset specific neurological events in only 4.2% (of 917 patients). Since the definition of neurological symptoms is vague and poorly defined, and since various studies have data extracted from electronic health records to patient descriptions, it is difficult to compare and interpret. Notwithstanding these difficulties, the huge number of COVID-19 cases and the expanding subset of neurological features with every recovering case needs special attention as the pandemic progresses and either declines or becomes an epidemic. In either case, the sheer number of 4.2% of the whole population affected with neurological affects will be humungous.

12.4 Pathogenesis of Neurological Manifestations in COVID-19

The neurological manifestations of COVID-19 might be due to the direct effect of the neurotrophic properties of the virus (meningitis, encephalitis, and encephalopathy), thrombotic complications (cerebrovascular ischemic events, cerebral venous sinus thrombosis, and deep vein thrombosis), blood pressure dysregulation (intracranial hemorrhage), inflammatory consequences (cytokine storm, thrombus formation, coagulation disorders, acute myelitis, Acute hemorrhagic necrotizing encephalopathy (ANE)), hypoxia (encephalopathy, headaches, dizziness, etc.). However, the exact underlying mechanism of some of the symptoms (hyposmia, hypogeusia, headaches, dizziness, diplopia, and ophthalmoplegia) remains elusive because of the lack of specific mechanistic research studies (Tables 12.1 and 12.2). Numerous studies have implicated two major mechanisms of these various neurological manifestations: (1) Due to the direct invasion of SARC-CoV-2 on the brain, neurons, or altered blood-brain barrier with little evidence of the virus in cerebrospinal fluid samples and (2) Indirectly due to an aberrant and exaggerated host immune response. However, the underlying molecular mechanisms specific to each presentation definitely warrant further large observational and mechanistic studies. Here we

Table 12.2 Neurological manifestations in COVID-19 and their etiologies

Neurological manifestations and etiologies in COVID-19	
Common manifestations	**Etiology**
Dysgeusia/Ageusia (loss of taste), Anosmia (loss of smell)	These form the main neurological symptom in covid-19 without significant rhinorrhoea or nasal congestion (Parma et al. 2020). Direct invasion of epithelial, support, and stem cells responsible for maintaining olfactory, gustatory, and chemesthetic systems, but not the primary neurons (Cooper et al. 2020)
Myalgia	Pathogenesis is evidenced by elevated Creatinine kinase (CK) and Lactate dehydrogenase (LDH) biochemically (Disser et al. 2020); the direct response of viral invasion of the skeletal muscles Uncontrolled immune response with elevated pro-inflammatory cytokines manifesting as cytokine storm subsequently causing muscle injury (Hamming et al. 2004; Lacomis 2020)
Headache	Nociceptive sensory neurons are triggered by the release of cytokines and chemokines by macrophages, T cells, and so on during various stages of COVID-19 infection (El-Ashmawy et al. 2021)
Altered mental status	Is considered a strong predictor of mortality in Covid-19 (Tyson et al. 2020) caused by acute hypotensive infarctions or embolic showers (Kenerly et al. 2021). Elevated neuron-specific enolase (NSE) levels in CSF and hypernatremia (Toklu et al. 2020)
Encephalopathy, Acute necrotizing encephalopathy (ANE), Acute disseminated encephalomyelitis (ADE)	Encephalitis (Moriguchi et al. 2020), cytokine storm, coagulopathy, direct neuroinvasion by the virus, endotheliitis, and possibly post-infectious auto-immune disorders. Systemic inflammatory response syndrome (SIRS) and cytokine storm are also thought to underlie in ANE (Virhammar et al. 2020). MRI findings showed demyelinating abnormalities for ADE ("Acute disseminated encephalomyelitis after SARS-CoV-2 infection" 2021)and peri-infectious immune-mediated syndrome, rather than direct viral encephalitis is significant (Zubair et al. 2020)
Dizziness, vertigo, and light-headedness.	These are among the most common symptoms in covid and are most probably due to direct invasion, hypoxia, hyper coagulopathy, as well as inflammation related to the viral infection, labyrinthitis (inflammation of the inner ear's labyrinth and vestibular nerve), and vestibular neuronitis (inflammation of the vestibular nerve) (Perret et al. 2021; Saniasiaya and Kulasegarah 2021)

(continued)

Table 12.2 (continued)

Neurological manifestations and etiologies in COVID-19	
Uncommon manifestations	**Etiology**
Ataxia and myoclonus movement disorders	Para-infectious direct invasion of the virus or secondary to host immunological response (Fernandes and Puhlmann 2021; Chan et al. 2021)
Focal motor and sensory deficits	Are rare and uncommon (Liotta et al. 2020) and most probably due to hypercoagulability and small infarcts
Impairment of consciousness, Coma	Sedation likely contributes to prolonged unconsciousness, given that sustained, high levels of sedation are often needed to ensure ventilator synchrony in COVID-19 patients with acute respiratory distress syndrome (ARDS) (Hanidziar and Bittner 2020). However Direct infection and damage of the brain parenchyma, toxic-metabolic encephalopathy, seizures, or demyelinating disease due to hypoxia/respiratory distress also contribute
Protracted inability to concentrate and decreased short-term memory loss referred to as "brain fog"	COVID-19 related capillary damage, pre-existing microvascular changes, and upstream vascular tone on tissue oxygenation in key organs (Ostergaard 2021)
Seizures	Are predominantly secondary seizures may be initiated after strokes, electrolyte imbalance, increased oxidative stress, and mitochondrial dysfunction (Nikbakht et al. 2020)
Syncope and orthostatic hypotension	Myocardial injury and diffuse acute myocardial inflammation (Luetkens et al. 2020; Oates et al. 2020)
Neuropsychiatric disorders (anxiety, mood disorders, sleep disorders, suicidal ideations)	Due to complications derived from an extended period of stay in the ICU, patients with a vulnerable mental status are prone to long-term psychiatric disorders. (elaborated in section on the neuropsychiatric disorder and in Table 12.3)
Polyneuropathy, polyradiculitis, Guillain-Barre syndrome/acute inflammatory demyelinating polyneuropathy (AIDP)/acute motor axonal neuropathy (AMAN)	Aberrant autoimmune response to peripheral nerve antigens. The antibodies produced by the immune system to fight the virus cross-react and bind to components of the peripheral nervous system, causing neuronal dysfunction (Abu-Rumeileh et al. 2021; Galassi and Marchioni 2021; Masuccio et al. 2020)
Acute transverse myelitis	Non-compressive myelopathy, myelitis (Chakraborty et al. 2020)
Cranial nerve impairment	Mechanism unknown (Cavalagli et al. 2020)
Ischemic and hemorrhagic stroke	Infection of the vascular endothelial cells and subsequent damage to the vasculature (Please see the section on Stroke)

summarize the most probable cause of the various manifestations in neuro-covid (Table 12.2).

The predominant clinical manifestations like fever, cough, shortness of breath are due to involvement of the upper respiratory tract which can lead to asymptomatic or mild-symptomatic respiratory illness, severe pneumonia, and acute respiratory distress syndrome requiring hospitalization and intensive care. It is interesting to note that, in humans, ACE2 is expressed sparsely in airway epithelia (Hikmet et al. 2020), but predominant in enterocytes, kidney tubule cells, small intestinal cells, gall bladder, cardiomyocytes, male reproductive system, placental trophoblasts, ductal cells, eye and vascular endothelia throughout the body and including CNS. SARS COV2 can affect the central and peripheral nervous system via various routes

- Endocytosis or Exocytosis of viral particles across endothelial cells expressing receptor ACE2.
- Virus-infected leukocytes can pass through the blood-brain barrier (BBB) termed the Trojan horse mechanism (Abassi et al. 2020). The systemic inflammation in COVID-19 likely increases the permeability of the BBB, thereby allowing infected immune cells, cytokines, and possibly viral particles to pass into the CNS.
- A pathologically dysregulated systemic immune response to SARS-CoV-2 has been strongly implicated in neuroinflammation (Muccioli et al. 2020).
- Encephalopathy in COVID is multifactorial, however, hypoxemia due to respiratory dysfunction or due to ventilators induces neurologic injury and is typically associated with encephalopathy, as are metabolic derangements due to organ failure and medication effects.
- By virtue of binding to ACE2, the SARS-CoV-2 virus may lead to secondary cardio- and cerebrovascular effects through a maladapted Renin-angiotensin system. However, the conundrum of the use of ACE inhibitors and ACE Receptor Blockers in COVID-19, and their potential protective role is hotly debated (de Miranda and Teixeira 2020).

There is a difference in the frequency and range of neurologic manifestations worldwide which can be attributed to genetic factors including polymorphism in the expression of the viral receptor angiotensin-converting enzyme 2 (ACE 2) in the nervous system, and possibly, SARS-CoV-2 strain variations. It has been observed that typically 80% of the patients with severe COVID disease, show nervous system involvement (Liotta et al. 2020). They are also prone to develop long-term sequelae. Severe complications like encephalopathy frequently occur in older people which is also associated with increased morbidity and mortality (Liotta et al. 2020). The major neurological manifestations and their possible etiologies are summarized in Table 12.2.

12.5 Stroke in COVID-19: Risk Factors, Pathogenesis, and Clinical Manifestation

A stroke is a sudden interruption of blood supply to the brain causing brain tissue to be deprived of oxygen and nutrients. Most strokes are ischemic strokes caused by blockage of the arteries supplying blood to the brain and hemorrhagic strokes are the other type of stroke caused by rupture of an artery in the brain. Ischemia occurs due to the thrombus formed by rupture of the atherosclerotic plaque, however, coagulation abnormalities leading to thrombotic microangiopathy may also lead to ischemia (Levi et al. 2020). Many patients with COVID-19 present with coagulation abnormalities and stroke associated with an increased risk of death (Tang et al. 2020). Further, two key risk factors for hemorrhagic stroke, hypertension, and diabetes were present in most of the affected patients who presented with ischemic stroke and COVID-19 (Wang et al. 2020b). Studies have shown the association of acute ischemic stroke, intracerebral hemorrhage, and cerebral venous sinus thrombosis with COVID-19 patients. Further, COVID-19-associated coagulopathy caused by inflammation and cytokine storm has been documented as pathogenesis of stroke in COVID-19 (Divani et al. 2020).

An increased D-dimer concentration, modest decrease in platelet count, and prothrombin time prolongation are typical findings in COVID-19 patients with coagulopathy. Increased D-dimer is the most typical finding in such patients and is associated with increased mortality (Table 12.3). The presence of thrombocytopenia, prolonged prothrombin time, and increased D-dimer indicate the presence of disseminated intravascular coagulation (DIC); however, as evident the pattern of DIC is distinct than that seen in sepsis, where thrombocytopenia is more profound and D-dimer levels do not increase to high values as found in COVID-19 patients (Thankam and Agrawal 2021). In addition to coagulopathy markers, COVID-19 patients showed increased concentrations of cytokines including interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α) which are involved in the suppression of endogenous anticoagulant pathways (IL-1 and TNF-α) and initiate coagulation activation and thrombin generation (IL-6) (Huang et al. 2020). Increased cytokines are a part of cytokine storm in COVID-19 (Thankam and Agrawal 2021). The secretion of these proinflammatory cytokines may be triggered by the binding of SARS-CoV-2 to ACE2 receptors in brain blood vessels leading to activation and extravasation of inflammatory cells such as lymphocytes, neutrophils, and macrophages which subsequently may lead to neurological complications including stroke (Wang et al. 2020b). The binding of the SARS-CoV-2 spike with ACE2 activates the platelets and activated platelets may contribute to thrombus formation and inflammatory responses in COVID-19 patients (Zhang et al. 2020b). Additionally, ACE2 expression on neurons could also be a significant factor precipitating ischemic stroke in COVID-19 (Newton et al. 2016). Further, angiotensin-converting enzyme (ACE) 2 downregulation in multiple organs (lungs, kidney, heart, liver, and brain) in SARS during 2003 was also reported. Downregulated ACE2 was also found in COVID-19 patients (Chen et al. 2020b) and it may lead to an increased risk of hemorrhagic stroke through increased angiotensin II (Ang II) levels leading to

Table 12.3 Coagulation profile dysregulation in COVID-19

Marker	Findings	Study references
D-dimer	Elevated D-dimer (>0.5 mg/L) in 260 out of 560 patients	Guan et al. (2020)
D-dimer PT Fibrinogen level Antithrombin	Increased mean D-dimer (2.12 mg/L) in patients who succumbed to COVID-19 compared to 0.61 mg/L in survived patients Mildly prolonged (15.6 s) in patients who died compared to patients who survived (13.6 s) Upper limits of normal during initial COVID-19 and then sudden decrease (<1.0 g/L) before death Lower percent of normal in COVID-19 non-survivors (84%) than in survivors (91%)	Tang et al. (2020)
D-dimer aPTT	Higher D-dimer (5.2 ± 3.0 vs. 0.8 ± 1.2 μg/mL) and Longer aPTT (39.9 ± 6.4 vs. 35.6 ± 4.5 s) in venous thromboembolism (VTE) patients compared to non-VTE patients	Cui et al. (2020)
D-dimer	Higher median D-dimer (2.4 mg/L) in patients admitted to ICU compared to those not admitted to ICU (0.5 mg/L)	Huang et al. (2020)
D-dimer	Increased D-dimer inpatient not survived (4.6) versus patients recovered (0.6) from COVID-19	Clinical characteristics of 113 deceased patients with coronavirus disease 2019: a retrospective study (2020)
D-dimer LDH	D-dimer >1 μg/L at the time of admission resulted in an 18 times increased risk of death Increased lactate dehydrogenase (LDH) in COVID-19 patients	Zhou et al. (2020)
D-dimer	D-dimer >2 μg/L at the time of admission resulted in an 18 times increased risk of death	Zhang et al. (2020a)
D-dimer LDH	D-dimer (2.6 vs. 0.3) and LDH (537 vs. 224) were markedly higher in severe compared to moderate COVID-19 cases	Chen et al. (2020a)
Platelet count	About 5% of patients present with a platelet count of $<100 \times 109$ cells/L 70–95% of severe COVID-19 patients had mild thrombocytopenia (platelet count $<150 \times 109$ cells/L)	Guan et al. (2020); Huang et al. (2020)
PT aPTT	Prothrombin time (PT) > 3 s and activated partial thromboplastin time (aPTT) > 5 s predicted the presence of thrombotic complications associated with increased incidence (31%) of thrombotic diseases	Klok et al. (2020)

(continued)

Table 12.3 (continued)

Marker	Findings	Study references
PT LDH D-dimer	Prolonged prothrombin time (13 s), elevated LDH (435 vs. 212) and D-dimer (414 vs. 166) in ICU admitted vs non-ICU patients	Wang et al. (2020a)
D-dimer	Increased D-dimer (2.27) in COVID-19 patients admitted to ICU	Helms et al. (2020b)
D-dimer	Increased D-dimer (3.5) in COVID-19 patients admitted to ICU	Ranucci et al. (2020)

increased blood pressure by acting on angiotensin II type 1 receptor (AT1R) or by decreased vasodilatory heptapeptide Ang 1–7 levels preventing its vasodilatory, growth-inhibiting, and antifibrotic actions (Wang et al. 2020b; Labo et al. 2020). Endothelial dysfunction in blood-brain barrier, decreased age-related ACE2 deficiency in older patients, inflammation, decreased immunity, stress, anxiety, and depression may also contribute to increased stroke incidence in patients with COVID-19 (Wang et al. 2020b) (Fig. 12.2).

COVID-19 patients, who are admitted to the hospital and have a longer non-ambulatory period may increase the chances of thrombus formation and thus might lead to stroke. Further, changes in coagulation profile in COVID-19 suggest the presence of a hypercoagulable state that might increase the risk of thromboembolic complications. However, there were reports of fewer patients with ischemic stroke at the casualty department during COVID-19 and it was suggested that this might be due to increased IL-6 concentrations (Morelli et al. 2020). Similarly, a less notable separation of mortality rates based on the D-dimer cutoff reported by Gris et al. (2020) suggested a selection bias in the studies. COVID-19 patients presenting with stroke symptoms had positive radiographic findings (Avula et al. 2020), altered mental status (Avula et al. 2020), or reduced level of consciousness (Oxley et al. 2020). Increased incidence of ischemic stroke, encephalitis, and polyneuropathy in COVID-19, as documented by various studies as mentioned in the previous section, is also supported by the presence of viral RNA in the CSF and previous findings of infected neurons in the cortex and hypothalamus and genomic sequences of SARS-CoV in these samples after a necropsy (Pennisi et al. 2020). Collectively, these studies suggest coagulopathy as pathogenesis of COVID-19 associated stroke.

Dysregulated coagulation profile was also found to be associated with other thrombotic complications including pulmonary embolism, deep-vein thrombosis (DVT), ischemic stroke, myocardial infarction, systemic arterial embolism, and venous thromboembolism (VTE) (Klok et al. 2020; Lodigiani et al. 2020; Mackman et al. 2020). An association between COVID-19 and cardiovascular disease (CVD) had a worse outcome and increased risk of death in patients with pre-existing CVD or risk factors for CVD such as hypertension and diabetes has been reported in various studies. COVID-19 by itself can induce or the drug-disease interaction in COVID-19 may lead to CVDs including myocardial injury, arrhythmia, acute coronary syndrome, and venous thromboembolism. Cardiac symptoms may also

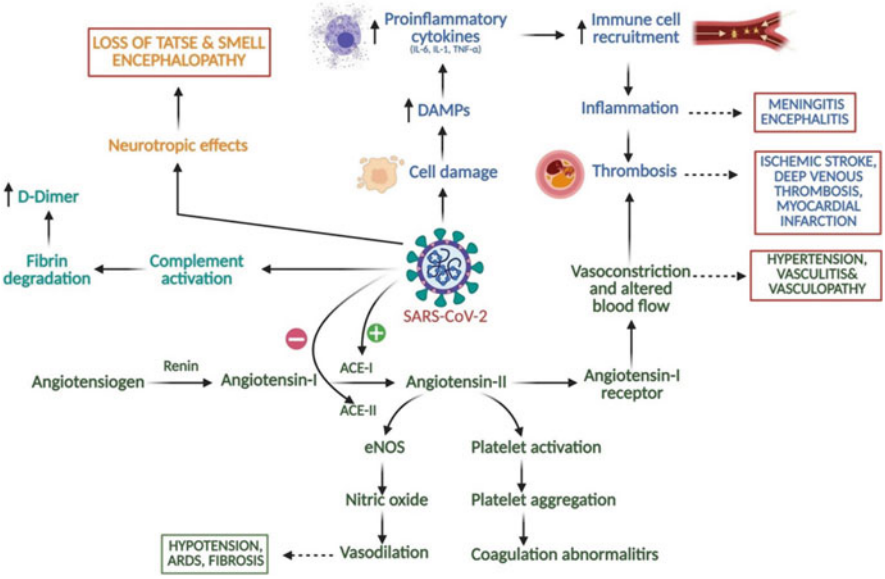

Fig. 12.2 Putative mechanisms of SARS CoV-2 in neuro-covid: Putative mechanisms of SARS CoV-2 in neuro-covid. In the RAS system, ACE-1 converts angiotensin I to angiotensin II. Angiotensin II is further cleaved to Angiotensin 1–7 with the help of ACE-2. SARS-CoV- interacts with ACE-2 and induces the signaling within the cell and causes various symptoms related to inflammation, thrombosis, and vasoconstriction. SARS-CoV-2 also binds to ACE-2 on the cell surface and essentially decreases its availability and ultimately strikes an imbalance between Angiotensin-II and Angiotensin 1–7. Simultaneously, SARS-CoV-2 also induces complement activation and fibrin degradation. *RAS* Renin-Angiotensin System, *ACE* Angiotensin-Converting Enzyme, *SARS-CoV* Severe Acute Respiratory Syndrome CoronaVirus

be the first symptom in a patient without the typical symptoms of fever or cough in COVID-19. Further, the drugs including hydroxychloroquine, azithromycin, lopinavir-ritonavir, and remdesivir may have associated pro-arrhythmic side effects (Nishiga et al. 2020). Acute myocardial injury (MI) with elevated levels of cardiac biomarker and electrocardiogram abnormalities were observed in 7–20% of patients with COVID-19 in China and was associated with increased mortality (37%). The mortality was higher in patients with underlying cardiovascular morbidities (69%). The reports for the typical features for myocarditis are limited with initial reports without typical sign-on echocardiography and/or electrocardiogram but later there were limited reports of typical features of myocarditis on cardiac MRI. Similarly, the histological evidence for myocarditis is also limited (Nishiga et al. 2020). These results suggest acute MI as a complication of COVID-19 and understanding the pathophysiology of acute MI in COVID-19 may help in determining the therapeutic strategies. COVID-19 patients also had thrombocytopenia, elevated PT, decreased antithrombin time, elevated fibrinogen levels, and increased tissue factors levels, the risk factors for thrombosis which might result in stroke and MI.

12.6 Neuropsychiatric Manifestations of COVID-19

The neurotropic nature of the SARS-CoV-2 virus (Fig. 12.2) causes a plethora of neuropsychiatric symptoms, of which some of them including headache, anosmia, hypogeusia, and dizziness have been discussed under neurological manifestations. Additionally, there are other potential acute and long-term neuropsychiatric sequelae of CoV-2 infection that can increase morbidity and worsen the quality of life. Mirfazeli et al. (Mirfazeli et al. 2020) reported that the neuropsychiatric manifestations of CoV-2 can be divided into three different categories: (1) anosmia and hypogeusia; (2) dizziness, headache, and limb force reduction; and (3) photophobia, mental state change, delirium, hallucination, balance disturbance, seizure, vision and speech problem, and stroke. Of these, stroke has been discussed above. Neuropsychiatric symptoms are heterogenous in the patient population and this heterogeneity may be due to different underlying pathogenesis of SARS-CoV-2. Further, it has been suggested that pre-existing conditions including cognitive deficits, age, schizophrenia, lack of stimulation, metabolic disturbances, urinary retention, constipation may also contribute to the neuropsychiatric manifestations or COVID-19 infection may exacerbate existing neuropsychiatric symptoms. The study by Mirfazeli et al.(2020) studying the population of 201 COVID-19 patients reported that 60.2% of patients suffered from muscle weakness and 75.1% of patients have at least one neuropsychiatric symptom. Limb force reduction was present in 40.3% of patients, anosmia in 33.8%, hypogeusia in 32.8%, and headache in 39.8% of patients with COVID-19 infection. Another study on 125 patients reported 57 patients with ischemic stroke, 9 patients with intracerebral hemorrhage, and one patient with CNS vasculitis, 39 patients with altered mental status comprising nine patients with unspecified encephalopathy and seven patients with encephalitis, and the remaining 23 patients with altered mental status (Varatharaj et al. 2020). Acute psychosis and manic disorders have been reported by two case reports (Fischer et al. 2020; Haddad et al. 2020). Delirium has been reported to be the most common acute symptom (McLoughlin et al. 2020) and cognitive and behavioral abnormalities in one-third of patients after discharge (Helms et al. 2020a) have been reported in COVID-19 patients. The presence of neuropsychiatric symptoms suggests the importance of a thorough neuropsychiatric evaluation in all COVID-19 patients was highlighted by this study to get an effective treatment and therapy.

Psychological and psychosocial stress with prolonged hospitalization may also be a precipitating factor for neuropsychiatric manifestations (Mirfazeli et al. 2020; Banerjee and Viswanath 2020; Jansen van Vuren et al. 2021). Delirium and confusional states dependent on the age group with few with elderly compared to younger population have been reported in COVID-19 patients (Liu et al. 2020). The neurotrophic effects and targeting of ACE2 during its pathogenesis may result in neuropsychiatric symptoms. Downregulation of ACE2 may lead to enhanced sympathetic activity, decreased tryptophan uptake, and reduced brain serotonin (5-hydroxytryptamine; 5-HT) levels (Jansen van Vuren et al. 2021). Dysregulation of these may lead to neuropsychiatric symptoms. Increased stress-related disorders such as anxiety may also contribute to hasten the onset of other neuropsychiatric

disorders including depression, bipolar disorder, schizophrenia, and substance abuse (discussed in detail in Jansen van Vuren et al. (2021)). The presence of neuropsychiatric symptoms in the CoV-2 patients may also have long-term sequalae, however, it can only be a perception based on previous CoV outbreaks. The long-term sequalae may be neuromuscular disorders including myopathy, GBS, multiple sclerosis, chronic psychiatric illness such as depression, anxiety, adjustment disorder, post-traumatic stress disorder, somatization, and obsessive-compulsive disorders, neurodegenerative disorders, and epilepsy (Banerjee and Viswanath 2020). An increased probability of the presence of long-term sequalae of COVID-19 is supported by the same trend of symptoms or findings as were present during previous coronavirus pandemic. The presence of low mood, anxiety, and fatigue in the acute phase of coronavirus spread previously and the presence of psychosis, mood disorders, catatonia, encephalopathy, and encephalitis in patients with COVID-19 support the possibility of long-term neuropsychiatric manifestations (Butler et al. 2020). The long-term neurological and neuropsychiatric manifestations may also be due to brain tissue edema, partial neurodegeneration, and neuron damage in patients with COVID-19. The long-term manifestations might also be an exacerbation of existing disorders such as multiple sclerosis and Parkinson's disease due to hypoperfusion of the frontotemporal lobe and neurodegeneration (Kumar et al. 2021).

The presence of multiple neuropsychiatric symptoms and the possibility of long-term manifestations suggest the need for the continuation of effective treatments and personalized therapy. However, due to a lack of in-depth understanding of the underlying pathogenesis of the SARS-CoV-2 virus in the nervous system, it is difficult to conceptualize a direct link between exacerbations of pre-existing psychiatric illnesses and the neurotrophic effects of CoV-2; and this might affect the treatment of such patients. Thus, more in-depth research and clinical studies are warranted for effective and personalized therapy. A team approach including physician, neurophysician, psychiatrist, and psychologist is needed to handle the neurological and neuropsychiatric manifestations and long-term sequalae.

12.7 Summary

Although COVID-19 is predominantly a respiratory disease, numerous studies have uncovered the nervous system as a predominant player in morbidity. Short-term and long-term complications of recovered and recovering COVID-19 patients have garnered serious attention. A majority of studies have implicated two major mechanisms of these various neurological manifestations: (1) direct invasion of SARC-CoV-2 on the brain and/or the spinal cord through the olfactory bulb, with inflammation assisting virus-carrying immune cells to cross the blood-brain barrier, and (2) Indirectly due to an aberrant and exaggerated host immune response maintaining and worsening the inflammatory phenotype. However, for 1, there is hardly any correlation between the extant of neurological involvement with viral presence in the cerebrospinal fluid and for 2, the specific players causing an aberrant

host response in the nervous system are not clear. In spite of these, the spectrum of manifestations seen in the nervous system compared to other organ systems is significantly higher warranting a closer look for the years to come, as the pandemic is nowhere near its end.

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13 Insights Into the Role of Angiotensin-Converting Enzyme 2 in the Onset of Severe Acute Respiratory Syndrome Coronavirus 2 Pathogenesis

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused the outbreak of pneumonia which originated in Wuhan, China, at the end of 2019 has turned into a global pandemic—now termed coronavirus diseases 2019 (COVID-19). Like previously reported SARS-CoV strains, the newly discovered SARS-CoV-2 was also found to initiate the pathogenesis by binding with the angiotensin-converting enzyme 2 (ACE2), a receptor produced by various organs in the human body. Hence, COVID-19 is a viral multisystem disease which particularly infects the vascular system expressing ACE2 and reduced the ACE2 function; this further complexed by organ-specific pathogenesis related to the damage of cells expressing ACE2, such as alveolus, glomerulus, endothelium, and cardiac microvasculature. Under these conditions, it was advocated that the upregulation of ACE2 expression in predisposing individuals with aberrant renin–angiotensin system (RAS) level to advanced viral load on infection and relatively a greater number of cell death. Recently, a significant role of decreased

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ACE2 production and inequality between the RAS and ACE2/angiotensin-(1–7)/MAS (mitochondrial Ang system) after the onset of SARS-CoV-2 infection was established as a key factor for multiple organ injury in SARS-CoV-2-infected individuals. Furthermore, restoration of this imbalance has been suggested as a therapeutic approach to attenuate organ injuries in SARS-CoV-2 infection. Based on available data, this chapter presents the updated mechanism of the multi-organ diseases causes by COVID-19 via ACE2 which can be further helpful in the development of specific therapeutics.

Keywords

RAS · MAS · Angiotensin-(1–7) · ACE2 · SARS-CoV-2 · COVID-19

13.1 Introduction

A novel coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged at the end of 2019 in Wuhan, Hubei Province, China, and since then has spread to become a global pandemic known as coronavirus disease 2019 (COVID-19; Huang et al. 2020a, b; Zhu et al. 2020). SARS-CoV-2 is reported as comparatively highly contagious by comparison to two major human pandemic coronaviruses, namely, severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East Respiratory Syndrome coronavirus (MERS-CoV). Since the outbreak of SARS-CoV-2, around 79 million cases of COVID-19 have been documented with nearly 1.7 million deaths worldwide by the end of December 2020.

Genetic analysis of SARS-CoV-2 using complete genome sequencing established that it shared 79.5% sequence similarity with SARS-CoV; pairwise protein sequence analysis for SARS-CoV-2 assisted in its classification under the category of SARS-related coronaviruses (Kirtipal et al. 2020; Yang et al. 2020). To note, both SARS-CoV and SARS-CoV-2 infect the host cells with the aid of a common cell receptor, namely, angiotensin-converting enzyme 2 (ACE2; Yang et al. 2020). Although the mean mortality rate for SARS-CoV-2 is lower than that of SARS and MERS; however, organ failures, such as acute cardiac injury, acute hepatic injury, acute kidney injury, and acute respiratory distress syndrome (ARDS), are widely recorded in serious SARS-CoV-2 infection. The receptor ACE2 is a homolog of angiotensin-converting enzyme (ACE) and spatially expressed in various organs and tissues as an essential factor for extensive biological activities; for instance, in many diseases, ACE2 is known to reduce the deleterious effect of the renin–angiotensin system (RAS; Donoghue et al. 2000; Patel et al. 2017; Santos et al. 2018). Surprisingly, the production of ACE2 protein is low in the lungs but high in type II pneumocytes—a cell type that also expresses TMPRSS2 (transmembrane protease serine 2) and also activates SARS-CoV-2 spike protein to dock with ACE2 receptor for transmission

into the host cell (Davidson et al. 2020). Hence, this chapter discusses the role of ACE2 in SARS-CoV-2 infection and provides more comprehensive information on the targeted organs expressing ACE2 which can be beneficial in epidemic management.

13.2 RAS and ACE2 Relation

RAS, a complex cell signaling network, is known for essential functions in the regulation of electrolyte and fluid homeostasis, blood pressure, and control the functions of several organs, including the blood vessels, heart, and kidneys (Santos et al. 2018). Angiotensin II (Ang-II)—a most demonstrative bioactive peptide in the RAS network—extensively contributes to the advancement of cardiovascular diseases, such as myocardial infarction, cardiac failure, and hypertension (Keidar et al. 2007). In classic RAS, renin digests the angiotensinogen as substrate and forms decapeptide angiotensin I (Ang-I) followed by removal of two amino acids by ACE from Ang-I at the carboxyl terminus to yield Ang-II (Fig. 13.1). Till now, three Ang-II receptors are discovered and hold common attractions in the nanomolar range toward Ang-II (Keidar et al. 2007). Among these receptors, the binding of angiotensin type 1 receptor (AT1R) with Ang-II results in extracellular matrix remodeling, cell division, inflammatory responses, vasoconstriction, and blood coagulation, while angiotensin type 2 receptor (AT2R) responds to the said effects facilitated by AT1R (Horiuchi et al. 1999). However, the catalytic activity of ACE2 on Ang-I and Ang-II produces angiotensin-(1–7), which connects to the MAS receptor to vascular protection, anti-proliferation, anti-fibrosis, anti-inflammation, and vasodilation. Moreover, Ang-II can also attach to AT2R to reduce the aforementioned consequences intermediated by AT1R (Yang et al. 2020). In 2000, two independent research groups discovered a homolog of ACE named ACE2 that forms the heptapeptide angiotensin-(1–7) by removing the carboxy-terminal phenylalanine in Ang-II (Donoghue et al. 2000, Tipnis et al. 2000). Besides, the formation of angiotensin-(1–7) was also suggested without interruption of Ang-II under the varying influences of ACE2 and ACE (Fig. 13.1). Here, Ang-I is initially hydrolyzed by ACE2 to form angiotensin-(1–9), which is subsequently dissected by ACE to produce angiotensin-(1–7). Additionally, Ang-I was also reported to produce angiotensin-(1–7) by direct activities of endopeptidases and oligopeptidases (Santos et al. 2018). Remarkably, the classical pathway of Ang-II to angiotensin-(1–7) is relatively widespread due to the high affinity between ACE and Ang-I (Santos et al. 2018). Furthermore, Angiotensin-(1–7) also acted as a ligand and attaches with the G-protein-coupled receptor MAS and generates effect against that induced by Ang-II; and thus, applies various events in multiple organs or systems (Patel et al. 2017; Santos et al. 2018).

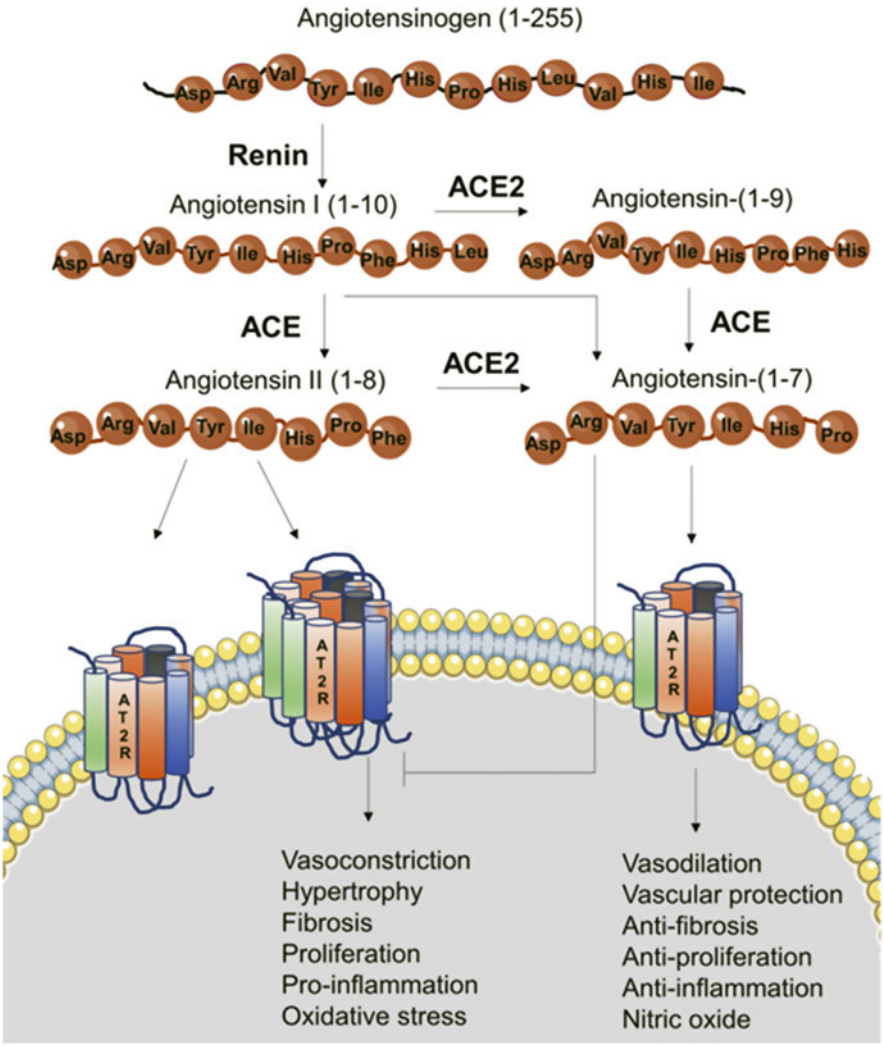

Fig. 13.1 Schematic presentation of the renin–angiotensin system (RAS) and ACE2/angiotensin-(1–7)/MAS axis (Yang et al. 2020)

13.3 ACE2 as Potential Receptors for SARS-CoV-2

The virus instigates the infection cycle primarily through attachment with the aid of functional receptors on the host cell; the functional receptor regulates the transmission, proliferation, and clinical symptoms in the infected individuals (Groneberg et al. 2005; Kuba et al. 2013; Xu et al. 2020a). Thus, such functional receptors are

projected as a key element in the avoidance and therapy of viral diseases. Under COVID-19, ACE2 has been recognized as the potential functional receptor required by SARS-CoV-2 for binding with host cells. However, some biological responses associated with viral receptors that could not be elucidated by ACE2 indicated that the functional receptor for SARS-CoV-2 binding is not quite clear. The coronaviruses contained spike protein (S-protein) for attachment with the host receptors; this S-protein shapes a trimer on the surface of virus structure, where each monomer contained a receptor-binding domain (RBD) that binds to the specific receptor on the membrane of the host cell (Li 2016). Interestingly, sequence analysis of S-protein RBD in SARS-CoV-2 suggested its high similarity to SARS-CoV's RBD than MERS-CoV's (Xu et al. 2020a). Furthermore, a close association of S-protein RBD in SARS-CoV-2 with human ACE2 molecules was confirmed by computer-guided homology modeling (Xu et al. 2020a). These results were also established by experimental studies using cell lines, for instance, HeLa cells with ACE2 expression were found sensitive to SARS-CoV-2 infection against cells deficient in ACE2 expression (Wan et al. 2020). Therefore, these results suggested the considerable role of ACE2 in SARS-CoV-2 infection and designated it as a potential functional receptor for SARS-CoV-2. Initially, SARS-CoV-2 S-protein structure in perfused conformation was determined using cryo-EM, where the prevalent state of S-protein trimer holds one of the three RBDs placed vertically in a receptor-accessible pose (Bian and Li 2021). Furthermore, structural and biophysical research revealed that the SARS-CoV-2 S-protein bound to ACE2 with a 10- to 20-fold higher affinity than the SARS-CoV S-protein, suggested its significant contribution to enhanced infectious behavior of SARS-CoV-2 against SARS-CoV (Wrapp et al. 2020), and hence, suggested as the significant factor that contributes to more contagious nature of SARS-CoV-2 against SARS-CoV. Subsequently, RBD in SARS-CoV-2 S-protein complexed with ACE2 crystal structure was also determined (Lan et al. 2020). This sequence of experimental work robustly reinforced the ACE2 protein in the host cells as the functional receptor of SARS-CoV-2 (Bian and Li 2021).

ACE2 receptor is nearly expressed with a varying degree in all the vital organs of the human body. The conventional immunohistochemical approach showed that the primary route of viral infection in the respiratory system is by type II alveolar epithelial cells that express ACE2. Besides, single-cell RNA-seq analysis revealed only weak expression of ACE2 on epithelial cells surface in the oral, nasal mucosa, and nasopharynx, directed to lungs as a main vulnerable organ for SARS-CoV-2 infection (Hamming et al. 2004; Zou et al. 2020a, b). ACE2 expression was also found on myocardial cells, bladder urothelial cells, and proximal tubule cells of the kidney as well as on enterocytes in the ileum of the small intestines (Hamming et al. 2004; Zhang et al. 2020; Zou et al. 2020a, b). Hence, it was suggested that cell-free and macrophage phagocytosis-associated virus can transmit to other organs of the host body with high expression of ACE2 from the lungs via blood circulation (Fig. 13.2). These assumptions are supported by the fact that ~67% of patients in the initial phase of SARS CoV infection suffered from diarrhea, while a considerable number of patients infected by SARS-CoV-2 also exhibited enteric symptoms

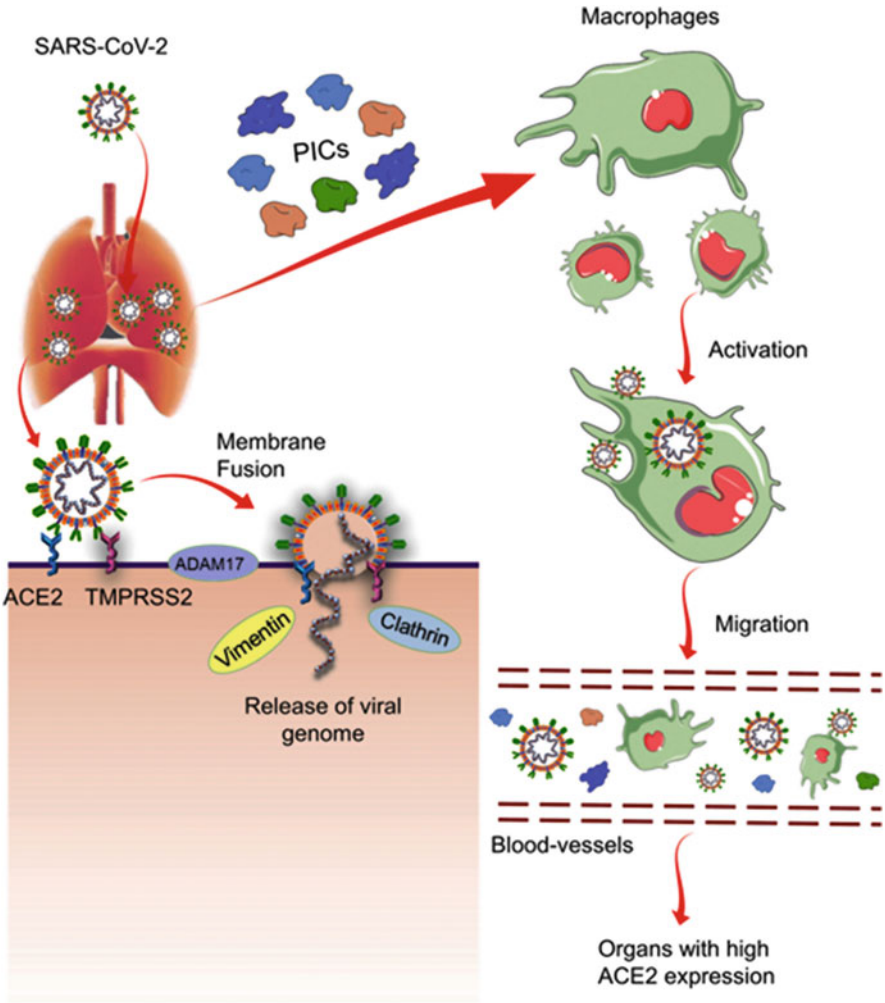

Fig. 13.2 Schematic representation for the SARS-CoV-2 infection on host cells expressing ACE2 receptor. Herein, initially, viral particles enter the lungs and bind with the cells via ACE2 cells with the aid of spike glycoproteins in the presence of other transmembrane proteinases, such as disintegrin metallopeptidase domain 17 (ADAM17) and transmembrane protease serine 2 (TMPRSS2), which assist the virus to enter into the cells allowing the virus to enter the cells. Following, the infected cells along with inflammatory cells agitated by the viral antigens results in the production of chemokines and pro-inflammatory cytokines (PICs) that further activate the inflammation and immunological reactions to prevent viral proliferation. Cell-free and macrophage-phagocytosed and cell-free viruses located in the blood are likely transferred to other organs of the host and typically afflict the cells abundant with ACE2 expression at local sites (Ni et al. 2020a, b)

(Leung et al. 2003; Liu et al. 2020b; Wang et al. 2020a). In this context, active viral replication has been documented in the enterocytes of the small intestine, and SARS-CoV-2 viral particles were also isolated from the fecal specimens of infected individuals (Cheung et al. 2020; Lamers et al. 2020).

13.4 Alliance of ACE2 with Multi-Organ Injury in SARS-CoV-2

The distribution of ACE2 in the different organs of the human body indicates the chances for SARS-CoV-like viral infection in the multiple organs. This is was supported by the autopsies of SARS-CoV-infected individuals, where damage in the organs such as the central nervous system, heart, liver, kidney, skeletal muscle, adrenal, and thyroid glands was observed apart from the mutilation in the lungs (Gu et al. 2005; Gu and Korteweg 2007). Likewise, the majority of seriously infected SARS-CoV-2 patients have had organ damage, including heart injury, acute lung injury, liver disease, acute kidney injury, and pneumothorax (Yang et al. 2020). Organ damaged, as seen in SARS and SARS-CoV-2, was commonly reported in MERS-CoV-infected patients, mostly in the gastrointestinal tract and kidneys, with the least occurrence of acute cardiac injury (Assiri et al. 2013; Alsaad et al. 2018; Hui et al. 2018; Hwang et al. 2019). To be evident, unlike SARS-CoV and SARS-CoV-2, MESR-CoV required Dipeptidyl-peptidase 4 (DPP4) as a functional receptor on host cells for entry, which expressed on activated leukocytes, multinucleated epithelial cells, pneumocytes, bronchial submucosal gland cells of the lungs, kidney epithelial cells, and small intestine epithelial cells (Boonacker and Noorden 2003; Lambeir et al. 2003; Raj et al. 2013). Moreover, DPP4 is sparsely expressed on the myocardial cells (Boonacker and Noorden 2003, Lambeir et al. 2003; Raj et al. 2013). Thus, these reports suggested the association of functional receptors with organ damage in the body (Fig. 13.3).

Conclusive results produced by a series of research on SARS-CoV suggested that pathogenesis of SARS-CoV-2 should be complex, including induction of inflammatory responses by the virus, excessive inflammatory cells recruitment, cytokines and chemokines expression, auto-antibodies formation, and insufficient interferon response (Gu and Korteweg 2007). To note, monocyte chemoattractant protein-1 (MCP-1), interferon-gamma-inducible protein 10 (IP-10), and chemokines plasma-like interleukin (IL)-1, IL-6, IL-12, IL-8, and pro-inflammatory cytokines (PICs) were observed with significant expression in the plasma of SARS-CoV-infected individuals (Wong et al. 2004, Zhang et al. 2004). These observations were also supported by the autopsy studies of SARS patients, where PICs and MCP-1 were noted in exceedingly expressed $ACE2^{+}$ cells infested by SARS-CoV when compared to noninfected $ACE2^{+}$ cells, indicating the role of virus-induced local immune-mediated damage (He et al. 2006). Considerably, similar elevated concentrations of PICs were discovered in the plasma of patients with a severe infection of SARS-CoV-2 (Huang et al. 2020a). Additionally, several studies documented that SARS-CoV caused downregulation of ACE2 expression in the infected cells and, hence, disrupts the physiological balance between ACE/ACE2

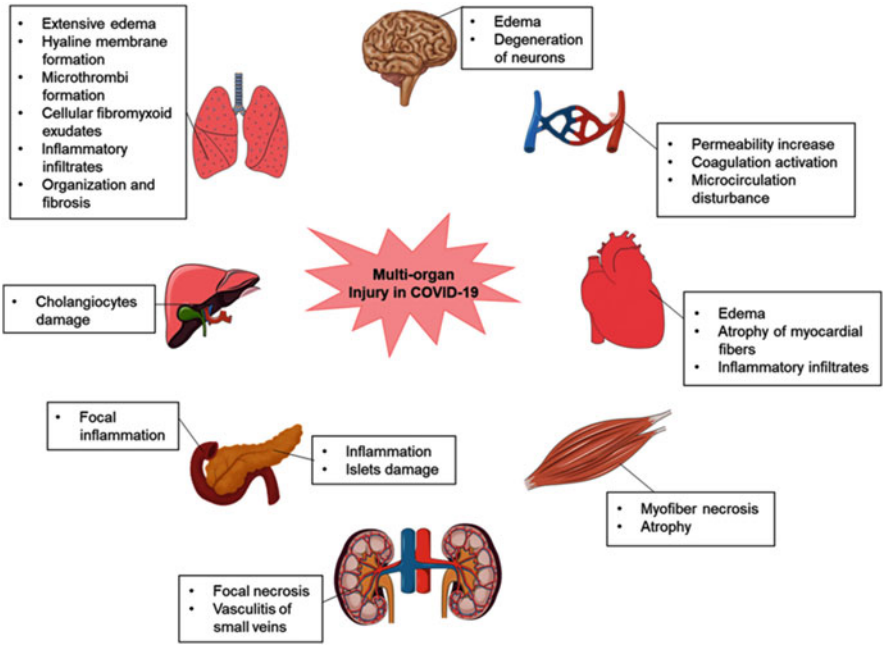

Fig. 13.3 Schematic representation for the multiple organ damage in SARS-CoV-2 infection (Ni et al. 2020a, b)

Ang-II/angiotensin-(1–7) consequently triggering severe damage to the organs in the body (Kuba et al. 2005, Haga et al. 2008, Oudit et al. 2009, Glowacka et al. 2010). Given that SARS-CoV-2 and SARS-CoV belong to the same family and used ACE2 as a functional receptor, ACE2 downregulation has been suggested as an essential factor to cause multiple organ damage in SARS-CoV-2-infected individuals (Fig. 13.3).

13.4.1 Acute Lung Injury

Although the mortality rate in SARS-CoV-2 infection is considerably lower in comparison to SARS-CoV and MERS-CoV, several SARS-CoV-2-infected individuals showed acute lung injury (ALI) (Leung et al. 2003, Yang et al. 2020). Remarkably, similar pathological features as observed in SARS and MERS, including severe diffuse alveolar damage, hyaline membrane formation, extensive edema, microthrombi formation, inflammatory infiltrates, organization, and fibrosis, were also noted in SARS-CoV-2 infection but relatively high cellular fibromyxoid exudates in the small airways and alveoli (Wichmann et al. 2020, Xu et al. 2020b). Interestingly, the clinical experiments have related the ACE insertion/deletion polymorphism along with the increased risk of ARDS (Marshall et al. 2002, Cruces et al. 2012), and elevated Ang-II levels in the lungs were associated

with an increase in vascular permeability resulting in pulmonary edema (Marshall et al. 2004, Wsd et al. 2018). Moreover, various reports have suggested the defensive role of the ACE2/angiotensin-(1–7)/MAS axis in the lungs against inflammation, pulmonary arterial hypertension, fibrosis, cancer cell growth, tumor angiogenesis, and tumor metastasis (Imai et al. 2005, Feng et al. 2010, Jia 2016, Santos et al. 2018). From various studies on animal models of ALI, ACE2-knockout mice showed increased vascular permeability, enhanced lung edema, accumulation of neutrophils, and demonstrated obstruction in lung function in comparison to wild-type mice (Imai et al. 2005). Interestingly, AT1R blockers or recombinant human ACE2 protein injection in the ACE2-knockout mice exhibited considerable decrement in the degree of ALI (Imai et al. 2005). Of note, SARS-CoV infection was also noted with a significant reduction in ACE2 expression in the lungs of the infected mouse (Kuba et al. 2005, Cruces et al. 2012). Subsequent experimental data revealed that recombinant SARS-CoV spike-Fc can significantly bind to human and mouse ACE2, which produces a downregulation in cell-surface ACE2 expression (Kuba et al. 2005). Moreover, deteriorated effects were observed in acid-induced ALI in wild-type mice treated with spike-Fc protein, while no alternations were recorded in the ACE2-knockout mice for the severity of lung failure, indicated that the effect of spike protein on ALI is specific to ACE2 expression (Kuba et al. 2005). However, the individuals infected with SARS-CoV-2 showed elevated levels of Ang-II in the blood plasma, which was directly proportional to the viral load and lung injury (Liu et al. 2020c). Based on these reports, RAS and ACE2 downregulation have been suggested as an essential factor that contributes to pathogenesis of lung injury in SARS-CoV-2 infection.

13.4.2 Endothelial Disease

In severe COVID-19, evidence of acute myocardial injury, that is, increased level of cardiac troponins have been observed as a common event and associated with impaired prognosis. Under the observation of tissue tropism of SARS-CoV-2 against ACE2-expressing cells, another major target of the body more liable to infection is vascular endothelium, where both small and large arteries and veins abundantly expressed the ACE2 receptor (Hamming et al. 2004, Monteil et al. 2020). Thus, endothelial dysfunction has been noted as a common feature to key comorbidities that elevated the risk for severe SARS-CoV-2 infection, including diabetes mellitus, obesity, hypertension, coronary artery disease, or heart failure. Interestingly, the preliminary results also revealed that vascular endothelial cells can be infected by SARS-CoV-2 as evident from inflammation and endothelial injury in advanced cases of SARS-CoV-2 infection (Nägele et al. 2020). However, the essential role of endothelial cells is well established to regulate and maintain the blood coagulation and vascular homeostasis; exacerbation of endothelial dysfunction induced by SARS-CoV-2 infection is therefore assumed to produce impaired organ perfusion and procoagulant state that causes both macro- and microvascular thrombotic events. In this context, ACE inhibitors, angiotensin receptor blockers (ARBs), and statins

were documented to improve endothelial dysfunction (Nägele et al. 2020). Moreover, endothelial injury and dysfunction have been suggested as results of direct infection by SARS-CoV-2, for instance, by stimulating intracellular oxidative stress and profound systemic inflammatory response (Kochetkov et al. 2018). Therefore, the potential relation of SARS-CoV-2 infection with endothelial injury projected a more severe SARS-CoV-2 infection in patients with preexisting endothelial dysfunction (Nägele et al. 2020).

13.4.3 Acute Cardiac Damage

Heart cells expressed a high concentration of ACE2, indicating its vulnerability to SARS-CoV-2 infection. For instance, autopsies of individuals diagnosed with SARS disclosed that 35% (7 of 20) of the SARS-CoV infected individuals had viral genome in cardiac tissue exhibited a comparatively high aggressive illness and earlier mortality rate (Oudit et al. 2009). Moreover, edema of the myocardial stroma, atrophy of cardiac muscle fibers, and inflammatory cell infiltration were also noted in patients with myocardial damage and SARS (Ding et al. 2003, Lang et al. 2003, Chong et al. 2004, Gu et al. 2005, Gu and Korteweg 2007). Of note, cardiac damage has been noted as a common feature in severely sick patients with SARS-CoV-2 while early acute myocardial damage was linked with an elevated risk of mortality (Ni et al. 2020a, b). Besides, ACE2/angiotensin-(1–7)/MAS axis positive role in the heart is already well established to induce vasorelaxation of coronary vessels, attenuate pathological cardiac remodeling, inhibit oxidative stress, and improve postischemic heart function (Jiang et al. 2014, Santos et al. 2018). It is important to mention that high expressions of ACE2 are normally observed at the initial stage of heart injury but gradually reduced with the progression of diseases (Keidar et al. 2007). For instance, knockout of ACE2 in mice causes myocardial hypertrophy and interstitial fibrosis, and hastens heart failure (Oudit et al. 2007, Zhong et al. 2010). Interestingly, both mice and humans infected with SARS-CoV exhibited a progressive downregulation in ACE2 expression on myocardial cells in the heart (Oudit et al. 2009). Recent studies also established hypertension as a comorbidity with severe disease in several patients (Ni et al. 2020a, b; Novel 2020*; Wang et al. 2020a). Thus, a considerable downregulation of ACE2 and upregulation of Ang-II in SARS-CoV-2 infected patients has been suggested to cause the overactivation of RAS and deactivation of angiotensin-(1–7) protective function that exacerbates and propagate cardiac injuries.

13.4.4 Damage to Digestive System

The gastrointestinal tract, particularly the intestine, has been documented as a potential target to SARS-CoV and SARS-CoV-2. For instance, unlike in the esophagus and stomach, SARS-CoV particles were only isolated from the epithelial cells of the intestinal mucosa (He et al. 2006, Gu and Korteweg 2007). Later, depletion of

mucosal lymphoid tissue was discovered as a major pathological discovery in the intestines of individuals infected by SARS-CoV, while only mild focal inflammation was observed only in the gastrointestinal tract of infected patients by SARS-CoV (Shi et al. 2005). These findings provide the explanation to the non-sever and non-transient nature of SARS-CoV-2 in the gastrointestinal tract. Moreover, several individuals infected by SARS-CoV-2 exhibit a minor to mild increment in the serum levels of aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) during the viral infection (Chen et al. 2020b, Wang et al. 2020a). Also, postmortems results for SARS patients showed cellular infiltration, hepatocyte necrosis, and fatty degeneration in the liver (Gu and Korteweg 2007). Nevertheless, most infected individuals infected by SARS-CoV particles showed no viral load in the autopsied hepatic tissue samples (Gu and Korteweg 2007). This can be explained by the fact that Kupffer cells, hepatocytes, and the endothelial lining of the sinusoids lack ACE2 expression as evident from both immunohistochemistry and single-cell RNA-seq analyses; only cholangiocytes were found positive for the expression of ACE2 receptor (Hamming et al. 2004, Chai et al. 2020, Zou et al. 2020a, b). Interestingly, a high expression level of gamma-glutamyl transpeptidase (GGT)—a marker of cholangiocyte damage—was documented in some patients infected by SARS-CoV-2 (Fan et al. 2020). These results reveal that most acute hepatic damage is not induced by the viral infection but is highly likely caused by other factors, including hypoxia, systemic inflammation, and drug hepatotoxicity. Hence, SARS-CoV-2 induced injuries in the bile ducts which shows high expression of ACE2 on cholangiocytes required further investigation to established SARS-CoV-2 hepatic pathogenesis.

13.4.5 Acute Kidney Injury

The expression of ACE2 is considerably high in the kidney, chiefly in the apical membranes of proximal tubular epithelial cells, and suggested that the kidney is an additional susceptible organ to SARS-CoV-2 infection (Chen et al. 2020a, Zou et al. 2020a, b). A disturbance in the equilibrium between Ang-II and angiotensin-(1–7) induced by deficiency of ACE2 deficiency was also suggested to intensify the vulnerability for the kidney to acute kidney injury (AKI) caused by other factors (Ortiz-Melo and Gurley 2016). For instance, SARS-CoV was isolated in epithelial cells of the distal tubules while viral genomes were also detected in urinary samples of several infected individuals (Chan et al. 2004, Farcas et al. 2005). To note, urinary samples of some patients were also discovered with viral particles of SARS-CoV-2 (Wang et al. 2020b). Moreover, a retrospective data analysis of 536 patients infected by SARS-CoV-2 showed the development of acute renal damage in 6.7% of patients during COVID-19 (Chu et al. 2005). Another large cohort study conducted in New York on SARS-CoV-2-infected individuals revealed a 36.6% incidence of AKI (Hirsch et al. 2020).

13.4.6 Other Organ and Tissue Injuries

13.4.6.1 Olfactory Epithelium (OE) and Brain Infection Through Nasal Cavity

During the clinical course of SARS-CoV-2 infection, symptoms range from asymptomatic infection to severe acute respiratory distress, including involvement of multiple organs and even death. The SARS-CoV-2 infection was also documented to cause extrapulmonary complications such as neurologic disorders and has been constantly reported in the literature (Abboud et al. 2020). Recent studies established total anosmia or partial loss of smell as an early symptom of SARS-CoV-2 infection; this occurrence can be caused by different unidentified factors, for example, "cytokine storm" started in some infected individuals or by direct injury in the olfactory receptor neurons (ORNs) situated in the olfactory epithelium (Butowt and Bilinska 2020). The latter possibility is most likely to occur under the fact that cells in the OE significantly expressed the functional receptor used by SARS-CoV-2 to cause infection in humans. In this context, several gene expression databases have been documented that show a considerable level of ACE2 and TMPRSS2 in human and murine olfactory mucosa (Butowt and Bilinska 2020). OE is a constantly rejuvenating multilayer structure in mammals that contained both non-neuronal and neuronal cells. Recent major RNA-seq (transcriptome) analysis on both human and murine OE consistently revealed the expression of ACE2 in non-neuronal cells (Kanageswaran et al. 2015, Saraiva et al. 2015, Olender et al. 2016). Besides, TMPRSS2 expression seems to be elevated by comparison to that of ACE2 and observed in both neuronal and non-neuronal cells in the OE (Kanageswaran et al. 2015, Saraiva et al. 2015). Also, RNA-seq analysis showed even expression of the majority of the genes, except intriguingly mosaic TMPRSS2 expression was recorded in the subpopulation of mature ORNs (Saraiva et al. 2015). These results indicated that some of the olfactory neurons in the OE are considerably vulnerable to viral infection by comparison to other morphologically similar ORNs. Furthermore, SARS-CoV-2 was suggested to bypass the olfactory axonal route and directly pass from non-neuronal OCE cells to cerebrospinal fluid covered by olfactory nerves—situated near the cribriform—and then migrate to most of the regions in the brain such as *medulla oblongata* which holds the cardiorespiratory controlling nuclei (Harberts et al. 2011). Thereof, SARS-CoV-2 infection in the brain poses a considerable threat as several neurological impairments are reported, including stroke, epilepsy, and encephalitis. Although, expression of ACE2 is considerably low but well documented in the glia and neurons of the brain, however, specific sites for SARS-CoV-2 indection are not identified (Harberts et al. 2011).

13.4.6.2 Pancreas Disability

Pancreatic cells substantially expressed the ACE2 receptors, thereof, a potential target to SARS-CoV-2 infection (Pal and Banerjee 2020). Recent reports also documented elevated serum amylase and lipase levels in ~16% of patients at a severe stage of SARS-CoV-2 infection accompanied by significant pancreatic changes in 7% of the infected patients on CT scans (Liu et al. 2020a). Additionally,

the clinical presentation of acute pancreatitis was also observed in the SARS-CoV-2-infected patients (Hadi et al. 2020). It is important to mention that ACE2/angiotensin-(1–7) display protective activity in diabetes by enhancing the pancreatic β cell survival, promoting insulin secretion, and decreasing insulin resistance (Santos et al. 2018). On this note, several SARS-CoV-infected patients with no diabetes and who had not been treated with steroids in comparison to non-SARS pneumonia patients developed insulin-dependent acute diabetes during hospitalization (Yang et al. 2006, Yang et al. 2010). Also, plasma glucose concentration and diabetes were observed as independent factors of mortality in SARS-CoV-infected patients (Yang et al. 2006). Moreover, postmortems of some SARS-CoV-infected individuals showed amyloid degeneration and atrophy in most of the pancreatic islets, indicating the islets damage induced by the virus (Lang et al. 2003). Thus, SARS-CoV-2 infection is stipulated to influence the pancreatic function as documented in SARS-CoV, and concentration of glucose levels should be diligently scrutinized in diabetic patients or under glucocorticoid treatment.

13.4.6.3 Skeletal Muscles Weakness

SARS-CoV-2 infection is also assumed to hold the ability to infect skeletal muscle, and a particular concern is the susceptibility of muscles connected with the respiratory pump, that is, the intercostal muscles and diaphragm (Ferrandi et al. 2020). Previous studies documented muscle weakness and high concentration of serum creatine kinase (CK) levels in ~30% of patients infected with SARS (Lee et al. 2003). Mild to moderate increases in CK levels were also recorded in the individuals infected by SARS-CoV-2 on hospitalization (Chen et al. 2020c). Although atrophy and myofiber necrosis were also noticed in the skeletal muscle tissues, electron microscopy analysis showed the absence of SARS-CoV particles (Leung et al. 2005, Gu and Korteweg 2007). Recently, a significant role of RAS was observed in the pathogenesis of several skeletal muscle disorders, and the ACE2/angiotensin-(1–7)/MAS axis was monitored to exert protective effects against muscle atrophy (Santos et al. 2018). Skeletal muscle myopathies are widely spread (D'Souza et al. 2013, Alway et al. 2014, Ryder et al. 2017, Maheshwari et al. 2020) and linked with certain populations defined at risk for SARS-CoV-2 infection (Guan et al. 2020, Wu and McGoogan 2020). However, infection of SARS-CoV-2 in the muscles and association of ACE2 downregulation with myopathy is not identified. The function of the diaphragm muscle is similarly obstructed in the course of aging caused by sarcopenia (Kelley and Ferreira 2017), while markers of systemic inflammation in chronic obstructive pulmonary disease (COPD) patients have been correlated with the severity of sarcopenic muscle loss (Byun et al. 2017). Therefore, the susceptibility of skeletal muscle to SARS-CoV-2 infection has been suggested to extend based on several myopathic circumstances correlated with comorbidities and aging (Ferrandi et al. 2020).

13.4.6.4 Damage to Central Nervous System Function

The neurons in the human brain widely express the ACE2 and contribute to the neural control of broad physiological functions, including, stress response and

neurogenesis, cardiovascular, and metabolic activities (Santos et al. 2018, Alenina and Bader 2019, Katsi et al. 2019). For instance, experimental studies with mouse models infected with SARS-CoV showed infiltration of viral particles into the brain through the olfactory bulb followed by transneuronal transmission to other regions (Netland et al. 2008). To note, gustatory and olfactory dysfunctions were also observed in several patients infected by SARS-CoV-2, indicating the involvement of the olfactory bulb in viral infection (Lechien et al. 2020, Luers et al. 2020). Moreover, SARS-CoV viral particles were discovered in the human brain tissue specimens (Gu et al. 2005, Xu et al. 2005), and autopsies showed focal degeneration and edema in the brains of the infected individuals by SARS-CoV (Gu et al. 2005, Gu and Korteweg 2007). Likewise, several patients (78/214) showed neurologic manifestations in SARS-CoV-2, and the virus was discovered in the cerebrospinal fluid of a patient with encephalitis (Huang et al. 2020b, Mao et al. 2020). With the fact that SARS-CoV-2 possessed a higher affinity for the ACE2 as a functional receptor by comparison to SARS-CoV, the former has been implicit to infect and damage the central nervous system.

13.4.6.5 Blood Vessels Damage

Endothelial cells of small and large blood vessels expressed elevated levels of ACE2, while the vascular endothelium expresses angiotensin-(1–7) (Hamming et al. 2004, Santos et al. 2018). Moreover, ACE2/angiotensin-(1–7)/MAS axis stimulates antiproliferative, vasodilatory, and antithrombotic effects in the vasculature (Santos et al. 2018). In this context, SARS-CoV RNA was detected in the endothelia of the small veins (Zhang et al. 2003). Likewise, substantial increased plasma D-dimer levels were observed in individuals under severe SARS-CoV-2 infection (Chen et al. 2020b, Huang et al. 2020a, Yang et al. 2020), while the incidence of disseminated intravascular coagulation (DIC) was not rare at the initial stage of the disease. Inflammatory responses and viral infection cause severe injury to the integrity of the vascular endothelium, and this results in elevated permeability, microcirculation disturbance, and coagulation activation that has been assumed to contribute to organ injury in SARS-CoV-2.

13.5 Conclusions

RAS and ACE2/angiotensin-(1–7)/MAS axis are well established for contribution in various pathophysiological and physiological events. Both SARS-CoV and SARS-CoV-2 utilize the ACE2 as a functional receptor to infect the host cells. Due to the considerably higher expression of ACE2 in various organs and tissues of the human body, SARS-CoV-2 has the ability not only to infiltrate the lungs but can also damage the other organs with high ACE2 expression. The pathogenesis of COVID-19 is exceedingly complicated involving multiple factors; in addition to the direct viral effects, inflammatory and immune responses against the viral invasion, imbalance of the RAS and ACE2/angiotensin-(1–7)/MAS axis, and downregulation of ACE2 can also subsidize to the multiple organ damages in

individuals infected by SARS-CoV-2. Thereof, the scientific community has recently engrossed its attention on ACE2 and ATIR, and their conceivable benefit/harms on the individuals infected by SARS-CoV-2 who experience pneumonia. However, some doubts still exist for the effect of respective inhibitors on SARS-CoV-2 infection. Thus, a potential approach to restoring the balance between RAS and ACE2/angiotensin-(1–7)/MAS has been looked upon as potential therapy to help diminish organ damages in patients infected by SARS-CoV-2.

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Immunology and Pathogenesis of COVID-19

14

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Abstract

The whole world is facing a pandemic situation ever since the outbreak of SARS-CoV-2 in Wuhan, China in December 2019. The coronavirus disease (COVID-19) presents a wide spectrum of clinical manifestations ranging from asymptomatic to severe pneumonia-like situations followed by multisystem failure leading to the death of the individual. Studies from the past coronavirus outbreaks, the SARS, and MERS-CoV, have helped us understand the current SARS-CoV2 to a large extent. Once the host encounters the virus, an innate immune response is generated which subsequently leads to activation of the adaptive immune response to eliminate the virus. However, this immune response is misbalanced in some individuals and is the main factor causing the pathological manifestation of COVID-19. In this chapter, we have addressed the humoral and cellular immune changes induced by the virus along with the role of cytokine storm in disease progression.

Keywords

SARS-CoV-2 · Immune response · Innate immunity · Adaptive immunity · Cytokines

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14.1 Introduction

December 2019 saw an outbreak of severe acute respiratory syndrome coronavirus-2 (SARS-CoV2) in Wuhan city of China, which was declared as a pandemic by the World Health Organization in late March 2020, due to its rapid transmission to almost all parts of the world. It is causing concerns globally due to the complications associated with it, such as acute respiratory distress syndrome (ARDS), pneumonitis, shock, respiratory failure, and death. In 1996, the first pathogenic coronavirus (CoV) was discovered that could cause interspecies infection. The CoV strains, E229 and OC43 came from rodents, and NL63 and HKU1 came from bats and infected the humans (Cao et al. 2020). These viruses remained limited in their spread causing mild seasonal common cold-like symptoms. In November 2002, an outbreak of severe acute respiratory syndrome (SARS) caused by a CoV from bats, also originated in Foshan city of China and was named SARS (now termed as SARS-CoV or SARS-CoV-1 to distinguish it from currently pandemic SARS-CoV-2). The SARS-CoV-1 epidemic lasted for over a year and ended in July 2003 (Wang et al. 2020b). The second CoV outbreak took place in June 2012, first detected in Jeddah, Saudi Arabia and so termed as Middle East Respiratory Syndrome (MERS), mimicked several clinical manifestations of SARS (Grein et al. 2020). The current spillover is the third one from animal CoVs to humans, first detected in Wuhan city of China in October 2019 and quickly spread over six continents of the world including 66 countries by March 1, 2020, when the World Health Organization (WHO) declared it as a pandemic. The novel coronavirus (initially termed as 2019-nCov and subsequently as SARS-CoV-2) causes severe coronavirus disease, now termed as COVID-19. All these coronaviruses (SARS-CoV-1, MERS, and SARS-CoV-2) have been found to have jumped to humans from bats, but some researchers believe that there is an intermediary host. In the case of SARS-CoV1, the intermediary animal is thought to be civet cats which are sold in abundance in the live-animal markets of China (Chen et al. 2020a). However, the origin of SARS-CoV2 is not clear but it shares around 96% of its genetic material with CoV found in bats (Lynch et al. 2016). These three coronaviruses share genetic and structural similarities but differ significantly at the epidemiological level. SARS-CoV and MERS-CoV have high lethality and low transmissibility while SARS-CoV2 has high transmissibility and the level of lethality has not yet been established globally. Thus, its tremendous spread has brought extreme pressure and disastrous consequences on the public health and medical setup worldwide.

14.2 General Characteristics of the Virus

Novel SARS-CoV2 has been placed under the family *Coronaviridae*. The virus contains single-stranded positive RNA of nearly 30 kbp as its genetic material. The genetic material is protected by an outer fatty layer of envelope containing spike –S, membrane –M and envelope –E- proteins. The subfamily *Coronaviridae*is further subdivided into four genera- the alpha, beta, gamma, and delta CoV. Viruses having

the ability to infect humans are categorized under α-CoV and β-CoV (SARS-CoV and MERS-CoV) and viruses infecting avians and pigs belong to ϒ-CoV and δ-CoV genera. The novel SARS-CoV2 has been placed under the genus β-CoV.

The entry of enveloped viruses into the host cells is usually mediated through the attachment of proteins expressed on the surface of host cells. To gain entry into the host cell, the S-glycoprotein present on the surface of the virus engages with the receptors on the host cells. Based on sequence similarities between the receptor-binding domains (RBD) in the S-protein of SARS-CoV-1 and SARS-Cov-2, it is now established that the angiotensin-converting enzyme 2 (ACE2), serves as the receptor for both the viruses. The ACE-2 is a type-1 transmembrane metallocarboxypeptidase molecule, which is highly expressed on vascular endothelial cells, the renal tubular epithelium, and Leydig cells in the testes. The ACE-2 is homologous to ACE, which is an enzyme and a key player in the Renin-Angiotensin system (RAS). For entry of the virus into the host cells, the spike protein on the surface of the virus, which binds to its receptor on the host cell, needs to be cleaved first.

The S protein is cleaved into subunits S1 and S2 during the infection stage, where the receptor-binding domain (RBD) present in the S1 subunit binds directly to the peptidase domain of the ACE2 molecule and the S2 subunit facilitates membrane fusion between host cell and virion (Kucharski et al. 2020). RBD-ACE2 binding induces conformational change on S-protein which exposes a cleavage site on the S2 subunit, which is cleaved by host serine proteases TMPRSS2. This step is a critical process mediating the fusion of the virus envelope with the cell membrane and thus allowing the viral RNA to enter into the target cell's cytoplasm (Hoffmann et al. 2020). Subsequently, viral RNA serves as a template for the translation of the polyproteins pp1a and pp1b that are cleaved into smaller proteins which join to form a replicase-transcriptase complex (RTC). In this complex, several copies of negative-strand RNA are made and used as the template to form complete positive-strand RNA. The structural proteins (S, E, M & N) are translated and are transported to the lumen of the intermediate compartment of the endoplasmic reticulum golgi intermediate complex (ERGIC). Along with the genomic RNA, virion formation occurs and mature virions are released from the cell by exocytosis, which then infect the neighboring healthy cells as well as released into the surrounding environment via respiratory droplets. The droplets carrying the infectious virus are highly contagious and cause the potential spread of the disease in healthy individuals (Fung and Liu 2019). Thus, ACE2 bearing cells are most vulnerable against SARS-CoV2. The majority of the ACE-2 bearing cells are alveolar epithelial type II cells, thus making the lung as primary target tissue and the most common entry route. Other extrapulmonary tissues expressing ACE-2 are kidneys, heart, endothelium, intestine, and tongue.

14.3 Clinical Manifestation of COVID-19

Depending on the individual immune response, the clinical manifestation ranges from asymptomatic (positive for SARS-CoV-2 virus but no symptoms), mild symptomatic (positive for virus with mild clinical manifestation), and severe (positive for the virus and a high degree of symptoms). In SARS-CoV-2, the mild symptomatic individuals present with dry cough, fever, and fatigue, difficulty in breathing, some have diarrhea, sore throat, congestion, and runny nose. The severe symptomatic patients have difficulty in breathing (greater than 30 times/minute), oxygen saturation less than 93%, the ratio between partial pressure of arterial oxygen and oxygen concentration in arterial blood is less than 300 mmHg) (Kimball et al. 2020). Critical individuals have a respiratory failure with the need for mechanical ventilation, organ failure, or need for treatment in the Intensive care unit (ICU) of a hospital. The wide range of clinical manifestations found in COVID-19 are associated with risk factors such as age and gender. Diabetes, cardiovascular diseases, high BP, lung diseases, treatments affecting the immune system are considered comorbidities that may result in the highest risk of severe disease and death in COVID-19 (Chen et al. 2020b) (Fig. 14.1).

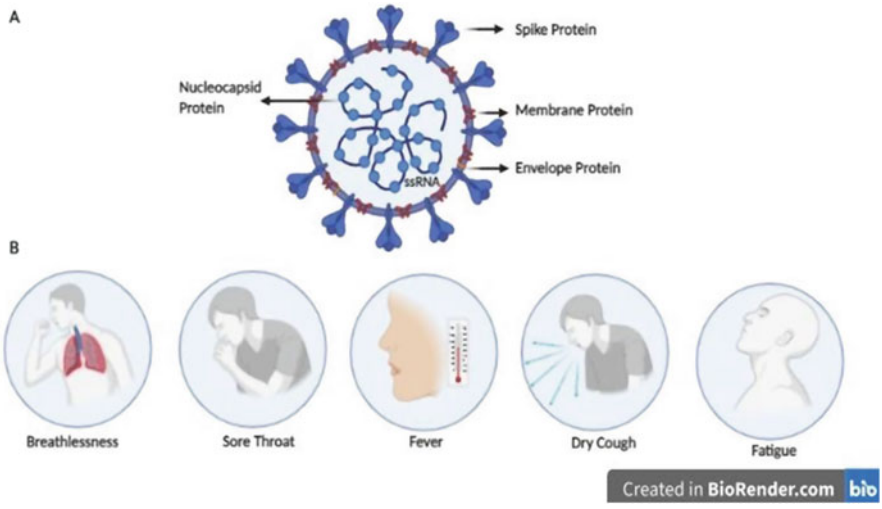

Fig. 14.1 (**a**) Schematic structure of Virion of SARS-CoV2 and its major structural proteins, (**b**) Symptoms of COVID-19

14.4 Immune Responses in COVID-19

14.4.1 Innate Immune Response

The innate immune system is made up of barriers that can prevent or limit the entry and spread of various foreign particles. Innate immune cells include DCs, neutrophils, macrophages, parenchymal cells like epithelial and fibroblast cells. Antigens related to viruses are recognized by receptors of innate immune cells known as pattern recognition receptors (PRR). The toll-like receptors (TLRs) recognizing pathogen-associated molecular patterns (PAMPs), RIG-I-like receptors (RLRs) recognizing nucleic acids, C type lectin-like receptors (CLRs), and NOD-like receptors (NLRs) are few other pattern recognition receptors that are responsible for identifying the viral antigens (Li et al. 2020). The RLRs and NLRs are usually expressed by epithelial cells and some local cells of the innate immune response like alveolar macrophages. Once the recognition of the pathogen is executed, the PRR's recruit adaptor proteins which lead to downstream activation of transcription factors like IFN, AP-1, NF-κB, which lead to the secretion of critically important type I and type III antiviral interferon (IFN). After activation of IFN signaling, an entire cascade of events occur that leads to the production of pro-inflammatory cytokines which further activate the endothelial cells, and produce chemokines. These chemokines attract other immune cells like monocytes, NK cells, dendritic cells, and polymorphonuclear leukocytes (PMN) including Neutrophils. These cells further release reactive oxygen species and directly kill the infected cells and produce more chemokines like monokine induced gamma interferon (MIG), Interferon-gamma inducible protein-10 (IP-10), monocyte chemotactic protein-1 (MCP-1), which recruit lymphocytes that can recognize the viral antigen presented by the DCs, thereby activating a pathogen-specific adaptive immune response (Dandekar and Perlman 2005). Apart from IFNs other cytokines like TNF-α, IL-1, IL-6, and IL-18 are also released. Thus, together these cytokines induce an antiviral defense mechanism and also activate the adaptive immune response. CoV infection can be limited in early stage if the IFNs are properly localized. The IFN-stimulated genes (ISG) like lymphocyte antigen-6 complex locus E (LY6E) interfere with S-protein mediated membrane fusion (Huang et al. 2011). However, in the later phase of infection, IFN can cause immune-pathogenesis by induction of other cytokines.

14.4.2 Immunopathogenesis of COVID-19 and Immune Escape Mechanisms

In-vitro infection of human lung explant by SARS-CoV2 leads to viral infection and replication in type I and type II pneumocytes and alveolar macrophages, although it failed to induce expression of IFN-I, IFN-II, and IFN-III (Chu et al. 2020). CoV has also evolved a mechanism to inhibit IFN-I induction and signaling. It has been observed that patients with severe COVID-19 have impaired IFN-I production as

compared to patients with mild symptoms (Blanco-Melo et al. 2020). Thus, it is inferred that CoV uses various evasion mechanisms like avoiding PRR sensing either by avoiding recognition or antagonizing PRR activation. For avoiding recognition by PRR, ssRNA CoV forms dsRNA-intermediate during replication, which is shielded by membrane-bound compartments and nonstructural proteins help in methylation of the viral RNA at 5′ end (Bouvet et al. 2010). In addition, in SARS-CoV2 it has been demonstrated that the viral proteins ORF9b, NSP13 & NSP15 interact with the downstream signaling molecules like Mitochondrial antiviral signaling proteins(MAVs), signaling intermediate like Tank-binding kinase-2 (TBK2) and Rosetta Net Implementation Framework (RNIF41), respectively, and they interfere with the TBK1 signaling complex (Gordon et al. 2020). SARS-CoV1 and MERS-CoV are also known to inhibit the TBK1 signaling complex (Gordon et al. 2020). MERS-CoV encodes matrix and accessory proteins from the open reading frame, ORF4a, 4b, and 5 which directly inhibit IFN promoter and nuclear localization of interferon regulatory factor-3 (IRF3) (Yang et al. 2013). Thus, pathogenic CoVs can block or inhibit IFN pathways but can also activate other inflammatory pathways that contribute to disease pathogenesis. For instance, SARS-CoV2 non--structural proteins, NSP-9 and NSP-10 might induce the IL-6 and IL-8 production and drive pathological inflammation. In fact, IL-6 has emerged as the dominant cytokine during immunopathogenesis. Thus, a decrease in innate antiviral response and hyper inflammation causing a “cytokine storm” could be a leading cause of COVID-19 severity.

14.4.3 Role of Innate Immune Cells in COVID-19 Pathogenesis

Neutrophils, monocytes, NK cells are the innate immune cells that play an important role in viral clearance as well as the pathogenesis of COVID-19. The cytotoxicity mediated by the NK cells is regulated by the expression of inhibitory and activating receptors expressed on them. The frequency of NK cells has been reported to be lower in severe COVID-19 cases as compared to mild cases. The NK cells from the peripheral blood of severe COVID-19 patients showed a reduced expression of CD107a (a marker for degranulation), Granzyme B (a marker of killer activity), and Ksp37 (a marker co-expressed with perforin, also a marker of killing activity), thereby indicating impaired cytotoxicity. In addition, there is impaired production of chemokines, IFN-ϒ and TNF-α (Zheng and Song 2020). The lower frequency of NK cells can be correlated with the increased concentration of IL-6 in the plasma. An in-vitro data suggests that stimulation by IL-6 and soluble IL-6 receptors can impair perforin and Granzyme B production in healthy donor NK cells, which can be restored by blocking IL-6R with tocilizumab (Wenjun et al. 2020). Further, this heterodimeric inhibitory receptor prevents NK cells from releasing IFN-ϒ. Thus, targeting this receptor by monalizumab can boost antiviral immunity. Reduced production of IFN-ϒ can also lead to the infiltration of the neutrophils in the alveoli and the high neutrophil to lymphocyte (N:L) ratio is an indicator of the severe stage of COVID-19. These accumulating neutrophils cause sustained neutrophil

extracellular traps (NET) formation which leads to stimulation of cascade of damaging inflammatory reactions. This has been observed in COVID-19 patients, with increased levels of NET-specific markers like myeloperoxidase DNA and citrullinated histone H3 (Kawasaki et al. 2018). In addition, the immune checkpoint molecule, NKG2A was also found to be increased in severe COVID-19 patients, indicating viral escape and NK cell exhaustion. Monocytes-derived DCs (Mo-DC), plasmacytoid DCs (PDC) also get dysregulated and potentially drive cytokine release syndrome (CRS), acute respiratory distress syndrome (ARDS), and lymphopenia. Single-cell transcriptome analysis done on the pulmonary tissue samples and peripheral blood samples of severe COVID-19 disease have revealed expansion of $CD14^{+}HLA\text{-}DR^{low}$ inflammatory monocytes, which are known to be an immunosuppressive phenotype of these monocytes and now clubbed as a broad category of Monocyte-derived suppressor cells or MDSCs. Thus, the innate immune system is unable to strike a balance to control the infection in a timely manner.

14.4.4 Complement Activation in SARS-CoV2 Infection

The complement system is an important part of innate immunity and acts as a bridge between innate and adaptive immunity. Complement recognizes a foreign pathogen and leads to the initiation of complement activation pathway using one of the three mechanisms. The classical pathway- characterized by C1q mediated antigen-antibody complex, the lectin pathway is mediated by mannose present on the microbial surface (also known as "Mannose-binding pathway") and third is the alternative pathway, which is mediated by spontaneous cleavage of C3 binding with pathogens' cell surface components. An imbalance in the complement system function can result in enhanced inflammatory and degenerative responses in various pathological conditions. Usually, C3a and C5a are the molecules that are increased in case of over-activation of complement pathways. The C5a being a chemoattractant, recruits inflammatory cells and leads to the production of granular enzymes and free radicals. It has been observed in past, that SARS-CoV leads to overactivation and production of C3a, which causes excessive alveolar air sac infiltration by neutrophils and monocytes and subsequently leads to SARS-CoV associated ARDS (Gralinski et al. 2018). In an animal model, these results were validated, wherein the C3 knockout mice were infected with SARS-CoV and a lower infiltration of neutrophils and monocytes was observed with a low level of cytokine and chemokine production in lungs as compared to wild-type mouse. However, the level of viral load was not changed in the lung alveoli (Bosmann and Ward 2014). In SARS-CoV2, a C5a mediated cell activation and cytokine release has been observed, which results in increased vascular permeability and epithelial cell degradation leading to an increase in the number of infiltrating cells in the lungs causing respiratory distress (Wang et al. 2015).

14.4.5 Adaptive Immune Response

The innate immune response invariably leads to an adaptive immune response against a pathogen, which is specific and longer lasting. Many immune cells like T cells, B cells, NK cells, all play a central role in the functioning of the immune system. The antigen-specific cell-mediated response, mainly governed by the helper T-cells and cytotoxic T cells is directed towards the elimination of the viral-infected cells, while the B cells help in antibody synthesis and long-lived memory cells are some of the components of the adaptive immune system. The pathogen taken up by the phagocytic cells (the DCs, macrophages, and neutrophils) of the innate immune system is processed and expressed on the surface of these cells in conjunction with molecules of major histocompatibiliy complex (the MHC-I and MHC-II). The antigens in this form are presented by these so-called antigen-presenting cells or APCs to the naive T cells in the lymphoid organs. So, the naive T-cells carrying specific T-cell receptors recognize the antigen along with the MHC molecule and get activated, which further differentiate into effector/helper CD4+ T cells (Th1, Th2, or Th17 cells). Subsequently, the activated helper T-cells further pass activation signals to antigen-specific B cells and CD8+ cytotoxic T lymphocytes (CTLs) through cell-to-cell interaction and release of cytokines. Activated B cells differentiate further to antibody-forming plasma cells, which can then produce antiviral antibodies and act through various mechanisms (opsonization, neutralization, complement activation) for clearance of the virus. In addition, activated CD8+ CTLs cause the direct lysis of the target infected cells. However, the dysregulated T-cell response can lead to the immunopathogenesis of the disease.

14.4.6 T-Cell Responses

In COVID-19 patients, particularly with moderate and severe disease, reduced frequency of CD4+ and CD8+ cells (lymphopenia) has been observed which correlated with disease severity and mortality (Wang et al. 2020a). Though this phenomenon is observed in other viral infections also but the exact mechanism is unclear since the direct infection of T cells by SARS-CoV2 has not yet been reported (Xiong et al. 2020). Few underlying mechanisms have been proposed for lymphopenia in COVID-19 patients, one such is the contribution of inflammatory cytokines. Increased serum levels of IL-6 have been correlated to lymphopenia in COVID-19, while in recovered patients the level of lymphopenia recovers to normal limits as the level of IL-6 in serum decreases. A similar trend was seen with other cytokines like TNF-α and IL-10 (Xiong et al. 2020). Multiple studies suggest that IL-6 leads to the down-regulation of HLA Class II molecules on CD14+ monocytes and B-cells (Wilk et al. 2020). While no such effect was seen on HLA Class I molecules. Thus, with low HLA class II molecules, the severe COVID-19 patients will not be able to mount a sufficient level of T cell response due to the decreased capacity of the antigen-presenting cells to present the antigen to the TCR, thereby such T cells are removed by apoptosis. These observations were reported in the

autopsy samples of spleen and hilar lymph nodes, where massive death of lymphocytes is linked to high IL-6 level and fas-induced apoptosis (Feng et al. 2020). Since CD8+ T cells require HLA-Class I for their activation via TCR and IL-6 doesn't affect the expression level of HLA-Class I molecules, so similar mechanism of apoptosis doesn't seem to operate in this condition. However, T cell exhaustion and activation can be the underlying mechanism for the elimination of CD8+ T cells (Diao et al. 2020). Higher levels of co-stimulatory and inhibitory molecules like CTLA-4, CD137, TIM-3, PD-1, NKG2a, etc. have been reported in several studies. In addition, the levels of these markers were found high in severe disease as compared to mild disease. Besides the frequency, the functionality of CD4 and CD8 T cells has also been shown to be altered, as these cells produced low quantities of IFN-ϒ and TNF-α on PMA stimulation and the CD8 cells expressed lower levels of cytotoxic molecules Granzyme B, Granzyme A, perforin, and CD107a (Yang et al. 2020). While the frequency of Th-17 cells is increased in the severe COVID-19 patients (He et al. 2020), the naive as well as induced T-regulatory cells, are reported to be decreased (Qin et al. 2020). Apart from effector T-cell subsets, the memory T-cells are also generated that fight against reinfection. Usually, the memory CD4+ T cells upon re-stimulation trigger B cells and other cells via cytokine production, and the CD8+ cytotoxic memory T cell destroy the virus-infected cells on re-exposure. In recent reports, the COVID-19 patients show a reduced level of memory T cells, which may aggravate the inflammatory response leading to cytokine storm and enhanced tissue damage and organ failure (Qin et al. 2020). In an animal model, when CD4+ memory T cells were administered through the intranasal route, it leads to a protective affect against human coronavirus. In addition, upon re-stimulation, these memory T cells were able to produce IFN-ϒ and also recruit CD8+ cells and cause rapid clearance of SARS-S366 peptides (Zhao et al. 2016). Thus, various studies reveal the dynamics of T-cell number and functional status during SARS-CoV2 infection and also indicate the important relationship between these parameters and the disease profile. Large-scale multi-centric studies are however warranted from different geographical locations in the world to precisely understand the role of T-cells in the pathogenesis of COVID-19.

14.4.7 B-Cell Responses

Apart from T-cell responses, humoral responses also play an important role in the clearance of viruses and it has been observed that B-cell responses start to appear within the first week following the onset of the COVID-19 symptoms. Similar to SARS-CoV1 infection, seroconversion occurs in COVID-19 patients between 7 and 14 days after the infection. The initial response occurs against the nucleocapsid (N) protein and later the antibody production continues to occur against the S-proteins (Dienz et al. 2009). Many studies support the fact that antibodies against the S-proteins can block the virus attachment to the ACE2+ cells (Tai et al. 2020). However, there are many questions, which are still unanswered with regard to the cross-reactivity of antibodies against alpha and beta coronaviruses, although it seems

that most likely the cross-reactivity seems to appear between SARS-CoV and SARS-CoV2, which share 90% of the amino acid sequence in S1 (Walls et al. 2020). IgM and IgA are usually detected within the first week of symptoms onset while IgG antibodies are detected around 14 days after the initial symptoms (Guo et al. 2020). Comparing the mild and severe cases, there was no significant difference in the production of IgG and IgA levels, while there was a slight reduction in the level of IgM in severe cases (Zhang et al. 2006). The B cell responses provide both immediate protection from the initial challenge besides exerting extended immunity against reinfection. Memory B cells can quickly respond to the reinfection by generating new high-affinity plasma cells while long-term protection is achieved through long-lived plasma cells and memory B cells. Thus, understanding how long the lifespan of B cells memory remains against SARS-CoV2 will be interesting. Due to the short time elapsed it has not been possible yet to understand but from the past human CoVs we have understood that in the case of SARS-survivors who were followed up for 6 years, the majority of patients had undetectable levels of anti-SARS CoV antibody and none of them showed the level of memory B cells. In contrast, the T-cell specific memory was present in about half of the SARS survivors (Abbasi 2020). In the case of MERS-CoV, the antibodies were detected in most of survivors after even 3 years of infection (Payne et al. 2016). Thus, studies from SARS-CoV1 and MERS-CoV indicate that virus-specific antibodies wane out with time and results in partial protection in case of reinfection. In SARS-CoV-2 infection, however, it has recently been shown that the virus causes an early disruption of germinal centers due to loss of Bcl6 expression on T-follicular helper cells (Tfh cells), which are absolutely required for the maintenance of long-living antibody-secreting plasma cells in the germinal center (Kaneko et al. 2020). This explains the dysregulated humoral immune induction in COVID-19 disease besides providing a mechanistic explanation for the limited durability of antibody responses in coronavirus infections. So, further long-term studies are needed to check on the degree and extent of the long-term protection.

A few research groups are beginning to question whether anti-SARS-CoV2 antibodies might be detrimental to the patients. It is postulated that antibodies can bind to viral fragments and can cause complement activation, which can exacerbate the disease. Secondly, antibody response to CoV might contribute to antibody-dependent enhancement (ADE). This phenomenon is observed when non-neutralizing virus-specific IgG antibodies facilitate the entry of the virus particles into the Fc receptor-expressing cells (macrophages and monocytes) and thereby leads to the activation of these cells. However, direct evidence for ADE in COVID-19 patients has not been reported so far. Thus, during the development of therapeutic targets for SARS-CoV2, the possibility of ADE should be considered (Fig. 14.2).

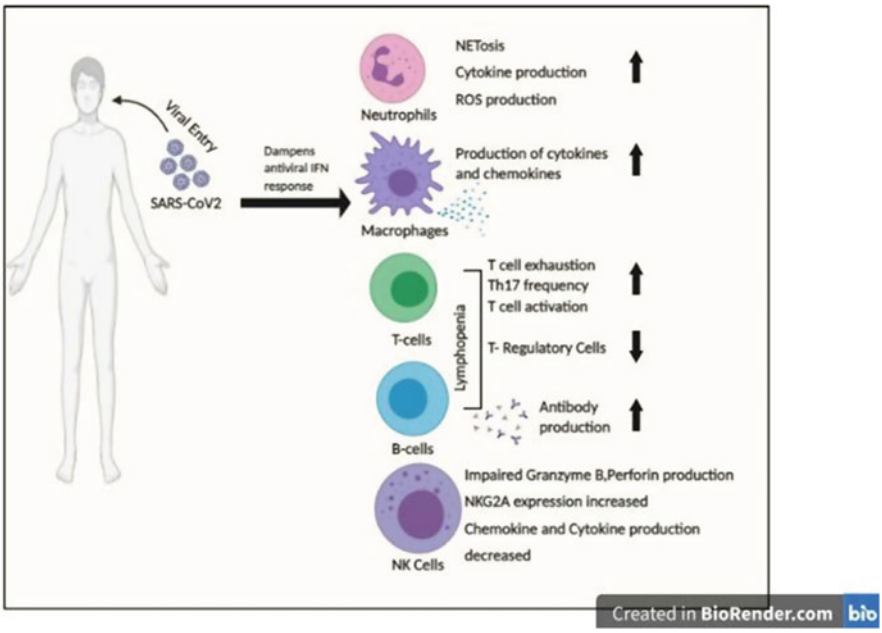

Fig. 14.2 Immunopathology of COVID-19: Numerous cells and molecules are involved in pathogensis of the disease

14.5 Viral Response During the Infection by SARS-CoV-2

14.5.1 Cytokine Storm in COVID-19

The term "Cytokine Storm" dates back to 1993 when it was initially reported in case of graft vs host disease (GVHD) and was used to describe an overactive immune response, which can be triggered by a variety of factors like virus infection, autoimmune disease, and immunotherapies. Under normal conditions, a balance is maintained between pro-inflammatory and anti-inflammatory cytokines, which can be broken under viral infection by abnormal activation of immune cells like dendritic cells, macrophages, and lymphocytes. These abnormally activated immune cells could lead to the overproduction of cytokines, among which the pro-inflammatory cytokines in a positive feedback loop mechanism could further activate more immune cells. Thus, the formation of a "cytokine storm" leads to a sort of suicidal attack that not only contributes to the elimination of pathogenic microorganisms but also causes tissue injury affecting many organs. The cytokine storm leads to a systemic inflammation syndrome known as cytokine release syndrome (CRS), which has previously been reported in SARS-CoV-1 and MERS-CoV patients, as well as in leukemia patients receiving engineered T cell therapy (Chen H et al. 2019). In SARS-CoV-1, immune cells triggered the production of pro-inflammatory

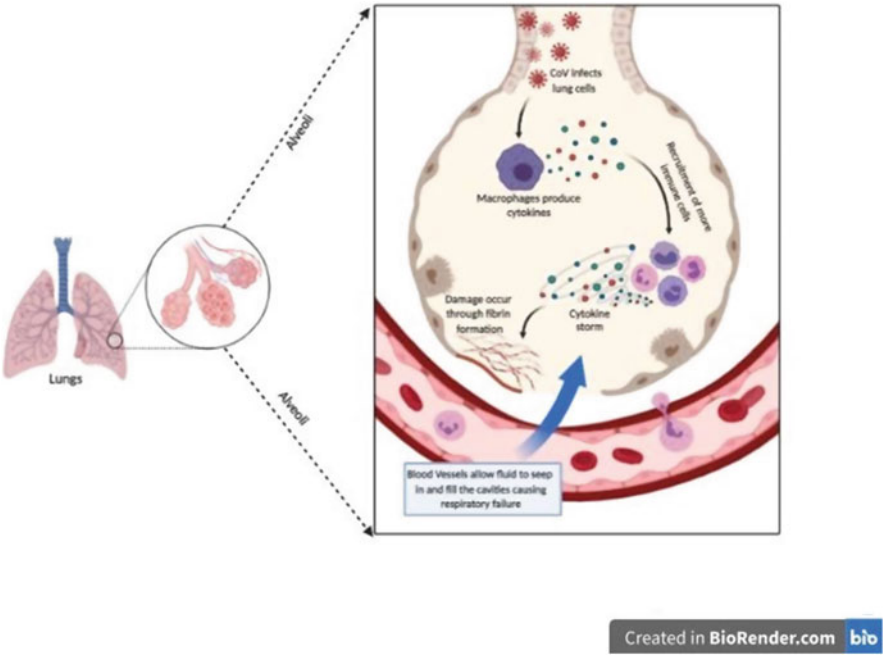

Fig. 14.3 Schematic Representation of the cytokine storm in COVID-19

cytokines like IL-6, IFN-α/γ, TNF-α and in MERS-CoV though delayed but increased production of IL-6, IL-8, IL-1β, IFN-α has been observed. In addition, one of the main causes reported for the deaths of patients with SARS-CoV and MERS-CoV was the "cytokine storm." A large number of factors are responsible for the dysregulated release of cytokines in case of these infections. It is assumed that once SARS-CoV2 binds to the ACE2+ epithelial cells in the lungs, rapid viral replication in the first stages of the infection results in high pro-inflammatory responses. In addition, the virus induces the release of large amounts of proteins that are known to delay IFN responses, which further provoke an accumulation of pathogenic inflammatory monocyte-macrophages. This, in turn, results in even higher production of cytokines in the lungs and the recruitment of more immune cells. The consequences of this hyper-inflammatory cascade are diverse, ranging from the dampening of T-cell responses, which leads to an even less controlled inflammatory response, to the apoptosis of epithelial cells, vascular damage, and ARDS (Fig. 14.3) (Channappanavar and Perlman 2017).

In the case of COVID-19 the patients in the critical stage, needing intensive care hospitalization and oxygen therapy, had higher levels of plasma inflammatory cytokines IL-2, IL-7, IL-10, G-CSF(granulocyte colony-stimulating factor), IFN-γ and MCP, and TNF-α circulating in the blood as compared to patients with mild or no symptoms, thus indicating a positive correlation between cytokine storm and disease severity. These listed cytokines suggest that both Th1 and Th2 responses are

involved in COVID-19 pathogenesis, unlike SARS-CoV infection. It is becoming evident from many published reports that the IL-6 and GM-CSF cytokines released by T lymphocytes and mononuclear cells are the key link of cytokine storm in COVID-19 in adult patients (Siddiqi and Mehra 2020). Although not much data is yet available from pediatric patients, yet the available literature indicates similar involvement of IL-6, IL-10, and IFN-γ in the severity of disease in children below 15 years of age. Thus, early recognition and prompt treatment can lead to better outcomes. Several biological agents targeting cytokines have been proposed for treating Cytokine storms. Anakinra, an IL-1 receptor (IL-1R) antagonist, which is used in the treatment of rheumatoid arthritis, has also proven to be helpful in cytophagic histiocytic panniculitis with secondary hemophagocytic lymphohistiocytosis, a disease associated with severe cytokine storm, is been proposed and under trial (NCT04324021). Tocilizumab, a recombinant humanized IL-6 receptor antagonist that interferes with IL-6 binding to its receptor and blocks signaling, being the other one. Tocilizumab is already being used in the treatment of rheumatoid arthritis, juvenile idiopathic arthritis, and has proven valuable in the treatment of Cytokine storm triggered by CAR-T cell therapy for hematological malignancies. Thus, tocilizumab can be used as a candidate drug in managing the cytokine storm accompanying COVID-19 (Xu et al. 2020). Sarilumab is another IL-6R blocker that is being investigated in SARS-CoV-2 infection, which may reflect a possible clinical benefit regarding early intervention with IL-6-modulatory therapies for COVID-19. Thus, targeting cytokines during the management of COVID-19 patients could improve survival rates and reduce mortality.

14.5.2 Emergence of Antigenic Drift in SARS-CoV-2

Several approaches and vaccines are being developed worldwide to combat the COVID-19 pandemic. The major area of focus has been toward antibody-based immunity to coronaviruses. In this context, based on some recent findings regarding the detection of some mutations in the viral genome that may lead to the emergence of viral species which are more infectious and highly transmissible in nature, the attention has diverted to the fact whether these viruses evolve to escape recognition by the immune system and if they do so what is the rate at which they change. Such kind of a phenomenon is known as antigenic evolution. For example, infection with the influenza virus produces antibodies that generally protect humans against that same viral strain for at least several decades. Unfortunately, the influenza virus undergoes rapid antigenic evolution to escape these antibodies, meaning that although immunity to the original viral strain lasts for decades, humans are susceptible to infection by its descendants within about 5 years (Couch and Kasel 1983). This continual antigenic evolution is the reason that the influenza vaccine is periodically updated. Similarly, in the case of SARS-CoV2, recently a new strain has been detected in a sizable number of infected individuals in the United Kingdom and has been given the code name B.1.1.7. Though only limited data is available on coronavirus antigenic evolution but a few studies conducted in 1980s on 229E strain

showed that individuals infected with one strain of 229E were resistant to reinfection with that same strain but partially susceptible to a different strain, which evolved from the original strain of virus (Reed 1984). Additional experimental studies suggest that sera or antibodies can differentially recognize spike proteins from different 229E strains (Shirato et al. 2012). Thus, consideration of antigenic drift in the different sub-strains of the virus is imperative in the design of a "one size fits all" universal vaccine to offer protection against the deadliest outbreak of this century. Since many leading SARS-CoV-2 vaccines use new technologies such as mRNA-based delivery that should make it easy to update the vaccine if there is antigenic evolution in spike. So for these reasons, the antigenic mutation in SARS-CoV-2 should be monitored as it might help in periodically updating the vaccine.

14.5.3 Vaccine Strategies

The widespread transmission of SARS-CoV2 infection across the globe has accelerated the development of vaccines using S-protein as the target antigen. Various vaccine platforms are being used, from traditional whole pathogen vaccine (live-attenuated vaccine, inactivated vaccine) to new generation vaccines (recombinant protein vaccine, vector-based vaccine) (Table 14.1). Some of these vaccines have already completed clinical trials showing a high level of immunogenicity and safety. Looking at the satisfactory levels of efficacy of these vaccines against the severe forms of the disease the manufacturers of these vaccines have submitted the interim analysis data to the regulatory bodies of different countries including WHO and got "Emergency use approval" in many countries world over. The vaccination drives have already started in these countries with the United States and India top-listing.

14.6 Summary

In December 2019, an outbreak of COVID-19 emerged in Wuhan city of China and soon it spread to different parts of the world. The rapid spread and increasing mortalities from this disease have alarmed the whole world and demanded urgency in both basic science and clinical research and each nation has met the current need with remarkable productivity. Within a short span of time, there has been a generation of enormous data which helped us to understand the basic pathophysiology of SARS-CoV2, but still, there remain many grey areas. Studies from the past on SARS-CoV and MERS-CoV have helped us better understand the current new virus but besides many similarities, there is considerable difference in the host immune response and disease profile as far as the SARS-CoV2 infection is concerned. The most devastating response to the viral infection studied so far is the excessive inflammation and cytokine release syndrome in COVID-19. Therefore, it is important that these immune mechanisms are further studied in larger group of patients so that better therapeutic strategies for COVID-19 can be developed.

Table 14.1 Overview of Current COVID 19 Vaccine Candidates

Vaccine Platform	Features	Limitations	Organizations
Live attenuated	Resembles natural infection Live pathogen with reduced virulence Stimulates immune system by inducing TLRs Strong immune response Long term immunological memory	Extensive testing Causes risk to immune-compromised patients Higher reactogenicity Reverse to the virulent form Requires great viral gene knowledge	Codagenix and Serum Institute of India are developing
Inactivated virus vaccine	Produced by inactivating or killing the pathogen Weaker immune response compared to live attenuated vaccine Adjuvants are needed	Booster dosage is required to maintain sustain the immune response	Wuhan Institute of Biological Products (VeroCell) Sinovac Biotech (PicoVacc) Beijing Institute of Biological Science (BBIBP-CoV) Bharat Biotech (Covaxin; BBV152A/B)
Protein sub-unit vaccine	Based on synthetic peptides/recombinant antigenic peptides S-protein and its antigenic targets most suited Safe with fewer side effects Cost-effective	Requires adjuvant to potentiate vaccine-induced immune response Memory for future response is doubtful	Novavax (NVX-CoV2373-nanoparticle-containing full-length spike glycoprotein) University of Queensland Univ. Of Pittsburg (PittoCoVacc-microarray needle-based vaccine) Premas Biotech India (Triple antigen vaccine-Spike, Envelope and Membrane protein)
Viral vectors based vaccine	Adenovirus vector-based Induced cellular and humoral immunity Safe and strong immune response Great potential for prophylactic use	Reduced efficacy in some cases due to prior exposure of the host against the vector	Univ. Of Oxford (ChAdOx1-nCoV19) Serum Institute of India (Covishield) Casino Biological Inc./ Beijing Institute of Biotechnology (Ad5-nCov) Johnson and Johnson (Ad26.CoV2-S) Gamaleya Research Institute (Gam-COVID-Vac)

(continued)

Table 14.1 (continued)

Vaccine Platform	Features	Limitations	Organizations
mRNA vaccine	Two kinds—nonamplifying mRNA and self-amplifying mRNA (saRNA) Translation occurs in the cytosol of the host cell Rapid vaccine development Flexible Mimics antigenic structure as seen in natural infection	Newer platform Previously not been in human clinical trials Needs formulation Shows instability	Moderna (mRNA-1273) BioNTech and Pfizer (BNT162) Imperial College of London (saRNA-based vaccine) Arcturus, Singapore (saRNA-based vaccine)
DNA vaccine	Encodes antigen and an adjuvant Develops at an accelerated pace and is cost-effective Generates cellular and humoral responses Safe to handle as it doesn't involve viral particles Thermally stable, fewer refrigeration requirements	Insertion of foreign DNA might trigger abnormalities in the host cell Might degrade due to the host enzyme activity	Inovio Pharmaceuticals (INO-4800) Osaka University, Takara Bio. (AGO301-COVID-19 and AGO302-COVID-19) Genexine Consortium (GX-19) Cadila Healthcare Limited (nCOV-Vaccine)

Currently, few vaccine candidates have been approved for administering in healthy volunteers but mass production and making the vaccine available world-over and administrating it globally is another major challenge, which is going to be faced in coming times. The success of the vaccine or any other therapy would further depend on the emergence of escape mutants of the virus. Hence, further investigations are required in order to reach precise and most efficient ways to combat this global health issue.

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mRNA Vaccine: An Advanced and Transformative Technology for Vaccine Development

15

Vikas Kushwaha, Anuprabha, and R. C. Sobti

Abstract

Throughout the world, billions of people are infected with various diseases and continue to suffer from them despite various treatments. Vaccination is commonly regarded as one of the most advanced approaches to disease prevention. RNA-based innovations have sparked widespread interest in the production of prophylactic and therapeutic vaccines over the last two decades. Because of their high efficacy, safe administration, and low manufacturing cost, mRNA vaccines have emerged as a promising tool for disease prevention. In animal models and humans, mRNA vaccines can induce a healthy, long-lasting cellular and humoral immune response. Furthermore, mRNA is an intrinsically secure vector that is just a transient carrier of information that does not interfere with the genome and provides full production versatility. Following the outbreak of COVID-19 in December 2020, mRNA-based vaccines made headlines in 2020. This chapter covers mRNA vaccines (both traditional and alternative), their delivery, immune responses elicited by them, and mRNA vaccines for infectious disease prevention.

Keywords

COVID-19 · Vaccines · Human models · mRNA vaccines

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15.1 Introduction

Vaccination is a powerful method to prevent disease progression every year or to reduce the occurrence of diseases like measles, smallpox, polio, hepatitis, mumps, rubella, classic swine fever, and cattle plague. Attenuated vaccines, inactivated vaccines, and subunit vaccines developed as conventional vaccines provide adequate defense against various infectious diseases. It is difficult to develop a vaccine against such pathogens which evade the host immune response. So, there is a need of more powerful and scalable platform to develop a vaccine. In the nineteenth century, nucleic acid therapeutics were seen as potential alternatives to conventional vaccines. Transcribed mRNA (IVT) and vasopressin-encoding mRNA were used in mice and rat but the findings were not promising (Wolff et al. 1990; Jirikowski et al. 1992). Thanks to substantial technological innovation and research support over the last decade, mRNA has emerged as a possible therapeutic mode in vaccine development. In comparison to DNA vaccines, subunit vaccines, and killed and live attenuated vaccines, mRNA vaccines are noninfectious, safe, more robust, and highly translatable. These vaccines have a distinct genetic vector and can be used several times. mRNA vaccines have the potential for quick, low-cost, and efficient production due to the high yields of in vitro transcription reactions (Karikó et al. 2008; Thess et al. 2015). Any protein can be encoded and expressed by mRNA, allowing for the development of prophylactic and therapeutic vaccines against any disease. Since changes to the encoded protein only change the sequence of the RNA molecule, its physicochemical properties remain relatively static, and a variety of products can be produced using the same existing manufacturing method, saving time and money compared to other vaccine platforms.

15.2 Advantages of mRNA Vaccines

mRNA-based vaccines have several advantages:

1. Many features of natural infections can be mimicked. mRNA enters in cells and produces antigen proteins within the cell through posttranslational modifications. mRNA vaccines have the ability to produce more viral proteins within a cell, and to increase immune responses, including B- and T-cell responses.
2. Multiple mRNA vaccines encoding multiple virus proteins allow the production of complex multimeric antigens. CMV vaccine, for example (mRNA-1647), includes six mRNAs, five of them coating five distinct proteins that form an immunologically critical CMV protein complex.
3. In silico designing of antigens and rapid production and testing of antigens cause advancement of mRNA vaccine program.
4. mRNA vaccine triggers a very strong T-cell response.
5. mRNA vaccine production does not require product-devoted production practices, facilities, and purification steps. mRNA production is an easy, cost-effective process that makes vaccines available to low-income people.

6. RNA is associated with RNases; mRNA produced in sterile conditions can be stored at −80 °C for months or even years.
7. In safety terms, mRNA is safe because of the transient nature of mRNA and the typical low doses of vaccination.

15.3 Concept and Form of mRNA Vaccines

The protein-encoding DNA is transcribed into mRNA, which is then translated into proteins. The production and delivery of RNA vaccines vary depending on the type of mRNA vaccines. The production of therapeutically useful in vitro-transcribed mRNA is optimally translated. In vitro-transcribed mRNA is synthesized from the linear DNA molecule, having an open reading frame consisting of the protein of interest, flanking UTRs, a 5′ cap, and a poly (A) tail. Further, it is processed into mature mRNA molecules in the cytoplasm of eukaryotic cells. In cytoplasm there are various extracellular RNases that degrade the mRNA. The cellular translation machinery transforms the transported mRNA into protein which then undergoes posttranslational modifications, resulting in a fully functioning protein (Weissman 2015).

The principle for developing an mRNA vaccine is surprisingly simple. The gene sequence of selected antigen from the targeted pathogen is synthesized and cloned into the plasmid DNA. In vitro-transcribed mRNA vaccine is administrated to the subjects which elicit potent humoral and cellular immune responses by using the host cell machinery. The signal peptide and transmembrane domain help in the transport of antigens to their cellular location or to the correct cellular compartment. As a consequence, the antigen may be expressed as a protein that is intracellular, secreted, or membrane bound (Maruggi et al. 2017). When the host's innate immune system recognizes any RNA sequences of viral origin, mRNA vaccines induce robust innate responses, which recruit chemokines and cytokines such as interleukin-12 (IL-12) and tumor necrosis factor (TNF-α), at the injection site (Pepini et al. 2017; Edwards et al. 2017). Currently, two types of mRNA vaccines are used: traditional mRNA/non-replicating mRNA with 5′ and 3′ untranslated regions (UTRs) flanking the antigen of interest, and self-amplifying mRNA derived from positive-stranded RNA virus genomes, encodes both the antigen and the viral replication machinery (Maruggi et al. 2019). Currently, there are two types of mRNA vaccines used:

15.3.1 Non-replicating mRNA/Conventional mRNA Vaccines

These vaccines carry ORFs having antigen-coding sequences flanked with 5′ and 3′ UTRs, cap, and poly (A) tail through which mRNA interacts with the translational machinery for the expression of antigen (Jansen 2001). Additionally, the codon substitution procedure has been employed in DNA, RNA, and viral vector vaccines to enhance protein expression by replacing uncommon codons with widely used

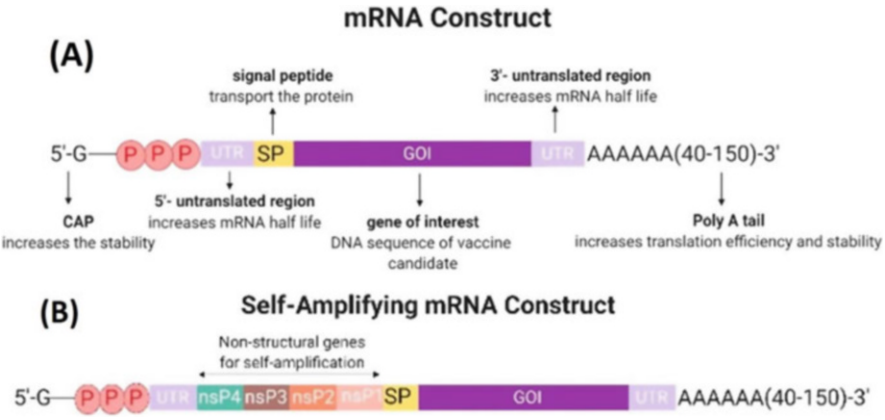

Fig. 15.1 (**a**) Convention mRNA construction with supporting coding sequences, (**b**) self-amplifying mRNA construct (source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6963228/)

compatible codons. However, a recent review suggests that codon optimization does not increase the production of proteins for mRNA therapeutics (Mauro and Chappell 2014). The uniformity and small size of the RNA molecule are advantages of the traditional mRNA vaccine approach (Fig. 15.1a).

Expression of the mRNA is limited due to its stability and activity under in vivo conditions. However, antigen expression and tenability can be improved by remodeling the structure of mRNA (Ross 1995). To elicit an antigen-specific immune response, mRNA vaccines must be expressed in situ; thus, vaccine optimization is important to understand the durability and consequences of antigenic expression following mRNA injections. mRNA formulation, route of administration, nucleoside modifications, and sequence optimization can influence kinetics and translational magnitude (Pardi et al. 2015). Intramuscular vaccination of unmodified mRNA induced protein expression in vivo, which lasted at least 6 days (Vogel et al. 2018).

Nucleoside base modification is an emerging fast modification approach for the production of modified mRNA that improves mRNA potency by reducing pattern recognition receptor (PRR) activation and dsRNA contaminant removal. Nucleoside modification enhanced protective immune response in mice and nonhuman primates (NHPs) against influenza and Zika viruses (Maruggi et al. 2019). Sequence-optimized mRNA (high GC content and optimized UTRs) formulated with lipid nanoparticles (LNPs) elicited robust effective antibody titers against rabies and influenza antigens in rodents (Thess et al. 2015). Another technology called RNActive technology enhances the adjuvant properties that can be beneficial for vaccination. A sequence-optimized, unmodified mRNA and a protamine-containing noncoding RNA complex serve as carriers in such adjusted vaccines (Rauch et al. 2017). In brief, the most advanced traditional mRNA vaccines (sequence-optimized mRNA, nucleoside-modified mRNA, and mRNA adjuvanted with

protamine-complexed noncoding RNA) have been currently studied in preclinical and clinical trials.

15.3.2 Self-Amplifying mRNA Vaccines

Positive-sense ssRNA viruses such as picornaviruses, flaviviruses, and alphaviruses have engineered RNA genomes that can be used to make self-amplifying mRNA vaccines. Venezuelan equine encephalitis viruses (VEEVs), SINV, Semliki Forest virus (SFV), and Sindbis virus are some of the well-studied self-amplified mRNA molecules. Self-amplifying mRNA replicons are made by substituting the viral structural genes with the interested antigen gene, and then delivering them to target cells' cytoplasm, where they can undergo RNA amplification and express the desired antigen in large amount (Fig. 15.1b). Due to the lack of endogenous viral structural genes, these replicons develop contagious virions or viruslike particles at the vaccination site, significantly diminishing the safety issues linked with a live attenuated virus vaccine. Self-amplifying mRNA could be generated in vitro or by delivering plasmid DNA containing the RDRP complex and the antigen of interest (Ljungberg and Liljeström 2015). After injecting self-amplifying mRNA or unmodified traditional mRNA into mice, the kinetics of antigen expression, immune responses, and protective efficacy were studied. They observed that the self-amplifying mRNA vaccine produced more potent immune responses and released much higher levels of the protein over a longer period of time than the standard mRNA vaccine (Vogel et al. 2018). The dsRNA amplification intermediates, as well as the magnitude and length of antigen expression parameters of self-amplifying mRNA, all lead to an increased immune response, which activates innate immunity, triggers host-sensing machinery, and confers an adjuvant effect (Magini et al. 2016).

The ability to encode several antigens in the same replica is another aspect of the self-enhancing mRNA vaccine platform. This may allow the development of vaccine expressing both a target antigen and an immunomodulating biological molecule for enhanced efficacy, vaccine encoding B-cell and T-cell antigen, single combo vaccine targeting multiple pathogens, or vaccine targeting multi-subunit complex antigens (Maruggi et al. 2019). Self-amplifying mRNA replicon expressing five full-length subunits (gH, gL, UL128, UL130, and UL131A) of the human cytomegalovirus (HCMV) pentameric complex elicits potent and broadly neutralizing antibodies in mice (Wen et al. 2014). The self-amplifying mRNA vaccine encoding HCMV gH/gL formulated with LNPs induced antibodies and CD4+ T cells. Similarly, self-amplifying mRNA vaccine (0.1–0.2 mg) encoding influenza M1 and/or NP elicits potent antigen-specific T-cell responses and protection from viral challenge in mice (Magini et al. 2016).

In summary, self-amplifying mRNA is a flexible technology that can be used to produce single- or multi-antigen vaccines with low doses, high antigen expression, and a powerful, intrinsic adjuvant effect. The rational design of improved self-amplifying mRNA vaccines will be possible with self-amplifying mRNA replicon

with innate immunity and consequent modulation of antigen-specific immediate response.

15.4 Delivery System for mRNA Vaccines

In order to achieve their target and perform well, mRNA vaccines need a delivery system because of the nuclease degradation and the large and negative burden of the naked RNA impedes the crossing of the cell membrane. Vehicles or delivery systems or methods for transporting RNA into cells are necessary. The vehicle (endosome) should cross the cell membrane of target cell after endocytosis and released and the mRNA cargo into the cytosol, where it is translated (Stanton 2018). Several major delivery vehicles are currently being investigated, some of which encapsulate mRNA molecules in particles and some polymers bind to RNA through charge interaction (Houseley and Tollervey 2009). For delivery of nonviral RNA, formulated lipid nanoparticles (LNPs) are commonly used as they increase the fusogenicity and endosomal escape. In addition, cationic ionizing amino lipids which condense with nucleic acid, poly(ethylene) glycol (PEG) lipids which provide steric stabilization, and cholesterol that enables vesicle stability in vivo are also used for RNA delivery (Cullis and Hope 2017). Several amino lipids were developed for siRNA delivery and the first therapeutic siRNA delivered by LNP was approved by the FDA (United States Food and Drug Administration) in 2018 (Alnylam Pharmaceuticals 2018) (http://investors.alnylam.com/news-releases/news-release-details/alnylam-announces-first-everfda-approval-rnai-therapeutic). Self-replicating mRNA vaccines delivered via polymer have shown a lot of considerable results. mRNA comprises high-amine-density molecule with branching structures eliciting CD8+ T-cell and antibody responses that protect mice from lethal challenge by Ebola virus, H1N1 influenza, and Zika viruses (Chahal et al. 2016; Chahal et al. 2017). The two-vial method was developed where the delivery formula can be produced and stored separately from the target mRNA. A delivery vehicle based on emulsion was used to bind with self-amplifying mRNA to the surface of the nanoparticle before immunization.

In mice, rats, and rabbits, self-amplifying mRNA delivered through cationic nanoemulsion elicited potent immune responses against viral, bacterial, and parasite infections (Bogers et al. 2015; Baeza Garcia et al. 2018). The effectiveness of mRNA is also influenced by the route of administration, which is a significant step in RNA expression. Vaccines are usually given as i.d., i.m., or s.c. injections into dendritic cell-rich tissue such as the skin and skeletal muscles. Optimized cationic lipids and lipoplex formation have been developed to enhance DC targeting by increasing the absorption, release, and translation of a self-amplifying mRNA molecule (Englezou et al. 2018). Vaccination of lipoplex-formulated self-amplifying mRNA-encoded influenza NP antigen elicited pro-inflammatory cytokines, humoral responses, and T cellular responses in mice. Vaccine thermal stability is another issue for storage and delivery in countries with inadequate cold-chain infrastructure.

15.5 Immune Response by mRNA Vaccines

Endosomal Toll-like receptors (TLRs) and retinoic acid-inducible gene-I (RIG-I) receptors are the two major RNA receptors involved in the activation of the innate immune system after mRNA recognition. TLRs (TLR-3, TLR7, TLR8, and TLR9) are located in the endosomal compartment of antigen-presenting cells (APCs), such as DCs, macrophages, and monocytes (Lee and Barton 2014). TLR3 detects dsRNA while TLR7 and TLR8 both detect ssRNA. TLR7 acts through the NF-kB pathway, whereas TLR7 and TLR8 lead to production of type I interferons (IFNα/β) and stimulation of B-cell response TLR3 recognizes dsRNA, while TLR7 and TLR8 recognize ssRNA. TLR7 activates the NF-kB pathway, while TLR7 and TLR8 activate the B-cell response by generating type I interferons (IFN/) (Takeuchi and Akira 2010; Ablasser et al. 2009). The RIG-I-like receptor family (RLR), which is found in the cytoplasm, recognizes single- and double-stranded RNA and stimulates IFN synthesis by recognizing ssRNA and dsRNA having a 5′ triphosphate (Sabbah et al. 2009). Type I interferons are primarily produced following the administration of an mRNA vaccine, which modulates antigen expression, APC activity, and T-cell differentiation (De Beuckelaer et al. 2017).

TCR elicited the desired T-cell response, including CD8 T-cell differentiation and proliferation into cytotoxic T cells, when they received the signals (Agarwal et al. 2009). STAT1 is triggered and anti-proliferation and pro-apoptosis events are initiated when IFN signaling occurs before the TCR is activated (Tanabe et al. 2005). As a result, it is proposed that the interferon type I response be controlled in order to enhance mRNA vaccine candidate translation and, as a result, vaccine potency (Pepini et al. 2017).

During mRNA processing, APCs present the mRNA-derived peptide through major histocompatibility complex (MHC) class I or II for activation and differentiation of T cell from naive T cells to effector cells. T-cell activation and differentiation also depend on the binding of co-stimulatory molecules, such as CD80 and CD86, by CD28 to T cells and secreted cytokines. The humoral response is recognized by antibodies secreted from B cells. mRNA vaccine-derived antigens bind to the B-cell receptors (BCR) and induce the production of antigen-specific antibodies (Charles et al. 2001; Lindgren et al. 2017). The accessibility of extracellular protein for B-cell recognition can be increased by using a secretion of signal peptide in the RNA sequence or by adding an MHC class II targeting sequence of a lysosomal or endosomal protein, such as lysosomal associated membrane protein (LAMP), in mRNA vaccines. mRNA-derived peptides are displayed on the B cell through MHC class II which bind to receptors of dendritic cell displaying the MHC II/peptide and release activation signals and cytokines. B cells proliferate and differentiate into memory B cells and antibody-secreting plasma cells (Lindgren et al. 2017). In conclusion, mRNA stimulates antigen-specific T-cell responses and increases the number of B cells, resulting in long-lasting antibodies (Pardi et al. 2018) (Fig. 15.2).

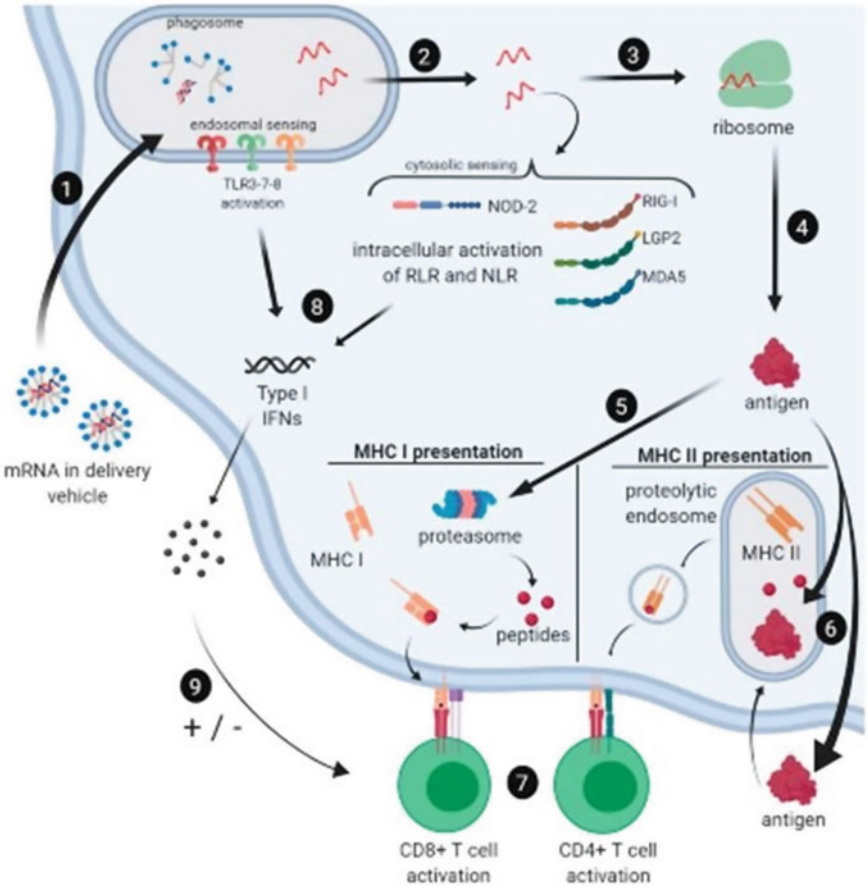

Fig. 15.2 Messenger RNA-encoded vaccine antigen processing in antigen-presenting cells (APCs) (source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6963228/)

15.6 mRNA Vaccines for Infectious Disease Prevention

mRNA vaccines have been studied extensively for the prevention of infectious diseases. mRNA vaccines against infectious diseases could be developed as prophylactic or therapeutic. T-cell and humoral immune responses are both induced when mRNA vaccines expressing antigens of infectious pathogens are used (Iavarone et al. 2017). When compared to whole microbe, live attenuated, and subunit vaccines, the development of mRNA vaccines is fully cell free, simple, and fast. Because of its pace and simplicity, mRNA is a promising product that may be able to bridge the gap between emerging infectious diseases and the urgent need for successful vaccines (Zhang et al. 2019).

15.6.1 mRNA Vaccines for Influenza Virus Infection

The most commonly used and FDA-approved mRNA vaccines against influenza virus were developed using conventional methods and showed promising results in a mouse model. During an outbreak of a deadly strain of H7N9 influenza in China in 2013, a self-amplifying mRNA vaccine encoding influenza HA antigen showed promising results (Krammer and Palese 2015). Unmodified conventional mRNA encoding various influenza virus antigens in combination with a protamine-complexed RNA adjuvant was found to be immunogenic and offered full defense against influenza virus in mice, ferrets, and domestic pigs (Petsch et al. 2012). T- and B-cell immune responses are induced by conventional or self-amplifying mRNA-based influenza vaccines formulated with LNP or CNE. Antiviral activity against influenza has also been found effective for chitosan, polyethylenimine (PEI), and dendrimer-based self-amplifying mRNA (Démoulins et al. 2016; Chahal et al. 2016). After i.d. and i.m. vaccination in humans, LNP-formulated N1-methyl-pseudouridine-modified traditional mRNA encoding HA of the H10N8 pandemic influenza strain displayed H10-specific memory B cells as well as a temporary presence of plasmablasts (Suan et al. 2017; Pilkinton et al. 2017). The first human trial of an mRNA-based influenza vaccine was recently released, combining nucleoside-modified traditional mRNA encoding an H10N8 HA antigen with LNP formulation (Bahl et al. 2017).

15.6.2 mRNA Vaccines for Zika Virus Infection

LNP-formulated nucleoside-modified traditional mRNA developed impressive neutralizing titers and provided protection against Zika virus challenge in mice (Pardi et al. 2017). Another study found that an mRNA vaccine encoding a modified Zika prM-E antigen with a mutated conserved fusion-loop epitope in domain II of the E protein protects the host from Zika virus infection while also increasing vaccine protection. Furthermore, this vaccine decreased antibody development, increasing dengue virus infection in cells or mice (Richner et al. 2017). A single i.m. vaccination with a Zika self-amplifying mRNA vaccine formulated with nanostructured lipid carriers was found to be adequate to improve immune responses and fully protect mice against a Zika virus challenge (Erasmus et al. 2018).

15.6.3 mRNA Vaccines for Rabies Virus Infection

A protamine-formulated and sequence-optimized traditional mRNA vaccine encoding rabies virus glycoprotein (RABV-G) induces protective immunity in mice and produces neutralizing antibody response in pigs (Schnee et al. 2016). Humans vaccinated with the rabies mRNA vaccine had a positive response with some side effects such as mild-to-moderate injection-site reactions, fever, fatigue, and pain. The delivery mechanism for the rabies mRNA vaccine was optimized to

achieve a long-term immune response (Lutz et al. 2017). The neutralization titer of the LNP-formulated rabies mRNA vaccine was found to be higher. A phase I clinical trial to test LNP-formulated sequence-optimized mRNA vaccine (ClinicalTrials.gov. NCT04380701 2020) has recently begun (CureVac 2018).

15.6.4 mRNA Vaccines for Cancer

Designing of cancer vaccines is mainly based on the tumor-associated gene of the target cell. Most of the vaccines developed till date are therapeutic vaccines that stimulate cell-mediated responses. For example cellular responses generated from CTLs reduce the tumor burden (122). Dendritic cells present the antigens that play a central role in generating antigen-specific immune responses. In 1996, Boczkowski and colleagues reported that electroporated DCs with mRNA induce robust immune responses against tumor antigens (124). Various immune-regulatory proteins have been discovered that enhance the strength of cancer vaccines. The immune-stimulatory function of DCs was significantly increased via production on IL-12 after electroporation with mRNAs encoding co-stimulatory molecules such as CD83, tumor necrosis factor receptor superfamily member 4 (TNFRSF4, also known as OX40), and 4-1BB ligand (4-1BBL) (125–128). Cocktail of mRNA encoding adjuvants CD70, TLR4, and CD40L enhances the DC activation and transposition of CD4+ T cells in T helper cells (Th1) (132–136). DC vaccines have also been used in several clinical trials to treat metastatic prostate cancer, metastatic lung cancer, renal cell carcinoma, brain cancers, melanoma, acute myeloid leukemia, and pancreatic cancer. The outcomes of mRNA vaccines depend on the route of administration (intradermal, intramuscular, subcutaneous, or intranasal) and some unconventional routes of vaccination (intranodal, intravenous, intrasplenic, or intratumoral). Intranodal vaccine administration involves the injection of mRNA directly into secondary lymphoid tissue directly at the site of T-cell activation that elicits potent prophylactic or therapeutic antitumor T-cell responses (62, 66). According to a more recent report, intranodal injection of mRNA encoding the E7 protein of the human papillomavirus (HPV) 16 with TriMix increased the number of tumor-infiltrating CD8+ T cells and inhibited the growth of an E7-expressing tumor model in mice (67). Intranasal vaccine administration of mRNA vaccine enables rapid antigen uptake by DCs. mRNA complexed with Stemfect LNPs delivered intranasally resulted in detained tumor onset and increased survival in prophylactic and therapeutic mouse tumor models (145). Intratumoral mRNA vaccination is another approach in which mRNAs encoding tumor-associated antigens are not introduced into cells rather than tumor-specific immunity is activated through immune-stimulatory molecules. In a more recent research, mice with OVA-expressing lymphoma or lung carcinoma received intratumoral delivery of mRNA encoding an engineered cytokine IFN-β fused with TGF-β, which inhibited tumor development (147). Intratumoral administration of TriMix mRNA stimulates CD8+ DCs and tumor-specific T cells, causing tumor growth to be delayed in mice (148).

15.6.5 mRNA Vaccines for Parasitic Infection

Plasmodium macrophage migration inhibitory factor (PMIF) is an orthologue of mammalian macrophage migration inhibitory factor (MIF), and secretion of PMIF from plasmodium attenuates the immune response of the host. The self-amplifying mRNA vaccine can provide protective immunity against malaria infection by neutralizing the plasmodium macrophage migration inhibitory factor (PMIF). Mice vaccinated twice with a self-amplifying mRNA replicon encoding PMIF had higher PMIF-specific CD4+ cells and anti-PMIF IgG titer, suggesting that the parasites were controlled and reinfection was prevented (Baeza Garcia et al. 2018). Vaccination with F2-RNA alone resulted in low antigen-specific Th1 responses and quite low IgG responses, while vaccination with mRNA replicon encoding for the LEISH-F2 gene formulated with glucopyranosyl lipid A in a stable oil-in-water emulsion induced very high IFN-y secretions and antigen-specific Th1 responses that significantly reduced the parasite burden in the liver of mice (Johanning et al. 1995). A lipid nanoparticle (LNP)-based self-replicating RNA encoding *T. gondii* nucleoside triphosphate hydrolase-II (NTPase-II) induces high specific immunoglobulin (IgG) titers and IFN production, resulting in increased survival time and rate of mice (Luo et al. 2017).

15.6.6 mRNA Vaccines for SARS-CoV-2

For the development of the mRNA coronavirus vaccine, the standard vaccine development approach was used, enabling new age of vaccine development. A lipid nanoparticle-encapsulated mRNA-base mrNA-1273, expressing the full-length SARS-CoV-2 stabilized spike protein(S), has been developed by the Moderna (Biotech company) with the National Institutes of Health (NIH) which induces robust neutralizing antibodies and increases T-cell response in nonhuman primates (Corbett et al. 2020). Clinical studies have shown that m-1273 induces high neutralization antibodies and Th1-biased CD+4 T human cell response, with 94.1% protective efficacy against severe COVID-19 cases and 100% protective efficacy against moderate COVID-19 cases (Jackson et al. 2020). Moderna's vaccine mRNA-1273 against COVID-19 has been approved for emergency use by the US Food and Drug Administration (FDA) and the WHO. This mRNA-1273 vaccine has been shown to be effective against two new SARS-CoV-2 variants, B.1.1.7 and 501Y.V2.

BioNTech and Pfizer have developed another mRNA-based vaccine candidate, BNT162 [BNT162b1: encodes an optimized SARS-CoV-2 receptor-binding domain (RBD) antigen and BNT162b2: encodes an optimized SARS-CoV-2 full-length spike protein antigen], having robust functional antibody titers with a 95% protective efficacy rate (ClinicalTrials.gov. 20202/NCT04380701 2020). Moderna BNT162 has received Emergency Use Authorization (EUA) from the US Food and Drug Administration. The LUNAR-COV19 (ARCT-021) vaccine with lipid-mediated delivery system developed by self-transcribing and replicating mRNA (STARRTM) technology induces strong IgG antibody response, with balanced Th1/Th2 CD4+

T-cell response, and increases protective efficacy, according to Arcturus Therapeutics Vaccines Company. Sanofi and Translate Bio are designing multiple mRNA constructs as COVID-19 vaccine candidates and have begun a phase I/II clinical trial (Sanofi and Translate Bio. Press release on 12 March 2021). Imperial College London is conducting a phase I/II clinical trial for a thermostable and immunogenic vaccine based on self-amplifying RNA technology (saRNA) and DNA/RNA stabilization technologies (Imperial College London). On June 15, 2020, a press statement was issued. The CureVac Company obtained approval from the German Paul-Ehrlich-Institute (PEI) and the Belgian Federal Agency for Medicines and Health Products (FAMHP) for clinical trials of an mRNA vaccine (CVnCoV), which encodes the SARS-CoV-2 spike protein (CureVac. Press release on 18 June 2020).

15.7 Conclusion

mRNA-based vaccines are a promising new platform that is highly versatile, scalable, and low cost and does not require a cold chain. They also bridge the gap between an emerging pandemic infectious disease and an abundant supply of successful vaccines. The preclinical and clinical findings indicate that mRNA vaccines are safe and well tolerated in animal models and humans, and that mRNA prophylaxis and therapy may be useful for preventing infectious disease and treating tumors. Future changes can also boost antigen-specific immune responses as well as the size of memory immune cell responses, such as memory B- and T-cell responses. We conclude that mRNA vaccine platform may be ideally suited for vaccine development, provided that production costs continue to decrease.

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Dampening of Inflammatory Markers and Understanding COVID-19 Viral Disease Development: A Combinatorial Approach

16

Anureet K. Shah and Jamil Momand

Abstract

One of the hallmarks of the global pandemic of coronavirus disease 2019 (COVID-19) is that it targets the immune system by producing inflammatory cytokines. COVID-19 has expedited investigations on numerous therapeutics to fight the disease-causing virus SARS-CoV-2, some without well-established safety or efficacy data. The severity of the disease depends on a number of factors, including genetic background and preexisting conditions. The difference in the genetic makeup makes everyone unique and the understanding of the COVID-19 cure arduous. To dampen these inflammatory markers and to understand the viral disease dynamics, accounting for genetic variability, a combinational three-way approach involving bioinformatics, nutrigenomics, and pharmacogenomics will give answers to many unanswered questions involving patient care. A futuristic approach to prevention and cure calls for continuous research with practice and training provision to the right group, accompanied with the awareness enhancing its utility.

Keywords

Inflammation · COVID-19 · SARS-CoV-2 · Bioinformatics · Nutrigenomics · Pharmacogenomics

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16.1 Introduction

The global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) underscores the need to deepen our understanding of the impact of viral infections on the immune system. Lymphocytes, consisting of B cells, T cells, and natural killer cells, are the first responders to the virus, triggering the immune response. T cells are the most crucial in responding to viruses and they are comprised of cytotoxic T cells (T_C cells) and helper T cells (T_H cells). T_C cells clear the infection by killing virus-infected cells, and T_H cells control the immune response by preventing its overreaction. In some viral attacks, the inability of T_H cells to effectively regulate the immune system can lead to the overproduction of inflammatory cytokines. Heightened levels of inflammation-associated molecules are frequently observed in COVID-19 patients (Mehta et al. 2020).

SARS-CoV-2 has many similarities to other disease-causing viruses, but is more adept at causing an inflammatory response. In addition to attracting lymphocytes, rapid viral replication signals the recruitment of macrophages and monocytes which induce the release of cytokines and chemokines, commonly referred to as the cytokine storm (Tay et al. 2020). The cytokine storm attracts T_H cells and activates other aspects of the immune response (Xu et al. 2020; Wu et al. 2020). A meta-analysis showed the association of inflammatory markers with the severity of SARS-CoV-2 infection, further substantiating this relationship (Zeng et al. 2020). Within the cell, the NF-κB pathway is critical for attracting an immune response (Deng et al. 2018). However, this pathway is suppressed when the cell is attacked by SARS-CoV-2, which allows for viral replication and disease progression (Okamoto and Ichikawa 2021). Henceforth, dampening these inflammatory markers (cytokines, chemokines, NF-κB pathway) to prevent progression and tissue damage is crucial. A study published recently stated how these pro-inflammatory cytokines causing severe lung damage are reversed with early dose of short-course corticosteroid (Kolilekas et al. 2020). As we cannot rely on steroids for longer duration because of deleterious side effects, we need to understand the genes involved and identify the bioactive compounds impacting this cytokine storm. Studies targeting inflammation-inducing pathways with nutrition are imperative and demand in-depth understanding of the markers. To understand the various markers involved, studies involving nutrition and bioinformatics will allow us to study the genes involved in the immune system responsiveness to viral attack.

16.2 Everyone Is Unique

Individuals who are immunocompromised do not have the same innate or adaptive immunity to fight viral infections as compared to their healthy counterparts and display disease symptoms ranging from mild to severe (Shah 2020). This current situation of vaguely known pathogenic mechanisms of SARS-CoV-2 makes it critical to find ways to identify prime molecular predictors of heightened inflammatory markers which can further be explored to understand the molecular

pathogenesis impacting humans (Lingeswaran et al. 2020). One possible predictor of the severity of COVID-19 symptoms is nutritional deficits.

Suppressed immune responses can result from nutritional deficits. Certain essential polyunsaturated fatty acid (PUFA) precursors (arachidonic acid [AA], eicosapentaenoic acid [EPA], and docosahexaenoic acid [DHA]) assist in activating receptors. Some combination of nutrients used in a timely manner demonstrates strong inhibitory effect on experimental inflammation targeting several mediators of inflammation and its related mechanisms (Ivanov et al. 2008). Terpenoid-based drugs and dietary compounds (zinc, vitamin D, vitamin C) have been shown to suppress certain molecular targets such as NF-kB pathway, which regulate inflammation (Ivanov et al. 2008). As demonstrated by Ivanov et al., including certain nutrient compounds as a component of nutrient mixture consisting of ascorbic acid, quercetin, naringenin, hesperetin, tea catechins, lysine, proline, arginine, and N-acetyl cysteine has a strong impact on controlling experimental *in vivo* and in vitro inflammation. We postulate that nutrition adjustments can dampen the inflammation response to viral infection, leading to better clinical outcomes. Integrating bioinformatics (study of biology and computer science to help analyze and interpret biological data), nutrigenomics (study of the intersection of human genome, nutrition, and health), and pharmacogenomics (study of the role of genome in drug response) should provide a novel approach towards combating tissue damage due to viral infection (Fig. 16.1).

Comparing and integrating data have been always essential to science, but the current global pandemic has made this practice more important. Accumulating evidence has suggested the association of several inflammatory markers associated with COVID-19 disease progression (Gao et al. 2020, Qin et al. 2020). Some of these markers include C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), procalcitonin (PCT), serum ferritin, interleukin-6 (IL-6), serum amyloid A

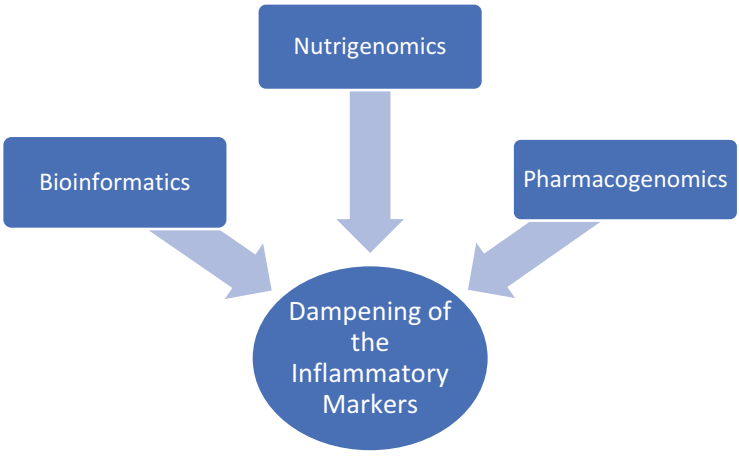

Fig. 16.1 The three-way approach to tackle futuristic care through dampening of the inflammatory markers

(SAA) lactate dehydrogenase (LDH), alanine transaminase (ALT), and TNF-α (Cheng et al. 2020, Xiang et al. 2020). Other possible targets involved in COVID-19 progression are the NFkB pathway, lack of type 1 IFNs, presence of specific autoantibodies, and an undefined marker on the X chromosome (Zhang et al. 2020; Guisado-Vasco et al. 2020; Tay et al. 2020).

16.3 Bioinformatics

A broad sampling of global bioinformatics data can be employed to gain insight into how COVID-19 causes life-threatening pneumonia. For example, relatively early in the pandemic it was observed that the percentage of men who die from COVID-19 is higher than women (Bakelmun 2020). For example, in India the ratio of men to women deaths is 1.8 and in Denmark the ratio is 1.3. There are several possible explanations for this outcome, including a higher frequency of preexisting conditions in men such as diabetes and heart disease (Sharma et al. 2020).

Another factor for the high death rate amongst men is the immune system. Interferons (IFNs) are protein cytokines that interfere with viral infection. Type I IFNs bind to the cell IFN-α/β receptors and block viral replication. In the case of SARS-CoV-2, type I IFNs block viral infection in vitro (Bastard et al. 2020). Bastard et al. (2020) decided to characterize the autoantibodies in COVID-19 patients. It was observed that 10.2% of patients with life-threatening COVID-19 pneumonia ($n = 987$) had neutralizing IgG autoantibodies against certain type I IFN family members (IFN-α, IFN-ω). These autoantibodies were not found in 663 individuals with mild or asymptomatic SARS-CoV-2 infection and were only found in 0.3% of healthy volunteers ($n = 1227$). Other data showed that the autoantibodies were present prior to infection, indicating that they select for disease progression. Thus, the severe disease is due to autoantibodies that inhibit IFN-α and IFN-ω, which allows the SARS-CoV-2 to replicate and cause COVID-19 pneumonia.

Interestingly, 97 out of 101 COVID-19 pneumonia patients with autoantibodies were men. This suggests that the production of autoantibodies against type I IFNs is linked to the X-chromosome. Future experiments that focus on the specific gene (s) on this chromosome will hopefully sort this out. It should be noted that SARS-CoV-2 is a poor inducer of type I IFN response and these IFNs are not part of the cytokine storm response. Yet, this low level of type I IFNs is protective against the virus.

Clinical applications of this finding are the following: (1) Prophylactic treatment of highly susceptible (i.e., immunocompromised) individuals with type I IFNs that do not have autoantibodies (such as IFN β) may be protective against SARS-CoV-2. (2) Patients shown to produce type I IFN autoantibodies should be treated with a class of IFNs which are not reactive to the autoantibodies to combat further viral infection. (3) Patients shown to produce type I IFN autoantibodies should be treated with drugs that inhibit the maturation of B cells that produce the autoantibodies. (4) Such patients should not be considered for donations of convalescent plasma, lest their autoantibodies aggravate COVID-19 in the recipients. From this interesting

study, one may consider altering the diet to lower the level of autoantibodies. This is certainly feasible given that we know that a gluten-free diet effectively lowers the level of several autoantibodies (the major one targeting transglutaminase 2) that appear to be causative for the majority of cases in celiac disease (Dieterich et al. 1997, Lebwohl et al. 2018).

16.4 Genomics

The success in mapping/sequencing of the human genome and development of sophisticated genomic analysis tools have further sparked the new era in personalized nutrition and medicine. Personalization, which is the need of the hour, has been brought more effectively into practice with in-depth genetic analysis making nutrigenomics and pharmacogenomics the imperative tool. How an individual is unique based on their genetic makeup and metabolic profile can be explored through research, practice, training, and awareness (Fig. 16.2). **Research:** Persistent research is needed so that we generate more and more evidence of the said science. This will help in enriching our understanding of the subject. **Practice:** Physicians and dieticians should implement the science in the clinic by understanding its scope and limitations. **Training:** Training about the science should be given to healthcare experts. **Awareness:** Awareness drives amongst common man are the key to enhancing the utility of nutrigenomics and pharmacogenomics.

The objective of both disciplines (nutrigenomics and pharmacogenomics) is to personalize medicine, diet, and nutrition as a prime component of patient care by aligning the drug or the nutrition to the individual genotype (Ghosh et al. 2007).

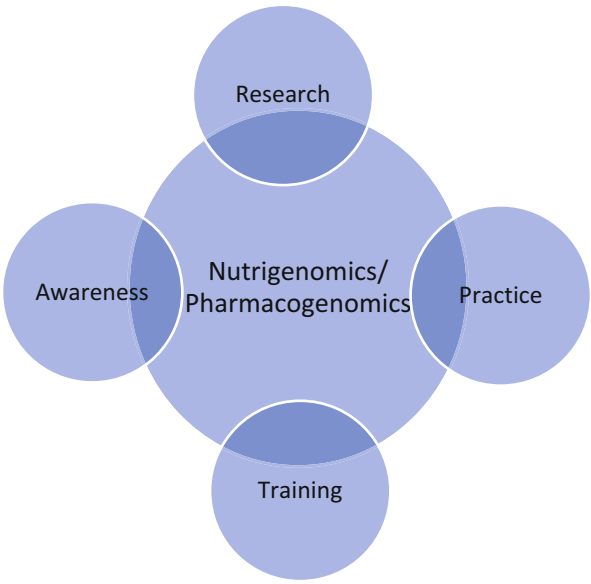

Fig. 16.2 Four key components to nutrigenomics/pharmacogenomics—futuristic approach to prevention and cure. Research, practice, training, and awareness

16.4.1 Genomics and Viral Susceptibility

The abundance of a specific type of protein receptor which is the direct target of virus in respiratory epithelial cells may make some people genetically more susceptible than others (Swanson 2020). Furthermore, the genetic basis of ACE2 receptor expression and function is still not fully understood in different populations. The differential allele frequency of ACE2 receptor in some individuals makes them more susceptible to the risk of infection and severity (Cao et al. 2020). Recent research shows that variation in the expression of these protein receptors amongst races is associated with differential responses to COVID-19 amongst different populations under similar conditions. The genetic analysis of expression quantitative trait loci (eQTLs) and potential functional coding variants in ACE2 amongst populations suggests fast spreading rate in East Asian (EAS) and other populations as compared to German people. This requires epidemiological investigations to further substantiate this abovementioned finding (Cao et al. 2020).

Nutrigenomics can be useful in understanding how immunity can be further enhanced in an individual by providing the right support to modulate metabolic activities such as oxidative stress, inflammation, and detoxification. Hence it can help in providing further personalization in improving the ability to resist and fight against infections. Nutrients such as resveratrol, vitamin B3, vitamin C, and vitamin D increase the expression of the ACE2 receptor (McLachlan 2020). There are many nutrient interventions which can directly or indirectly influence the cellular activity of ACE 2 receptor. About 25 selenoprotein genes (GPX family) have been identified in human genome known to protect from oxidative stress and free radical production during viral attack (Guillin et al. 2019). A knockout GPX model of mice, mice with selenium deficiency (Beck et al. 1998), and mice with vitamin E deficiency exhibited higher pathogenic effects of coxsackievirus infection (B3 (CVB3/0)) as compared to the control mice (Beck et al. 2003). The best small animal for testing the efficacy of diet and nutrition on COVID-19 disease is the ferret (Muñoz-Fontela et al. 2020). It would be worthwhile to determine if diets supplemented with vitamin E lower the level of inflammation in the alveoli and decrease ferret-to-ferret transmission rates.

16.4.2 Pharmacogenomics and Combating Virus

Varied responses to drug, from no effect to high toxicity in COVID-19 patients, suggest that human genome variabilities play a role in the drug effectiveness. Pharmacogenomics will undoubtedly increase our understanding of efficacy and safety of drugs being used for COVID-19 treatment. In one study, researchers showed several drug-gene variant pairs that alter the pharmacokinetics of the drugs being used and the adverse effects with their usage (Takahashi et al. 2020). For example, individuals who are G6PD (glucose-6-phosphate dehydrogenase) deficient are relatively at higher risk of hemolytic anemia (Rosenberg et al. 2020) and/or retinopathy (Allikmets et al. 1997) upon treatment with hydroxychloroquine or chloroquine. In regard to the family of IFNs, IFN-ß1b is currently being

investigated for the treatment of COVID-19 alone or in combinational therapy like lopinavir/ritonavir which had previously demonstrated some efficacy against SARS and MERS coronaviruses (Bhimraj et al. 2020). Furthermore, antibiotic treatment showed different responses based on pharmacokinetics. Azithromycin, an antibacterial drug with anti-inflammatory properties, showed up to twofold lower peak concentrations in 20 healthy volunteers with genetic variation in ABCB1, after a single dose (He et al. 2009). As azithromycin exhibits lower number of interactions with proteins, another study demonstrated lesser drug-drug interactions with the usage of azithromycin as opposed to other macrolide antibacterial agents like erythromycin or clarithromycin (Fohner et al. 2017). The in vitro studies suggest that remdesivir (a RNA polymerase inhibitor) is a substrate for drug-metabolizing enzymes CYP2C8, CYP2D6, and CYP3A4, and is a substrate for OATP1B1 and P-glycoprotein transporters (Fact sheet 2020, Seithel et al. 2007). To date, there is no pharmacogenomic data in patients to decide whether these metabolizing enzymes alter the effectiveness of remdesivir. Although remdesivir became available for severe COVID-19 in the USA via FDA Emergency Use Authorization on May 1, 2020 (FDA Letter 2020), the known variants of the abovementioned genes (CYP2C8 PharmVar Consortium 2020; CYP2D6 PharmVar Consortium 2020; CYP3A4 PharmVar Consortium 2020) could theoretically affect the pharmacokinetics for its continued use and better outcomes.

16.5 Conclusion

Hyperactivated T cells can cause severe immune injury due to activation of the cytokine storm and generation of heightened inflammatory markers. Suppressing or inhibiting these markers may be a step towards improving the response to the virus. We discussed a few examples where COVID-19 disease severity can be traced to the genetic background of the patients.

1. Men exhibit a higher frequency of COVID-19 pneumonia than women because they produce autoantibodies to type I virus-fighting interferons. This is likely linked to the X-chromosome.
2. ACE2 gene variants and allelic frequency are associated with increased virus spread in East Asian populations and low virus spread in German populations.

Given that diet and nutrition alter metabolism and gene expression, it is imperative that we use this knowledge in combination with genomic data to provide personalized treatment of COVID-19 patients.

The unique genetic makeup of individuals substantiates the use of a three-way approach to dampen the inflammation for better cure and recovery. This approach involving bioinformatics, nutrigenomics, and pharmacogenomics will provide insight to our understanding of the COVID-19 disease variability. Although no substantial pharmacogenomics data is available for COVID-19 patients at this time, the genetic determinants may play a role in combating and understanding the

right cure. Combining bioinformatics data with genomics is more important than ever to improve the efficacy of the treatment ensuring safety. This further calls for continual research, practice, training, and awareness around the globe. In-depth research into this will enhance our ability to accelerate answers as a global community.

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Covid-19: New Use of Therapeutics

17

V. P. Kamboj

Abstract

Severe acute respiratory syndrome novel virus (SARS-CoV-2), after its origin from probably bats in Wuhan, China, has spread all over the world within around 2–3 months of origin, called as Covid-19 pandemic. The virus has infected nearly 10 million people globally with over 500,000 deaths. Industry and academia globally are involved in developing repurposed drugs and vaccines as also developing new drugs, monoclonal antibodies and vaccines. Over 1200 molecules (including monoclonal antibodies and stem cells) and 180 plus vaccines are in clinical trials or under development. Hydroxychloroquine, remdesivir, favipiravir and a number of antivirals are in clinical use to save lives. Dexamethasone, a life-saving drug, has also been used in critical patients on support system. A monoclonal antibody and about five vaccines have reached Phase II–III clinical trials; one vaccine in India has entered Phase I clinical trial. BCG, mycobacterium W and polio vaccine are also under trial to treat Covid-19.

Keywords

Covid-19 · Repurposed drugs · Antimalarial · Antiviral · Monoclonal antibodies · Protein antagonists · Immunomodulators/cytokine disruptors

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17.1 Problem

It is evident as to how insecure the human beings are feeling during this pandemic and do not know how to effectively manage and control the coronavirus disease, Covid-19. The virus has virtually defeated our knowledge of molecular/structural biology or bioinformatics to speedily deal with the situation. It also shows the inadequacy of our knowledge about the pathology of infectious disease-causing pathogens in general and coronavirus in particular.

17.2 Introduction

The Covid-19 pandemic has happened after a century of the earlier influenza pandemic (known as Spanish flu pandemic) of 1918–1919. That pandemic was caused by H1N1 virus and was much more severe than the current Covid-19 pandemic. The origin of the virus was not precisely known but had genes of avian origin. It was spread worldwide during the years 1918–1919. Nearly 1/3rd (about 500 million people) of the world population was infected with casualty of more than 50 million people worldwide and maximum mortality of children of less than 5 years of age; about 675,000 people died in the USA alone. It is difficult to visualise the situation at that time when drugs or vaccines were not available. There were no antibiotics for secondary bacterial infections. The management was only by isolation, quarantine, good personal hygiene, disinfectants, social distancing, etc. In India, the Spanish flu pandemic outbreak was first observed in Mumbai probably through the soldiers returning from Europe by ships in 1918 after the First World War and then spread all over the country and lasted from 1918 to 1920. About 5% (i.e. about 12 million people) of India's population died (Centres for Disease Control and Prevention 2006; Taubenberger and Morens 2006; Chandra et al. 2012; Arnold 2019).

SARS coronavirus-2 (SARS-CoV-2), after origin from probably bats to humans in Wuhan, China, has spread all over the world due to free and fast travel of people amongst the countries. The disease caused by this coronavirus is called Covid-19 and the first case was observed in Wuhan on 31st December 2019. Within a couple of months of its origin, the virus infection virtually engulfed majority of the countries of the world and was declared as a pandemic by the WHO on 11th March 2020. The disease, till 30th June 2020, has already infected people in at least 188 countries (out of 195) of the world. It has infected nearly 10 million people globally and the death toll is over 500,000 persons with maximum deaths in the USA; in India, the infection rate has crossed 566,000 mark with casualty of nearly 2.5% which is much less than the global figure. The vaccines and drugs are therefore urgently needed to manage and control this pandemic.

17.3 Situation Management

The age-old strategy to identify, isolate and treat the infected subjects and their contacts is being followed. The use of face masks and social distancing (at least 6 ft apart) is being advocated. Social gatherings are prohibited by law. There has been countrywide lockdown in India and also air, train and bus services were cancelled within the country and amongst the countries and these were gradually lifted from the end of June 2020 to revive economy. Other countries too have enforced partial or complete lockdowns and have gradually lifted.

Unprecedented efforts by academia, public sector institutions and industry all over the world to develop prophylactic/therapeutic vaccines, therapeutic monoclonal antibodies, repurposing existing vaccines, drugs and phytopharmaceuticals after determining their efficacy against coronavirus in cell cultures in vitro have been undertaken. Ayurvedic and homoeopathic drugs for the control of Covid-19 are also in use. People are advocating the use of vitamins D and C, ketogenic diets and herbal medicines for protection from coronavirus or to treat mild/moderate disease due to it, albeit without any scientific data of their efficacy (Medical News Today 2020). The broad targets for the drugs are to prevent the entry of virus into the cell, to prevent virus replication in cells and to minimise damage that the coronavirus causes to the body and organs or treat Covid-19.

A number of pharma/biotech companies are also developing monoclonal/polyclonal antibodies from patient plasma which are capable of generating immune response and protection against coronavirus infection or treating Covid-19 patients; nanoparticles, nano-viricides, etc. are also under exploration. In fact, there is a race amongst the pharma companies to capture the potential market speedily to win dividends. But there is no accelerated way to develop a new therapeutic or vaccine. The time needed is 10–12 years and it costs more than two billion dollars. Pandemic is spreading so fast that to develop a new therapeutic/vaccine is only wishful thinking. Thus, the right approach adopted is to repurpose drugs/monoclonal antibodies and vaccines to contain the Covid-19 disease with less people infected and deaths than that happened with influenza/Spanish flu pandemic in 1918–1919.

However, for the disease for which no therapeutic/vaccine is available and only palliative (management) therapy can be provided, regulatory authorities of countries have provided rules and regulations for fast-track or accelerated approvals for rare or orphan diseases or orphan drugs. Here it falls in neither of the category. Because the disease is killing thousands of people and regulatory authorities have lived up to the situation to fast-track approvals to save humanity, such a provision is also provided in the rules and regulations for drugs/vaccines in different countries including India. Based on the safety and efficacy data of vaccines in animals, the regulators have conducted rolling reviews and given fast-track permission to conduct Phase I/II clinical trials simultaneously. If found to be safe in humans and to generate good immunity after two injections, 7, 15 or 21 days apart, based on antibody titres against SARS-CoV-2 infection in a limited number of cases (say 100 volunteers in Phase I and 500 volunteers in Phase II), further trials are permitted. Phase I/II trial of vaccine is in normal human volunteers and by monitoring antibody tires at 2 months or so

and if good titres are observed, permission for Phase III trial with vaccine (randomised double blind with placebo control in one group and vaccine in another group) in large population, say 20,000–50,000 people, is considered and granted permission. If safe and effective, Emergency Use Authorisation is given within the country. Simultaneously, Phase III clinical trial or emergency use permission is processed in other countries. Likewise for new drugs, if found to be effective and safe in animal models, Phase I safety trial in about 50 normal volunteers can be granted. If safe, Phase II trial in early and critical Covid-19 patients (about 200 of each type) is considered and permitted. If effective in Phase II clinical study, Phase III trial in about 5000–10,000 patients or more is permitted to pick up rare side effects before granting marketing permission. Pharmacovigilance is continued after marketing, for at least 5 years, to monitor rare adverse effects.

There are over 1200 molecules registered under clinical trial at various stages of development and primarily with repurposed drugs since these drugs have the advantage of having proven safe clinically (InvivoGen 2020). As per the International Vaccine Initiative webinar, Seoul (14 June 2020), over 180 (now 194) vaccines are under development, 15 are under clinical trial and 168 are under preclinical development and it has shown how South Korea, Hong Kong and China have effectively managed Covid-19. As per the WHO, there are more than 115 teams working to develop a drug or vaccine to treat or provide prophylaxis against coronavirus infections and treat mild/moderate/severe disease.

As per Milken Institute report of 2nd June 2020, there are 29 monoclonal antibodies under clinical trial and 40 are under preclinical development; 17 antivirals are in clinical trial and another 17 are in preclinical development. In addition, 10 cell-based, 10 RNA-based and 93 repurposed drug therapies are in in clinical trial and 6 RNA-based therapies are in preclinical development (Anonymous n.d.-a). However, as per Biopharma reports, 61 antibodies, 22 antivirals, 15 cell-based compounds, 10 RNA-based compounds, 18 scanned repurposed compounds and 86 drugs with anti-inflammatory, antimalarial, interferon, protein-based, antibiotics, receptor modulators, etc. accounting to a total of 293 compounds are under development in May 2020 (Anonymous n.d.-b). This shows that large-scale efforts are being made by the industry to find a therapeutic for the treatment of Covid-19 patients.

Repurposed drugs being clinically used or evaluated to manage/treat coronavirus-infected patients are listed below:

Therapeutic targets against the coronavirus exist at different stages of infection, namely (a) viral attachment at S1 receptor—ACE2, (b) membrane infusion at S2 receptor—TMPRSS 2, (c) viral entry—endocytosis, (d) viral proteolysis—3CLpro & PLpro, (e) viral replication by the replication process including RNA-dependant RNA polymerase (RdRp) and (f) host cytokine response-IL-6R. Repurposed drugs that have been shown to act at different stages of infection are chloroquine at ACE2; chloroquine and hydroxychloroquine at endosomal membrane by its acidification; imatinib at endocytosis; remdesivir and favipiravir at replication complex; nafamostat and camostat at membrane fusion (are clinically active against MERS-CoV); lopinavir, ritonavir and disulfiram at viral proteolysis; cyclosporin A at

calcineurin-NFAT pathway and transcription of (−) strand; IFN-B at antigen-presenting cells; and sarilumab and tocilizumab at IL-6R (InvivoGen 2020).

A number of reviews on repurposed drugs have appeared from March to June which give you the number of approved existing drugs and those in clinical trials which have been identified based on efficacy against the coronavirus in cell cultures in vitro and with which clinical trials against Covid-19 mild/moderate/severe disease have been initiated (InvivoGen 2020; Anonymous n.d.-a, n.d.-b; Mark and Middleton 2020; Craven 2020). One can get a brief profile of all the drugs in clinical trials for the treatment of coronavirus disease in reviews (InvivoGen 2020; Anonymous n.d.-a, n.d.-b; Mark and Middleton 2020; Craven 2020). The compounds under preclinical development have not been listed.

17.3.1 Antimalarial Drugs

Hydroxychloroquine and chloroquine phosphate are drugs for malaria and rheumatoid arthritis. These drugs significantly inhibit SARS-CoV-2 infection in vitro in Vero E6 cells but the former is more effective and less toxic (Yao et al. 2020). The search of electronic database shows six studies, three randomised controlled trials and three observational studies with chloroquine and hydroxychloroquine (with azithromycin) but the methodology used is very poor (Gautret et al. 2020). Two placebo-controlled studies have been conducted in China with hydroxychloroquine. The Wuhan study enrolled 31 age-matched coronavirus patients each in the drug treatment and placebo groups. All patients have relatively mild disease with CT scan showing pneumonia. The dose of hydroxychloroquine is 200 mg twice daily for 5 days. The patients in treatment group showed significant improvement in fever, cough and pneumonia (by CT scan). The Zhejiang study is in 15 patients of mild diseases each in drug treatment and placebo groups. The dose of the drug is 400 mg once daily for 5 days. This study shows no response. It is difficult to suggest whether the difference between the two doses is due to single dose versus two doses, one wonders (Gao et al. 2020). A small study in France using hydroxychloroquine along with azithromycin, zinc and intravenous vitamin C showed efficacy in Covid-19 patients (Frei and Gbinigie 2020; Owens 2020). A retrospective analysis of 1061 Covid-19 patients who have been treated with hydroxychloroquine plus azithromycin before complications occurred has shown good clinical outcome, safety and low fatality rate in patients (Million et al. 2020). However, a number of international trials mainly in Europe, especially the UK, have shown no clinical benefit in the treatment of Covid-19 patients with hydroxychloroquine. At least one trial has been closed due to adverse effects. Hydroxychloroquine used with (113 patients) or without azithromycin (97 patients) has been seen to reduce the use of mechanical ventilation in Covid-19 patients as compared to placebo controls (158 patients). In post-exposure prophylaxis, hydroxychloroquine is not effective in preventing the development of Covid-19 disease after taking the drug within 4 days of high-risk exposure (Craven 2020).

The US Food and Drug Administration (FDA) initially approved limited emergency use of hydroxychloroquine but subsequently withdrew it after European (UK) study results.

The results with hydroxychloroquine appear to be equivocal and planned trial in mild and medium disease and prophylactic use by relations and healthcare workers handling Covid-19 patients are indicated. Government agencies may like to test hydroxychloroquine in Covid-19 patients as no industry will be interested in giving this cheap drug a fair trial. Other antiviral drugs are also planned to be fast-tracked for testing against coronavirus infections/disease.

Most of the data on the mechanism of action of chloroquine and hydroxychloroquine is based on in vitro studies which have not yet been validated by in vivo studies in animals or clinically. The base drug interferes with lysosomal activity and autophagy. It accumulates in lysosomes, destabilises lysosomal membranes, inhibits lysosomal function and enhances release of lysosomal enzymes within cells. Alteration of lysosomal function may inhibit lymphocyte function and show immunomodulatory or even anti-inflammatory effects. Hydroxychloroquine is also approved for the treatment of rheumatoid arthritis and lupus disease. It inhibits stimulation of toll-like receptors which induce inflammatory response by activation of innate immune system. This drug may act as a prophylactic against coronavirus by stimulation of innate immunity of the user. It has shown antiviral effects against a number of viruses in in vitro and in cell cultures (Fantini et al. 2020).

ICMR study on the sustained prophylactic use of hydroxychloroquine with personal protection equipment (PPE) led to a significant decline in the number of healthcare workers getting coronavirus (SARS-CoV-2) infection. The study has been carried out in 549 RT-PCR SARS-CoV-2-positive participants. The only side effects are nausea (8%), headache (5%) and diarrhoea (4%). The protective effect of hydroxychloroquine cannot be concluded from this study (Chatterjee et al. 2020). However, clinically protective property with prophylactic use of this drug against SARS-CoV-2 infection has yet to be demonstrated.

17.3.2 Antiviral Drugs

Remdesivir, an adenosine nucleoside triphosphate analogue, is a broad-spectrum antiviral drug against RNA viruses. SARS-CoV-2 replication depends on viral RNA-dependant RNA polymerase, which is the likely target of remdesivir. In earlier studies it has been demonstrated to be effective against Ebola virus and MERS coronavirus. It is found to be ineffective against coronavirus-2 (Wang et al. 2020a). A randomised, double-blind, placebo-controlled, multicentre trial has been conducted at ten hospitals in Hubei, China. The trial involves 158 severe Covid-19 patients on remdesivir treatment and 79 in placebo group. There are no significant clinical benefits with remdesivir and the trial had to be stopped due to adverse events of the drug (Wang et al. 2020b). However, in another trial on 61 SARS-CoV-2 patients, the drug appears to have improved the clinical condition (Wang et al. 2020b). Three separate Phase III trials with remdesivir in Covid-19 patients by

NIH's ACTT in 1059 cases; Capital Medical University's randomised, double-blind, placebo-controlled multicentre trial in 237 subjects; and SIMPLE trial in 19 cases showed time to clinical improvement from 14 to 10–11 days but no improvement in mortality of patients. Japan is the only country where this drug under the trade name Veklury is approved for the treatment of Covid-19 patients. The US-FDA has also allowed the use of remdesivir based on ACTT trial in treating Covid-19 patients (Craven 2020; Furuta et al. 2017) and has also approved its inhaler formulation under emergency clause. However, the results so far in the treatment of Covid-19 patients with remdesivir are equivocal.

Nevertheless, health officials from the WHO have noted that remdesivir has demonstrated efficacy in treating the coronavirus infection. The above-referred studies do not support the conclusion of the WHO. However, the basis of the WHO observation is not mentioned. A large multi-country trial is in progress in many hospitals (Craven 2020) and its results will determine the status of remdesivir, which has already been approved for marketing in many countries on the basis of the WHO indication, and the USA has purchased its entire current stock (Newspaper reports, TOI, 30 June, 2020).

Favipiravir is an existing antiviral drug which prevents replication of influenza virus. It is also a broad-spectrum antiviral drug against RNA viruses. It selectively inhibits RNA-dependant RNA polymerase (RdRP) of RNA viruses (Furuta et al. 2017). This drug is marketed for flu and influenza in Japan and China and is a promising drug against untreatable viral infections. Japan is now sending this drug to 40 countries for clinical trials for the treatment of Covid-19 patients (Hornyak 2020). Its trade name "Favilavir" has been approved in China for the treatment of coronavirus patients. It has shown efficacy in a clinical trial involving 70 coronavirus patients with minimal side effects in Shenzhen, China (China Daily 2020). Another trade name "Avifavir" of this drug has been approved in Russia for the treatment of coronavirus patients. The trial has been initiated in several hospitals in June 2020 (Hilotin 2020).

Favipiravir has also been approved for clinical trial in India for the treatment of Covid-19 patients (Shukla 2020).

Galidesivir, another nucleoside RNA polymerase inhibitor antiviral drug, shows broad-spectrum activity against a wide range of pathogens including coronavirus. It inhibits and disrupts the process of viral replication in RNA viruses. A randomised double-blind clinical trial for the treatment of coronavirus has been initiated on 9th April 2020 to determine its safety and efficacy (BioCryst Pharmaceuticals and NIAID 2020).

The drug has already shown survival benefits in patients against deadly filovirus infections such as Ebola, Zika, Marburg, and yellow fever (Rodgers 2014).

Umifenovir (Arbidol) is a broad-spectrum antiviral drug. This drug is already used in Russia and China for prophylaxis and treatment of influenza. A prospective, randomised controlled, open-label multicentric trial comparing umifenovir and favipiravir in Covid-19 patients has been conducted in China. There is no difference in clinical recovery rate on day 7 between the two drugs; however, the latter drug significantly improves fever and cough (Gregory 2020; Chang et al. 2020). India has

initiated clinical trials with favipiravir and umifenovir to treat Covid-19 patients (Mascarenhas 2020). CSIR-CDRI, Lucknow, has got the permission (TOI, 18th June, 2020) to initiate Phase II clinical trial in Covid-19 patients with umifenovir in three hospitals in Lucknow (India), namely, KGMU, RMLINMS and Era's Lucknow Medical College (CDRI, Lucknow 2020).

OYA1, a potent antiviral compound, has shown efficacy against coronavirus in laboratory essays. It is found to be more effective than chlorpromazine HCl in inhibiting SARS-CoV-2 from replicating in cell culture. This drug is found to be effective against cancer and is being repositioned for treating coronavirus in the USA (Focus News 2020).

17.4 HIV Antiviral Drugs for Coronavirus Treatment

Lopinavir, an antiretroviral drug, is HIV protease inhibitor and this drug in combination with ritonavir is being evaluated for the treatment of MERS and SARS coronaviruses. Ritonavir inhibits metabolising enzyme P450 3A and therefore increases half-life of lopinavir. In a preliminary study, this combination has been found to reduce the risk of severe hypoxia or death in 41 SARS-CoV-2 patients (Sheahan et al. 2020).

The combination is listed in the WHO list of essential medicines. Lopinavir is believed to act on the intracellular processes of coronavirus replication and has demonstrated reduced mortality in the non-human primate (NHP) model of the MERS virus.

Lopinavir/ritonavir (Kaletra) in combination with ribavirin has shown reduced fatality rate and milder disease course during an open clinical trial in patients in the 2003 SARS outbreak (Chan et al. 2003; Chu et al. 2004).

A randomised, controlled open-label trial involving 199 SARS-CoV-2 hospitalised patients has been conducted in China. No benefit is observed with this combination treatment (Cao et al. 2020). When this combination was administered along with interferon beta-1B to 127 mild-to-moderate infection Covid-19 patients in six hospitals in China, the response was better than lopinavir-ritonavir in relieving disease symptoms and duration of virus shedding (Hung et al. 2020).

Cipla is also reportedly planning to repurpose its HIV drug LOPIMUNE, which is a combination of protease inhibitors, lopinavir and ritonavir, for the treatment of coronavirus disease.

Darunavir, another protease (2 nucleotide reverse transcriptase inhibitor), is suggested to be a potential antiviral agent against Covid-19. This compound has been approved only for use with a boosting agent, and in combination with other antiretrovirals, for the treatment of HIV-1. The drug is being evaluated in vitro for activity against coronavirus. An open randomised clinical trial with darunavir/corbistat in combination has been initiated (in February 2020) in Wuhan, China, to determine the efficacy and safety with conventional treatment containing thymosin alpha 1 in adult patients with 2019-nCoV infection (Liping and Xinghuan 2002).

17.4.1 Monoclonal Antibodies

Actemra (Tocilizumab or Atlizumab), an interleukin-6 inhibitor, a humanised monoclonal antibody drug, is under clinical trial in China for the management of severe complications of coronavirus. It is an immunosuppressive drug for the treatment of rheumatoid arthritis and juvenile idiopathic arthritis. This drug is known to prevent cytokine storms or overreaction of immune system which is probably the main reason of organ failure leading to death in some coronavirus patients. Treating patients with drugs like tocilizumab is "like cutting the branches off a tree", said Dr. Louis Staudt of NCI, USA. It has been approved by the US-FDA in March 2020 for the treatment of critically ill patients post-Covid-19 infection; trials were initiated in April 2020 (Parsons 2020). In a randomised placebo-controlled trial in 129 patients, tocilizumab treatment produced significant clinical improvement in pneumonia-associated Covid-19 patients and reduction in mortality. A trial in Italy in 65 Covid-19 patients also showed significant clinical benefits and reduction in mortality (Craven 2020).

Kevzara (Sarilumab) is a fully humanised monoclonal antibody that binds and blocks IL-6 receptor. IL-6 may play a role in activating inflammatory response in patients who are critically ill with Covid-19 infection. A preliminary study in China has already confirmed this with another monoclonal antibody. The US-FDA has granted permission to the company on 16th March to initiate Phase II/III clinical trial in Covid-19 patients. This drug is approved for the treatment of rheumatoid arthritis. First patients outside the USA have been treated in global Kevzara (sarilumab) clinical trial program for patients with severe Covid-19 (Sanofi 2020).

REGN3048 and REGN3051, two more monoclonal antibodies, are under safety studies in humans before initiating efficacy studies. Both the antibodies bind to S-protein of MERS coronavirus. These monoclonal antibodies have been found to induce high level of neutralisation in the mouse model of MERS coronavirus in blood but with reduced viral loads in lungs. Phase I human clinical trial is supported by NIAID, USA (Duddu 2020).

TJM2, a neutralising antibody, is being developed as a treatment for cytokine storm in patients suffering from a severe form of coronavirus infection. The drug targets the human granulocyte-macrophage colony-stimulating factor (GM-CSF), which is responsible for acute and chronic inflammation. It has already been found safe in Phase I clinical trial in China and is now in Phase II clinical trial in rheumatoid arthritis patients. Phase I clinical trial has been approved by the USA and subsequent trials in severe coronavirus cases are planned. The company has already filed an application with China and South Korea regulators for permission to initiate treatment of cytokine storm (clinical trials) in hospitalised patients with severe or critical Covid-19 (Duddu 2020; I-Mab 2020).

Gimsilumab, a human monoclonal antibody drug targeting granulocyte-macrophage colony-stimulating factor (GM-CSF), which is a pro-inflammatory cytokine found in high levels in the serum of Covid-19 patients is also under development. Targeting GM-CSF is expected to reduce lung damage and reduce mortality rate in Covid-19 patients. The company has dosed its first Covid-19 patient

with lung injury or acute respiratory distress syndrome in April 2020 in the USA. It has been safe and well tolerated in Phase I clinical trial (Roivant Sciences 2020). The company on 13th May 2020 has reported that the results of its randomised, double-blind, placebo-controlled, multicentre pivotal trial evaluating the impact of intravenous (IV) treatment with gimsilumab on mortality in Covid-19 patients with lung injury or ARDS are satisfactory (Roivant 2020).

TZLS-501, a monoclonal antibody, is a human anti-interleukin-6 receptor (IL-6R) under development for the treatment of Covid-19 patients. It prevents lung damage and elevates levels of IL-6. The drug binds to IL-6R and reduces the level of IL-6 circulating in the body, thereby reducing chronic lung inflammation. The company is expediting the development of this molecule in China in Phase IIb trials. The US-FDA has cleared its Phase I clinical trial (Duddu 2020; Tiziana Life Sciences plc 2020).

17.4.2 Protein Antagonists

APN01, an ACE2 protein antagonist, is under evaluation in China in a Phase I pilot trial as a treatment for Covid-19 patients. APN01 is based on the research conducted by a professor at the University of British Columbia for treating SARS. ACE2 protein has been considered as the main receptor for the SARS virus. This compound is also in Phase II clinical trial in 200 severe coronavirus-infected hospitalised patients in Denmark, Germany and Austria to determine its efficacy (Duddu 2020; McGrath 2020).

Ifenprodil (NP-120), an N-methyl-d-aspartate (NDMA) receptor glutamate receptor antagonist, has in repurposing shown efficacy in improving survivability in mice infected with H5N1. It is planned to be tested clinically to find out whether it will reduce viral load in patients. The USA and South Korea have granted permission to conduct Phase IIb/III trial to determine the safety and efficacy of NP-120 in the treatment of Covid-19 (Duddu 2020; Algermon Pharmaceuticals 2020).

17.4.3 Immunomodulators/Cytokine Disrupters

AT-100 (rhSP-D), an engineered version of a novel human recombinant protein named AT-100 (rhSP-D), is being explored for the treatment of coronavirus. This drug in preclinical studies reduces inflammation and infection in the lungs and also generates an immune response against various respiratory diseases. It has the potential to be effective against Covid-19. The company is upscaling its production for clinical trials (Duddu 2020; OyaGen 2020).

BPI-002 is a small molecule indicated for treating various infections including Covid-19. It has been found to stimulate CD4+ helper T cells and CD8+ cytotoxic T cells, thereby generating an immune response in the body. In combination with a Covid-19 vaccine, the molecule has the ability to generate long-term protection against viral infections (Duddu 2020; Hospimedica Beyond Spring 2020).

CD24Fc, a new antiviral immunomodulator, is also under trial at a number of sites. The company has obtained Phase III trial permission in the USA to treat hospitalised Covid-19 patients (OncoImmune Inc. 2020).

SNG001 is an inhaler formulation of naturally occurring interferon-β directly delivered to the lungs. It has been developed to treat asthma and chronic obstructive pulmonary disease. It is also planned to be evaluated to reduce the severity of coronavirus infection in the lungs. The company planned for a double-blind placebo-controlled trial in 100 Covid-19 patients by launching a clinical trial in May 2020 (Duddu 2020; Synairgen 2020).

Calquence, a cancer drug also called acalabrutinib, can cut off the cytokine supply at its source. Coronavirus is known to affect the cytokines.

Dr. Louis Staudt, a scientist at the National Cancer Institute who is one of the lead investigators of the study, said, "Acalabrutinib is going for the trunk of the tree".

The study is in some subjects only and without a control group, but the results are promising. After about 2 weeks of treatment, 8 of 11 people who needed supplemental oxygen and 2 of 8 people who were on ventilators could breathe on their own and left for their homes. Another two on ventilators were able to come off the machines, and two others died. The company got permission to treat Covid-19 patients. The compound exerts its action by treating cytokine storms that manifest in severely ill Covid-19 patients (AstraZeneca 2020).

17.4.4 Other Drugs/Prospective Molecules

Dexamethasone, a life-saving drug, has been found to be effective in critical cases in a number of small clinical trials. Low doses of this drug reduced the death rate of critical ventilated patients by 1/3rd in RECOVERY trial in more than 2100 Covid-19 cases in multiple hospitals in the UK (Oxford University 2020). The WHO has now also recommended the use of dexamethasone in critically severe Covid-19 patients.

Brilacidin is a drug used for inflammatory bowel disease and oral mucositis in cancer patients. It is a defensin mimetic drug candidate and may be a potential treatment for coronavirus as it has shown antibacterial, anti-inflammatory and immunomodulatory properties in several clinical trials. It is being planned to be developed as a coronavirus drug (Duddu 2020).

BXT-25, designed to be 5000 times smaller than blood cells, has been found to efficiently transport oxygen through the body for a period of 9 h before it is processed by the liver. The drug can help in supplying oxygen to the vital organs and enables the patient to recover and survive. It is being planned as a treatment for acute respiratory distress syndrome (ARDS) in late-stage patients infected with the coronavirus. The diffusion of oxygen to the blood is compromised in patients suffering from ARDS leading to fluid build-up in the lungs (Duddu 2020; Bioxytran 2020).

17.4.5 Vitamins C and D

Vitamin C: In six clinical trials (three placebo-controlled in China, Canada and the USA and three with no control in Italy, the UK and the USA) severely ill Covid-19 patients in hospitals are being treated with intravenous vitamin C. Likewise, with regard to **vitamin D**, in several Phase II–IV clinical trials Covid-19 patients who are vitamin D deficient are being treated with oral vitamin D to prevent or treat infection. However, there is no scientific data to support their preventive or curative effects.

17.4.6 Others

A number of monoclonal antibodies and other drugs including convalescent plasma are in early clinical trial stage for the treatment of Covid-19 patients.

17.5 Efforts in India

The PSA to the PM of India Dr. K. Vijay Raghavan (TOI. 29 May, 2020) in press release has stated that in India the academia, public sector and industry have pooled efforts in developing vaccines and drugs. Thirty groups are involved in developing a vaccine. Four categories of vaccines, namely mRNA vaccines, attenuated vaccines, inactivated vaccines and adjuvant vaccines, are under development. Some companies in India are at the late preclinical trial stage. In addition, the following products are under various stages of clinical trial. These therapies are favipiravir, itolizumab, phytopharmaceuticals, mycobacterium W, convalescent plasma, Arbidol, ACQH, hydroxychloroquine, remdesivir and BCG vaccine (Vijay Raghavan 2020).

The companies involved in the development of vaccine are Serum Institute of India, Zydus Cadila, Bharat Biotech and Biological E (Vijay Raghavan 2020).

17.6 Summary

Pharmaceutical industry, biotech companies and academia all over the world are in a race to develop a new drug/monoclonal antibody/vaccine to contain the novel SARS-CoV-2, called as Covid-19 pandemic, to reduce the misery of infected people, their families and deaths. The accelerated approach adopted is to repurpose drugs/monoclonal antibodies/vaccines after testing their virus-killing efficacy in virus cell cultures. There are more than 1200 molecules registered for clinical trial. These include 29 monoclonal antibodies, 17 antivirals, 10 RNA-based therapies, 10 cell-based therapies and 86 drugs. The ones clinically used as therapy are favipiravir, remdesivir, dexamethasone and in selected critical patients convalescent plasma as well. Hydroxychloroquine has yielded equivocal results and deserves evaluation as a prophylaxis for contacts of Covid-19 patients and healthcare workers treating/

attending them in hospitals. Some prospective antivirals, monoclonal antibodies, protein antagonists, immunomodulators/cytokine disrupters and two prospective candidate molecules are under development. The therapies under clinical evaluation in India have also been included. Age-old practices that have been followed at the time of influenza pandemic, seen a century ago, that is, use of face masks, social distancing, use of personal hygiene (frequent hand wash for 20 s with soap), patient identification, isolation, quarantine and treatment of Covid-19-positive cases (those needing hospitalisation), are continued to be followed, after discharge. The use of face masks, social distancing and personal hygiene has to be practiced till the Covid-19 pandemic is fully controlled/eradicated.

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Pharmacological Agents Targeting Coagulopathy in COVID-19: A Review

18

Kanchan Gupta, Shalini Arora, and Vandana Kaushal

Abstract

Infection with severe acute respiratory syndrome coronavirus (SARS-CoV-2) and the resultant syndrome COVID-19 has wrecked the entire world. The disease mostly manifests as mild viral pneumonia but in a small proportion of patients it can produce an intense inflammatory and prothrombotic state leading to multi-organ failure and even death. Varying incidences of venous thromboembolism (VTE) have been found in COVID-19 patients. This review describes the role of various pharmacological agents used prophylactically as well as therapeutically for thromboembolism in such patients. The anticoagulants which are administered as antithrombotic therapy can be used parenterally (heparin and direct thrombin inhibitors) or orally (direct oral thrombin inhibitors). The mechanism of action, pharmacology, usage, and adverse effects of such agents has been discussed especially in the context of ongoing COVID-19 pandemic. As a result of various completed and ongoing clinical trials, scientific community has collected promising evidence and formulated guidelines regarding the role of anticoagulants in COVID-19 patients.

Keywords

COVID-19 · Thromboprophylaxis · Anticoagulants · Heparin · DOAC · Prophylactic dose · Therapeutic dose

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18.1 Introduction

COVID-19 has crippled the healthcare system of many countries in the world. It has a detrimental effect on various systems of the body including respiratory system, cardiovascular system, and immune system. One of the major complications of COVID-19 is the development of a pro-thrombotic state. Endothelial injury and proinflammatory cytokines activate the coagulation cascade and thus it results in thromboembolic events. The risk of venous thromboembolism is even higher if the patient is immobilized because of severe disease. This has evoked an interest in the role of anticoagulants in the prevention and treatment of coagulopathy-related complications in COVID-19. Anticoagulant therapy has a role to play as it helps to reduce fibrin deposition and formation of microthrombi and prothrombotic state in such patients.

Recommendations and guidelines issued by various societies dealing with the management of coagulopathy in COVID-19 patients recommend the use of antithrombotic thromboprophylaxis in hospitalized patients (Hajra et al. 2020; Marchandot et al. 2020). Before discussing the recommendations of these guidelines in detail, the pharmacological agents which target various steps of the coagulation cascade (Fig. 18.1) will be elaborated.

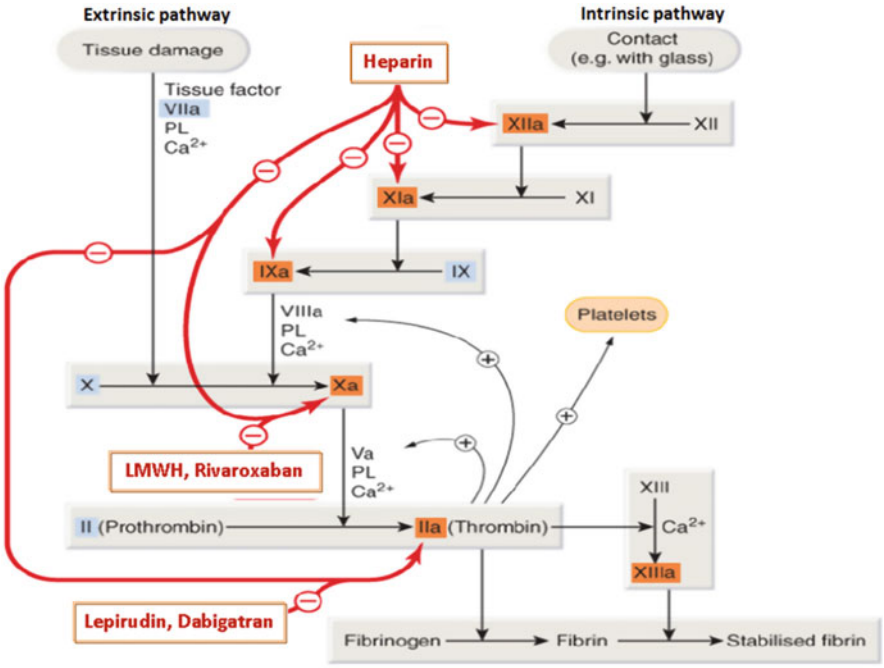

Fig. 18.1 Coagulation cascade depicting the site of action of various anticoagulants (Ritter et al. 2019)

18.2 Classification of Anticoagulants

1. Parenteral anticoagulants
   (a) Heparin and its derivatives
      - Unfractionated heparin (UFH)
      - Low-molecular-weight heparin (LMWH)
      - Fondaparinux
   (b) Other parenteral anticoagulants
   Direct thrombin inhibitors (DTIs)
      - Lepirudin
      - Desirudin
      - Bivalirudin
      - Argatroban
2. Oral anticoagulants
   (a) Coumarin derivatives, e.g., warfarin
   (b) Direct oral anticoagulants (DOACs)
      - Direct thrombin inhibitors
        – Dabigatran
      - Direct factor Xa inhibitors
        – Rivaroxaban
        – Apixaban
        – Edoxaban

18.3 Anticoagulant Drugs

18.3.1 Heparins

Heparin is present in the secretory granules of mast cells. On getting released from mast cells, it gets ingested and destroyed by macrophages and cannot be detected in plasma under normal circumstances. Unfractionated heparin (UFH) chains have a mean molecular weight of 15,000 (range 5000–30,000). Low-molecular-weight heparin (LMWH) is prepared from UFH and consists of smaller fragments of heparin and has a mean molecular weight of about 5000 (range 2000–8000). Fondaparinux is a synthetic heparin derivative and congener of the pentasaccharide sequence in heparin with a molecular weight of 1728 (Hogg and Weitz 2018).

18.3.1.1 Mechanism of Action

The anticoagulant effect of heparin is mediated by an endogenous component of plasma—antithrombin. Antithrombin is an endogenous anticoagulant and a plasma protease inhibitor, synthesized in the liver. It is a suicide substrate for the target enzymes. Antithrombin neutralizes the clotting factor proteases, particularly thrombin and factor Xa, by forming stable complexes with them. Heparin acts by

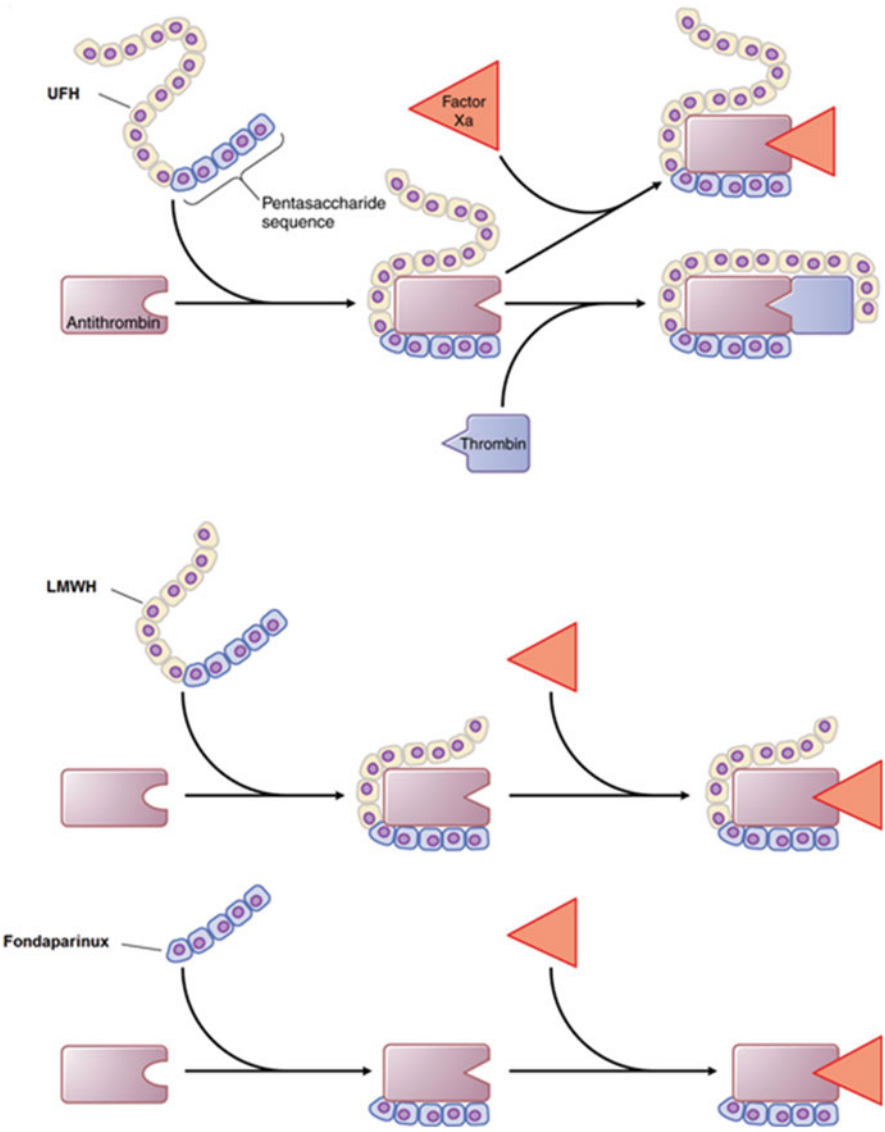

Fig. 18.2 Mechanism of action of UFH, LMWH, and fondaparinux

increasing the rate of these, or otherwise through slow reactions by about 1000-fold. It binds to antithrombin through its unique pentasaccharide sequence, thus inducing a conformational change that accelerates the interaction of antithrombin with factor Xa. Heparin should simultaneously bind to both antithrombin and thrombin to potentiate thrombin inhibition as shown in Fig. 18.2. This bridging can be achieved only by unfractionated heparin which has at least 18 saccharide units with a

molecular weight of 5400 (Hogg and Weitz 2018). Almost all chains of UFH have the capacity to inhibit thrombin. Thus, UFH has equal capacity to enhance the inhibition of both thrombin and factor Xa.

LMWH exerts its anticoagulant activity by selectively inhibiting factor Xa with little effect on thrombin. With less than 18 saccharide units, LMWHs are of insufficient length to catalyze the inhibition of thrombin. However, these are able to accelerate factor Xa inhibition by antithrombin as it is mainly because of the conformational change in antithrombin brought about by binding of the pentasaccharide sequence. Thus, LMWH has its anticoagulant effect largely because of factor Xa inhibition (Fig. 18.2).

Fondaparinux accelerates only factor Xa inhibition by antithrombin, by inducing conformational change, as the pentasaccharide sequence is short and cannot bind antithrombin and thrombin together (Fig. 18.2).

18.3.1.2 Pharmacology

UFH, LMWH, and fondaparinux are administered by parenteral routes of administration as these cannot be absorbed by the GI mucosa. UFH is given by continuous or intermittent intravenous infusion (IV) or subcutaneous (SC) injection. When heparin is given IV it has immediate onset of action but subcutaneous injection has a delayed onset of about 1–2 h and variable bioavailability. UFH has dose-dependent clearance and half-life. At low doses, heparin has a short half-life as it binds to the endothelium. The half-life increases with increasing doses as the endothelium becomes saturated and clearance of heparin is reduced. Intravenous heparin in doses of 100, 400, or 800 units/kg has a half-life of about 1, 2.5, and 5 h, respectively. Also, certain heparin-binding proteins in the plasma reduce the anticoagulant activity of heparin. The levels of plasma proteins vary from patient to patient, thus resulting in a variable anticoagulant activity of heparin. These proteins also include certain acute-phase reactants in ill patients and platelet factor 4 released from activated platelets. Consequently, heparin therapy requires monitoring to achieve optimal therapeutic response. This can be done by monitoring the activated partial thromboplastin time (aPTT) or by anti-factor Xa level. A two- to threefold rise in aPTT usually denotes adequate therapeutic levels of heparin.

LMWH is usually given subcutaneously. Because of shorter chains, LMWHs bind weakly to the endothelium, macrophages, and heparin-binding proteins. Therefore, it has dose-independent half-life and clearance. The plasma half-life of LMWH is nearly 4 h. The bioavailability on subcutaneous injection is about 90% with a predictable dose response. It can be administered once or twice daily and does not require any monitoring. This makes it a suitable anticoagulant for nonhospitalized patients. In case monitoring is required it can be done using anti-factor Xa levels. LMWH gets excreted mainly by kidneys and can get accumulated in renal insufficiency. Such patients may require monitoring with anti-factor Xa levels. Monitoring of LMWH should also be done in obese patients, pregnancy, infants, children, and patients with mechanical heart valves.

Fondaparinux is administered by subcutaneous route, once a day. Monitoring is not required. It does not bind to the endothelial cells or plasma proteins. The plasma

half-life is 17 h and the clearance is dose dependent. However, it is excreted unchanged in urine. It is recommended that LMWH or fondaparinux should not be given to patients with creatinine clearance <30 mL/min (Hogg and Weitz 2018; Leavitt and Minichiello 2019).

18.3.1.3 Administration and Dosage

Prophylactic dose of **UFH** is 5000 units subcutaneously, twice or thrice a day. This dose does not require monitoring. For therapeutic purpose, weight-based heparin nomograms are utilized to standardize the dose of heparin and to achieve therapeutic aPTT in a short time. An initial bolus dose of 5000 units or 70 units/kg of heparin is administered followed by an infusion of 12–15 units/kg/h.

LMWH doses for prophylactic or therapeutic purpose vary depending upon the LMWH preparation. For prophylaxis, usually a dose of 4000–5000 units subcutaneously is administered once a day or 2500–3000 units subcutaneously twice a day. For therapeutic purpose, dose of LMWH in venous thromboembolism (VTE) is 150–200 units/kg, given once daily.

Fondaparinux is given at a dose of 2.5 mg once a day for prevention of VTE. For established VTE, the dose is 7.5 mg once daily (Weitz 2018).

Adverse Effects

1. Bleeding: This is a major complication with these drugs, although UFH carries the highest risk. Risk increases with increasing doses of heparin and any underlying cause, e.g., recent surgery, trauma, peptic ulceration, underlying hemostatic defects, and antiplatelet or fibrinolytic drugs. Elderly women and patients with renal dysfunction are more prone. Mild bleeding can usually be controlled by discontinuing the drug but life-threatening hemorrhage may require administration of heparin antagonist protamine sulfate, through slow intravenous infusion. For every 100 U of heparin remaining in the patient, 1 mg of protamine sulfate at a rate not exceeding 50 mg in 10 min can be used. Excess amount of protamine should be avoided as it has anticoagulant effect of its own. Also, slow infusion is recommended to reduce the risk of anaphylactoid reaction. Protamine is less effective as an antidote for LMWH and not effective at all against fondaparinux as it can bind only to longer heparin chains.
2. Thrombocytopenia: Heparin-induced thrombocytopenia (HIT) is seen in 1–4% of patients treated with UFH for at least 7 days. It is a hypercoagulable state in which the platelet count is <1,50,000/mL or <50% from pretreatment value. This is an antibody-mediated process resulting in platelet activation and generation of platelet microparticles and thus promotes thrombin generation. There is generation of immunoglobulin G antibodies formed in response to the heparin-platelet factor 4 complexes. LMWH has much lower risk of thrombocytopenia as it binds weakly to platelets, thus causing less platelet factor 4 releases. Fondaparinux does not cause HIT as it does not bind to these proteins. HIT requires discontinuation of heparin and replacement with direct thrombin inhibitors or fondaparinux (Thachil et al. 2020). LMWH should not be used for replacing UFH for HIT in view of the cross-reactivity shown by the antibodies (Zehnder 2018).

3. Osteoporosis: Bone density is reduced with heparin therapy given for a duration of over 1 month. Prolonged therapy has been found to be associated with spontaneous fractures. Heparin reduces the bone formation as well as increases bone resorption. Osteoporosis risk is lesser with LMWH.
4. Enhanced level of transaminases: Mild and reversible elevation in serum transaminase levels without a concomitant rise in serum bilirubin may be seen with heparin therapy (Weitz 2018).

Comparison of Pharmacological Effects of UFH and LMWH

- Given subcutaneously, LMWH has better bioavailability and longer half-life than UFH.
- UFH has dose-dependent half-life and clearance and thus requires frequent dosage adjustments, in contrast to LMWH.
- UFH has less predictable anticoagulant response and therefore requires regular monitoring with aPTT or anti-factor Xa level. LMWH need not be monitored for its anticoagulant activity.
- LMWH has lesser adverse effects including thrombocytopenia and osteoporosis, so it is safer than UFH especially for long-term use.

18.3.2 Parenteral Direct Thrombin Inhibitors

Lepirudin and desirudin are recombinant forms of hirudin. They are eliminated by the kidneys; the half-life is about 2 h after subcutaneous administration and about 10 min after IV infusion. Desirudin is used for thromboprophylaxis in patients undergoing hip replacement surgery. Both of these are also used for the treatment of thrombosis in patients with HIT (Kelton et al. 2013). Lepirudin can be given by IV infusion if rapid anticoagulation is desired; otherwise it is given by subcutaneous route. Caution should be exercised in patients with impaired renal function. Daily monitoring of serum creatinine and aPTT needs to be done.

Bivalirudin is administered intravenously. It is the shortest acting parenteral DTI with a plasma half-life of 25 min. It is partially excreted via kidneys; dosage reductions are recommended for patients with renal impairment. aPTT can be used to evaluate its activity. Bivalirudin is approved as an alternative to heparin for patients who undergo percutaneous coronary intervention (PCI). It has also been found to be useful in HIT patients who require PCI or cardiac bypass surgery (Barria Perez et al. 2016).

Argatroban is administered intravenously with a half-life of 40–50 min. It undergoes hepatic metabolism and is excreted in bile. Therefore, it is safe in patients with renal impairment, but dose needs to be reduced in patients with hepatic dysfunction. It is approved both for prophylaxis and treatment of patients who have or are at risk of developing HIT (Grouzi 2014). The anticoagulant effect is monitored using aPTT.

18.3.3 Direct Oral Anticoagulants (DOACs)

For a long time, vitamin K antagonists such as warfarin were the only option available as oral anticoagulant. However, the scenario changed with the arrival of direct oral anticoagulants, which include dabigatran, rivaroxaban, apixaban, and edoxaban. DOACs have consistently shown equivalent antithrombotic efficacy and lower bleeding rates when compared with conventional warfarin therapy. However, one should be cautious that owing to their short half-life, patient noncompliance will quickly lead to loss of anticoagulant effect and risk of thromboembolism.

18.3.3.1 Advantages of DOACs Over Conventional Oral Anticoagulants

The new direct oral anticoagulants represent a significant advance in the prevention and therapy of thrombotic disease. Their advantages include

- Predictable pharmacokinetic parameters (including bioavailability) which permit fixed dosing and also predictable anticoagulant response which makes routine coagulation monitoring unnecessary
- The quick onset of action of these agents which allows for immediate anticoagulation
- Fewer drug and dietary interactions in comparison with warfarin, which is known to have multiple drug-drug and drug-food interactions
- Convenience of once- or twice-daily oral dosing

18.3.3.2 Direct Thrombin Inhibitor

Dabigatran is the only oral direct thrombin inhibitor approved by the FDA.

Mechanism of Action: It acts by the blockade of the active site of free as well as clot-bound thrombin in a competitive and reversible manner. This further blocks thrombin-mediated conversion of fibrinogen to fibrin and hence feedback activation of coagulation cascade.

Pharmacology: The maximum effect occurs in 2 h with a half-life of 12–14 h. The oral bioavailability is 3–7%. Dabigatran is 35% bound to plasma proteins. Renal impairment leads to prolonged drug clearance. Hence, dose reduction is needed in the presence of severe renal insufficiency (creatinine clearance 15–30 mL/min) (Hogg and Weitz 2018).

18.3.3.3 Direct Factor Xa Inhibitors

Mechanism of Action: Rivaroxaban, apixaban, and edoxaban inhibit factor Xa in the final common pathway of clotting leading to reduction in generation of thrombin. Hence, this leads to suppression of platelet aggregation and formation of fibrin.

Pharmacology: Bioavailability of rivaroxaban is 80% with maximum effect seen in 3 h. Its half-life is 7–11 h. If given with meals, its absorption is enhanced. It is 95% plasma protein bound. Dose reduction is required in patients with renal impairment or severe hepatic dysfunction as levels are increased in such patients.

Apixaban has oral bioavailability of 50% with peak onset of action 1–3 h after ingestion. Its absorption is not affected by the presence of food.

Edoxaban has oral bioavailability of 62% and peak levels are observed 1–2 h after ingestion. Food has no effect on its absorption. The drug does not undergo hepatic metabolism, and hence can be used safely in patients with liver disease. The plasma level is increased in the presence of renal dysfunction and low body weight. Therefore, the dose should be reduced in patients with a creatinine clearance between 15 and 50 mL/min, in those with a body weight of 60 kg or less (Hogg and Weitz 2018).

Indications for DOACs

1. To prevent stroke in patients who have nonvalvular atrial fibrillation: For this indication, they have been compared with warfarin in four randomized trials that enrolled 71,683 patients. The results demonstrated that DOACs have a better benefit-to-risk ratio compared with warfarin, and have comparable relative efficacy and safety among a broad spectrum of patients with atrial fibrillation (Zehnder 2018).
2. DOACs were compared with conventional anticoagulants in patients with acute venous thromboembolism in a large group of patients. The results showed that DOACs are non-inferior to well-managed warfarin for the treatment of VTE, but are linked with significantly less bleeding episodes. Parenteral anticoagulant therapy has to be given for a minimum period of 5 days before starting dabigatran. But edoxaban, rivaroxaban, and apixaban can be administered as an all-oral regime starting with a higher dose for 21 days and 7 days, respectively (Weitz 2018).
3. In some countries, lower dose regime of once-daily dabigatran is approved for thromboprophylaxis after knee or hip arthroplasty. Rivaroxaban and apixaban are also licensed for the same indication (Weitz 2018).

Adverse Effects and Contraindications of DOACs

1. As with any anticoagulant drug, bleeding is the major adverse effect. In elderly patients with atrial fibrillation, the risk of major bleed with DOACs is comparable to that with warfarin. Nevertheless, the risk of intracranial bleed is markedly lower as compared with warfarin. The only exception is GIT; incidence of GI bleed with dabigatran, rivaroxaban, and edoxaban, but not apixaban, is higher than with warfarin. This might be due to the ability of unabsorbed drug in the GIT to promote bleed from preexisting lesions (Zehnder 2018). The risk of bleeding is higher if patient is also taking antiplatelet or nonsteroidal anti-inflammatory agents.
2. Dyspepsia is observed in up to 10% of patients on dabigatran but taking the drug with food reduces the incidence. Dyspepsia is not common with rivaroxaban, apixaban, and edoxaban.
3. DOACs are contraindicated for prevention of stroke in patients with mechanical heart valves.
4. All DOACs, being small molecules, can easily pass through placenta. Hence, they are contraindicated in the setting of pregnancy, and if used by women of childbearing potential, appropriate contraceptive methods should be employed.

They should be avoided in lactating mothers and their safety in children is yet to be established.

18.3.3.4 Drug Interactions of DOACs

All DOACs are substrates for P-glycoprotein efflux pump; so drugs that inhibit P-glycoprotein like verapamil, dronedarone, quinidine, ketoconazole, and clarithromycin may increase their plasma levels whereas concomitant administration of rifampicin which induces P-glycoprotein may decrease their plasma levels. Rivaroxaban and apixaban are metabolized by CYP3A4 enzymes, whereas edoxaban undergoes only minimal CYP3A4-mediated metabolism. Therefore, serum levels of rivaroxaban and apixaban may be altered by concomitant administration of CYP3A4 inducers such as carbamazepine and phenytoin or inhibitors like erythromycin and ketoconazole (Preston 2019; Anderson and Smith 2019).

18.3.3.5 Administration and Dosage

For stroke prophylaxis in patients with nonvalvular atrial fibrillation, dabigatran is given 150 mg twice a day with a dose reduction to 75 mg twice a day if creatinine clearance is between 15 and 30 mL/min; rivaroxaban is administered 20 mg once a day with a dose reduction to 15 mg once a day in patients with a creatinine clearance of 15–49 mL/min; apixaban is given 5 mg twice a day with dose reduction to 2.5 mg twice a day in patients with at least two of the following criteria: age 80 years or more, body weight of 60 kg or less, and serum creatinine >1.5 g/dL; and edoxaban is administered 60 mg once a day with a dose reduction to 30 mg once a day for patients with a creatinine clearance between 15 and 50 mL/min and body weight of 60 kg or less, or receiving concomitant potent P-glycoprotein inhibitors.

For treatment of VTE, dabigatran is administered 150 mg twice daily after a minimum duration of a 5-day course of heparin or LMWH; rivaroxaban is given 15 mg twice a day for 21 days, and then the dose is reduced to 20 mg once a day; apixaban is started 10 mg twice a day for 7 days and then for next 6 months, dose is reduced to 5 mg twice a day after which the dose can be further reduced to 2.5 mg twice a day; and edoxaban is administered at a dose of 60 mg once a day after a minimum duration of a 5-day course of heparin or LMWH. The dose of edoxaban is reduced to 30 mg once daily for patients with a creatinine clearance of 15–50 mL/min and body weight of 60 kg or less, or receiving concomitant potent P-glycoprotein inhibitors.

For thromboprophylaxis after hip or knee replacement surgery, rivaroxaban is administered 10 mg once a day while apixaban is given 2.5 mg twice a day. The drugs are usually given for 14 days after knee arthroplasty and for 35 days' duration after hip arthroplasty (Weitz 2018).

The major characteristic features of various DOACs are highlighted in Table 18.1.

18.3.3.6 Assessment and Reversal of DOACs' Effects

For qualitative assessment of anticoagulant activity, prothrombin time can be utilized for factor Xa inhibitors and aPTT for dabigatran. Rivaroxaban and edoxaban

Table 18.1 Comparison of the features of DOACs

Feature	Dabigatran	Rivaroxaban	Apixaban	Edoxaban
Prodrug	Yes	No	No	No
Bioavailability (%)	3–7	80	50	62
Half-life (h)	12–14	7–11		
Renal excretion (%)	80	33	25	35
Drug interactions	P-gp	CYP 3A4/P-gp	CYP 3A4/P-gp	P-gp
Dosing regimen	Twice a day	Once a day	Twice a day	Once a day
Antidote	Idarucizumab	Andexanet	Andexanet	Andexanet

Abbreviations: *P-gp* P-glycoprotein, *CYP* cytochrome p450

prolong the prothrombin time more than apixaban. Reversal of anticoagulant effect should be considered in patients with life-threatening bleeding, like intracranial bleeding, if bleeding continues despite supportive measures or urgent surgery is required. Idarucizumab, a humanized monoclonal antibody, is a specific reversal agent for dabigatran. The recommended dose is 5 g as an intravenous bolus injection. If bleeding recurs, a second dose may be given. Andexanet alfa is a reversal agent for various oral Xa inhibitors. By sequestering circulating factor Xa inhibitors, it quickly reverses the anti-factor Xa activity and restores thrombin generation. It is administered as an IV bolus followed by a 2-h infusion. Higher doses are required to reverse rivaroxaban or edoxaban than apixaban. Ciraparantag, a potential reversal agent for all DOACs, is at an early stage of development (Hogg and Weitz 2018). Until these reversal agents are available, prothrombin complex concentrate can be considered for reversal of the oral factor Xa inhibitors in patients with life-threatening or ongoing bleeding.

18.4 Anticoagulant Therapy in COVID-19 Patients: The Present Scenario

There are more than ten international guidelines and guidance statements that emphasize the role of anticoagulants in COVID-19. The major ones include the 2020 CHEST COVID-19 Guidelines (COVID-19 Guidelines 2020), the International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee (SSC) COVID-19 clinical guidance, the American Society of Hematology (ASH) 2021 guidelines, the Anticoagulation (AC) Forum interim clinical guidance, and the American College of Cardiology (ACC) clinical guidance.

The ISTH-SSC guidance in hospitalized patients with COVID-19 suggests that both critically ill patients (who develop respiratory or cardiovascular failure normally requiring advanced clinical support in the ICU or CCU) and acutely ill patients (who require hospital admission without advanced clinical support) should receive standard prophylactic doses of LMWH or UFH, although intermediate-intensity LMWH may be considered for patients at high VTE risk. The convenient dosage schedule of once-daily administration of LMWH gives it an edge over UFH which

has to be injected twice or thrice daily and has a higher incidence of heparin-induced thrombocytopenia (Zhai et al. 2020). The ISTH-SSC suggests that therapeutic-intensity anticoagulation should not be used until clear-cut scientific evidence is available in its favor. Although certain DOACs are approved for prophylaxis in hospitalized patients, they should be used cautiously in COVID-19 patients in whom co-administration of immunosuppressant, antiviral, and other experimental drugs may interfere with their action. The guidelines also suggest the duration for which thromboprophylaxis should be given in hospitalized COVID-19 patients. In high-VTE-risk medically ill patients, as in pneumonia or sepsis, this risk may extend up to 6 weeks posthospital discharge. Based on the recent data, it is recommended that extended-duration thromboprophylaxis for nearly 4 weeks with prophylactic dose LMWH (e.g., enoxaparin, dalteparin) or a DOAC (e.g., rivaroxaban) should be given. For confirmed VTE in hospitalized COVID-19 patients, LMWH should be preferred in patients when they are hospitalized followed by DOACs at the time of discharge (Spyropoulos et al. 2020).

The American Society of Hematology (ASH) 2021 panel has recommended using prophylactic-intensity over intermediate-intensity or therapeutic-intensity anticoagulation for patients with COVID-19-related acute illness and critical illness without a suspected or confirmed VTE. Pertaining to the choice of a specific agent, not enough clinical trials have been conducted comparing different anticoagulants. The drug (for example low-molecular-weight heparin, UFH) may be chosen based upon its availability, cost, facilities, familiarity, and effort to minimize PPE usage or reduce the exposure of hospital staff to COVID-19-infected patients. Various patient-specific factors for example renal function, history of HIT, and issues regarding GIT absorption should also be taken into consideration (Cuker et al. 2021).

The anticoagulant regimens have been classified on the basis of intensity as prophylactic, intermediate, and therapeutic intensity as shown in Table 18.2.

Similar to the views expressed in other guidelines (Singhania et al. 2020), the expert panel report from CHEST also recommends the use of anticoagulant thromboprophylaxis in both acutely ill and critically ill patients. These guidelines favor LMWH or fondaparinux over UFH and these drugs over DOACs in acutely ill patients. In critically ill patients, order of preference for thromboprophylaxis is LMWH followed by UFH and then fondaparinux or DOACS. LMWH has the advantage of limited staff exposure as compared to UFH. The panel cautions against the use of DOACs especially in critically ill patients because of hemodynamic instability, chances of possible drug interactions with other adjunctive therapies, and high risk of acute kidney injury. Moreover, the use of DOACs carries a high risk of rapid clinical deterioration and gastrointestinal disturbances. The other issues of concern with DOAC use are cost implications and/or lack of reversal agents in some hospitals (Bikdeli et al. 2020). As per the guidelines, inpatient thromboprophylaxis only is recommended over extended thromboprophylaxis after hospital discharge (Moores et al. 2020).

The AC Forum document also includes suggestions on thromboprophylaxis in pregnant women and monitoring strategies for parenteral anticoagulation therapy (Barnes et al. 2020).

Table 18.2 Classification of anticoagulant regimens by intensity (Cuker et al. 2021)

Prophylactic intensity
Apixaban 2.5 mg, orally, twice a day
Dabigatran 220 mg, orally, once a day
Dalteparin 5000 Unit, subcutaneously, once a day
Enoxaparin 40 mg (4000 Unit), subcutaneously, once a day
Fondaparinux 2.5 mg, subcutaneously, once a day
Unfractionated heparin 5000 Unit, subcutaneously, twice a day
Unfractionated heparin 5000 Unit, subcutaneously, thrice a day
Rivaroxaban 10 mg, orally, once a day
Intermediate intensity
Enoxaparin 0.5 mg/kg (50 U/kg), subcutaneously, twice a day (if CrCl >30 mL/min)
Enoxaparin 0.5 mg/kg (50 U/kg), subcutaneously, once a day (if CrCl <30 mL/min)
Unfractionated heparin 7500 Unit, subcutaneously, thrice a day
Dalteparin 5000 Unit, subcutaneously, twice a day
Therapeutic Intensity
Apixaban 5 mg, orally, twice a day
Apixaban 10 mg, orally, twice a day
Argatroban, intravenous to target aPTT therapeutic range as per institutional guidelines
Bivalirudin, intravenous to target aPTT therapeutic range as per institutional guidelines
Dabigatran 75 mg, orally, twice a day (if CrCl 15–30 mL/min)
Dabigatran 150 mg, orally, twice a day (if CrCl >30 mL/min)
Dalteparin 100 Unit/kg, subcutaneously, twice a day
Dalteparin 150 Unit/kg, subcutaneously, once a day
Edoxaban 30 mg, orally, once a day (≤60 kg, CrCl 15–50 mL/min)
Edoxaban 60 mg, orally, once a day (weight ≥60 kg and CrCl >50 mL/min)
Enoxaparin 1 mg/kg (100 Unit/kg), subcutaneously, twice a day (for CrCl >30 mL/min)
Enoxaparin 1.5 mg/kg (150 Unit/kg), subcutaneously once a day (for CrCl >30 mL/min)
Enoxaparin 1 mg/kg (100 Unit/kg), subcutaneously, once a day (for CrCl <30 mL/min)
Fondaparinux 5 mg, subcutaneously, once a day (if weight <50 kg and CrCl >50 mL/min
Fondaparinux 5 mg, subcutaneously, once a day (if weight 50–100 kg and CrCl 30–50 mL/min)
Fondaparinux 7.5 mg, subcutaneously, once a day (if weight 50–100 kg and CrCl >50 mL/min)
Fondaparinux 7.5 mg, subcutaneously, once a day (if weight >100 kg and CrCl 30–50 mL/min)
Fondaparinux 10 mg, subcutaneously, once a day (if weight >100 kg and CrCl >30 mL/min)
Unfractionated heparin 250 Unit/kg, subcutaneously every 12 hourly
Rivaroxaban 15 mg, orally, twice a day
Rivaroxaban 15 mg, orally, once a day (for GFR 15–50 in AF patients)
Rivaroxaban 20 mg, orally, once a day

Abbreviations: *AF* Atrial fibrillation, *aPTT* Activated partial thromboplastin time, *CrCl* Creatinine clearance, *GFR* Glomerular filtration rate

Heparin, in addition to anticoagulant effects, has been found to be especially useful in COVID-19 patients by virtue of its additional anti-inflammatory (Young 2008) and antiviral properties (Mukhopadhyay et al. 2010; Ghezzi et al. 2017). Inhaled forms of heparin have also been tried as COVID-19 ARDS is known to

cause significant pulmonary injury in the form of diffuse alveolar damage with extensive pulmonary coagulation activation (Thachil et al. 2020). UFH prevents SARS-CoV-2 from binding with ACE-2 and thus infecting cells. Also, as an antiviral, if administered via inhalational route, it may prevent disease progression (Van Haren et al. 2020).

Although none of the societies recommend routine use of DOACs for anticoagulant thromboprophylaxis (Flaczyk et al. 2020; Schulman et al. 2020) there are very few published studies which favor the use of DOACs for thromboprophylaxis. A retrospective, observational cohort study was conducted by Wenzler et al. (2020) to describe the safety and efficacy outcomes of the use of therapeutic dose of apixaban in critically ill ICU patients with severe COVID-19. The results indicated that apixaban appeared safe and efficacious in this high-risk population. None of the participants had any major bleeding event or any other serious adverse effect in spite of the patient cohort suffering from high incidence of renal dysfunction and severe respiratory illness leading to ARDS. Hence, apixaban may be considered as a safe and effective alternative to UFH or LMWH in hospitalized patients with COVID-19, including those with severe disease. The major advantages of apixaban are that it can be given twice a day and requires less frequent monitoring. Also, the exposure of hospital staff and PPE use is minimized. Further, apixaban has an additional anti-inflammatory property similar to that of UFH and LMWH through inhibition of plasma-evoked superoxide generation (Ishibashi et al. 2014).

18.5 Conclusion

Ever since the onset of the pandemic, a lot of clinical trials are being conducted on various strata of population based on different set of criteria. Consequently, a plethora of scientific literature addressing varied issues (including coagulopathies) related to COVID-19 has been emerging over time. Anticoagulants, by acting on various steps of coagulation cascade, have been found to be extremely useful for prophylaxis and treatment of thromboembolism. Various internationally recognized societies have issued guidelines regarding management of COVID-19-associated coagulopathies. These guidelines have significant similarities as well as differences but they all unanimously agree that thromboprophylaxis should be given in all hospitalized patients with COVID-19 infection. There are a number of issues including choice of anticoagulant, dose and duration of thromboprophylaxis, and postdischarge treatment, for which studies are ongoing and the scientific literature is being updated continuously. The clinicians need to keep themselves apprised of the latest guidelines for optimal management and benefit of patients.

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Gut Microbiome in COVID-19: New Insights 19

A. K. Sharma

Abstract

The last discovered organ of the human body is microbiome which is present at different sites in it. Gut microbiome consists of about 1000–1500 bacterial species and as regulated by genetic makeup, lifestyle, and environmental conditions, the gut microbiota of a healthy individual can comprise approximately 160 species of bacteria. Majority of gut microbiome consists of Firmicutes, Actinobacteria, Bacteroidetes, and to a lesser extent Proteobacteria, Euryarchaeota, Fusobacteria, and Verrucomicrobia. The gut-lung axis is involved in the migration of immune cells from gut to respiratory tract through circulation and encourages the host's ability to fight infections. The gut regulates the responses in lungs via host-acquired inflammatory mediators in the circulation. Dendritic cells located in the Peyer's patches of the intestine, macrophages, and Langerhans cells are the major antigen-presenting cells that play a vital role in the modulation and development of innate immune response. Gut microbiota interacts via the regulation and development of adaptive immune response. B and T lymphocytes are the key players of adaptive immunity. CD4 + T cells after activation differentiate into four major kinds of cell classes: (1) regulatory T cells (Treg), (2) Th2, (3) Th1, and (4) Th17 cells. Gut microbial interactions can induce the production of various types of immune cells as demonstrated by various studies. For instance, Clostridia induces the formation of Treg cells. Likewise, Bacteroides fragilis inhabiting the gut can incite the production of Th1 cells and production of T17 cells is stimulated by segmental filamentous bacteria. Gut microbiota also plays a vital role in the physiology and metabolism leading to the synthesis of various immunoregulatory metabolites such as SCFAs, antimicrobial

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peptides (AMPs), amino acids, and polyamines. SARS-CoV-2 virus entry to the cell is via ACE2 receptor present in respiratory epithelium and gut epithelium. This receptor is highly expressed (100 times more than in the lung) in the epithelial cells of the stomach, duodenum, ileum, and rectum as well as cholangiocytes and hepatocytes. High level of ACE2 receptor expressing in the gastrointestinal epithelial cells along with high-level co-expression of TMPRSS2 (cellular serine peptidase) causes coronavirus to infect gastrointestinal tract along with lungs leading to altered intestinal permeability and enterocyte malabsorption with symptoms of diarrhea in patients of COVID-19. Hence, COVID-19 patients with gastrointestinal symptoms have significantly longer duration of illness and viral clearance time than patients without any gastrointestinal symptoms. Obese patients with gut dysbiosis have decreased population of Bacteroides species. COVID-19 patients with type 2 diabetics have increased population of Fusobacterium, Ruminococcus, and Blautia with decreased population of Bacteroides, Bifidobacterium, Faecalibacterium, Akkermansia, and Roseburia. Diet with low fiber, high fat, and high carbohydrate causes gut dysbiosis. Intake of high-fiber diet consisting of whole grains, vegetables, and fruits induces growth of Bifidobacterium, Bacteroides, and Lactobacilli. Probiotics are non-pathogenic live organisms which are safe to be taken as dietary supplements. The major genera of probiotics are Lactobacillus, Bifidobacterium, and Saccharomyces. These probiotics increase the activity of T cells, NK cell, and polymorphonuclear cells. Prebiotics in the form of maize fiber, inulin, and polydextrose improves digestion and immunity. Hence, healthy gut microbiome with its strong immune intervention may bring recovery in COVID-19 patients. However, so far no published studies have reported that probiotics can be used as an adjunctive therapy in our fight against the SARS-CoV-2 infection. A far-reaching approach should consist of randomized, multicenter, controlled trials to explore the potential benefits of gut microbiome and how changes in dietary habits can be used as an add-on strategy against the COVID-19 pandemic.

Keywords

Firmicutes · Actinobacteria · Bacteroidetes · Gut-lung axis · ACE2 receptor · Dysbiosis · Probiotics

19.1 Introduction

The last discovered organ of the human body is microbiome which is present at different sites in it. The microbiome of the human body is probably one of the significant factors playing a major role in the COVID-19 epidemic. The composition of microbiome in humans is trillions of microbes mostly consisting of tiny fauna, fungi, viruses, and other living entities which inhabit every part of the body. Gut microbiome consists of multispecies commensals having a strong impact on host immune homeostasis in the gut. The count of microbes residing in gastrointestinal

tract (GIT) has been predicted to be more than 10^{14} and altogether their genomic content is reported to be 100 times the amount of total human genome (Bäckhed et al. 2005). Gut microbiome consists of about 1000–1500 bacterial species and as regulated by the genetic makeup, lifestyle, and environmental conditions, the gut microbiota of a healthy individual can comprise approximately 160 species of bacteria. Firmicutes and Bacteroidetes are among the most predominant genera found in the gut while lung microbiota is predominantly composed of Proteobacteria, Bacteroidetes, and Firmicutes (Zhang et al. 2020). Gut microbiota offers numerous benefits to its host which include direct inhibition of pathogens, maintaining gut integrity, metabolizing undigested compounds especially certain carbohydrates, as well as developing and strengthening the mucosal barrier along with intestinal epithelium (Natividad and Verdu 2013). Complex network of interactions exists between the gut microbiota and human immune system as approximately 70–80% of body's total immunological components exist in the gut. Majority of gut microbiome consists of Firmicutes, Actinobacteria, Bacteroidetes, and to a lesser extent Proteobacteria, Euryarchaeota, Fusobacteria, and Verrucomicrobia. This community of microorganisms have evolved along with human species over millions of years. Due to this coevolution of prokaryotic bacteria and eukaryotic cells, the genomic functional complementarity with genetic reduction could occur among themselves. It is important to note that organs such as the lungs, stomach, esophagus, and intestine, also populated by the microbiota, are all embryologically derived from same germline endoderm. So, it is not surprising that these developmentally homologous organs are connected to each other in gut-lung axis. It is a bidirectional communication which means that microbial metabolites and endotoxins from gut can affect the lungs and vice versa (Dhar and Mohanty 2020). The gut-lung axis is involved in the migration of immune cells from gut to respiratory tract through circulation, where it encourages the host's ability to fight infections. The gut regulates the responses in lungs via host-acquired inflammatory mediators in the circulation. Some studies have shown that changes do occur in the gut microbiota of mice during its infection of lung by respiratory syncytial virus infection (Groves et al. 2020). The interactions between gut and lung axis are caused either by the involvement of immune cells or via gut microbes or their products. However, microbes and their products which enter the intestinal mucosa are phagocytosed by the APCs and then moved to mesenteric lymph nodes which in turn might activate B and T cells. After activation, both B and T lymphocytes can either move to their original site, i.e., intestinal mucosa, or migrate to a different action site, e.g., lungs. The second proposed mechanism is that surviving bacteria or any bacterial products can enter the circulation and reach lungs either via blood or via lymphatic system generating a local or general immunological response that can further damage the lungs (Bingula et al. 2017). A study conducted by Fagundes et al. (2012) highlighted the impact of gut microbiota on lungs. In this study, mice lacking intestinal microbiota displayed much lower pathogenic clearance from lungs (Fagundes et al. 2012). Similarly, another study reported that intratracheal administration of lipopolysaccharide (LPS) might disturb the lung microbiota, which in turn can cause disruption of gut microbiota as well as increases the bacterial load (Sze

et al. 2014). Various changes in the composition of microbiome in the gut and also of the lungs are connected to the immune response alterations and disease development in COVID-19 patients. For example, chronic obstructive pulmonary disease (COPD) typically occurs together with chronic diseases of the gastrointestinal tract. COVID-19 patients have symptoms of myalgia, fever, cough, fatigue, and pneumonia which may lead to acute respiratory distress syndrome with or without multiorgan dysfunction (Musa 2020). A lot of studies have reported the appearance of gastrointestinal symptoms (nausea, vomiting, abdominal pain, and diarrhea) as mild or severe during the course of COVID-19 infection. These symptoms can be caused either because of the ability of SARS-CoV-2 to directly bind, invade, and infect the enterocytes via gut-lung-microbiota axis or through immune-regulatory mechanisms (Dumas et al. 2018).

19.2 Gut-Lung Axis

Any change in gut microbiome is linked to a bidirectional deviation in the interaction between the gut and human organs which eventually may cause severe disease symptoms in COVID-19 patients. An increase in Clostridia species and reduction in Bifidobacteria in gut microbiome are linked to asthma in early life (Anand and Mande 2018). The gut-lung axis also causes the migration of immune cells from gut to respiratory tract through blood circulation, which helps the host's ability to fight infections. Thus gut is able to regulate the immune responses in lungs via host-acquired inflammatory mediators (e.g., IL-6, TNFα) in the circulation. The elevated levels of these inflammatory mediators detected in the serum of patients with gut disorders influence immune responses in the lungs. The viral infections of respiratory tract can alter the intestinal microbiome in situations where the intestinal microbiome develops the adaptive immune responses against the respiratory pathogens which is necessary for priming the innate immune responses against those pulmonary infections. It is common during respiratory viral infections that the level of macrophage response to the respiratory viruses depends on the presence of healthy intestinal microbes (Hanada et al. 2018). This suggests that the lung and the gut are closely linked organs that affect each other's homeostasis via an immunological coordination between them. Emerging data identifies the role of gut microbiota in improving the antiviral immunity (He et al. 2020). Certain reports also suggest the role of gut microbiota modulation in reducing enteritis and ventilator-associated pneumonia and reversing the side effects of antibiotics so as to reduce the replication of influenza virus in lung epithelium. However, at present no clinical evidence is available of gut microbiota modulation as a therapy for treating COVID-19. Few emerging reports have shown the role of targeting the gut microbiota as an adjuvant therapeutic option.

19.3 Gut Microbiome and Immunity

Gut microbiome has a role in the pathogenesis of various multifactorial diseases like inflammatory bowel disease (IBD), chronic heart diseases and kidney diseases, and type 2 diabetes mellitus (Wu and Wu 2012). The common microbial species found in gastrointestinal tract are Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria for providing nutrients and maintaining homeostasis in the host. Human intestine is exceptional in its own way as it possesses an outstanding mechanism for the development of host immune system. The immune system in gastrointestinal tract is an intestine mucosa immune system or gut-associated lymphoid tissue (GALT) with three different structures: epithelial and mucosal barrier, lamina propria, and Peyer's patches (PPs). The epithelial and mucosal barriers are the small intestinal epithelium and Paneth cells which secrete antimicrobial peptides (AMPs). Immune cells of mucosal barrier contain innate lymphoid cells, natural killer or NK cells, intraepithelial lymphocytes (IEL), cytolytic and immunoregulatory αβ+ and γδ+ T cells, DCs, and Tregs (Johnson et al. 2019). The lamina propria and lower layer of intestinal epithelial cells carry a large number of innate lymphoid cells (ILCs) and B, NK, and T cells (γδ+ T, Th17). The key role of T cells seated in the lamina propria is to rapidly respond to the lumen signals and generate an appropriate anti-inflammatory response through secreting cytokines (IL17, IL22, IFN-γ, and IL26) and also inducing defensins and chemokine (Montalban-Arques et al. 2018; Bhagat et al. 2008). Intestinal homeostasis is achieved by the interaction and coordination of intestinal innate and adaptive immunity, both having mutually advantageous relationship among them. Gut microbiota exhibits their regulatory effect on innate immunity mainly via the antigen-presenting cells (APCs), for example, dendritic cells located in Peyer's patches, Langerhans cells, and macrophages. These cells however have some immunogenic properties which enable them to be tolerant towards the gut microbiota. For instance, macrophages develop "inflammation anergy" (Smythies et al. 2005). Mast cells and NK cells are the major components of innate immune system that interacts with the gut microbiome cells. B and T lymphocytes are the major kinds of cells induced and involved whenever gut microbiome interacts via adaptive host immune response. B cells of gut are found mostly in the Payer's patches. Gut microbiome may also have a role in the development of plasma cells since a study on germ-free mice revealed that they have lower levels of plasma (Ivanov et al. 2009). T cells constitute an essential component of adaptive immune response. CD4+ (cytotoxic) T cells after getting activated differentiate into four major types, namely Th1, Th2, Treg (regulatory t cells), and Th17 cells (Smythies et al. 2005). Gut microbial interactions can induce the production of various types of immune cells as demonstrated by various studies. For instance, Clostridia induces the formation of Treg cells. Likewise, Bacteroides fragilis inhabiting the gut can incite the production of Th1 cells and production of T17 cells is stimulated by segmental filamentous bacteria (Mazmanian et al. 2005; Nagano et al. 2012). Many studies have reported that synthesis and count of CD25+, CD4+, and NK cells and mononuclear leukocytes can be increased by Bifidobacterium lactis (Levy et al. 2017). In addition, gut microbiota plays a vital

role in the physiology and metabolism leading to the synthesis of various immunoregulatory metabolites such as SCFAs, AMPs (antimicrobial peptides), amino acids, and polyamines (Gill et al. 2001). Therefore, gut microbiota plays a vital role in the development of adequate host immune responses. As mentioned before, many patients infected with SARS-CoV-2 display gastrointestinal symptoms due to the invasion of intestinal enterocytes by virus indicating that SARS-CoV-2 might be interacting and disturbing the balance of healthy gut microbiome.

19.4 Pathogenesis and COVID-19

COVID-19 is caused by SARS-CoV-2, a positive-sense, single-stranded RNA virus of family Coronaviridae and genus Betacoronavirus. The structure of SARS-CoV-2 virus consists of four structural proteins (membrane (M), envelope (E), nucleocapsid (N), spike (S)), 15 mature nonstructural proteins (nsp1−10 and nsp12−16), and 9 accessory proteins (Prates et al. 2020). The SARS-CoV-2 infection occurs by the entry of virus through ACE2 receptor present on the epithelial cell lining of lung, gut, and other organs. This receptor is highly expressed (100 times than that in the lung) in the epithelial cells of the stomach, duodenum, ileum, and rectum as well as in cholangiocytes and hepatocytes of the liver. However less expression of ACE2 receptor is found in esophageal mucosa. The high number expression of ACE2 receptor in the absorptive enterocytes of the ileum and colon suggests a reason for digestive symptoms such as diarrhea found in many COVID-19 patients. Butyric acid is a short-chain fatty acid (SCFA) produced by beneficial gut bacteria Clostridia species along with propionic and acetic acids (a fermentation product of dietary fiber) that plays a pivotal role in gut microbial metabolism. In elder persons altered gut microbiota predisposes COVID-19 patients to severe symptoms of diarrhea due to disrupted gut barrier (Kim 2021, Fig. 19.1). Next the new virions assembled in the enterocytes are released into the gastrointestinal tract (Dahiya et al. 2020; Vuille-Dit-Bille et al. 2020). These newly released virions SARS-CoV-2 destruct more epithelial cells, induce strong immune responses, and trigger cytokine storm in patients. The viral spike protein S1 mediates the attachment of virus to the host cell membrane and S2 spike protein favors the fusion of the cell membranes. This process also needs priming by host cellular enzyme serine proteases (TMPRSS2) which enables viral S-protein cleavage, thereby controlling the entire mechanism (D'Amico et al. 2020). Evidence also suggests that ACE2 receptor has protective anti-inflammatory effects and S-protein of the virus downregulates its expression via increased release of pro-inflammatory chemokines (MCP1, IP10, and MIP1α) and cytokines (TNF, IL-1β, IL-6, IL-8, G-CSF, and GM-CSF), thereby causing inflammation and increased vascular permeability in the lungs (Bonafè et al. 2020). By integrating the proteome structural analyses with the multi-omics data, a recent study suggests that COVID-19 not only involves a direct binding of SARS-CoV-2 to ACE2 for cell entry but also causes an imbalance of various other components of RAS. The expression profile of RAS genes from cells of bronchoalveolar lavage samples of COVID-19 patients suggested significantly upregulated angiotensin, renin, MAS,

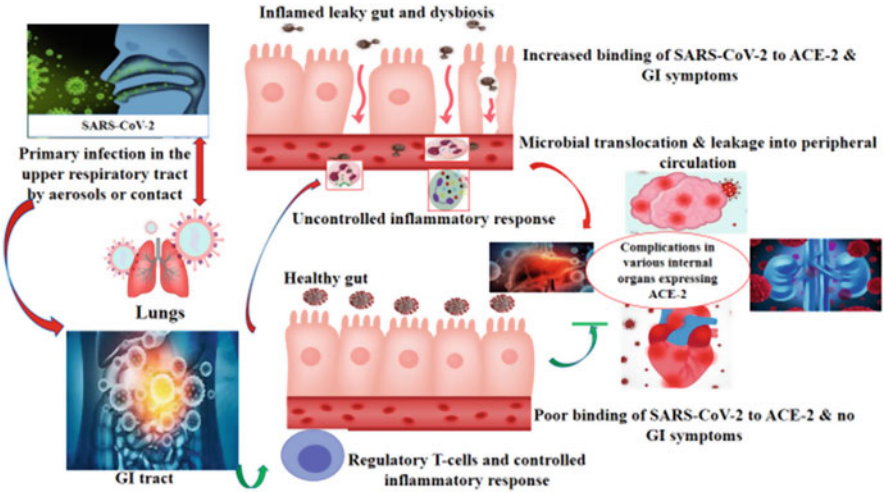

Fig 19.1 COVID-19 and gut pathogenesis

ACE, and ACE2. In addition, the expression data from several sources and tissues also suggest that the SARS-CoV-2 targets the host gastrointestinal system (Prates et al. 2020). Another study also showed presence of ACE2 receptor-rich lining in epithelial cells of the lower digestive tract (Wadman et al. 2020). Hence high level of ACE2 receptor expressing in the gastrointestinal epithelial cells along with high-level co-expression of TMPRSS2 (cellular serine peptidase) causes coronavirus to infect gastrointestinal tract along with lungs. This may lead to altered intestinal permeability and enterocyte malabsorption. Another role of intestinal ACE2 is involvement in dietary amino acid uptake and regulation of the expression of antimicrobial peptides (AMPs) to promote intestinal homeostasis (D'Amico et al. 2020). Evidences suggest that ACE2 plays a crucial non-catalytic role in the modulation of intestinal microbiota composition suggesting that the beneficial effects of ACE2 are partially mediated through the alteration in intestinal microbiome. For instance, ACE2-KO animals display altered gut microbial composition, decreased expression of AMPs, and declined levels of neutral amino acids in their serum with specific impairment of tryptophan (Trp) uptake, which can be restored by the tryptophan usage. To support this, the probiotics are shown to reduce the oxidative stress, positively alter the cholesterol levels, release vaso-deleterious ACE2-inhibiting peptides, and reduce stress-induced hyper-permeability (Cole-Jeffrey et al. 2015). ACE2 regulates innate immunity and also influences the composition of host intestinal microbiota (Perlot and Penninger 2013). Host tryptophan metabolites such as the melatonin, serotonin, and kynurenines and the bacterial tryptophan metabolites like indole, indolic acid, tryptamine, and skatole have effects on intestinal microbial composition, microbial metabolism, host immune system, host-microbiome interface, and host immune system-intestinal microbiota interactions. Thus, ACE2 has a role in regulating intestinal amino acid homeostasis,

expression of AMPs, innate immunity, and gut microbial ecology. Another pathway for virus entry inside host cell is that the S-protein of SARS-CoV-2 can bind to another surface molecule, i.e., CD147 of the host cell. CD147 is mainly found on hematopoietic cells including red blood cells (RBCs) and neuronal and epithelial cells in humans. N-protein of SARS-CoV also binds to cyclophilin A in ACE2-expressing infected host cells. To validate it was found that in vitro inoculation of human lung epithelial cells with SARS-CoV-2 produces cytopathic effects of the lung epithelial cells (Gubernatorova et al. 2020). Hence, a strategy to develop therapeutics against the SARS-CoV-2 is by blocking the ACE2 or TMPRSS2 using compounds like baricitinib and ruxolitinib for ACE2 and camostat mesylate and nafamostat mesylate for TMPRSS2 or using monoclonal antibodies targeting the S-protein of SARS-CoV-2 that may inhibit the virus entry or membrane fusion into the host cell (Tay et al. 2020). The gene expression study via mRNA sequencing has found that the SARS-CoV-2 infection elicits specific cytokines and interferon-stimulated genes (ISGs) for type I and III interferon responses (Lamers et al. 2020).

19.5 Dysbiosis

Patients of COVID-19 with gastrointestinal symptoms have illness of longer duration with delayed viral clearance time than those patients without gastrointestinal involvement. Certain factors including poor diet, inadequate sanitation, superimposed infections, and antibiotic uptake may cause dysbiosis. Dysbiosis is also caused by host factors which are decreased immune function, impaired absorption, mucosal barrier failure, and pro-inflammatory response. This dysbiosis may cause over-reactive or under-reactive immune response leading to increase in severity of the infection. A shotgun sequencing and analysis of stool samples of 15 COVID-19 patients revealed that their fecal microbiome has undergone drastic changes as compared to controls and at least 23 bacterial taxa were associated with COVID-19 severity. The amount of beneficial microbes reduced and opportunistic pathogens have drastically increased in count. The gut dysbiosis in patients has not reversed even after the clearance of SARS-CoV-2 from throat swabs. It was found that at baseline, 23 taxa of bacteria were associated with COVID-19 severity and mainly belong to phylum Firmicutes (15 out of the 23 taxa). Among these, 8 taxa had displayed a positive correlation and 7 taxa had displayed a negative correlation with the severity of disease. Coprobacillus, Clostridium ramosum, and Clostridium hathewayi were the major bacterial genera which had a positive correlation with the severity of disease (Forrester and Spain 2014). Alistipes onderdonkii and Faecalibacterium prausnitzii were negatively correlated with COVID-19 severity. Faecalibacterium prausnitzii is considered to have anti-inflammatory properties and Alistipes plays a pivotal role in the maintenance of gut immune homeostasis. Also, Bacteroides dorei, Bacteroides ovatus, Bacteroides thetaiotaomicron, and Bacteroides massiliensis had a significant negative impact on fecal viral load in SARS-CoV-2 illness. All of these organisms are associated with the downregulation of ACE2 expression in the colon (Geva-Zatorsky et al. 2017). This study has

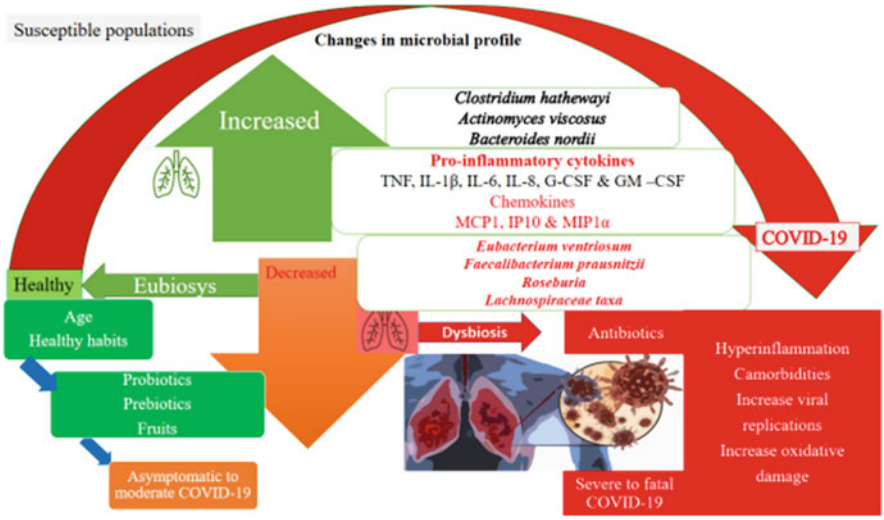

Fig 19.2 How SARS-CoV-2 virus infection causes dysbiosis

demonstrated that when the count of certain species increases, expression of ACE2 receptors is upregulated while other species downregulate the ACE2 expression in COVID-19-infected individuals. There may also be a decrease in beneficial microbial species involved in gut-immune homeostasis and generation of anti-inflammatory responses required for the suppression of COVID-19 infection. Hence, in comparison to the healthy individuals, gut dysbiosis might increase the severity of illness in COVID-19 patients. Another study with analysis of 30 patients showed that the opportunistic pathogens such as Rothia, Streptococcus, Veillonella, and Actinomyces outnumbered the beneficial organisms during COVID-19 infection in patients (Yang et al. 2020; Gu et al. 2020). Obese persons of COVID-19 have gut dysbiosis with decreased population of Bacteroides species whereas type 2 diabetics have increased Ruminococcus, Fusobacterium, and Blautia. In them there is decreased population of Bacteroides, Bifidobacterium, Faecalibacterium, Akkermansia, and Roseburia. In elderly COVID-19 patients Bacteroidetes are increased and Firmicutes are decreased (Hu et al. 2021, Fig. 19.2). In the management of patients with preexisting digestive diseases such as IBD, COVID-19 infection may mimic IBD exacerbations. Hence, IBD patients should be tested for SARS-CoV-2 before assuming it a flare. It is also assumed that IBD patients are at higher risk of developing COVID-19 complications as the ACE2 expression and activity of host cell trypsin-like proteases are increased in inflamed gut of these patients (Queiroz et al. 2020).

19.6 Diet, Probiotics, and COVID-19

The content of low fiber, high fat, and abundant carbohydrate in daily diet leads to gut dysbiosis (Trompette et al. 2014). This may change the homeostasis with alteration in immune response of an individual. A study on mice showed that intake of high-fiber diet leads to higher levels of circulating short-chain fatty acids which protects against allergic inflammation in lungs. Hence a diet rich in fiber regulates gut microbiota as well as lung microbiota which shows an effect on lung immunity (Valdes et al. 2018). Intake of whole grains, vegetables, and fruits induces growth of Bacteroides, Bifidobacterium, and Lactobacilli in the gut. Gut microbiome is more stable and achieves microbial environment resembling the adults with greater resistance towards infections by 3 years of age (Heiman and Greenway 2016). It has been found that consumption of dietary fibers lowers down the serum levels of C-reactive protein (CRP), tumor necrosis factor-alpha (TNFα), interleukin IL-6, and IL-18, and thus has an inverse correlation with disease severity. High-fiber diet lowers blood glucose level and increases plasma concentrations of adiponectin (an insulin-sensitizing adipo-cytokine with anti-inflammatory properties) (Williams 2010). Hence, a balanced diet rich in cereals, legumes, fruits, whole grains, unsaturated fatty acids, and green vegetables is generally recommended to fight against SARS-CoV-2 infection and for those who are in quarantine as well as for patients who are asymptomatic or have mild symptoms to improve their chances of recovery. Thus, COVID-19 patients should adopt a diversified diet with high-fiber and plant-based foods (Trottein and Sokol 2020) to strengthen intestinal epithelial barrier, lower down the pro-inflammatory state, and increase the intestinal motility (Trottein and Sokol 2020).

Probiotics are the nonpathogenic live organism spores which are safe and can be supplemented with diet. The major classes of probiotics belong to the genera Bifidobacterium, Lactobacillus, and Saccharomyces. Probiotics increases the activity of T cells and NK cells and phagocytic activity of polymorphonuclear cells. Hence, probiotics have a significant role in maintaining the immunogenic homeostasis of the gut. Other functions of probiotics are to maintain the pH of the intestine and to lower the invasion or colonization of the pathogens in the gut. Probiotics have been found to be helpful in the recovery against various diseases, for instance ulcerative colitis, antibiotic-associated diarrhea (AADs), as well as infectious diarrhea and hepatic encephalopathy (Wilkins and Sequoia 2017). It has been found that probiotic species like Bifidobacterium breve and Lactobacillus rhamnosus are beneficial to mice in terms of modulating the innate immune response as well as maintaining the balance of inflammatory responses in mice. Probiotics intake influences the ACE2 receptors as their microbial fermentation produces ACE inhibitory peptides leading to decreased production of angiotensin-2 (Dave et al. 2016). Human cathelicidin, LL-37, is a human antimicrobial peptide with a broad range of activities against the bacterial and viral pathogens (Mookherjee et al. 2020). The immunomodulatory and protective effect of cathelicidin upregulates the count of Lactococcus lactis (Wong et al. 2012) which can be a step ahead in helping combat the COVID-19 infection by using antimicrobial peptide-expressing probiotics

delivery system (Wong et al. 2012). Empirical use of antibiotics in the early phase of COVID-19 patients may lead to more unfavorable dysbiosis. Hence, in the early phase of COVID-19, patients can suffer from dysbiosis of gut microbiome. Therefore, intake of probiotics may restore the balance of colonic microbiota which may reduce the incidence of secondary coinfections. It was found that the dosage of probiotic species (Bacillus subtilis, Enterococcus faecalis, and Lactobacillus rhamnosus GG) to the ventilator-ridden patients with severe COVID-19 illness resulted in minimizing their ventilator requirements in comparison to placebo (Li et al. 2019). The increase in production of intestinal butyrate using probiotics is helpful to strengthen the gut epithelial cell health. The use of Bifidobacteria and Lactobacilli is considered beneficial for butyrate production. Similar role of F. prausnitzii in stimulating butyrate along with anti-inflammatory properties in the intestine has been found in facilitating the treatment for inflammatory bowel disease (Lopez-Siles et al. 2017).

Prebiotics like maize fiber and polydextrose are known to improve digestion and boost immunity via reconstructing the gut microbiome symbiosis especially among the elderly people. Prebiotics act closely in the growth and function of probiotics in the gut. Gut microbes, particularly belonging to probiotic genera, act upon prebiotics (fructan, glucan, arabinoxylan) and utilize them as their growth substrates to produce short-chain fatty acids (SCFAs), butyric acid, and propionic acid. These end products affect the differentiation or functions of T cells, macrophages, and dendritic cells. Prebiotics regulate various pro- and anti-inflammatory cytokines. They promote maturation, differentiation, and reproduction of macrophages and lymphocytes and activate reticuloendothelial cells. Complex carbohydrates found in whole grain are known to decrease the concentration of pro-inflammatory cytokines (IL-6). Likewise, butylated high-amylose maize starch has been shown to enhance the concentration of anti-inflammatory cytokines (IL-10) (Collado et al. 2018; Johnson et al. 2019). Gut microbes metabolize prebiotics and various dietary components and produce short-chain fatty acids (SCFAs), elevating their intestinal concentration, which in turn regulates the lymphoid (gastrointestinal as well as secondary) tissues (Agans et al. 2011). SARS COVID-19 virus after entering lungs activates lung immune system as well as through gut-lung axis does immune activation in gut microbiome. Healthy gut microbiome with its strong immune intervention may bring recovery in COVID-19 patients with correction of dysbiosis. Due to complex gut microbiota ecosystem, a single food item may not be helpful to dramatically shift its overall composition. The role of the Mediterranean diet has been found to boost immunity with a regular supply of dietary fibers in adequate quantity. Mediterranean diet is characterized by high consumption of whole grains, vegetables, legume, nuts, extra-virgin olive oil (rich in polyphenols), and fats mostly in the form of unsaturated fatty acids and low consumption of processed meats and refined sugars. This diet has favorable modulation of relative gut microbial abundance with diversity to maintain its homeostasis (De Filippis et al. 2016). On the other hand, paying attention to the effects of microbial-diet-host interactions, diet consisting of diversified food items such as fermented dairy products, vegetables, whole grains, and fermented soybeans naturally enriched with probiotics such as Lactobacillus bulgaricus and

Streptococcus thermophilus is vital and beneficial in numerous ways as it creates a nurturing environment that helps maintain the host immunity (Ghosh et al. 2019). At this time when various drugs are being tested for the cure of COVID-19 disease with no clear success as yet the role of probiotics in various clinical trials is being analyzed to lessen the severity and incidence of COVID-19 infection. It may be that inter-individual variation in gut microbiome will have different efficacy on suggested various interventions for using gut microbiota in our fight against SARS-CoV-2 infection. So far, the International Scientific Association of Probiotics and Prebiotics (ISAPP) has mentioned that scientists and clinicians globally are studying to gain further insights of the relationship between the healthy gut microbiome and susceptibility to COVID-19 with assessment of the role of various probiotic strains to lower the viral load in patients. A multicenter randomized, double-blind, placebo-controlled phase 2 trial is being done to assess the role of probiotic Lactobacillus rhamnosus GG in the prevention and treatment of COVID-19 infection (Hu et al. 2021). More studies are being done to understand the host immunity to overcome and recover from COVID-19 illness either via manipulating the gut microbiota composition or via enhancing vaccine response. Diversified dietary strategies directed to restore beneficial microbiota may possibly suppress viral infection in the elderly and those with underlying health problems. A far-reaching approach should consist of randomized, multicenter, controlled trials to explore the potential benefits of gut microbiome and how changes in dietary habits can be used as an add-on strategy against the COVID-19 pandemic.

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COVID-19 Pandemic and Mental Illness: Impact of Gut Microbiota

20

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Abstract

The world is currently facing a global pandemic caused by SARS-CoV-2. Although COVID-19 is primarily a respiratory illness, various studies have demonstrated the ability of this virus in spreading to extrapulmonary sites, thereby leading to multiorgan failure and eventually death in highly susceptible individuals. The scourge of the virus world over has led to a severe impact (direct and indirect) on the mental health of individuals belonging to all age groups. In this context, the role of gut microbiota in influencing mental health via the gut-brain axis holds immense significance. Recent evidences have highlighted the possible link between COVID-19 infection, gut dysbiosis, and various psychological and neurological abnormalities. Thus, maintenance of a healthy gut microbiome becomes imperative given the absence of a definite cure to such a dangerous illness as COVID-19. Various strategies such as regular intake of a healthy diet and personalized nutrition, co-supplemented with probiotics, prebiotics, and psychobiotics, should be adopted wherein gut microbiota profile can be manipulated for providing multiple benefits to the host. Religiously following such practices will not only enrich the gut with beneficial microbes and boost host immunity but also prove to be a strong prophylactic measure in reducing the incidence/severity of diseases such as COVID-19 virulence and result in improved prognosis of infected individuals.

Keywords

COVID-19 · Mental health · Gut microbiota · Probiotics · Psychobiotics

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20.1 Introduction

A pneumonia outbreak in Wuhan, Hubei province of China, in late December 2019, quickly spread to the rest of the world and was declared a pandemic by the WHO on March 11, 2020 (Wang et al. 2020). The pandemic was reported to be caused by a novel coronavirus, named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belonging to the family of RNA viruses (Lake 2020; Singhal 2020). The highly contagious virus has been known to primarily invade the pulmonary epithelium via receptor-binding interaction between spike (S) protein and angiotensin-converting enzyme (ACE2) receptors expressed abundantly on type II alveolar epithelial cells (Hoffmann et al. 2020) resulting in clinical manifestations ranging from asymptomatic, mild flu-like illness to severe pneumonia and fatal acute respiratory distress syndrome (ARDS). Interestingly, expression of ACE2 receptor can also be seen on renal cells, neuronal cells, endothelial cells, and gastric tissue, primarily intestinal enterocytes in the ileum and colon, paving the way for it to invade and occupy these extrapulmonary sites, thereby leading to multiple-organ failure and eventually death (Zhang et al. 2020a). The disease's severity depends on the age group, location, underlying comorbidities, and chronic health conditions, like diabetes, obesity, asthma, and cardiovascular diseases (Huang et al. 2020; Hassan et al. 2020).

Inspite of almost a year since its emergence, the virus continues to plague the nations world over. An overburdened healthcare setup and the crippling economic status of countries amidst the pandemic have resulted in social, emotional, and psychological impact on individuals across generations. A population's mental health and psychological state greatly influence their pandemic-fighting ability, with great implications for its incidence and transmission. The stress caused by confirmed COVID-19 cases among patients and their relatives, along with the emotional stress and anxiety experienced by the people due to implementation of strict preventive measures, has led to various short- and long-term consequences (Rajkumar 2020). In general, pandemic-associated mental health issues and their detrimental impact are often overlooked. Mental health disorders can impair host immunity and vice versa, thereby increasing the frequency of infection, resulting in permanent psychological impairment, especially among subpopulations which are at higher risk of contracting this infection (Salari et al. 2020; Wu et al. 2020).

Increasing evidence has highlighted the role of gut microbiota and its impact on mental health. Studies pertaining to neurological disorders such as Alzheimer's and Parkinson's have increasingly associated abundance/deficit of specific microbes, thereby giving more credence to this assertion. Strategies such as administration of probiotics and intake of a healthy, balanced diet directly influence the composition of the gut microbiota. Moreover, the ability of these microbes to then modulate the gut-brain axis, thereby ameliorating any adverse psychological effect experienced by the host, can provide multifaceted gains in the present scenario where a robust immune system is quintessential for combating a deadly virus such as SARS-CoV-2.

Therefore, there is an urgent need to address COVID-19-associated psychological health issues and to promote awareness programs and public mental health interventions in order to prevent the manifestation of such severe psychological consequences including depression, personality disorders, anxiety, stress, fear, denial, behavioral changes, and post-traumatic stress (Duan and Zhu 2020).

20.2 COVID-19 and Mental Health

20.2.1 Mental Health Issues Among Healthcare Professionals and Personnel

Healthcare professionals, mainly frontline doctors and workers, are at a higher risk of developing psychological distress due to increased workload, lack/extended use of personal protective equipment (PPE), lack of training to cope with the psychological crisis, overburdened healthcare facilities, regular witnessing of COVID-19 emergencies, infected patients, deaths, and fear of becoming infected and risking the lives of family members (Tsamakis et al. 2020; Zhang et al. 2020c). All this has led to a high pervasiveness of anxiety, depressive behavior, insomnia, stress, and post-traumatic stress disorder (PTSD) among the healthcare personnel (Lai et al. 2020; Carmassi et al. 2020).

20.2.2 COVID-19-Associated Psychological Issues Among Infected Patients and the General Public

Physical isolation of infected or suspected patients from family members during hospital treatment or home quarantine has been reported to result in immediate psychological problems and morbidities (Zhang et al. 2020b). This might be due to disease progression, medications, fear of viral transmission to close contacts, physical health issues, uncertainty about the treatment, and repeated exposure to devastating news (Vindegaard and Benros 2020). Simultaneously, the fear of getting infected, lack of specific treatment options, sudden lockdowns, countrywide closures, strict guidelines, unpredictability of endpoint of the disease, unemployment, socioeconomic crisis, etc. have created panic, fear, anxiety, depressive behavior, loneliness, boredom, phone addiction, and stress among the general population (Pfefferbaum and North 2020; Brooks et al. 2020). Serious restrictions on social gatherings and outings, along with work from home culture, have further resulted in sedentary lifestyle which has increased the risk of contracting lifestyle diseases such as obesity and diabetes. Social media platforms and news channels have further magnified it by repeatedly displaying reports of severely ill patients and those that succumbed to the disease, leading to an unusual syndrome known as "headline stress disorder," which refers to a highly intense emotional response (stress, anxiety) to constant reports on social media and news coverage (Dong and Zheng 2020). All this has seriously impacted the psychological health of individuals.

20.2.3 Mental Health Issues Among Elders and Those with Pre-existing Psychological Disorders

Older individuals have been reported to be highly susceptible to COVID-19 and its associated mental health impairments, due to (i) weakened immune response and (ii) underlying comorbidities due to old age. Ever since its emergence, COVID-19 outbreak has been projected as the "disease of elders" creating distress, social stigma, negativity, and age-based discrimination among elderly people, which has additionally been responsible for triggering psychiatric and psychological issues (Yang et al. 2020a; Michel et al. 2020).

COVID-19-associated shutdowns significantly reduced the availability of caregivers for elders and those with pre-existing psychological disorders. Aggressive curtailment of in-person counseling and psychotherapy sessions further intensified the psychiatric and psychological disorders among patients with established psychopathological conditions, thereby triggering panic, psychosis, aggression, self-harm tendencies, and suicides (Pan et al. 2021; Gautam et al. 2020). Mental health patients constitute a highly vulnerable group as they have a low life expectancy and poor health conditions, putting them at a higher risk of contracting COVID-19 infection and comorbidities associated with COVID-19 (Pinkham et al. 2020).

20.2.4 Mental Health Issues Among Adolescents and Young Children

Adolescents and children comprise an extremely vulnerable group. During the initial phase, they had been continuously exposed to stressful situations, such as strict lockdown, forceful stays at home, reduced physical activity, social distancing, and detachment from peers (Loades et al. 2020). Subsequently, the closure of schools and universities further impacted their daily routine, creating social gaps and behavioral changes, boredom, loneliness, and phone addiction that strongly impact mental health resulting in anxiety, depression, and distress among adolescents and young children, particularly those with intellectual disabilities (Liu et al. 2020).

20.2.5 Mental Health Issues Among Homeless People, Migrants, and Daily-Wage Workers

The pandemic has been extremely unfortunate for the homeless populations and unemployed workers inflicted with health and economic crisis due to sudden lockdown. Majority of them being already subjected to chronic mental and physical health issues have had reduced access to healthcare facilities (Liem et al. 2020). COVID-19-induced stressful conditions further exacerbated the already existing psychological issues and in certain cases induced new psychological disorders among this population (Tsai and Wilson 2020; Subbaraman 2020).

In such conditions, there are growing evidences that indicate the possibility of managing such stress-induced mental illnesses by a healthy gut microbiome.

20.3 Gut Microbiota and Mental Health

Gut microbiota refers to the complex and dynamic community of microbes, particularly bacteria, that colonize the gastrointestinal tract (GIT) of humans. The microbial phyla *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* dominate the gut microbiota of healthy individuals. Colon harbors the most significant amount and diversity of bacteria, most prevalent being the *Firmicutes* (*Eubacterium*, *Lactobacillus* spp.) and *Bacteroidetes* (*Bacteroides*, *Prevotella* spp.) (Thursby and Juge 2017; Villanueva-Millán et al. 2015). Several studies have revealed an association between the gut microbiota and mental well-being of the host. In this regard, the gut-brain axis (GBA) holds special significance. The latter is a complex network of interaction that results in bidirectional cross talk between the gut and the central nervous system (CNS). This axis, along with the host gut-microbiota, plays an essential role in maintaining GI tract and brain homeostasis and has been proven to be crucial for the host's psychological well-being (Cryan et al. 2019; Martin et al. 2018). The enteric nervous system (ENS), also known as "second brain of the body," is a branch of the autonomic nervous system and communicates with CNS through various signaling pathways that can be modulated by the gut microbiota (Rao and Gershon 2018; De Vadder et al. 2018). The route of communication involves neural pathways, particularly vagus nerve and enteric nervous system (ENS), chemical pathways (neuroactive compounds), neuroendocrine pathways (gut hormones, hypothalamus-pituitary-adrenal (HPA) axis), and immunological pathways (cytokines, chemokines) (Carabotti et al. 2015). These molecules along with microbial metabolites, such as SCFAs (butyric acid, propionic acid), interact with the host immune system, enter the circulation, or act on ENS and vagus nerve that relay signals to the CNS, and thus influence the host behavior (Dalile et al. 2019). In this context, various studies have shown that patients suffering from depression often display a significant change in the gut microbiota composition (Sherwin et al. 2018). Such patients have an abundance of *Prevotella*, *Klebsiella*, *Enterobacteriaceae*, and *Alistipes* in their gut and the abundance of SCFA producers belonging to *Lachnospiraceae* family and beneficial bacteria such as *Lactobacillus*, *Bifidobacteria* spp., and butyrate producers (*Faecalibacterium*, *Coprococcus*) have been found to be reduced in such patients (Valles-Colomer et al. 2019; Huang et al. 2019). Studies have indicated that gut dysbiosis has a negative impact on GBA resulting in hyperactivation of immune system resulting in a very high concentration of pro-inflammatory cytokines and dysregulation of HPA axis, which is known to be associated with major depressive disorders (Bastiaanssen et al. 2020). In patients suffering from general anxiety disorder (GAD), an increase in the abundance of *Fusobacterium*, *Escherichia-Shigella*, and *Ruminococcus* and reduction in *Faecalibacterium*, *Sutterella*, and *Eubacterium* have been observed (Jiang et al. 2018; Yang et al. 2019). Similarly, the gut microbiome of patients suffering from

post-traumatic stress disorder (PTSD) has an abundance of *Enterococcus*, *Escherichia*, and *Shigella*, while the count of *Actinobacteria*, *Lentisphaerae*, *Verrucomicrobia*, and beneficial microbes *Ruminococcaceae* and *Lachnospiraceae* is greatly reduced (Bajaj et al. 2019). Gut microbiota regulates the secretion of serotonin produced by chromaffin cells of gastrointestinal tract. Serotonin and other neurotransmitters, dopamine, and gamma-aminobutyric acid (GABA) affect the sleep quality and regulate the normal sleep-wake cycle. It is observed that disturbances in gut microbiome impact the functions of CNS, disturb the circadian rhythm, and can cause insomnia (Li et al. 2018).

The gut microbiota also plays a role in neurodegenerative disorders, namely Alzheimer's disease with studies highlighting their role in the accumulation of amyloid plaques (Vogt et al. 2017; Harach et al. 2017). Individuals with AD display a shift in the gut microbiota composition with an increased abundance of *Bacteroidetes*, *Escherichia*, and *Shigella* spp. associated with increased expression of amyloid proteins in AD and decreased abundance of *Firmicutes*, *Eubacterium rectale*, and *Bifidobacterium* spp. (Cattaneo et al. 2017). Studies on different animal models of AD have demonstrated marked changes in gut microbiota and its effect on amino acid metabolism (Wang et al. 2019), learning and memory (Mezö et al. 2020), and disease progression and severity (Minter et al. 2016). Likewise, the association between gut and Parkinson's disease, which is the second most common neurodegenerative disorder after AD, was postulated by Braak's hypothesis, which suggests that before affecting the brain, the pathology of PD starts in the gut in some cases (Braak et al. 2003) and further studies have elaborated on this concept with reports on GI symptoms and early accumulation of phosphorylated α-synuclein in the ENS and vagus nerve before they appear in brain (Challis et al. 2020; Kim et al. 2019). Furthermore, recent studies have shown that individuals with PD have altered composition of gut microbiota and metabolic profiles (tryptophan and SCFAs) along with loss of commensals linked with anti-inflammatory properties and abnormal levels of *Prevotellaceae*, *Faecalibacterium*, and *Ralstonia* and increased levels of *Enterobacteriaceae* (Barichella et al. 2019; Cenit et al. 2017; Scheperjans et al. 2015; Bedarf et al. 2017; Unger et al. 2016; Sampson et al. 2016). Similar studies in patients suffering from autism spectrum disorder (ASD) have also reported a deviation from the normal gut microbiota with alterations in the Bacteroidetes/Firmicutes ratio, and *Prevotella* and *Clostridium* (Shaaban et al. 2018), and an increased abundance of *Clostridium*, *Roseburia*, and *Barnesiella* and decreased abundance of *Faecalibacterium*, *Prevotella*, *Dialister*, and *Bifidobacterium* in ASD patients (Bundgaard-Nielsen et al. 2020). Likewise, for multiple sclerosis (a disease characterized by degeneration of neuronal signaling), the fecal specimens from the patients reveal alterations in the abundance of *Akkermansia*, *Blautia*, *Pseudomonas*, *Dorea*, and *Mycoplasma* spp. as compared to healthy individuals (Mangalam et al. 2017). Thus, gut microbiota plays a significant role in various mental health conditions ranging from stress and mood disorders to more severe neurodegenerative diseases.

20.4 Gut Microbiota and COVID-19

Several studies have brought attention to the composition and possible association of gut microbiota with the incidence, severity, and outcome of COVID-19 cases. At the onset of illness, patients manifest mild flu-like symptoms like fever, sore throat, chest pain, headache, body ache, cough, cold, loss of taste and smell, difficulty in breathing, and fatigue (Huang et al. 2020). However, various studies have shown that about 5–10% of infected individuals also exhibit gastrointestinal symptoms, such as diarrhea, emesis, nausea, anorexia, and abdominal pain, possibly due to the intestinal infection caused by SARS-CoV-2 as the virus can invade and replicate within the enterocytes displaying ACE-2 receptors (Villapol 2020; Jin et al. 2020). Additionally, viral RNA has also been detected in the fecal specimen of COVID-19 patients (Wu et al. 2020; Lin et al. 2020). In a study conducted by Lin et al. (2020) among the 95 evaluated cases of COVID-19, 58 cases presented GI symptoms, and fecal specimens of 52.4% patients were tested positive for viral RNA. RNA from SARS-CoV-2 was also found in rectum, duodenum, esophagus, and stomach of severely affected patients. It has been concluded that GI tract might be an extrapulmonary target site and a possible transmission route for SARS-CoV-2. It has also been observed that the most severely affected individuals, i.e., older people (>60 years) and those with underlying comorbidities (chronic inflammation, hypertension, cardiovascular diseases, obesity, diabetes mellitus, etc.), have a higher prevalence of gut dysbiosis and reduced diversity of commensal microorganisms (Roncon et al. 2020; Siordia Jr 2020). Gut microbiota composition is dynamic and changes in response to various factors like nutrition, lifestyle, genetics, location, environment, age, antibiotic intake, and stress (Thursby and Juge 2017; Hasan and Yang 2019). Gut dysbiosis refers to imbalance or alteration in the composition of normal gut microbiome and its associated functions. It has been closely linked to many immune-related and multifactorial diseases, such as inflammatory bowel disease (IBD), diabetes mellitus, and cardiovascular diseases, and as per recent reports might play a role in disease severity of COVID-19 patients as well (Xu et al. 2020).

SARS-CoV-2 interactions with gut microbiome can affect the functions of host immune system and enhance the production of pro-inflammatory cytokines that incite cytokine storm, thereby leading to higher induction of immune cells. Exaggerated immune response can damage lungs and other organs, leading to multiple-organ failures and, eventually, death (Yang et al. 2020c). Dysbiosis also increases the host's susceptibility to secondary infections, further worsening the clinical status of patients (Hanada et al. 2018). Therefore, a healthy gut microbiome is essential to generate an optimal immune response and to effectively fight infections. Examination of alterations in gut microbiome of 15 COVID-infected patients compared with 6 pneumonia controls and 15 healthy controls (HCs) by Zuo et al. (2020) revealed that in comparison to HCs, the fecal microbiome of COVID-19 patients was enriched with opportunistic pathogens (*Clostridium hathewayi*, *Actinomycetes viscosus*, and *Bacteroides nordii*) and deficient in beneficial symbionts (*Faecalibacterium prausnitzii*, *Eubacteria*, and *Lachnospiraceae*

bacterium). In fact, the gut dysbiosis was found to persist even after the clearance of SARS-CoV-2 from previously infected patients, which indicates that COVID-19 can cause a long-term detrimental impact on the composition and functions of gut microbiome. Further, it was seen that 23 bacterial taxa were significantly linked with COVID-19 severity, out of which 15 belonged to phylum *Firmicutes*. *Coprobacillus*, *Clostridium ramosum*, and *Clostridium hathewayi* presented a positive correlation with COVID-19 severity, while *Faecalibacterium prausnitzii* with anti-inflammatory properties and *Alistipes onderdonkii* known to maintain gut-immune homeostasis were found to be inversely associated with disease severity. Also, *Bacteroides dorei*, *Bacteroides massiliensis*, *B. ovatus*, and *B. thetaiotaomicron*, which are known to suppress ACE2 expression in the murine gut, exhibited a negative correlation with fecal SARS-CoV-2 viral loads, while *Firmicutes* spp. demonstrated a strong positive correlation with them (Zuo et al. 2020). In another similar study conducted by Yeoh et al. (2021) to decipher gut microbiome's association with dysfunctional immune responses, disease severity, and outcomes in 100 COVID-infected patients, commensal microorganisms such as *Bifidobacteria* spp., *Eubacterium rectale*, and *Faecalibacterium prausnitzii*, known to exhibit immunomodulatory functions linked to anti-inflammatory host responses, were reduced, and levels of inflammatory cytokines, chemokines, and blood markers, such as IL-8, -6, and -10 and C-reactive proteins (CRP), were elevated in infected patients. All these studies reflect the association of gut microbiota composition with the magnitude of host immune response in COVID-19; an overaggressive response can cause widespread tissue damage which in turn regulates the magnitude of disease severity (Yeoh et al. 2021).

Although the mechanism of neurological damage caused by SARS-CoV-2 remains unclear, it is most likely that the SARS-CoV-2 approaches and affects the brain via this microbiota-gut-brain axis (Esposito et al. 2020). In addition to the pulmonary alveolar epithelial cells, ACE2 viral receptors are also expressed on the cells of gut epithelia and enteric nervous system (ENS) (Deffner et al. 2020). After the ACE2-mediated entry of SARS-CoV-2 into the intestinal epithelial cells, it can alter the composition of gut microbiota, which can affect the integrity, permeability, and functions of the intestinal epithelial barrier and blood-brain barrier, thereby allowing the entry of intestinal bacteria, microbial toxins, and metabolites into the brain via blood circulation, finally affecting the brain function (He et al. 2020; Cryan et al. 2019). In the intestine, SARS-CoV-2 can also invade ENS via ACE2 receptors, reach the brain through the vagus nerve, and affect CNS functions. ENS injury can lead to abnormal intestinal blood flow and damage the gut barrier causing leakage of toxins and bacterial metabolites into blood, further increasing the brain damage (Deffner et al. 2020). Although the former mechanisms have been proposed, the exact mechanism of neuroinvasion of SARS-CoV-2 remains unclear, and further investigations are required.

20.5 Diet-Induced Composition of Microbiota and COVID-19

The importance of diet in shaping the gut microbiome remains undisputed. Therefore, maintenance of a healthy balanced gut microbiome through regular intake of healthy diet, supplemented with personalized nutrition, becomes essential. The SARS-CoV-2 pandemic has made us realize the importance of home-cooked food and a balanced diet enriched with macronutrients (proteins, fats, and carbohydrates), polyphenols, dietary fibers, vitamins, minerals, and trace metals (zinc, iron, etc.) that enhances the growth of "good bacteria" in the gut and maintains gastrointestinal symbiosis, which strengthens the gut-immune axis and boosts host immunity (Rishi et al. 2020). The latter helps the host in fighting various infections including COVID-19 by reducing severity, thereby resulting in better prognosis and outcome of such clinical manifestations. It has been observed that the consumption of plant-based fiber-rich diet promotes the growth of *Bifidobacteria*, lowers the Firmicutes-by-Bacteroides (F/B) ratio, and maintains a more diverse and stable gut microbiome within the host (Avila-Nava et al. 2017). Among macronutrients, intake of carbohydrates like oligofructose and arabinoxylan enriches the gut with probiotic bacteria such as *Bifidobacteria* and *Lactobacillus*, influences F/B ratio, and confers various health benefits to the host. Consumption of unsaturated fats reduces the count of harmful microorganisms (e.g., *E. coli*, *Streptococcus* spp.) and promotes the growth of beneficial microbes, such as *Akkermansia* and *Bifidobacteria* in the gut. In contrast to this, high intake of saturated fats and oil-based food reduces the abundance of beneficial bacteria (*Faecalibacterium*) and increases the F/B ratio, which can promote pro-inflammatory responses in the host. Therefore, the types of fats consumed and their quantity both are essential to maintain the gut microbiome health (Yang et al. 2020b). Likewise, the quality and quantity of proteins affect the balance of gut microbiota. It has been reported that the consumption of animal-based proteins, such as fish, eggs, and meat, increases the abundance of deleterious microbes in the gut. Whey protein, when consumed in low concentration, enhances the growth of *Bifidobacteria* and high concentration of whey protein reduces the abundance of *Bifidobacteria*. The intake of vitamin and mineral supplements or food items enriched with zinc, such as nuts; milk products (Zackular et al. 2016) and vitamin-rich foods, such as lemon, spinach, broccoli (enriched with vitamin C); eggs and fish (rich in vitamin D); and almonds, broccoli, and olive oil (rich in vitamin E) can maintain gut symbiosis by enhancing the growth of beneficial bacteria such as *Lactobacillus* and *Roseburia* spp. and lowering the F/B ratio (Yang et al. 2020b).

Whole grains are rich in antioxidants, nondigestible carbohydrates (resistant starch and fibers), oligosaccharides, and carbohydrates. Nondigestible carbohydrates undergo fermentation by resident microbes in the colon and produce short-chain fatty acids (SCFAs); therefore the consumption of whole grain-rich diet confers various health benefits and helps in disease prevention (De Filippis et al. 2016; Den Besten et al. 2013). The consumption of raw vegetables, fruits, cereals, drinks such as wine and coffee, being rich in polyphenols (e.g., phenol, flavonoids), inhibits the growth of pathogenic bacteria, such as *H. pylori* and enriches the gut with beneficial bacteria such as *Faecalibacterium, Lactobacillus, and Akkermansia* (Williamson

2017). It is suggested that the consumption of a balanced, home-cooked diet enriched with raw vegetables and fruits rich in antioxidants, micronutrients, and minerals helps reduce the chances of getting infected with SARS-CoV-2. Healthy nutrition and intake of such functional food help in maintaining gut symbiosis, inversely correlate with the level of inflammatory cytokines, and boost host immunity, which is a potential weapon against the SARS-CoV-2 infection. In addition to this, synbiotics (probiotics and prebiotics) are considered as key modulators of gut microbiota composition and offer a promising prophylactic approach against COVID-19 (Rishi et al. 2020).

20.6 Strategies for Manipulation of Host Gut Microbiota

20.6.1 Probiotics as Immune Enhancers

Supplementation of probiotics is yet another option to bring about desired changes in the composition of gut microbiota. Probiotics are live microorganisms which when administered in adequate quantity confer health benefits to the host by restoring a healthy gut microbiota. They are generally recognized as safe (GRAS) and can be consumed in the form of fermented foods such as cultured milk products, yoghurt, and kefir or supplements (Wieërs et al. 2020). Major probiotic genera that colonize the gut of healthy individuals are *Bifidobacteria* (e.g., *B. breve*, *B. longum*, *B. bifidum*) and *Lactobacillus* (e.g., *L. acidophilus*, *L. plantarum*, *L. fermentum*, *L. paracasei, L. rhamnosus*). Probiotics act as immunomodulators, maintain immunogenic homeostasis, restore gut symbiosis, strengthen the intestinal epithelial barrier, and prevent GI tract infections by competing with pathogens (Mak et al. 2020; Wilkins and Sequoia 2017). Recent studies have also substantiated the role of probiotics in ameliorating the pathological, physiological, and psychological conditions of mice suffering from *Salmonella*-induced brain infection by manipulating various aspects of the GBA (Kaur et al. 2020). Similarly, probiotics have been used in the treatment of antibiotic-associated diarrhea and different chronic inflammatory diseases (Mortaz et al. 2013). The possible mechanism of action of probiotics against SARS-CoV-2 may include the attachment of probiotics to epithelial surfaces, thereby causing steric hindrance and thus blocking the binding of virus to ACE2 receptors (Singh and Rao 2021).

The efficacy of probiotics has been studied previously against various respiratory tract infections caused by viruses and it has been observed that administration of probiotic bacteria reduces the incidence and severity of viral RTIs. A study by Ji et al. (2019) demonstrated that administration of probiotics to mice infected with respiratory syncytial virus (RSV) increased the abundance of SCFA producers in the gut leading to higher SCFA production, which in turn promoted the growth of *Lactobacillus* and *Corynebacterium* spp. in lungs and activated pulmonary immunity. Verma et al. (2019) demonstrated that expression and secretion of human ACE2 receptors by *L. paracasei* and binding of these secreted receptors with spike (S) proteins of SARS-CoV-2 could inhibit viral invasion and thus reduce the chances

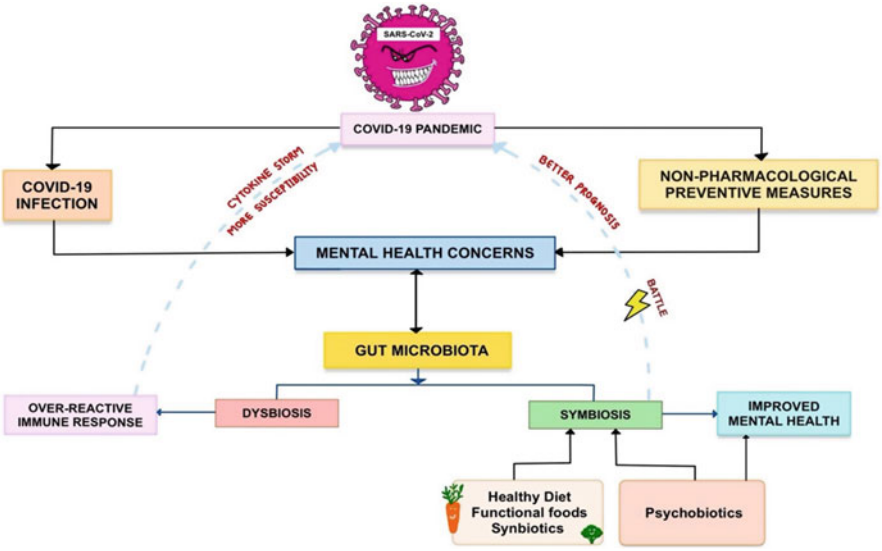
of COVID-19 infection (Rizzo et al. 2020). Likewise, it has also been observed that some microbial fermentations produce peptides with ACE2 inhibitory activity and thus probiotics may influence the ACE2 receptors that are utilized by SARS-CoV-2 for invasion, which can prevent serious COVID-19 infection (Li et al. 2019). Recently, Fanos et al. (2020) reported that probiotics comprising *Bifidobacteria* and *Lactobacillus*, particularly *L. gasseri*, can be used as a therapeutic measure against SARS-CoV-2 infection (Fanos et al. 2020). Singh and Rao (2021) proposed that *L. rhamnosus*, *B. bifidum*, *L. gasseri* SBT2055, *Bacillus subtilis*, *L. casei* DK128 (based on clinical studies in mice), *L. fermentum*, *B. lactis* Bb12, *L. rhamnosus* GG, *L. casei*, *B. infantis*, *L. reuteri* ATCC 55730, and *L. paracasei* (based on clinical studies in humans) can be further explored as a promising strategy in the management of SARS-CoV-2 infection. In a recent study by Li et al. (2021), analyzing the effect of probiotics on 311 severely infected COVID-19 patients, it was found that probiotics were effective in enhancing immune functions and simultaneously reducing the incidence of secondary infections in such patients (Li et al. 2021).

All these findings suggest that probiotics can be used as promising immunity boosters and a prophylactic strategy to reduce the severity and shorten the duration of COVID-19 disease (Arshad et al. 2020). However, due to the paucity of research evidence and clinical data, the prospect of using probiotics and its effect on COVID-19 still remains uncertain. In the wake of absence of a proven antiviral therapy against SARS-CoV-2, prevention is the only measure; therefore, intake of different types of immunity-boosting foods, probiotics, and prebiotics would provide immense benefit to the host along with protection from the disease.

20.6.2 Probiotics as Psychobiotics

In addition to healthy dietary habits, co-supplemented with probiotics, a special class of probiotics called "psychobiotics" might offer a possible solution to ensure psychological well-being of individuals in the given COVID situation. Psychobiotics are live organisms which when administered to host in adequate amount deliver mental health benefits with a potential to treat various neurological and neuropsychiatric disorders (Dinan et al. 2013). The term psychobiotics has now been expanded to include prebiotics (indigestible food materials that promote the growth of bacteria with psychobiotic potential) and other dietary supplements, exercise, and antipsychotics that indirectly modulate the gut microbiota and support the psychobiotic microbes (Sarkar et al. 2016). The most commonly used microorganisms with psychobiotic potential are *L. reuteri*, *L. rhamnosus*, *L. helveticus*, *L. acidophilus*, *L. plantarum*, *L. paracasei*, *L. bulgaricus*, *L. lactis*, *L. gasseri*, *B. longum*, *B. bifidum*, *Clostridium butyricum*, *Streptococcus thermophilus*, and *Bacillus coagulans* (Cheng et al. 2019; Gualtieri et al. 2020). Psychobiotics affect the CNS function by regulating various aspects of GBA and promote psychological well-being by synthesizing or inducing the synthesis of a variety of neurotransmitters via the metabolism of indigestible fibers, such as GABA, serotonin, norepinephrine, acetylcholine, dopamine, glucagon-like

peptide-1 (GLP-1), peptide tyrosine tyrosine (PYY), neuropeptide Y (NPY), GLP-2, SCFAs, and anti-inflammatory cytokines (Dinan et al. 2015; Cheng et al. 2019; Ansari et al. 2020). Thus, they can be used to treat mood disorders, sleep disorders, depressive behavior, anxiety, and various other psychiatric health issues (Yamamura et al. 2009; Miyazaki et al. 2014). In a preclinical study conducted by Allen et al. (2016) it was observed that administration of *B. longum* 1714 led to stress reduction and improvement in memory and behavioral and physiological response in stressed mice. Other studies have also reported similar findings wherein administration of *Faecalibacterium prausnitzii* in one and *B. breve* CCFM1025 in the other resulted in significant anxiolytic and antidepressant effect along with restoration of microbial symbiosis (Hao et al. 2019; Tian et al. 2020). Clinical studies have also shown that 49 male university students aged between 18 and 22 years recovered from fatigue, anxiety, and depressive behavior on daily administration of *L. gasseri* CP2305 (Sawada et al. 2019) and similar observations were recorded after daily administration of a combination of *L. helveticus* R0052 and *B. longum* R0175, thereby substantiating the findings (Messaoudi et al. 2011). All these findings suggest that psychobiotics can be used as a potential therapeutic approach to ameliorate the mental health of patients suffering from such diseases.



20.7 Concluding Remarks

After a year since its emergence, the spread of SARS-CoV-2 remains unabated. In spite of the tremendous amount of effort, resources, and lightening-paced research, the treatment to this disease remains far beyond the horizon and prophylactic

measures such as vaccines have not proven to be foolproof. In this scenario, the most vital armor that can be harnessed against such deadly diseases is the natural host immunity and strategies that can help in immune system augmentation and further strengthen the physiological, pathological, and psychological conditions of the host. Herein, daily intake of a healthy, balanced diet co-supplemented with probiotics/synbiotics/psychobiotics can enhance the beneficial gut microbiome and thereby provide multiple benefits via the gut-brain axis. The notion "We are, what we eat" seems to have come a long way, in reducing the incidence, severity, and associated mental illnesses, thereby emphasizing the importance of revisiting our traditional food habits. More such strategies should be designed that provide holistic advantages to the host which can help in testing times such as the ones experienced during the ongoing COVID-19 pandemic.

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The Psychological Impact of COVID-19

21

Sandeep Grover and Swapnajeet Sahoo

Abstract

COVID-19 pandemic has emerged as one of the most dreadful nightmares which humanity had to witness in the recent times. The pandemic has affected every human being in one way or the other. The psychological impact of COVID-19 on general public includes uncontrolled fear and pervasive anxiety of getting infected and getting quarantined or admitted in COVID care facilities alone, leading to disabling loneliness, frustration, and boredom due to reduced socialization, monotonous daily routine, and worries related to income and livelihood. The pandemic has led the healthcare workers to work beyond normal working hours for months together. They have been going through the feelings of anxiety, and fear of getting infection from the patients while discharging their duties. The frontline healthcare workers are also facing issues like remaining isolated during their duty periods, undergoing quarantine for several weeks, remaining away from their families, and staying alone in hotels/accommodation provided by the employers. Similarly the pandemic has led to significant negative impact on the life of women, children and adolescents, elderly, and persons with various illnesses. As the pandemic is about to stay for a while, it is expected that there can be more long-standing mental health effects of the pandemic on human beings in the near future. Therefore, strengthening mental health resources, taking timely mental health support and help from mental health professionals, destigmatizing mental illnesses, and implementing the awareness programs of mental health are the need of the hour.

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Keywords

COVID-19 · Psychological impact · Depression · Anxiety

21.1 Introduction

The COVID-19 pandemic can be said to be one of the most dreadful nightmares which humanity had to witness in recent times. It is now well known to every individual how the COVID-19 pandemic started, progressed, the havocs it created for months together and how it is still continuing in many parts of the world. Such is the infectivity potential of the SARS-n-CoV-2 virus that it brought the entire world to a standstill, i.e., complete lockdown in many countries. The COVID-19 pandemic challenged and tested the strength of the economy, financial and healthcare system capability, and social empowerment of every country (developed as well as developing). It was seen that even the most powerful and developed countries had to face several crises due to the ongoing pandemic. The entire burden of the COVID-19 screening, treatment, seclusion/isolation/quarantine, and management was shouldered by the health care workers (HCWs) and security forces of every country. Several drastic decisions were taken by the government and public health authorities to control the spread of the infection and to safeguard the public at large. One such decision was lockdown of the country, i.e., restriction of movement of people and closure of shopping malls, offices, schools, institutions, business sectors, etc. The people were forced to stay indoors with minimum movement outside their homes and many psychological consequences of the lockdown came to the limelight in few weeks to months of lockdown.

The pandemic affected different group of people in one way or the other. In subsequent sections, we would examine the psychological impact of the pandemic on different groups in the society. The psychological impact of COVID-19 on general public can be understood by the uncontrolled fear and pervasive anxiety of getting infected, getting quarantined or admitted in COVID care facilities alone, which led to disabling loneliness, frustration, and boredom due to reduced socialization, monotonous daily routine, worries related to income and livelihood, significant loss due to closure of business and travel, etc.(Serafini et al. 2020).

21.1.1 General Public

The COVID-19 pandemic situation evolved very rapidly and drastically altered people's lives in multiple aspects, i.e., social, personal, and economical. The people were forced to stay indoors with immediate effect from a certain date in most of the countries, i.e., lockdown was imposed. Initially, the lockdown measures were not welcomed by the general public and therefore the governments had to take severe steps to control public movements forcefully with the help of security forces and imposed monetary fines for not following COVID-19 protocols (social distancing,

wearing masks, travelling outside, etc.). Further, there were rising levels of misinformation driven by fear of contamination, stigma, and blame due to prevailing myths in the society (Boston 677 Huntington Avenue 2020; Sahoo et al. 2020a) which created lots of chaos and violence against HCWs engaged in contact tracing (Devi 2020). Further, there were anti-religious sentiments propagated against a specific community gathering event, which aggravated more stigma in the general public (Devi 2020). As the duration of lockdown progressed from initial 1 month to several months (5–6 months), the impact of the lockdown on the psyche of the general public gradually started to appear. An online survey conducted under the aegis of the Indian Psychiatric Society in the month of April 2020 revealed that about two-fifths (40.5%) of the surveyed people were experiencing common mental disorders (depression—10.5% and anxiety—38.5%) due to lockdown and the prevailing pandemic situation (Grover et al. 2020a). A systemic review ($n = 19$ studies) of available studies from different parts of the world reported relatively high rates of symptoms of anxiety (6.33–50%), depression (14–48%), post-traumatic stress disorder (PTSD) (7–53%), and psychological distress (34–38%) in the general population during the COVID-19 pandemic in eight countries (China, Spain, Iran, Italy, the USA, Turkey, Nepal, and Denmark) (Xiong et al. 2020). The review also identified the common risk factors associated with mental distress during the pandemic, some of which were female gender, younger age, having chronic medical illnesses or psychiatric illness, unemployment status, student population, and frequent exposure to COVID-19-related news or social media (Xiong et al. 2020). Many reports of suicides and self-harm attempts were reported from several parts of India (Sahoo et al. 2020b; Thakur and Jain 2020) and the world during the lockdown period and prevailing pandemic, which were attributed to the ongoing stress and COVID-related anxiety which were quite alarming and have been a matter of concern, which requires increasing mental health awareness and interventions for the general public(Gunnell et al. 2020; Hawton et al. 2021). The general public also had to go through the grief and separation from loved ones who succumbed to the COVID-19 infection (Ingravallo 2020).

21.1.2 Healthcare Workers (HCWs)

The main burden of management of patients infected with COVID-19 infection fell on the healthcare workers. Starting from screening, contact tracing, treating the sick patients, and looking after needs of the quarantined patients, the healthcare workers had to work beyond normal working hours for months together. Additionally, there were problems related to the usage of personal protective equipment (PPEs) for a long duration. They have been going through the feelings of anxiety, restlessness due to PPEs, and fear of getting infection from the patients while discharging their duties. The frontline HCWs faced many other issues, such as they had to remain isolated during their duty periods; had to undergo quarantine for several weeks; were unable to meet their families; and had to stay alone in hotels/accommodation provided by the employers. All these further added to the burden of caring for

COVID-19 patients. Many doctors, nurses, and other paramedical staff also acquired the COVID-19 infection while discharging their duties and had to get admitted in COVID care facilities. Across the world, there have been several reports of deaths of HCWs who were actively involved in patient care during the pandemic (Green 2020; Kapoor and Kapoor 2020; Zhan et al. 2020). It is estimated that in India, till September 10, 2020, 2714 doctors got infected with the COVID-19 infection and 382 died due to the viral infection, which implied a case fatality rate of 16.7% among Indian doctors, which is 10 times higher than the case fatality rate of the general population, i.e., 1.7% (Kapoor and Kapoor 2020). Several studies from across the world have also revealed a great degree of perceived stress (29–63%), depression (12–55%), anxiety (24–68%), PTSD, and insomnia among the HCWs (Vizheh et al. 2020). Some of the risk factors identified for COVID-19-related psychological impact among the HCWs include working in a high-risk department such as anesthesia and critical care, having a family member diagnosed with COVID-19 infection, improper use of PPEs, close contacts with patients (>12 times/day), long daily contact hours, and unprotected exposure to COVID-19 patients (Shaukat et al. 2020). Nurses, female HCWs, and younger medical staff reported greater psychological distress than other HCWs (Vizheh et al. 2020). An Indian study found one in every seventh HCWs involved in COVID-19 patient care to be suffering from diagnosable mental disorder (Mehra et al. 2020a). Due to all these, COVID-19 pandemic has been rightly labelled as a bigger crisis for HCWs than that for the public (Grover et al. 2020b). All these factors led to widespread feeling of burnout and emotional as well as physical exertion in the HCWs which can have long-standing effects (Launer 2020). Therefore, adequate psychological first aid for the HCWs is the need of the hour (Gupta and Sahoo 2020).

21.1.3 Sanitary Staff

As the only effective method to prevent the spread of infection was thorough maintenance of proper hand hygiene and cleanliness in the environment, all over the world, the sanitary staff and housekeeping staff have been engaged in cleaning and sanitization of roads, buildings, offices, hospitals, etc. since the beginning of the pandemic. This group had their own concerns related to the pandemic. Some of these people came from a low socioeconomic background, and had been staying in crowded places/homes, which increased their risk of getting infected and transmitting the infection to others. They had greater risk of infection while handling biomedical waste materials related to COVID patient care, and difficulty in understanding the various aspects of using the PPEs. A survey done at Post Graduate Institute of Medical Education and Research, Chandigarh, showed that a substantial proportion of sanitary staff had mild anxiety (11%), mild depressive symptoms (21%), and several negative emotional states as well as family-related concerns while performing COVID-19 duties (Sahoo et al. 2020c).

21.1.4 Security Staff/Police

Security/police services played a very significant role in controlling the pandemic worldwide. To keep the lockdown strategies effective, the police personnel were carried out extra duties in addition to their usual duties of maintaining law and order. They were given responsibilities to ensure strict movement restrictions of public, avoid crowding at public places and market areas, and ensure the safety of HCWs on duty, etc. round the clock with the added fear of getting infected with COVID-19 infection. All these were quite stressful and had a significant impact on the mental health of the police personnel during COVID-19 pandemic. Further, there have been few reports of violence against police personnel while delivering COVID-19 duties (Grover et al. 2020c). A study from India, which evaluated the psychological impact of COVID-19 duties during lockdown on police personnel ($n = 623$) from Northern part of India, reported that about 10% of police personnel had significant anxiety, 18% had significant depressive symptoms, and many reported high level of perceived stress (Grover et al. 2020c).

21.2 Special Populations

21.2.1 Women

The impact of the pandemic on women mental health has been multifaceted. Available data suggest that, compared to males, females have experienced significantly higher level of stress, anxiety, depression, and post-traumatic stress symptoms in most of the studies (Ahmed et al. 2020; Wang et al. 2020). Many studies have shown that females had a greater risk of developing psychiatric problems (anxiety, depression, PTSD, insomnia, loneliness, etc.) (Li and Wang 2020; Liu et al. 2020a) during the pandemic. Worries related to pregnancy and childbirth during the pandemic added extra psychological burden to the ongoing stress of lockdown. Pregnant women have been more preoccupied with issues such as vulnerability to infection during pregnancy, increase in pregnancy-related complications if infected with COVID-19 during pregnancy, fear of transmission of infection to the fetus/baby during labor and childbirth, fear of getting infected during antenatal hospital visits, deciding about the place of delivery and mode of delivery, etc. (Almeida et al. 2020). Taking care of all family members during lockdown, attending to online classes of the children, working for more hours due to lack of domestic helps, and having less free self-time for relaxation added to the stress encountered by the females during the pandemic. There was an added burden of domestic violence. It was reported that domestic violence/intimate partner violence rates increased exponentially during the lockdown in many countries due to forced coexistence, economic stress, increased interpersonal conflicts, and excess household work (Evans et al. 2020; Vora et al. 2020). An online survey-based study from Tunisia which surveyed 751 females reported psychological abuse to be the most frequent type of violence experienced during the lockdown (96%) with more than half of the participants (57.3%) reporting

extremely severe distress due to the same (Sediri et al. 2020). An online survey from India, that explored the spousal violence during lockdown in the country ($n = 560$), revealed the rate of current spousal violence to be 18% with rates of physical, sexual, verbal, and emotional violence being 34%, 10%, 65%, and 43%, respectively (Pattojoshi et al. 2020).

21.2.2 Children and Adolescents

The COVID-19 pandemic also had a significant impact on the mental health of children and adolescents across the world. Lockdown and the subsequent closure of schools and colleges led to home confinement. Subsequently, they were hooked up to online classes and subsequent increased internet use. This raised severe concern among the parents with respect to the unsupervised internet exposure and handling children/adolescents at home throughout the day (Singh et al. 2020). Indian parents reported online classes to be less satisfactory (78%) and less comfortable (82%) for them. They also reported that their child had poor attention and concentration (80%) (Grover et al. 2020d). Other mental health issues among the children during the pandemic included increased irritability, inattention, clinging behavior, feeling uncertain, disturbed sleep, nightmares, poor appetite, and other behavioral problems (Jiao et al. 2020; Viner et al. 2020). Older children and adolescents were found to be anxious due to cancellations of their examinations, and were worried about their future career and academic events (Lee 2020). Those children with special needs (autism, ADHD, cerebral palsy, intellectual disability, developmental delay, etc.) had more aggravation of behavioral problems due to lockdown and restriction of activities and parents had a tough time in handling them (Cortese et al. 2020). Further, children and adolescents who got infected with COVID-19 or were under quarantine, although they were mostly asymptomatic, developed feelings of sadness, anxiety, fear of death, fear of parents' death, and fear of being isolated in hospital (Liu et al. 2020b; Sahoo et al. 2020d).

21.2.3 Elderly

The elderly were the most vulnerable of getting infected and are at higher risk of mortality and having more severe infection. This group of population had problems in following COVID protocols. Social distancing, home confinement, fear of getting infection, hearing news of deaths of near ones of contemporary age groups, staying alone (in case of widowed or separated from family), lack of knowledge to use smart phones for connecting with family, lack of domestic help, etc. were some of the stressful situations which made the elderly more susceptible to numerous mental health problems during the pandemic (Vahia et al. 2020). There have been several reports of worsening or relapse of previous mental illness among the elderly during the pandemic (Mehra et al. 2020b). Also there were significant concerns about the

mental health issues in elderly with multiple medical comorbidities and with dementia (Mehra and Grover 2020).

21.2.4 Migrant Population

COVID-19 pandemic and subsequent immediate enforcement of lockdown brought the lives of migrant workers to a standstill. They encountered loss of employment, problems in acquiring adequate food and proper shelter, and difficulty in travelling back to their native places. In a country like India, thousands of migrants started to walk to their native places on foot which was heart wrenching (Singh 2020). This created tussle with the government and every possible effort was made to provide adequate support to the migrants. All these created significant mental distress among the migrant population. Available studies reported psychological morbidity, i.e., depression and/or anxiety, in about three-fourths (73.5%) of the sample (Kumar et al. 2020). They also had the risk of acquiring as well as spreading infection to others due to resource as well as space limitations and difficulty in understanding and following COVID containment rules. Many such factors affected the migrant population psychologically.

21.2.5 Persons with Mental Illnesses

People with mental illnesses have also been significantly affected by the pandemic. Lack of accessibility to routine psychiatric services due to lockdown across the world led to several problems in handling persons with mental illnesses (Bojdani et al. 2020). Many mental health facilities in the government sector as well as private sector were providing limited patient care and it affected the routine care of the needy individuals with mental illness (Grover et al. 2020e, f). Telepsychiatry services were started to cater to the needs of the patients with mental illness across the country which gave some respite and improvement in patient care. Many patients had to discontinue the medications due to difficulty in procuring medications, leading to relapse of their illnesses. There was also a rise in newly diagnosed mental health issues in the general population as well as a sharp rise in self-harm and suicide cases (Pathare et al. 2020). Further, there are studies to suggest that patients with mental illnesses are at greater risk of acquiring COVID-19 infection and also had greater mortality, which was independent of known physical health risk factors for COVID-19 (Taquet et al. 2020; Wang et al. 2021).

21.3 Conclusions

The COVID-19 pandemic had affected all sections of population adversely in many aspects. Psychological impact of the COVID-19 pandemic in all sections was seen in the short duration of lockdown in the form of increase in mental health issues in

every section of the society. As the pandemic is about to stay for a while, it is expected that there can be more long-standing mental health effects of the pandemic on human beings in the near future. Therefore, strengthening mental health resources, taking timely mental health support and help from mental health professionals, destigmatizing mental illness, and implementing the awareness programs of mental health are the need of the hour.

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Potential Influence of Parasitic Interactions on COVID-19 Pathology and Epidemiology

22

Neelima Gupta and Siddhartha Kumar Mishra

Abstract

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and coronavirus disease 2019 (COVID-19) has affected the human lives worldwide. The emerged disease and pandemic have challenged the public health particularly in the countries with middle or low economic condition including India. The transmigration of this zoonotic virus from bats and pangolins to humans in a process of genetic drift has targeted the lungs and respiratory and cardiovascular damages. Analyzing the infection of SARS-CoV-2 and COVID-19 as a parasitic component in the human physiology, it drew considerable attention to analyze the impact of other residential parasitic entities. This adds to the involvement of the infection and proliferation of other human parasites especially malaria, soil-transmitted helminths (STH), and schistosomes. The coevolution mechanisms pertain to manage the growth and proliferation of pathogenic and nonpathogenic parasites in a cellular micromilieu. One of the key factors that have appeared is COVID-19-induced cytokine storm in cells which is characterized as a physiological state of cells with a hyperinflammatory response. In case of SARS-CoV-2 infection, this phenomenon has been even critically implicated especially with an exacerbated response of IL-6. Herein, this perspective analysis explores the potential influence of parasite coinfection in COVID-19 infection especially the interaction of parasites of malaria, STH, and schistosomes. In a nutshell, the correlation analysis between COVID-19 and other human parasites and diseases presented globally has shown a negative correlation with the COVID-19 cases. Thus, extensive large-scale epidemiological studies would be required to further establish the correlation between parasitic

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interactions with COVID-19 and to resolve the ambiguity existing in variable repertoire.

Keywords

Coronaviruses · SARS-CoV-2 · COVID-19 · Cytokine storm · Parasitic interaction

22.1 Introduction

Emerging diseases and epidemics have challenged the public health worldwide and particularly in the countries with middle or low economic condition including India where the abundance of population itself increases the risk of pathogen-transmitted diseases (Lindahl and Grace 2015). The growing numbers of human population are also occupying more space in terms of biological and environmental ecosystem and simultaneously, they are increasingly being infected with emerging infectious diseases in both human and animal lives (Pandey et al. 2020). Viruses, such as the agents of influenza, dengue, chikungunya, Zika, and yellow fever, have kept on infecting thousands of people worldwide. Infectious diseases, such as severe acute respiratory syndrome (SARS), represent a major threat to public health (Zhu et al. 2020).

The novel human coronavirus (SARS-CoV-2) emerged in December 2019 in Wuhan, China, and was declared as a pandemic by the World Health Organization (WHO). Over 105 million cases of SARS-CoV-2 infection are reported worldwide with over 2.2 million deaths in about 200 countries in about a year of pandemic declared by the WHO. The figures in India are larger in world context with over 10 million cases of SARS-CoV-2 infection and over 154,000 deaths (World Health Organization 2021). The COVID-19 pandemic has turned the clock back by years in the fight against other diseases. It has interrupted research, trials, and other efforts to ease the public health burden. It is reported that in the last 20 years, we have had six significant threats, SARS, MERS, Ebola, avian influenza, and swine flu; we dodged five bullets but the sixth got us, SARS-CoV-2 (Gill 2020).

Coronaviruses are naturally hosted and evolutionarily shaped by bats. Indeed, it has been postulated that most of the coronaviruses in humans are derived from the bat reservoir (Cui et al. 2019). Although the specific route of transmission from natural reservoirs to humans remains unclear yet several studies have shown that pangolins may have provided a partial *spike* gene to SARS-CoV-2 (Cui et al. 2019; Wei et al. 2020). SARS-CoV-2 is a member of β-coronaviruses and is genetically related to the human SARS and the Middle East respiratory syndrome-human coronavirus, MERS-CoV (Chan et al. 2015; Docea et al. 2020). Infection with this virus leads to respiratory damage, which can progress to pneumonia or damage to the whole body (Guan et al. 2020).

Seeing the COVID-19 through a parasitic interaction view, it fetches larger interest as to how the virus would cope up with other parasitic entities in human

physiology. This could lead to a favoring response towards increasing COVID-19 infection or towards fighting against infection. This has been sought from the nature of infection and proliferation of other human parasites especially malaria, soil-transmitted helminths (STH), and schistosomes. Countries with poor resources such as in tropical regions may be harder hit by the COVID-19 pandemic than other areas, yet a variable trend is observed which indicates the intervening interaction of other parasitic entities in the cellular micromilieu (Guan et al. 2020; Siles-Lucas et al. 2021; Ssebambulidde et al. 2020). Taking notes from the severity of COVID-19 and with not so clear etiology, it has been apprehended that COVID-19 may cause long-lasting impacts through pathogenic interactions so that deaths related to HIV could increase by 10%, tuberculosis by up to 20%, and malaria by 36% over the next 5 years (Hogan et al. 2020).

This purview has drawn attention of researchers for emphasizing on the potential influence of parasitic interactions with SARS-CoV-2 infection and COVID-19 pathology and epidemiology. This contemporary chapter aims to analyze such plausible interactions and to propose a suggestive viewpoint for further course of disease management in a coherence manner considering the importance of parasitic diseases in nature.

22.2 Coronavirus Disease 2019 (COVID-19): An Overview

SARS-CoV-2 is a β-coronavirus which is comprised of crown-like, enveloped, positive-sense single-stranded RNA (þssRNA) virus with an envelope. The non-structural proteins of SARS-CoV-2 are RNA polymerase, helicase, and proteases which are similar to 3-chymotrypsin and papain (Islam et al. 2020). The coronavirus nucleocapsid protein (NP) packages the genomic RNA to form a helical nucleocapsid (Masters 2019). The coronavirus membrane (M) proteins are located at the intracellular membrane structure and bind to internal NPs to form the virus core structure. Further, the viral envelope (E) and the M proteins and the spike (S) protein interact with each other and form a viral envelope (Schoeman and Fielding 2019).

The genome sequence length of SARS-CoV-2 is about 30 kb ssRNA, with a 5′-cap structure and 3′-poly-(A) tail which is enveloped by a complex of structural proteins that form a crown-like enveloped virus (Chen et al. 2020a). Upon entry into the host cells, the genomic RNA serves as a template and directly translates polyprotein (pp) 1a/1ab which is processed into 16 nonstructural proteins (NsPs) by proteolytic cleavage. Most NsPs are assembled in the form of replication and transcription complexes (RTCs) and assist in the transcription and replication of the virus (Chan et al. 2020). Viral structural proteins surrounding the RNA strand are the most important, of which S protein has the function of binding to the conversion enzyme of angiotensinogen II (ECA2); thus, ECA2 acts as a receptor for SARS-CoV-2 binding on cell surface (Reid et al. 2015) while the viral S protein is modified by transmembrane proteinase serine 2 (TMPRSS2) which acts for the modification facilitating the entry of viral particles into the cell (Farsalinos et al. 2020; Guo et al. 2020a; Meo et al. 2020).

The COVID-19 outbreak reported from live animals in "wet markets" of Wuhan in South China was a spread of SARS-CoV-2 transmission to humans from pangolins and earlier reported to be transmigrated from other wild animals (Zhang et al. 2020a; Frutos et al. 2020). The pathophysiological characteristics and the mechanism of SARS-CoV-2 spread still remain unclear. SARS-CoV-2 is mostly transmitted via inhalation as the lung epithelial cells are the primary target of the virus and can be manifested as an asymptomatic infection or mild-to-severe pneumonia (Guan et al. 2020). The virus-infected individuals develop COVID-19 and may experience abnormal respiratory events, higher leukocyte influx, and increased levels of plasma pro-inflammatory cytokines. It may also cause leukopenia, increased level of C-reactive protein (CRP), high erythrocyte sedimentation rate, and extreme increase in inflammatory cytokines and chemokines (Huang et al. 2020). It also causes an elevation of serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT), procalcitonin, and ferritin levels (Huang et al. 2020; Chen et al. 2020b); decreased lymphocytes; and elevated fibrinogen, neutrophil, lactic dehydrogenase, fibrinogen, and acute hypoxic respiratory failure (Han et al. 2020). The immunopathology thus imparts a crucial role in the development of disease severity in SARS-CoV-2-infected patients. SARS-CoV-2 virus could also be transmitted by the fecal-oral route along with coughing and sneezing (Liu et al. 2020a). Diarrhea is an additional gastrointestinal disorder associated with COVID-19 diseases along with nausea and vomiting, abdominal discomfort or pain, and mild or moderate damage of the liver and pancreas (Liu et al. 2020a).

22.3 SARS-CoV-2-Induced Inflammatory Cytokine Storm

Cytokine storm is a physiological state of cells and tissues characterized by an acute hyperinflammatory response which may be responsible for critical illnesses including viral infections, cancer, sepsis, and multi-organ failures (Weaver and Behrens 2017). In case of SARS-CoV-2-infected patients, this phenomenon has been implicated in critically ill patients and they exhibit a poor course of prognosis and increased fatality rate (Bhaskar et al. 2020). Cytokine storm in SARS-CoV-2-infected patients appears to be an important aspect of pathogenesis and is associated with several severe manifestations of COVID-19. Some of such manifestations include acute respiratory distress syndrome, thromboembolic diseases like acute ischemic strokes due to large-vessel occlusion and myocardial infarction, encephalitis, acute kidney injury, and vasculitis (Hojyo et al. 2020).

The earlier report from the first cohort study of 41 COVID-19 patients in Wuhan led to the discovery of the novel SARS-CoV-2 virus and revealed a cytokine profile which was much similar to that of secondary hemophagocytic lymphohistiocytosis (sHLH) which is a hyperinflammatory condition triggered by viral infection (Huang et al. 2020). The COVID-19 patients showed higher levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ)-induced protein 10 (IP10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1 alpha (MIP1α), and tumor necrosis factor alpha

(TNFα) (Huang et al. 2020). Further observations from another cohort study of 150 patients in Wuhan showed that those who died of COVID-19 complications had extensively higher levels of serum CRP, IL-6, and ferritin, and this suggested a fundamental hyperinflammatory process (Ruan et al. 2020). Another cohort study demonstrated that COVID-19 patients experiencing cardiac comorbidities have a relatively higher level of troponin T (TnT) and significantly higher CRP and procalcitonin levels that were associated with increased morbidity and mortality (Guo et al. 2020b). The COVID-19 patients who died due to experiences of endothelial cell infection and an endotheliitis affecting many organs were early examples of cytokine modulatory responses (Bhaskar et al. 2020; Varga et al. 2020). Those immunological markers in combination may be used as prognostic markers to determine COVID-19 severity and may help in designing the further course of disease management.

The S protein of SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) while attaching on to the host cells. Most COVID-19 patients presented with respiratory symptoms have expressed ACE2 in vascular endothelial cells of the lower respiratory tract at a higher level (Guo et al. 2020a). The severe COVID-19 cases showed hypercytokinemia in the lungs that caused diffused alveolar damage, hyaline membrane formation, thrombus formation, fibrin exudates, and fibrotic healing (Dolhnikoff et al. 2020). Forty percent of COVID-19 patients experienced proteinuria and hematuria which suggested kidney infection and injury (Rabb 2020). COVID-19-related kidney injury occurs because ACE2 receptors are found in the kidney in the brush border of proximal tubular cells. Although the postmortem examination of kidneys of COVID-19 patients reveals SARS-CoV-2 antigens in the proximal tubules, the role of cytokine storm in causing kidney injury is not yet clear (Perico et al. 2020).

The consequences of cytokine influx in elderly patients, especially older males, with comorbidities, have been shown to rapidly increase and affect the disease prognosis and increase the risk of severe condition or even fatality from COVID-19 (Wang et al. 2020). This accords with the notion that aging is associated with a decline in immune function or immunosenescence (Aw et al. 2007; Del Giudice et al. 2018). The immune system presents a series of changes in the process of aging which are characterized by immunosenescence markers such as a decline in the generation of CD3+ T cells, an inversion of the CD4 to CD8 (CD4/CD8) T-cell ratio, an increase in regulatory T cells (Treg), and a decrease in B lymphocytes (Li et al. 2019). COVID-19 has been postulated to induce cytokine storm which may contribute to the poor prognosis in elderly patients because immunosenescence per se T lymphocytes can be potentially infected by the virus and their number may be reduced due to apoptosis (Westmeier et al. 2020).

Hypercytokinemia is an unregulated hyperinflammatory response in cells due to the systemic spread of a localized inflammatory response due to bacterial or viral infection. Cytokine influx further elevates endothelial dysfunction, vascular damage, and paracrine/metabolic dysregulation and leads to multiple-organ system damages. The acute response of cytokines (TNFα and IL-1β) and chemotactic cytokines (IL-8 and MCP-1) starts to elevate to notable level in early hypercytokinemia, and these

responses facilitate a sustained increase in IL-6 (Heinrich et al. 2003). IL-6 further binds to IL-6 receptor and forms a complex that acts on gp130 and regulates levels of IL-6, MCP-1, and GM-CSF via JAK-STAT signaling pathway, and thereby disseminates the inflammatory processes. This has extrapolated with the inflammatory cytokine storm-induced COVID-19 especially in critically ill patients with other comorbidities (Castelli et al. 2020).

The infection of SARS-CoV-2 initiates the chain reaction involving macrophage activation, dendritic cell-triggered early immune response, and lymphocytosis and cytokine release (Schulert and Cron 2020). This inflammatory response further leads to the destruction of lymphocytes attempting to block SARS-CoV-2 infection. The resultant lymphopenia results especially in patients severely affected who show rapidly dysregulated, damaging cellular responses in different organs especially the lungs and then kidneys, heart, blood vessels, and brain (Diao et al. 2020). COVID-19-induced cytokine storm is associated with a cascade of damages beginning with disruption of the epithelial barrier in the lungs. It causes activation of NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP-3) inflammasome and the relative blunted response of histone deacetylase 2 on nuclear factor-kappa beta (NF-κB) complex that has been suggested to assist in epithelial barrier disruption (Shah 2020).

Thus, exploring the pathogenic mechanism of cytokine storm may help in not only unravelling risk factors associated with COVID-19 complications but also designing the therapeutic strategies to modulate the immune response. This will also assist in special management aids for improving the disease outcomes in critically ill COVID-19 patients at high risk for severe disease. The comprehensive information on the mechanism of SARS-CoV-2 infection and consequent immunological signature and disease impact is represented in Fig. 22.1.

22.4 Diagnostics and Therapeutic Management of COVID-19

The laboratory-based diagnostics of SARS-CoV-2 virus infection applies serological tests using enzyme-linked immunosorbent assay (ELISA) or Western blotting that detects specific SARS-CoV-2 proteins, while molecular approaches utilize real-time-polymerase chain reaction (RT-PCR) for viral RNA amplification and detection, and Northern blot hybridization targeting specific SARS-CoV-2 genes (Corman et al. 2020). Direct immunofluorescence assay (IFA) also applies for detecting viral antigens present in the specimen, whereas the total lymphocyte count (TLC) and chest CT examination are used for analyzing the impact of viral infection on lungs (Han et al. 2020).

An effective therapeutic management of SARS-CoV-2 requires following up among the strategies like inhibition of essential functional enzymes or proteins in virus survival pathway; inhibition of viral structural proteins for preventing host cell interaction; inhibition of human cell receptors acting for virus attachment; and stimulation of human immunity against viral pathophysiology (Wu et al. 2020). The prospective treatment modalities for SARS-CoV-2 infection include existing

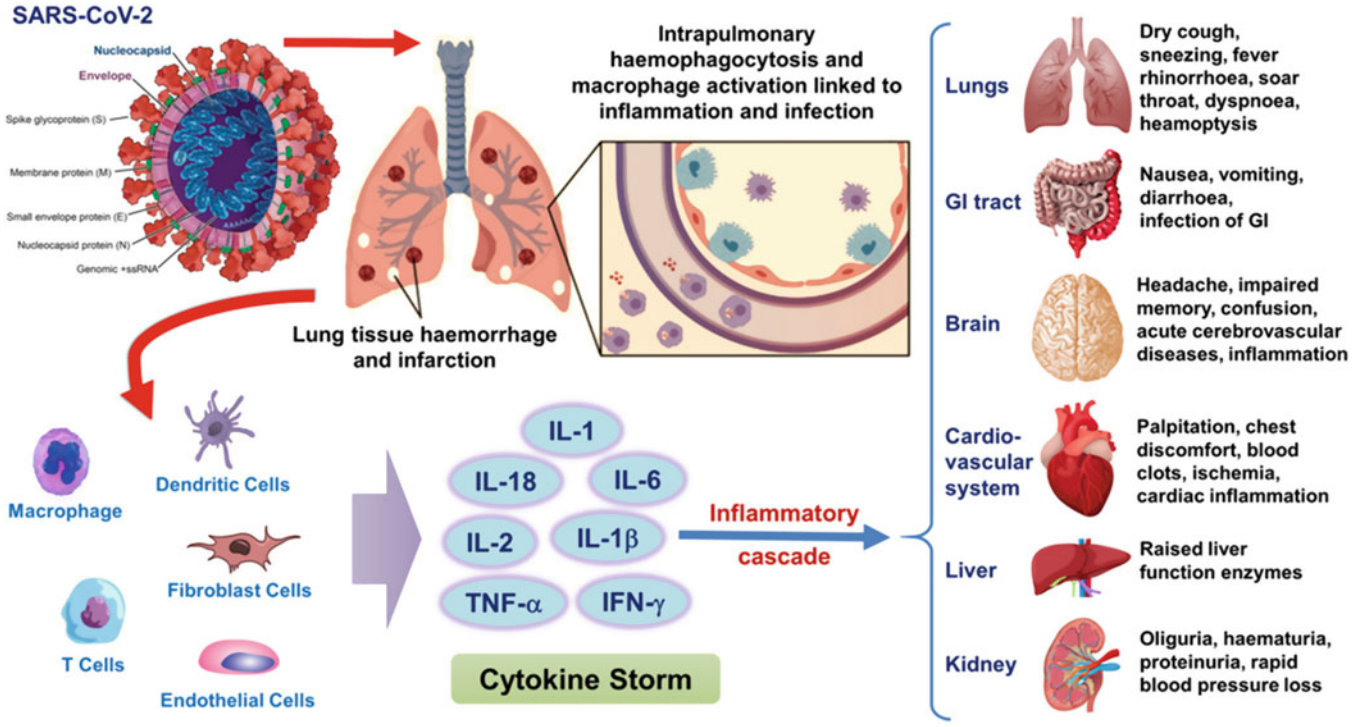

Fig. 22.1 Schematic demonstration of the events after SARS-CoV-2 infection. The infection of SARS-CoV-2 to lungs induces a series of events causing cytokine storm through activation of macrophages, dendritic cells, T cells, fibroblast cells, and endothelial cells. These cellular activations lead to hyperactivation of cytokines mainly IL-6, IL-1β, TNF-α, and IFN-γ. This immunopathological inflammatory cascade causes acute respiratory distress syndromes (ARDS) and opens the fronts for multiple microbial infections, especially bacterial, followed by sepsis and tissue injuries. Events of multiorgan failure are initiated which affect mainly dysfunction of lungs, liver, and kidney, and its variables with comorbidities of patients. Image components are not to scale. Figure is conceptually redrawn from Islam et al. (2020); Bhaskar et al. (2020); Hojyo et al. (2020); and Castelli et al. (2020)

and novel created antiviral candidate drugs, immune-potentiating therapies, therapeutic antibodies, and vaccines that have entered into several preclinical and clinical trials in the year 2020 through various experimental settings in cell culture, animal, and early human trials (Islam et al. 2020; Gautret et al. 2020).

Among the therapeutic measures underway are the searches of candidate drugs for blocking the entry of virus into the cells. It is known that SARS-CoV-2 can bind only onto the ACE2 receptor which is highly activated in people with chronic diseases as compared to healthy individuals (Islam et al. 2020). This is eased by TMPRSS2 protease binding to the S (spike) receptors of the virus to ACE2 receptors. SARS-CoV-2 needs the TMPRSS2 protease in the cell, as present in the human cells, to enter into cells; thus, this protease can be targeted to block as a potential target for therapeutic interventions. In this regard, several candidate drug molecules that could block ACE2 have been identified and ruxolitinib has appeared as a lead molecule and entered into clinical trials (Chen 2020). ACE targeting has also been attempted by a recombinant human ACE2 (rhACE2), studied in acute respiratory distress syndrome (ARDS), showed beneficial effects on patients with COVID-19 by lowering viremia (due to limited binding to the ACE2 receptor), and reduced the ARDS symptoms in lungs and respiratory complications (Apeiron Biologics 2020).

Chloroquine [(N4-(7-chloro-4-quinolinyl)-N1,N1-diethyl-1,4-pentanediamine)] is a conventional drug used in several countries for treatment of malaria and recently it has been utilized in several studies to target SARS-CoV-2. An early study using chloroquine showed effectiveness to prevent the spread of SARS-CoV-2 in vitro (Yao et al. 2020). Chloroquine could elevate the endosomal pH and interfere with terminal glycosylation of the ACE2 receptor and cause inhibition of the virus-receptor binding to host cells. Chloroquine was also effective against the MERS strains OC43 (HCoV-OC43) and inhibited virus replication in HRT-18 cells (Gordon et al. 2020). The molecular mechanism of the action of chloroquine and hydroxychloroquine was mediated by changing the pH of the surface of the cell membrane and thus inhibited the fusion of the virus with the cell membrane. Comparatively, hydroxychloroquine was reported to be less toxic than chloroquine and more effective in inhibiting SARS-CoV-2 infection in vitro (Liu et al. 2020b). The two derivatives were also associated with the inhibition of viral nucleic acid replication, viral protein glycosylation, virus assembly and transport of virions, and release of the new virus particles from the infected cells (Liu et al. 2020b). The other candidate antiviral drug mesylate camostat inhibited the TMPRSS2 protease, blocked the entry of virus particles into the cell, and prevented the infection of SARS-CoV-2 infection (Uno 2020). A camostat derivative has been approved for the treatment of inflammation of the pancreas in Japan, yet the human clinical trials against SARS-CoV-2 are needed (Zhou et al. 2015). The Ebola-failed antiviral drug, remdesivir, inhibited RNA-dependent RNA polymerase prematurely blocking RNA transcription in SARS-CoV-2. It showed broad antiviral spectrum with efficacy against coronaviruses in both in vitro and in vivo studies with a superior efficacy of the lopinavir/ritonavir/IFNβ combination in several studies. The US-FDA has authorized the use of remdesivir in severe SARS-CoV-2 infection through the

Special Emergency Use Authorization (SEUA) (Beigel et al. 2020). A combination of lopinavir/ritonavir (LPV/RTV) has shown potent antiviral efficacy for HIV as a protease inhibitor. The combination has shown some degree of activity against SARS-CoV-2 (Cao et al. 2020) yet some studies failed to identify a reduction in the viral clearance in patients (Lim et al. 2020). Umifenovir serving as an antiviral for influenza viruses has shown to block the entry of the virus into cells (acting as fusion inhibitor) and the immunomodulatory effects in patients with uncomplicated pneumonia in COVID-19. The combination of umifenovir with LPV/RTV resulted in a faster rate of virus clearance at the nasopharyngeal level and a faster regression of lung infection as compared to LPV/RTV monotherapy (Lian et al. 2020). With regard to the cytokine storm and anti-inflammation-based therapies, tocilizumab (a monoclonal antibody targeting the IL-6 receptor) showed potent immunomodulatory effects as IL-6 receptor antagonist in a subset of patients with severe COVID-19 with excessive activation of inflammation. The combination of tocilizumab with corticosteroids was even favorable in terms of viral clearance and suppression of disease signatures (Rodríguez-Baño et al. 2020).

The recent trend and growing researches are focusing on vaccine development against SARS-CoV-2. Based on the immunopathological basis of the virus, several vaccine candidates in several countries are under trials and open delivery through SEUA. There are three COVID-19 vaccines for which certain country-specific regulatory authorities have been issued yet none have received WHO EUL/PQ authorization (Rawat et al. 2021). The leading vaccine candidate is an adenovirus-vectored vaccine ChAdOx1 nCoV-19 (AZD1222) with a trade name COVISHIELD^{TM} that has been under human deliveries (Knoll and Wonodi 2021). The COVAXIN^{TM} is an indigenous, inactivated vaccine, developed using whole-virion-inactivated Vero cell-derived platform technology. The inactivated vaccine claims to be not replicating and is therefore unlikely to revert and cause pathological effects (Ella et al. 2020). Thus, with the growing scenario more incumbent vaccines may be readily available for human trials and delivery as well as the therapeutic regimens acting as antiviral drugs may also be introduced.

22.5 Interaction of Parasites in COVID-19 Cellular Micromilieu

The COVID-19 pandemic has altered not only human behavior in profound ways but also the ecological system within and outside the human body. This fits into a growing microbial ecological paradigm prevailing in the human body and implies on to the host-parasite interactions (Piña-Vázquez et al. 2012). The constant threats to humans from parasitic infections have been engaged by a plethora of surface and secreted molecules from parasites to enter and modulate mammalian cells' function. The pathogenic parasitic interactions between species are likely to be geographical distribution dependent yet they systematically share a connection between species. The interactive connection between species in the representation of genetic transmission and drifts between species is further associated with the infectious diseases of humans and animals (Wardeh et al. 2015). This adds to the three key points

interconnected to infectious and parasitic diseases involving interaction between viruses with other microbes, animals, and humans such as COVID-19. Viruses may also infect protozoa, fungi, helminths, and insects and can interfere with their biological properties and further infections (Shen et al. 2019).

Recently, the parasitology research community has taken careful attention on investigating the plausible influence of parasite coinfections on the outcomes of COVID-19. The parasitic infections have greatly impacted the host cell response and parasite-mediated immunomodulation and inflammatory disorders which remain critical factors in the pathophysiology in hosts (Cooke 2012). COVID-19 is known to induce a vast immune response marked by elevated pro-inflammatory cytokines and chemokines especially IL-6, IL-1β, IL-2, and TNF-α and is commonly referred to as cytokine storm (Bhaskar et al. 2020; Castelli et al. 2020). Explaining the correlation and influence of parasitic infection with COVID-19, there are three major points of interest (de Souza 2020). Firstly, infectious and parasitic diseases are interconnected and virus-borne diseases like COVID-19 strongly interfere with the immune system (cytokine storm). This creates ideal conditions for other coinfections especially by fungi, protozoa, and helminths. Reports from Brazil indicate the high levels of infection by *Plasmodium vivax* and *P. falciparum*, *Trypanosoma cruzi*, *Leishmania*, and *Toxoplasma gondii* among other parasitic agents, yet a dramatically higher number of COVID-19 cases is reported from Brazil. Secondly, some viruses also infect protozoa, fungi, helminths, and insects that transmit various parasitic protozoa. They additionally interfere with their biological properties as shown by the presence of viral particles in several members of the Trypanosomatidae family, and in *Giardia, Trichomonas,* and *Cryptosporidium* (Charon et al. 2019). Thirdly, the critical manifestations like SARS-CoV-2 infection have abilities to enter into cells through a mechanism of endocytosis that is similarly used by prokaryotic and eukaryotic microorganisms such as *T. cruzi* and *T. gondii* (Barrias et al. 2019). The virus interacts with the cellular structures and organelles of the host cell and creates a condition for the assembly of new virions for release outside the cell through a mechanism usually achieved by exocytosis. Again, most of these steps resemble the processes used by intracellular protozoan to exit the cellular boundaries. Therefore, mechanisms of membrane transport and localization of parasites may also affect the virus entry and replicative process in the cell.

Parasitic protozoa are among the most important pathogens worldwide that has caused dreaded diseases like malaria, leishmaniasis, amoebiasis, giardiasis, trichomoniasis, and trypanosomiasis. Parasites including *Plasmodium* and *Schistosoma* and various STH such as *Ascaris*, hookworm, and *Trichuris* have a long life span in the infected humans who are even asymptomatic (Ssebambulidde et al. 2020). Parasites are proficiently known to modulate the immune system as they induce an immune tolerogenic response in infected individuals via an insurgent imbalance between pro-inflammatory and anti-inflammatory cellular responses. Such immunomodulatory potentials are exploited through the use of parasitic excretory secretory pathways towards the treatment of inflammatory conditions such as multiple sclerosis and inflammatory bowel disease (Fleming et al. 2011; Benzel et al. 2012). Considering the immunomodulatory and inflammatory responses altered by parasitic

infections, it is plausible that parasitic infections could potentially influence the incidence and clinical severity of COVID-19 in different subsets of patients in different geographic regions. A cross-sectional ecological study evaluated the epidemiological data for COVID-19 patients from the parasitic epidemic regions like malaria, schistosomiasis, and STH (Ssebambulidde et al. 2020). Among the globally studied 215 countries, 42% of countries were endemic for malaria, 32% of countries were endemic for schistosomiasis, and 47% of countries were endemic for STH. From the geographic distribution aspect, the Americas had the largest proportion (42%) of the global COVID-19 cases and 33% of COVID-19 deaths, with 0.4% of malaria cases, 0.7% of schistosomiasis cases, and 5.4% of the STH. Africa had the smallest proportion (0.9%) of the global COVID-19 cases and 0.5% of COVID-19 deaths, with the highest proportion (93%) of malaria cases, 90% of schistosomiasis cases, and the second largest proportion (25%) of the STH. India had the higher proportion (14%) of the global COVID-19 cases and 9% of COVID-19 deaths, with a higher proportion (47%) of malaria cases, 1% of schistosomiasis cases, and a larger proportion (22%) of the STH (Ssebambulidde et al. 2020).

The study further elaborated that malaria, schistosomiasis, and soil-transmitted helminth-endemic countries had significantly lower median COVID-19 cases than their non-endemic counterparts. The median number of COVID-19 cases was 315 in malaria-endemic countries (comparably lower), it was 192 in schistosomiasis-endemic countries (even lower), and 270 in STH-endemic countries (lower) (Ssebambulidde et al. 2020). Collectively, these data suggested a negative correlation between COVID-19 and malaria, schistosomiasis, and STH. Another study has reported that individuals in malaria-endemic regions may be protected from COVID-19 based on the hypothesized ecological protection involving molecular and genetic variations associated with malaria that make hosts less susceptible to SARS-CoV-2 infection (Napoli and Nioi 2020). In this line, another recent study suggested that BCG vaccination can be correlated with low COVID-19 cases and deaths (Miller et al. 2020). This correlation was based on the postulation that the nonspecific immune stimulation by BCG can lead to protection against other microbes other than *Mycobacteria* spp. and that may also potentially cross-protect from COVID-19 through a not-well-defined mechanism. Additionally, severe COVID-19 ill patients may be related to an overly activated pro-inflammatory state which was improved by subsequent blocking by IL-6 antagonist (Zhang et al. 2020b). Based on the distribution of COVID-19 cases in endemic and non-endemic zones, parasite-induced immunomodulatory mechanisms were among individuals who emigrate from parasite-endemic regions. The coevolution of parasites has been with humans and thus become proficient immune modulators by inducing cytokine (IFNγ, IL-4, IL-5, and IL-13) response. In case of schistosomiasis, granulomas and immunoregulatory cytokines IL-10 are dampened as compared to other parasitic infections (Jenkins et al. 2005; McManus et al. 2020).

In case of malaria, the individuals having detectable malaria parasitemia may remain clinically asymptomatic as the clinical immunity to malaria has been attributed to immunoregulatory mechanisms involving regulatory CD4+ T cells that secrete IL-10 and TGF-β (Gonzales et al. 2020; Doolan et al. 2009). These

two cytokines are master immunomodulators that block the effects of pro-inflammatory cytokines and induce an immunotolerance remarked by anti-inflammatory cytokines and Tregs (Omer et al. 2000; Cavalcanti et al. 2019). In case of helminth-infected individuals, the immune tolerogenic state is typically defined in parasite-endemic areas to reduce vaccine efficacy in the settings of parasitic infection in comparison to that without parasitic infections (Ssebambulidde et al. 2020). An earlier cohort study from Ethiopia demonstrated that IFN-γ responses following BCG vaccination were lower in individuals with an active helminth infection. In addition, anti-helminthic treatment caused improvement in the responses to BCG vaccination possibly via clearance of immunomodulatory parasites (Elias et al. 2001). These approaches shall be applied in a general consideration as the cellular and immunological responses are in a close cellular micromilieu in which other parasites are also localized.

Recently an approach to analyze the effect of the trematode *Fasciola hepatica* as a modulator of SARS-CoV-2 infection and COVID-19 pathology was postulated (Siles-Lucas et al. 2021). Helminth parasites have coevolved and adapted with their hosts since long which usually results in chronic diseases yet with low mortality and variable morbidity. This evolutionary coadaptation with the vertebrate hosts has contributed to the modulation of several cellular and immunopathological mechanisms and impacted the physiological growth. Helminth parasites have triggered a modulated T helper (Th)2 response in humans which resulted in an immune reaction with closely regulated inflammatory modules like inhibition of pro-inflammatory cytokines and induction of a hyporesponsive state via IL-10 and Tregs (Maizels and McSorley 2016). Following the coevolutionary legacy, the hygiene hypothesis furthermore proposes that the absence of helminth infections in the populations, such as in developed countries, may lack the helminth-induced immunological stimuli especially at childhood. This could further pacify the incumbent autoimmune response and disease with an aggravated inflammatory response in the forms of allergy, asthma, and rheumatoid arthritis (Jackson et al. 2009). Bradbury et al. (Bradbury et al. 2020) exclusively discussed the potential role of helminth coinfections in the modulation of hyperinflammatory responses against SARS-CoV-2 and COVID-19 pathophysiology, and showed the presence of an aggravated immune response affecting the lungs (Bradbury et al. 2020). This exacerbated innate immune response to lungs could cause an ARDS featuring rapid onset of generalized inflammation in the lungs and subsequent death by respiratory distress (Zhou et al. 2020). This is also further associated with the "cytokine storm" in which pro-inflammatory cytokines, mainly IL-6, dominate the pathophysiology (Bhaskar et al. 2020; Castelli et al. 2020). In this context, tocilizumab targeting the IL-6 receptor that is known to cause general immunosuppression shall be limited to use in the current COVID-19 pandemic. Similarly, other immunosuppressive drugs that have shown beneficial impacts on COVID-19 shall be critically analyzed for the risk of higher viral replication and secondary infections pertaining to immunosuppressive mechanism (Rodríguez-Baño et al. 2020; Zhang et al. 2020b).

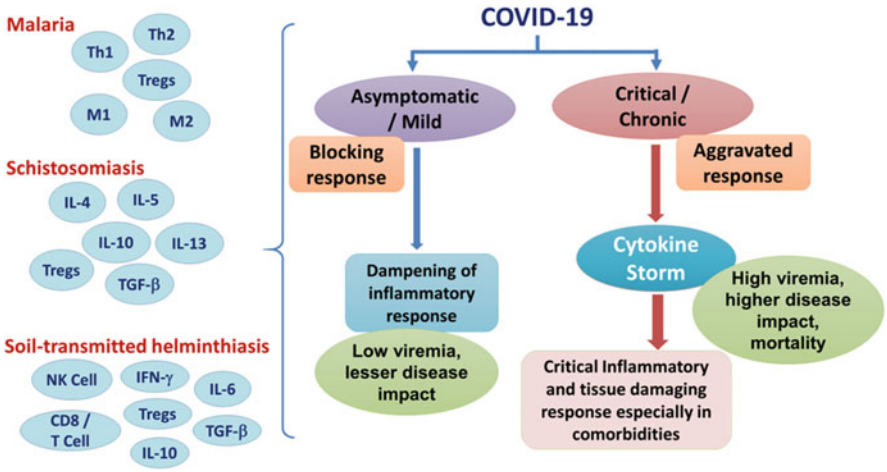

Fig. 22.2 Interactive influence of parasitic infections with COVID-19. The acute and chronic infection of SARS-CoV-2 at lower viremia causes asymptomatic characteristics while in chronic or critical infections cytokine storm causes further severe damages (parasitic diseases like malaria, schistosomiasis, and soil-transmitted helminthiasis)

Helminth parasites are proposed to possibly change the outcome of COVID-19 infections especially in areas of the world with high helminthic infections via eliciting a modified Th2 response. Notably, in countries of Africa and Latin America, where helminth infections are prevalent, the numbers of COVID-19 cases and deaths are substantially lower than those regions with high-income countries and low helminthic infection (Siles-Lucas et al. 2021; Bradbury et al. 2020). The severity of COVID-19 in helminth-endemic areas needed a supportive hypothesis because of the ambiguous animal coinfection studies showing both favorable and unfavorable effects (Maizels and Gause 2014). The eosinophilic link in allergic diseases involving the Th2 immune system is characterized which can alternatively activate M2 macrophage and eosinophils as immune defense against pathogenic infections including viruses (Ariyaratne and Finney 2019). These may also serve as a tool to combat against RNA viruses including SARS-CoV-2 and COVID-19. The interaction between preexisting helminth infection and the subsequent severity of COVID-19 may or may not be a negative correlation yet the theoretical and empirical evidences suggest that helminth-infected population may indeed have a beneficial mitigation over COVID-19 (Hays et al. 2020). Furthermore, considering the SARS-CoV-2 as a dreaded infectious condition in cells which causes severe immunopathological perturbances, the conditions of other intracellular parasites and microbes need to be addressed, as well as this may provide a favorable or critical adversity in the cell which should be considered when designing the therapeutic and preventive management strategies (Fig. 22.2).

22.6 Conclusion and Future Perspectives

The COVID-19 pandemic has greatly affected the human health worldwide and has altered not only the physiological systems but also the cellular microenvironment. These perturbations have affected the cellular immunological states and pathophysiology. This also has involved a critical role of microbial ecological paradigm prevailing in the human physiology especially the host-parasite interactions. Cytokine storm induced by COVID-19 has created a critical condition in cells to consider for the response of other coinfections and the cellular parasites especially the interaction of parasites of malaria, STH, and schistosomes. COVID-19 induces a vast immune response, marked by elevated pro-inflammatory cytokines and chemokines especially IL-6, IL-1β, IL-2, and TNF-α, and is called as "cytokine storm." Parasites including *Plasmodium* and *Schistosoma* and various STH like *Ascaris*, hookworm, and *Trichuris* have a long life span in the infected humans who are even asymptomatic. Parasites are proficient immune modulators and they may induce an immuno-tolerogenic state in infected individuals by striking a balance between pro- and anti-inflammatory responses. Parasites have immuno-modulatory potential and are targets of treatment of inflammatory conditions as the case of COVID-19. Correlation between COVID-19 and other human parasites and diseases was presented globally which has shown interesting characteristic features. Among the globally COVID-19-infected countries, the countries endemic for malaria, schistosomiasis, and STH showed a negative correlation with COVID-19 cases. Africa had the highest burden of parasitic infections globally yet the lowest global proportion of COVID-19 cases. In turn, this raises a thought whether parasitic interactions are influencing us favorably and raise curiosity to further explore the plausible mechanisms and molecular, immunological, and epidemiological levels globally.

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Plausible Impacts of SARS-CoV-2 on Human Reproductive System

23

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Abstract

SARS-CoV-2, a member of the family Coronaviridae, is a positive-stranded RNA virus with the spike glycoproteins present on its envelope. ACE2 serves as the entry mediator of SARS-CoV-2 as it attacks mainly the organs of the respiratory, cardiovascular, digestive, and urinary system showing high expression of ACE2 or TMPRSS2. ACE2 is found to have significant differential expression in all the reproductive tissues, thus posing the reproductive system vulnerable to the adverse effects of SARS-CoV-2 infection. Previous coronavirus attacks (SARS-CoV and MERS) have also been known to impose adverse effects on the reproductive system. Therefore, there is a dire need to safeguard the reproductive system against COVID-19 as it not only bothers the present generation but may also affect the well-being of future progeny. Since the inception of pandemic, several scientific studies have been carried out to assess its impact; yet there are research lacunas to claim reproductive system as a potential target of this deadly virus. To avoid the detrimental effects of the current pandemic on reproductive sustainability, well-planned large-scale and multicentric cohort follow-up studies are mandatory for accurate evaluation of the enduring effects of SARS-CoV-2 infection on human fertility and pregnancy outcomes.

Keywords

SARS-CoV-2 · ACE2 · Testis · Ovary · Uterus · Vertical transmission · Psychological stress

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23.1 Introduction

SARS-CoV-2 has stirred the scientific community to the core. It is believed to be a new β-coronavirus and causes the coronavirus disease 2019 (COVID-19). The World Health Organization declared this disease as a global pandemic on 11 March 2020 (Lai et al. 2020; Puliatti et al. 2020). COVID-19 was previously thought to be confined only to the respiratory symptoms such as sore throat, rhinorrhea, cough, and dyspnea, to pneumonia and in more severe cases to acute respiratory distress syndrome. But new insights highlight that other organs may also fall prey to the infection of SARS-CoV-2 owing to the presence of ACE2 receptors on them (Tu et al. 2020). Currently, the scientific community is engrossed in the characterization of SARS-CoV-2 virus, unfolding the pathology of COVID-19 and designing the efficient vaccines and drugs to combat the virus. Till date, only a handful of studies have investigated the possible impact of SARS-CoV-2 infection on human reproductive systems. If the fertility of male/female or the gametes are infested with the viral traces, the neonates may not be able to contend with the infection. The risk of fetus getting infected in utero from the COVID-19-positive mother can still not be denied. Therefore, shielding the reproductive systems from this lethal virus is the need of the hour because these serve as the link between the present and the future generations.

23.2 SARS-CoV-2: Origin and Pathogenesis

Coronaviruses (CoVs) are positive-stranded, round-shaped RNA viruses falling under four genera: alfa, beta, gamma, and delta (Li 2016; Pal et al. 2020). The newly discovered SARS-CoV-2 shares at least 70% genetic homology with the previous severe acute respiratory syndrome coronavirus (SARS-CoV) and approximately 50% homology with the Middle East respiratory syndrome (MERS) coronavirus (Hui et al. 2020). Earlier known as 2019 novel coronavirus infection (2019-nCoV), it was named COVID-19 by the World Health Organization (WHO) on February 11th, 2020, and simultaneously the nomenclature of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was done by the International Committee of Taxonomy of Viruses (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(CoVid-2019)-and-the-virus-that-causes-it).

Previous outbreaks of coronaviruses include the SARS-CoV (severe acute respiratory syndrome) epidemic in 2003 (Lau et al. 2005) and the MERS-CoV (Middle East respiratory syndrome) in 2012 (Zaki et al. 2012). SARS-CoV-2 imitates SARS-CoV as they both use the cellular receptor angiotensin-converting enzyme 2 (ACE2) to gain entry into the target host cell and protease transmembrane protease serine (TMPRSS2) for priming with S protein (Hoffmann et al. 2020; Lu et al. 2020; Lukassen et al. 2020) (Fig. 23.1). Other host proteases like TMPRSS2 (type II membrane serine proteases), 4, 11A, 11D, and 11E have also been reported to

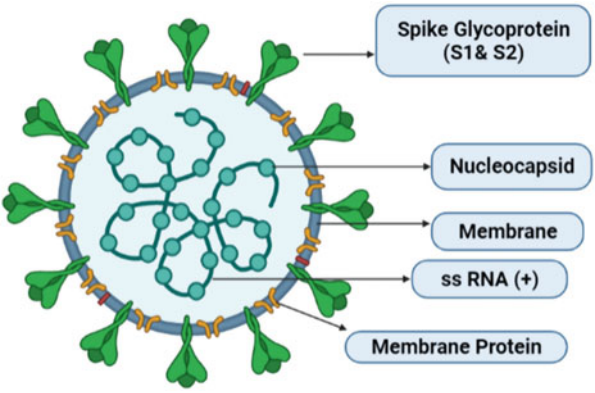

Fig. 23.1 Morphology of SARS-CoV-2

facilitate the protein spike (S) priming so that the viral envelope can fuse with the cell membrane (Hoffmann et al. 2020; Ou et al. 2020).

The prime way of transmission is via respiratory droplets and direct and indirect contact while the other less common ways include hidden transmission through biological samples, such as stool, urine, saliva, blood, and body fluids (including seminal fluid). Various studies have reported the multi-organ effect of SARS-CoV-2 on intestine, stomach, liver, pancreas, kidney, brain, heart and blood vessels, eyes, nose, etc. (Akhmerov and Marban 2020; de-Madaria et al. 2020; Lin et al. 2020; Mukherjee et al. 2020; Wang et al. 2020a; Wu et al. 2020a). This multi-organ involvement is responsible for poor prognosis of the COVID-2019 and often leads to hospitalization and in severe cases admittance to intensive care unit (ICU).

23.3 Role of ACE2 in Mediating the Infection

ACE2 is a fundamental module of the renin-angiotensin-aldosterone system (RAAS), which in addition to modulating the cleavage of angiotensin II (Ang II) and Ang (1–7) also serves as the receptor for SARS-CoV-2 (Kai and Kai 2020). During the course of COVID-19, majority of the ACE2 receptors bind to the virus which results in the increased availability of unconverted angiotensin II (Dimitrov 2003). This excess angiotensin II is responsible for the pulmonary symptoms that attribute to COVID-19. After cell invasion by SARS-CoV-2, the expression level of ACE2 downregulates which causes disruption of RAAS system and increased pro-inflammatory response generated by Ang II (Gheblawi et al. 2020; Kai and Kai 2020). Three strains of coronavirus, SARS-CoV, NL63, and SARS-CoV-2, can enter the host's cell membrane with the aid of angiotensin-converting enzyme 2 (ACE2) receptors (Verdecchia et al. 2020). The efficient binding of the spike (S) viral protein which is a 1273-amino acid-long protein to the angiotensin-converting enzyme 2 (ACE2) receptors mediates the entry of SARS-CoV-2 into the cells (Hoffmann et al. 2020; Walls et al. 2020).

First of all, the N-terminal portion of the viral protein S1 binds to the pocket of the ACE2 receptors which is followed by the cleavage of S1 and S2 proteins by the receptor TMPRSS2, a member of the hepsin/TMPRSS subfamily and stoichiometrically adjoining to ACE2 receptor (Glowacka et al. 2011). This cleavage of the viral protein by TMPRSS2 is believed to be a very crucial step because once the S1 protein gets detached, the remaining viral S2 unit undergoes a conformational rearrangement which leads to the completion of fusion between the viral and cellular membrane. Once the virus enters the cell, it releases its content, replicates, and infects other cells (Hoffmann et al. 2020).

Taking this theory further, it can be estimated that organs with upregulated ACE2 or TMPRSS2 are more likely to fall prey to SARS-CoV-2 infection. Currently, the list of vulnerable organs includes the respiratory, cardiovascular, digestive, and urinary system (Zou et al. 2020). Other than that, reproductive tissues were also found to express *ACE2* RNA with higher level in testis and lower levels in the prostate gland, vagina, endometrium, cervix, fallopian tubes, and ovary. *TMPRSS2* RNA has also been reported in most of the male reproductive tissues such as ductus deferens, seminal vesicles, and prostate with major expression (Pan et al. 2013; Zupin et al. 2020).

SARS-CoV-2 has a high affinity to ACE2 receptors and thus the hypothesis that a little ACE2 deficiency can prevent viral infection stands void (Hoffmann et al. 2020; Walls et al. 2020). On the other hand, a scenario of ACE2 downregulation by virus infection can lead to the imbalance between the angiotensin II *and* angiotensin$_{1\text{-}7}$-mediated pathways. In the lungs, these kind of dysregulations can facilitate the advancement of inflammation and hyper-coagulation (Akhmerov and Marban 2020; Mehta et al. 2020).

ACE2, Ang II, and Ang (1–7) control the normal working and function of the male and female reproductive systems. In the females, they carry out folliculogenesis, steroidogenesis, oocyte maturation, ovulation (Reis et al. 2011), and endometrial regeneration (Vaz-Silva et al. 2009). In the males, testicular ACE2 may regulate testicular function (Douglas et al. 2004) and maintain sperm viability and quality (Köhn et al. 1998; Gianzo et al. 2018). The reproductive cells and/or tissues seem to be prone to SARS-CoV-2 infection due to the presence of ACE2 receptors to facilitate the viral entry by binding with them and hence could get disturbed physiological functions.

23.4 Effect of SARS-CoV-2 on Male Reproductive System

In humans, reproductive health is necessary to maintain the quality and quantity of successive generations and includes a range of aspects such as physical, mental, and social well-being.

It has been reported that SARS-CoV-2 shows a sexual dimorphism and age-based discrimination as the male elderly subjects are at higher risk of infection and mortality (Chen et al. 2020a; Huang et al. 2020). It has been observed that mortality due to COVID-19 increased in the presence of comorbid conditions such as diabetes,

hypertension, obesity, chronic pulmonary disease, cardiovascular diseases, and cancer (Guan et al. 2020; Rothan and Byrareddy 2020).

A gene present on X chromosome (Xp22.2) regulates the expression of ACE2; thus it can be speculated that there may be some differences in the expression of ACE2 between males and females (Tipnis et al. 2000). According to the epidemiological data of gender-based clinical differences in SARS-CoV-2, it has been observed that mortality rates were higher in male patients, especially in elderly patients (Bwire 2020) as compared to female patients. In a study conducted by Wu et al. in 2020 on SARS-CoV-2 patients, it has been observed that men comprised 63.7% of the total patients with their mean age being 51 years with hypertension (19.4%), diabetes (10.9%), and cardiovascular disease history (4.0%) as most frequently observed comorbidities (Wu et al. 2020b).

A study on pathological changes in testes from 12 deceased COVID-19 patients revealed that in majority (90%) of the cases, no evidence for the virus was found in the testes by RT-PCR. In 9 out of 11 cases, Sertoli cells and seminiferous tubules showed moderate-to-severe injury with a significant reduction of Leydig cells and mild inflammatory infiltrates in the interstitium (Yang et al. 2020a). Wang and colleagues reported that spermatogonia and spermatids were the main sites of TMPRSS2 expression, whereas in spermatogonia, Leydig, and Sertoli cells, ACE2 expression was widely observed. Some histopathological and immunohistochemical studies also support the effects of the SARS-CoV-2 infection on the testicular cells. As per these reports, novel coronavirus infection resulted with the presence of inflammation in the seminiferous tubules as witnessed by permeates, IgG deposition in seminiferous epithelium, interstitium and degenerated germ cells, Sertoli cells, and Leydig cells (Abobaker and Raba 2020; Wang and Xu 2020) (Fig. 23.2).

A study conducted by Li et al. reported that SARS-CoV-2 was present in the semen of 6 COVID-19 patients out of total 38 patients tested (Li et al. 2020a). This study heaves the concern that SARS-CoV-2 can survive in the semen of infected male patients and could even persist in patients recovering from infection. Hence, the reproductive function of recovered male patients should be regularly monitored for a long term to eliminate the chances of sexual transmission of this deadly virus.

The impact of COVID-19 on male reproductive hormones was assessed by two studies. In one study, sex-related hormone levels of 119 SARS-CoV-2 infective men of reproductive age were compared with 273 age-matched controls (Ma et al. 2020). They observed a higher luteinizing hormone (LH) level and a lower ratio of testosterone (T) to LH in the serum of COVID-19 group (Ma et al. 2020). In the second study, Rastrelli et al. checked the hormone levels in COVID-19 male patients admitted to the respiratory intensive care unit ICU. Clinical status of the patients worsened with a progressive reduction in T levels and higher LH levels. But concluding remarks cannot be made based on these results as the sex hormone analysis in these patients before infection was not observed (Rastrelli et al. 2020).

Also, as per these reports, SARS-CoV-2 infection does not cause secondary hypogonadism because gonadotrophin levels were reported to be high; rather it causes primary hypogonadism by damaging steroidogenesis in Leydig cells, thereby decreasing testosterone levels. It can thus be concluded that the increased LH, lower

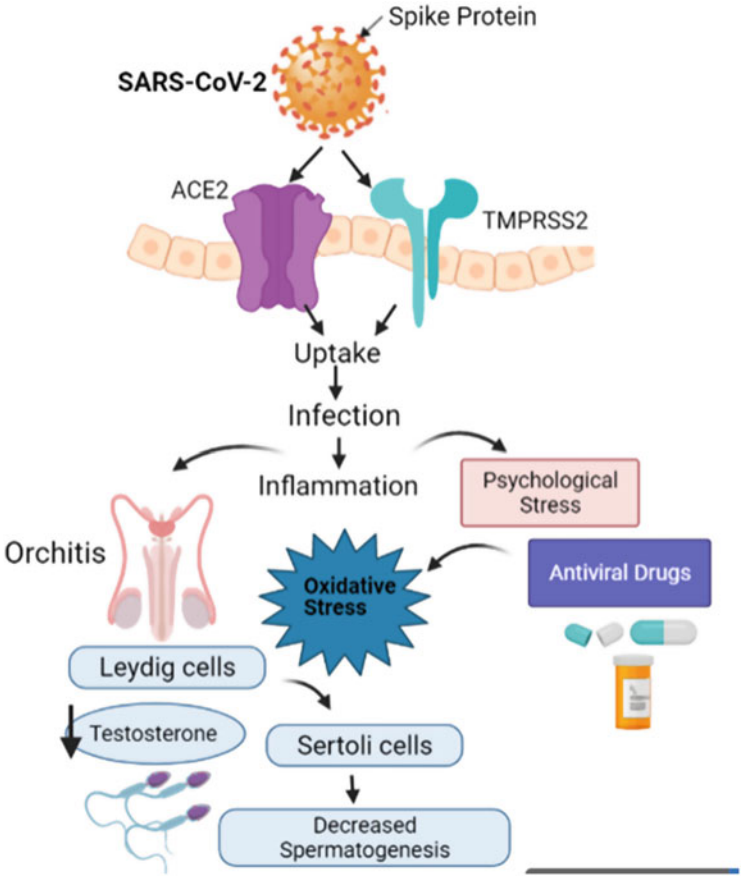

Fig. 23.2 Probable pathway followed by SARS-CoV-2 to deter male reproductive functions

serum testosterone levels, and reduced T:LH ratio could be due to testes dysfunction as a consequence of plausible damage of Leydig cells by SARS-CoV-2.

In a recent study, it was found that 8 out of 11 COVID-19-healed sexually active male patients were crypto-azoospermics and 3 were oligospermics. Although this observation nullifies the risk of SARS-CoV-2 sexual transmission after recovery, still caution should be taken during assisted reproduction and cryopreservation concerning the semen of healed COVID-19 patients (Gacci et al. 2021).

As per the available evidence on the association of SARS-CoV-2 with reproductive hormones, it can be suggested that reduction in testosterone level in COVID-19 patients is because of secondary immune reactions rather than due to viral influence on the hypothalamus-pituitary-testicular (HPT) axis as observed by increased levels of gonadotrophins. Moreover, Tian and Zhou in their very recent study concluded that the presence of virus in testicular tissues is not entirely credited to the viral infection but may occur because of high viral load in blood, local inflammation,

hyperpyrexia, and inefficient blood-testis barrier (BTB). Thus, it may be possible that the patients with mild COVID-19 infection may not experience any side effects (Tian and Zhou 2021).

Hence, before arriving at a definitive conclusion on the impacts of SARS-CoV-2 on male fertility and testicular functions, more comprehensive studies are required with reference to detailed physiological as well as pathological examinations of the male reproductive system in COVID-19 patients during infection and post-recovery.

23.5 Effect of SARS-CoV-2 on Female Reproductive System

Previous viral attacks on humankind have been known to infect the female reproductive system, for example, the Ebola virus (Rodriguez et al. 1999) and the Zika virus (Prisant et al. 2016). ACE2 is the crucial mediator of regulating the Ang II and Ang (1–7) levels and thus indirectly responsible for steroid secretion (Shuttleworth et al. 2002; Hayashi et al. 2003), follicle development (Shuttleworth et al. 2002; Ferreira et al. 2011), and oocyte maturation (Yoshimura et al. 1992; Giometti et al. 2005; Stefanello et al. 2006). It also affects ovulation (Kuji et al. 1996; Acosta et al. 2000; Guo et al. 2012) and modulates luteal angiogenesis and rupture (Sugino et al. 2005). On the other hand, Ang-(1–7) enhances ovulation by accelerating the production of estradiol and progesterone (Costa et al. 2003; Muthalif et al. 1998; Viana et al. 2011) and resuming the meiotic division in the oocyte (Honorato-Sampaio et al. 2012). Hence, it can be estimated that the nonavailability of ACE2 due to SARS-CoV-2 may hinder female fertility by affecting oocyte quality and function.

Recently, mRNAs of the SARS-CoV-2 gene receptors like ACE2, TMPRSS2, Basigin (BSG), and others were found expressed in the human female reproductive tract. High expression has been recorded in the ovary (cumulus cells) and endometrium and even during the early developmental stages of the human embryo (Henarejos-Castillo et al. 2020; Hikmet et al. 2020; Stanley et al. 2020; Weatherbee et al. 2020). Although in a cohort study a negligible amount of viral RNA was detected in the 16 oocytes isolated from two asymptomatic women (Barragan et al. 2021), this study left a trace of probability that the symptomatic women may shelter viral particles in their oocytes, which may affect the future progeny. All the above findings hint towards a minute yet credible vulnerability of the female reproductive tissues for SARS-CoV-2 infection.

Qiu and co-workers in 2020 reported the case where the presence of SARS-CoV-2 in vaginal fluid was assessed in ten patients, but none were found to be positive for SARS-CoV-2 as observed by RT-PCR (Qiu et al. 2020). Studies related to the presence of SARS-CoV-2 in the oocytes of infected or recovered females are pretty scarce. Because of the limited data available on the vulnerability of the female reproductive system towards SARS-CoV-2, no definitive statement can be made yet. However, more research should be conducted on women going through infection and recovery phase to generate more inference on the topic and precautions must be taken to avoid the unforeseeable circumstances and health hazards.

23.6 Vertical Transmission

Pregnancy represents a vulnerable period that may be easily influenced by COVID-19 pandemic and its outcomes. The pregnant women thus constitute a group that requires special care concerning the SARS-CoV-2 risk. As per an established definition of vertical transmission of SARS-CoV-2, an innate neonatal infection can be confirmed if the amniotic fluid collected before the rupture of membranes or the blood drawn early in life shows the presence of the virus (Shah et al. 2020) (Fig. 23.3).

The following criteria are obligatory for successful transplacental transmission of the virus, namely—(a) SARS-CoV-2 must make its way to the placenta and (b) presence of ACE2 receptors in placenta to facilitate the viral entry. The first condition is fulfilled by several research papers reporting the presence of SARS-CoV-2 in placental tissue. Specifically, the placental alteration has been observed in histopathological studies of pregnant women having SARS-CoV-2 infection, in addition to inflammation and abrupted vascular supply (Baergen and Heller 2020; Baud et al. 2020; Kotlyar et al. 2020; Patanè et al. 2020; Whittaker et al. 2020). Concerning the second condition, several studies reported the ACE2 receptors expressed in the placenta, ovaries, uterus, and vagina (Valdés et al. 2006; Cui et al. 2020; Jing

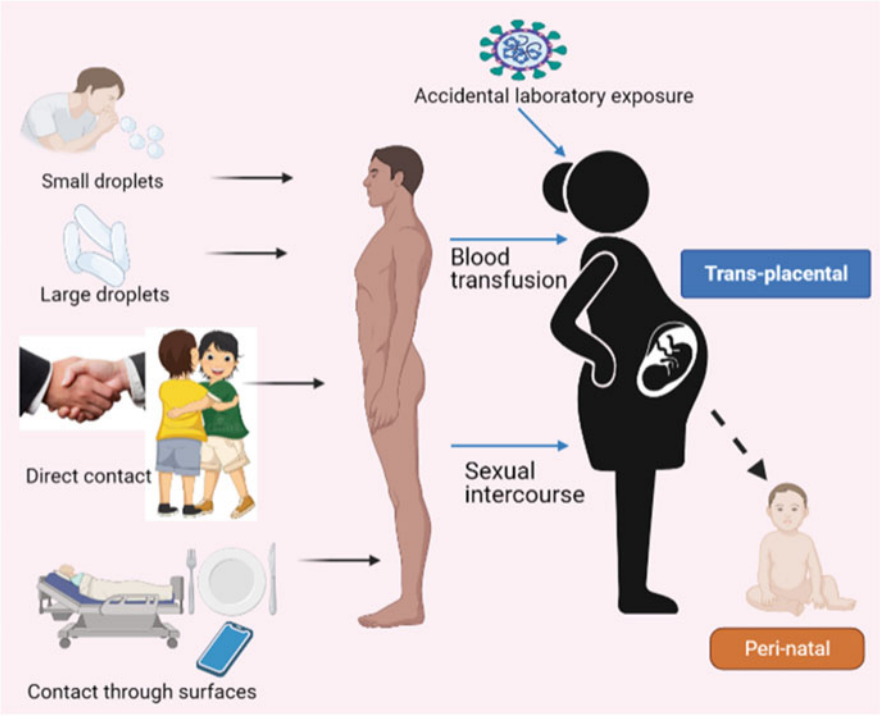

Fig. 23.3 Risk of vertical transmission of SARS-CoV-2

et al. 2020). However, various studies have reported that SARS-CoV-2 was found missing in breast milk, amniotic fluid, or cord blood (Rasmussen et al. 2020; Sighaldeh and Kalan 2020; Yang et al. 2020b).

As an inference drawn from studies, SARS-CoV-2 affects pregnant and nonpregnant women equally. Both underwent similar symptoms like fever, cough, shortness of breath, diarrhea, malaise, muscle pain, chills, sore throat, headache, loss of taste and smell, etc. as a consequence of novel coronavirus infection. Although the severity of most symptoms is reduced and the symptoms occur less frequently during pregnancy (Allotey et al. 2020; Juan et al. 2020; Zaigham and Andersson 2020), pregnant women need intensive care more often for proper ventilation as compared to nonpregnant women of reproductive age. Seventy-three deaths of COVID-19-positive pregnant women have been reported in data compiled from 26 studies conducted on 11,580 women (Allotey et al. 2020).

The possible risk areas of vertical transmission include the placenta and maternal-neonatal at the time of delivery and lactation. Only a single study (Chen et al. 2020b) tested the presence of SARS-CoV-2 in amniotic fluid or umbilical cord blood of COVID-19-positive women; however no viral traces were found. Vivanti and co-workers reported a case of transplacental transmission of SARS-CoV-2 from an infected woman to fetus during late pregnancy (Vivanti et al. 2020). While a few studies failed to detect SARS-CoV-2 in neonates, some were able to report the specific antibodies in them (Dong et al. 2020; Zeng et al. 2020a; Zhu et al. 2020). Nevertheless, a few studies found the virus in the neonate samples, but lack the data regarding the possible route of transmission since the placenta, amniotic fluid, and maternal/newborn blood were not tested systematically (Alzamora et al. 2020; Lorenz et al. 2020; Yu et al. 2020; Zeng et al. 2020b).

Although a few studies reported viral traces in the breast milk of COVID-19-positive mothers (Buonsenso et al. 2020; Groß et al. 2020; Wu et al. 2020c), the mother's milk cannot be considered as a significant source for COVID-19 infection. It contains specific protective antibodies that could prevent a possible newborn infection by SARS-CoV-2 (Davanzo 2020). Given the evident benefits of breastfeeding, the World Health Organization (WHO 2020) strongly recommends that women with COVID-19 be encouraged and supported to breastfeed, wear masks, and adopt contact precautions.

Most of the case reports and case series studies reported the maternal infection in the third trimester of pregnancy without any fatalities and good neonatal clinical outcomes. Although the methodology varied among studies, usually the neonatal samples included serum and pharyngeal swabs besides breast milk, placenta, amniotic fluid, and umbilical cord blood for detailed laboratory analysis (Chen et al. 2020c; Fan et al. 2020; Liu et al. 2020; Loffredo et al. 2020; Vaira et al. 2020; Wang et al. 2020b; Zeng et al. 2020b). Among these, only one study reported a SARS-CoV-2-positive pharynx swab in a newborn of 36-h age (Wang et al. 2020b). Besides, high levels of IgM along with IL-6 and IL-10 in neonatal blood further substantiate the vertical transmission of SARS-CoV-2 (Dong et al. 2020; Zeng et al. 2020a).

Hence, the detection of IgM and IL-6 in neonatal serum is suggestive of possible intrauterine infection. The presence of SARS-CoV-2 in neonatal nasopharyngeal swabs cannot be a clear evidence since the samples were collected hours or days after birth which can be due to nosocomial infection as the virus was not detected in amniotic fluid, placenta, or breast milk (Simões et al. 2020).

23.7 Psychological Stress Concerning COVID-19

A minimal amount of data is available till now regarding the psychological influence of the COVID-19 pandemic, which will probably linger for many months and years to come. Psychological stress due to SARS-CoV-2 infection triggers the hypothalamic-pituitary-adrenal (HPA) axis and primes the depression, anxiety, psychiatric disorders, and post-traumatic stress disorder (PTSD) (Li et al. 2020b; Steenblock et al. 2020).

The isolation, anxiety, fear, death of a kin or friend, loss of job, and financial uncertainty pertaining to COVID-19 can cause psychological stress. COVID-19 not only is responsible for increased psychological stress but paves the way for other types of stress as well like restraint stress comprising lockdown implications, psychiatric and neuropsychiatric glitches in the patients, and people related to them (Park et al. 2020; Rogers et al. 2020).

Women fall more vulnerable prey to SARS-CoV-2 infection owing to their caring instincts; ignorance towards self, sexual, and reproductive health; and increased domestic violence cases. The reduced production and nonavailability of contraceptive supplies have further worsened the condition of women, comprising unwanted pregnancies accompanied by stress and depression (Sharma and Borah 2020). It is a well-known fact that gender differences occur between women and men regarding COVID-19 infection (Cai 2020). This differential risk attributes to breaching of social isolation, social liabilities, psychological stress, poor quality of life, financial instability, etc. On the basis of this theory and earlier studies, Prasad and colleagues speculated a stress-induced pathway resulting in the augmentation of reactive oxygen species (ROS) in the ovaries. Once ROS levels cross the physiological limit, oxidative stress is caused to hinder the growth of follicles and induce the apoptosis of oocytes, eventually instigating poorer reproductive outcomes (Prasad et al. 2016). Like their male counterparts, women with psychological disorders can have sexual dysfunction (Yehuda et al. 2015; Brotto et al. 2016; McCabe et al. 2016).

COVID-19 has caused high mortality worldwide, due to the lack of proper vaccination against the virus. The elderly and people with underlying conditions or immune deficiencies lie at a much greater risk. Moreover, the health professionals who work tirelessly to help the infected people are also exposed to a higher viral load.

Most pregnant women undergo a various change in the emotions, which may lead to anxiety and depression. Conditions such as extreme stress, conflict situations, poor access to hospitals, and natural disasters can impose perinatal mental health morbidity risks. Henceforth, even if not infected pregnant women are vulnerable to

mental ill effects during the COVID-19 pandemic. Although the presence of SARS-CoV-2 in a second-trimester placenta has been demonstrated, the risk of miscarriage as an outcome of SARS-CoV-2 infection remains unclear (Baud et al. 2020). These uncertainties further add to psychological stress which itself may become reason for increased rates of pregnancy terminations.

The prolonged pandemic chaos will inevitably lead to economic consequences, uncertainties related to reproductive health, and emotional stresses. All these collectively may intensify the psychological burden and the mental well-being of infected patients, pregnant women, and new mothers. Strict public health measures directed towards mitigation of viral spread and securing people's mental health are necessary. The patients on the verge of psychological distress should be identified and counselled with psychosocial help and support.

23.8 Concluding Remarks

SARS-CoV-2 is no longer a novel topic of concern for humankind as we have completed one year of the pandemic, and the spread is still not completely stopped. The number of studies concerning the impact of SARS-CoV-2 on the reproductive systems is pretty scarce. It is quite clear that males are more prone to getting infected than females due to abundant ACE2 receptors in the male reproductive tract. No definitive statement can be made yet regarding the safety of the female reproductive system, as only a few studies have been carried out. Those, too, showed the negligible impact of SARS-CoV-2 on females. Most importantly, there have been no long-term implemented studies on the substantial effects of this virus on reproductive functions; therefore, the probability of risk cannot be excluded as yet. There is a dire need for in-depth research to evaluate the causal mechanisms of how COVID-19 impacts men and women's health and fertility. Future research should include comprehensive assessment of ovarian function in females and spermatogenesis and testicular function in males during the acute and recovery phases so that the reproductive systems could be protected to the maximum extent.

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An Insight into Association Between COVID-19 Infection and ABO Blood Group

24

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Abstract

Coronavirus disease 2019 (COVID-19), a pandemic that is triggered by a novel coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or 2019-nCoV, causes primarily respiratory discomfort along with other mild symptoms/no symptoms, leading to severe illness and death, if proper care is not taken. At present, COVID-19 is the resilient reason for a large number of human casualties worldwide as well as a cause of crucial economic loss posturing global threat. There is a necessity of intensive research to elucidate the pathogenic mechanisms of COVID-19, which would assist in understating the susceptibility towards the infection as well as prompt development of effective prevention and treatment strategies. Over the years, clinical studies have indicated the risk of various pathogenic infections prejudiced due to preexisting chronic diseases as well as ABO blood group types to a larger extent. In line of this, current COVID-19 infection-associated clinical studies intensely endorse the relationship of blood group type of individual and risk of COVID-19 infection. In this chapter, various clinical studies from January 2020 to June 2020 have been summarized to highlight the eminence of ABO blood group and COVID-19 infection susceptibility in human population. These reports evidently support the fact that individuals with A histo blood group were found to be more vulnerable to COVID-19 infection whereas individuals with blood group O were less likely to get infected with virus. To get deeper insight in this fact, many more studies are desirable in order to further explicate the promising protective role of the blood

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group O and it will be supportive for designing and planning several additional countermeasures against COVID-19 infection.

Keywords

SARS-CoV-2 · COVID-19 · ABO blood groups · Susceptibility · Genetic loci

24.1 Introduction

The Austrian pathologist Karl Landsteiner discovered the ABO blood group system in 1901, who categorized the blood groups on the basis of the presence of A and B antigens on the surface of the red blood cells (RBCs) after observing the patterns of agglutination during blood transfusion (Shereen et al. 2020). There are various accumulating evidences which reflect that ABO blood group system is profoundly associated with numerous human diseases such as cardiovascular disorders encompassing hyperlipidemia, atherosclerosis, heart failure, etc. along with diabetes and other pathological conditions (Cooling 2015; Dean and Dean 2005; Ruvoën-Clouet et al. 2000). Few more studies also conferred that individuals with blood group A and B are at higher risk of developing thromboembolic diseases, when compared against O blood group individuals (Issitt and Anstee 1998). The footprint of ABO blood group is also found in individuals with type 1 and type 2 diabetes (Mollicone et al. 1988; Hoffmann et al. 2020). Even in more complex scenarios of different cancers, the involvement of ABO blood group has been proved. Individuals with blood group A are more vulnerable to develop pancreatic cancer, nasopharyngeal carcinoma, and lung cancer, while individuals with blood group O are less likely to develop the same (Park et al. 2020; Zhou et al. 2020; Watanabe et al. 2019).

Human ABO blood group system comprises the three main antigens (A, B, and H) and these antigens make the four phenotypes of the blood group (A, B, AB, and O) by the sequential addition of carbohydrate units to the oligosaccharide precursor (Shereen et al. 2020). Antigen H (blood group O) is the precursor form with a backbone of galactose, NAG, galactose, and fucose. Further addition of N-acetylgalactosamine and D-galactose to the precursor backbone makes the blood groups A and B, respectively, shown in Fig. 24.1 (Cooling 2015; Ruvoën-Clouet et al. 2000). Antibodies produced against the missing ABO blood antigens are shown in Table 24.1 (Cooling 2015; Mollicone et al. 1988). Apart from RBCs, ABO blood group antigens are present on the lymphocytes, different organs, mucosal surfaces, and exocrine secretions (Cooling 2015; Issitt and Anstee 1998; Mollicone et al. 1988). The genetically inherited traits like ABO histo blood group are distributed variably among various populations. The distribution frequency of these blood groups in a certain population also decides the occurrence of some particular blood groups. For example, O and AB blood types have the most and least prevalence in many populations, respectively.

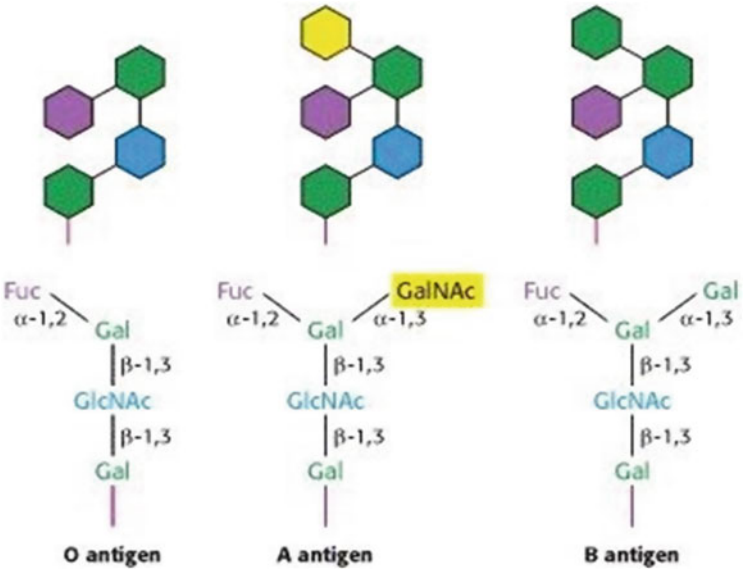

Fig. 24.1 Structure of the H, A, and B antigens of ABO blood groups

Table 24.1 Different blood groups with different antigens, different antibodies, and different genotypes

Blood group	Antigen(s)	Antibodies	Genotypes
A	Antigen A	Anti-B	AA, AO
B	Antigen B	Anti-A	BB, BO
AB	Antigens A and B	–	AB
O	–	Anti-A and -B	OO

24.2 SARS-Cov-2 Invasion Via ABO (H) Blood Group-Determining Carbohydrates

The SARS-CoV-2 enters human target cells via its viral transmembrane spike (S) glycoprotein which is a trimeric class I fusion protein and consists of two subunits, namely S1 and S2. The S1 subunit mainly facilitates the attachment of the virus and subsequently the S2 subunit allows the fusion of the viral and human cellular membranes (Hoffmann et al. 2020; Park et al. 2020; Zhou et al. 2020). Each subunit of S protein is highly glycosylated with both N-linked and O-glycosylation sites. The glycosylation pattern of the spike protein plays an important role in protecting the amino acid residues and other epitopes from cells and antibody recognition, which enables the coronavirus to evade both the innate and adaptive immune responses (Park et al. 2020).

On the other side, O-glycosylation of spike protein of SARS-CoV-2 and ACE-2 receptor plays an important role in the entry of virus in humans which is similar to the mechanism of SARS-CoV-1 which was found 14 years back. Watanabe suggested that SARS-CoV-2 needs to form an intermediate hybrid network with ACE-2 to invade the human cell (Watanabe et al. 2019). It binds to the ACE-2 with the help of viral spike(s) protein to make an ACE-2-S protein complex, which is subsequently cleaved by Cathepsin-L, and then the virus enters the host cell by receptor-mediated endocytosis (Inoue et al. 2007; Simmons et al. 2005). The crucial step in the whole host-viral entry is the mobilization of the viral serine molecule that is carried out by host TMPRSS2 (transmembrane protease serine subtype 2) (Wang et al. 2008; Matsuyama et al. 2010). After the association of disease severity of individuals with blood group A or other non-O blood groups it was pointed out that ABO(H) blood group phenotype-determining sugars were proved to be the crucial glycosidic targets in SARS-CoV-2 infection.

The serine residues preserved on the viral spike protein are present in the involvement of host's transmembrane serine protease TMPRSS2 (Hoffmann et al. 2020), while the ACE protein performs a hybrid binding by mediating the ABO (H) glycan-transferring activity (Cidl et al. 1996). Different pathogenic viruses and human ABO(H) glycans have been known to have interacted for decades. For example the infectivity of a human rotavirus has been known to have specifically abrogated by anti-A antibodies (Lee et al. 2018). The anti-histo blood groups have also left a significant mark as a defense mechanism against the SARS-CoV outbreak back in 2002 (Hong Kong) which has also been elaborated earlier (Guillon et al. 2008). It was explained in animal model which showed that the SARS-CoV S protein/ACE2-dependent adhesion was explicitly inhibited by either a monoclonal anti-A or a natural plasma anti-A antibody. This model had also shown that the main function of the natural anti-histo blood group antibodies is to slow down and delay the epidemic. So, this study concluded that anti-histo blood group antibodies could have a strong effect (full protection) by delaying the duration of first-case occurrence and the total development of the outbreak which ultimately may lead to a huge impact on human life. It is also connected to the frequency of occurrence of O individuals in the population. There have been a number of other significant studies in the past which showcased the importance of numerous other large molecules like mannose or lectins binding to the glycan (viral S protein) and disrupting the viral/ACE2 interaction (Keyaerts et al. 2007).

Till date, several plant lectins have been identified which are very much specific for the human ABO blood group glycosides and have been used for the purpose of blood typing for many decades. Another study found out similarities between SARS-CoV-2 spike protein and lima bean lectin where the lima bean agglutinin (LBA) is specific to blood group A and selectively binds to the terminal N-acetyl-galactosamine of the group A polysaccharide (Singh and Walia 2018) (Fig. 24.2).

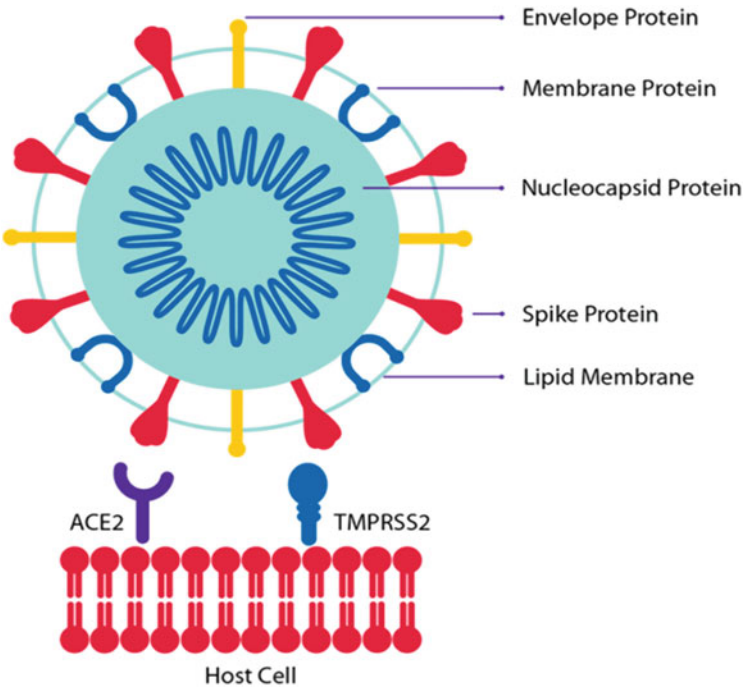

Fig. 24.2 Interaction between SARS CoV 2 and the host cell, respectively

24.3 ABO Group Types and SARS-Cov-2 Infection Rate

There have been many clinical reports in the last few months which show blood group information of COVID-19-infected patients along with healthy individuals. In this particular chapter, we have included the data from January 2020 to June 2020 to highlight the importance of blood group specificity and COVID-19 infection susceptibility in vivid human population (Table 24.2). Zhao et al. compared blood groups of 23,386 healthy individuals with 285 SARS-CoV-2-infected patients in Shenzhen city and 3694 healthy individuals with 1775 patients in Wuhan city, China (Zhao et al. n.d.). The percentages of blood groups A, B, AB, and O in controls were 28.77%, 25.14%, 7.32%, and 38.77% versus 28.77%, 29.12%, 13.68%, and 28.42% in patients in Shenzhen city. Similarly, the study carried out in Wuhan city showed 32.16%, 24.90%, 9.10%, and 33.84% of A, B, AB, and O blood groups versus 37.75%, 26.42%, 10.03%, and 25.80%, respectively, in patients, which showed frequency of blood group A to be more prevalent in patients than in control ($p < 0.001$). These results corresponded to a significantly increased risk of COVID-19 for blood group A with an odds ratio of 1.27 (95% CI = 1.13–1.44) and decreased risk of COVID-19 for blood group O with an OR of 0.68 (95% CI = 0.59–0.77). In both the studies, it was found that blood group A people are

Table 24.2 List of studies that were carried out to compare the relation between SARS-CoV-2 and ABO blood types

SN	Studies	Controls/patients	Controls (%)				Patients (%)			
		(*n*)	A	B	AB	O	A	B	AB	O
1	Zhao et al. (Zhao et al. n.d.)									
	Study 1	3694/1775	32.16	24.90	9.10	33.84	37.75	26.42	10.03	25.80
	Study 2	23,386/285	28.77	25.14	7.32	38.77	28.77	29.12	13.68	28.42
2	Juyi Li et al. (Li et al. 2020)	3694/2153	32.2	24.9	9.1	33.8	38.0	26.1	10.2	25.7
3	Ghada Ali et al. (Aljanobi et al. 2020)	5291/72	23.60	23.87	3.91	48.62	23.62	33.33	9.72	33.33
4	Abdollahi et al. *(*Abdollahi et al. 2020*)*	500/397	36	21	5	38	40.3	22.4	9.3	28
5	Goker et al.*(*Göker et al. 2020*)*	1881/186	38	–	–	37.2	57	–	–	24.8
6	Y. Wu et al. *(*Wu et al. 2020*)*	1991/187	27.47	32.35	9.99	30.19	36.90	33.69	7.49	21.92
7	Minfei Peng et al. *(*Peng et al. 2020*)*	82/138	26.8	28.0	7.3	37.8	–	–	–	–
	Severe	32	–	–	–	–	34.4	34.4	12.5	18.8
	Non-severe	106	–	–	–	–	28.3	27.4	11.3	33.0

more susceptible to get COVID-19 infection, whereas O blood group people were the lowest to get the hit. The blood groups were also checked in dead patients ($n = 206$) as well to have a total view of the situation. The percentages of A, B, AB, and O blood groups in dead patients infected with SARS-CoV-2 infection were 41.26%, 24.27%, 9.22%, and 25.24%, respectively. In this case as well, blood group A had much higher frequency compared to other non-A blood groups with OR = 1.48 (95% CI = 1.11–1.97, $p = 0.008$).

In another study, Juyi Li et al. included 2153 patients diagnosed with COVID-19 infection from February 1st, 2020, to March 25th, 2020, and 3694 healthy controls. ABO blood group analysis showed 38.0% patients with blood group A, 26.1% with B, 10.2% with AB, and 25.7% with blood group O compared to 32.2% (A), 24.9% (B), 9.1% (AB), and 33.8% (O) in control ($n = 3694$) (Keyaerts et al. 2007; Li et al. 2020). Proportion of blood group A in COVID-19-positive patients was very high than that in healthy controls (38.0% vs. 32.2%, $p = 0.017$), while the proportion of blood group O in patients was lower than that in healthy controls (25.7% vs. 33.8%, $P < 0.01$). Guillon et al. found that natural anti-A antibodies inhibited the S protein/ACE-2-dependent adhesion of these cells to an ACE-2-expressing cell line that might block the interaction between virus and its receptor which explains why blood group O is less susceptible to COVID-19 infection (Guillon et al. 2008).

The first Middle Eastern study by Ghada Ali Aljanobi et al. of Qatif Central Hospital, Eastern Province, Saudi Arabia, demonstrated the relationship between ABO blood groups and COVID-19 infection wherein blood group O is the least affected blood group against the viral invasion among the Saudi patients (Aljanobi et al. 2020). Another study conducted in Tehran University of Medical Sciences, Iran, acquired the similar approach on 500 controls and 397 patients to find the relation between ABO blood group in patients and COVID-19 infection (Abdollahi et al. 2020). They performed binary logistic regression analysis to assess the combined effect of various blood groups on COVID-19 susceptibility. The percentages of A, B, AB, and O blood groups in the patients were 40.3%, 22.4%, 9.3%, and 28%, whereas in controls it were 36% (A), 21% (B), 5% (AB), and 38% (O). This study also suggested that individuals with O blood group are less susceptible to SARS-CoV-2 infection. After carrying out the ABO blood group analysis in the same study, all patients were segregated into ICU and non-ICU category and the percentages of blood groups A, B, AB, and O found in ICU patients and non-ICU patients were 40.2%, 22%, 7.9%, and 29.9% and 40.4%, 22.6%, 10%, and 27%, respectively. Patterns of outbreak in Iran and China might be different due to the discrepancy of the unequal distribution of ABO blood group. AB is the least prevalent blood group among populations and the individuals with blood group A are on the higher side. This could be a reason in the present situation as per the limited clinical reports that the percentage of Iranian people getting infected with SARS-CoV-2 is less likely compared to China (Abdollahi et al. 2020). This study shows that the percentage of O blood group in COVID-19 patients was lesser in Iranian population.

Goker H. from Hacettepe University, Turkey, published a study regarding the relationship between ABO blood grouping and severity of SARS-CoV-2 infection in which out of 1881 controls, 38% of individuals were with blood group A and 37.2% of individuals with blood group O, while in 186 patients, blood group A was found to be more frequent and blood group O was the least affected (Göker et al. 2020). Wu et al. carried out a study with 1991 controls and 187 patients to demonstrate the relation between ABO blood group and COVID-19 infection, and the distribution of A, B, AB, and O blood groups in controls was 27.47%, 32.35%, 9.99%, and 30.19% versus 36.90%, 33.69%, 7.49%, and 21.92% in patients (Wu et al. 2020). Keeping this line of investigation, Peng and team (Peng et al. 2020) conducted a study on the distribution of ABO blood type in COVID-19-positive patients (138) and controls (82). They further segregated the positive cases into severe ($n = 32$) and non-severe (106) categories. In this study they observed that the blood group O was associated with the lower risk possibility.

A retrospective study of SARS-CoV-2 disease showed data from 105 countries till 13th April 2020 and it was further analyzed by statistical approaches in order to assess the associations between ABO blood group and COVID-19 (Alkout and Alkout 2020). To carry out this specific study, the world meter website (www.worldmeter.info) was used to collect data on SARS-CoV-2 cases from various countries and it was found that the frequency of blood group A was the highest in COVID-19-positive patients compared to the non-A blood group and the blood group O was negatively correlated with COVID-19 disease. Discrepancy in host susceptibility to various infections may be related to differences in blood group antigen expression. As the probability of the inhibition of the adhesion between SARS-CoV S and ACE-2 was earlier discussed by either a monoclonal anti-A or a natural plasma anti-A antibody (Guillon et al. 2008), the process of opsonization that leads to the complement-mediated neutralization could also be utilized as another way of blocking the interaction between the virus and its receptor by the body's natural antibodies (Eagan et al. 2007).

Recently, a case-control genome-wide association study (GWAS) was conducted which consisted of COVID-19 patients ($n = 1980$) and healthy subjects ($n = 2381$) from seven hospitals of Italy and Spain. Researchers found that there is a presence of genetic predisposition to severe COVID-19 which may be due to the involvement of two loci, i.e., rs11385942 insertion–deletion GA or G variant at locus 3p21.31 (comprising *SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6,* and *XCR1*) (odds ratio [OR], 1.77; 95% confidence interval [CI], 1.48–2.11) and the rs657152 A or C single-nucleotide polymorphism (SNP) at locus 9q34.2 (ABO blood group) (OR, 1.32; 95% CI, 1.20–1.47), which persisted after adjusting on the basis of sex and age, and was constant when investigated in Spain and Italy independently. With respect to ABO blood group locus, higher risk was associated with blood group A persons compared to patients with other blood groups (OR, 1.45; 95% CI, 1.20–1.75) and on the other side a protective effect for blood group O was observed as compared with the other blood group (OR, 0.65; 95% CI, 0.53–0.79) individuals. However, this study is also having its own limitations; therefore further efforts are required to

establish the genetic link of ABO blood types with COVID-19 (Ellinghaus et al. 2020).

Based on plenty of recent medical-scientific literature on laboratory-confirmed COVID-19-infected hospitalized patients it was consistently observed that clinical implication of ABO blood group types is one of the key factors for COVID-19 infection. Hence, ABO blood group typing could be an intriguing area to explore further any relationship between the ABO blood group and the COVID-19 susceptibility.

24.4 Conclusion

In the current pandemic situation, a number of studies are appearing across the world elucidating noteworthy link of ABO blood group and SARS-CoV-2 susceptibility which portrayed that individuals with A histo blood group were found to be more vulnerable to viral attack and blood group O individuals were less likely to get infected. More research work is needed to get deep insight into the detailed mechanistic regulation and involvement of ABO blood group for adhesion of viral protein and subsequent entry inside the human host. Individuals with blood group A are generally the target for the viral transmission so there is necessity to strengthen their personal protection in order to reduce the chance of infection. On the other hand, blood group A patients need to receive more aggressive treatment and vigilant surveillance. In view of all clinical reports, ABO blood typing could be introduced to manage the SARS-CoV-2 infection severity by clinicians and healthcare workers in all hospitals. This pandemic necessitates advancement of striking approaches to comprehend the SARS-CoV-2 infection which will help to untangle the facts about possibly additional relevant and significant functions of numerous genes located at locus 9q34.2 related to ABO blood group. One of the grave concerns in present COVID-19 infection scenarios is the widespread increase in asymptomatic infection rather than symptomatic infection and to combat it, extensive comprehensive investigations are required to characterize transmission modes, infection rate, and clinical spectrum of the disease for improvement and advancement of all possible strategies in all clinical practices.

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Herbal Formulations for the Treatment of COVID-19

25

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Abstract

COVID-19 has emerged as the most severe pandemic with rapid transmissibility. Initially the pathophysiology was not clear and the drugs and their basis to be used have been a great challenge. Even after over 1 year of COVID-19, strategies of the usage of drugs for the treatment have been to use existing drugs that have been repurposed and/or have been given "Emergency Authorization." Herbal medicines also emerged like other repurposed medicines in allopathy. The major focus for these drugs in various parts of the world has been prior evidence of antiviral or immunomodulatory activity. Some of the formulations could be developed from Africa, China, and India. Although the usage of such formulations started in different conditions without proper scientific studies or clinical trials, some formulations could be developed with limited but systematic clinical trials and it is expected to have its place in the world market and open new vistas of knowledge and research for COVID-19 and related diseases. It is suggested that however such herbal drug formulations must be promoted not only when allopathic drugs fail or one has a trust on traditional medicines but also if there is scientific basis for targets of COVID and evidences through clinical trials.

Keywords

COVID-19 · Herbal formulations · Immunomodulators · Antivirals

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25.1 Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)-induced coronavirus disease-19 (COVID-19) emerged as the most severe pandemic after the Spanish Flu of 1918. This pandemic has severely challenged humankind because of its rapidity. It produces transmission without any intermediate vector, and the clinical prognosis leads to death within less than a month. Even after 1 year, a drug or vaccine that regulatory agencies have appropriately and fully approved worldwide is not available. All those currently approved have been approved under "Emergency Use Authorization." Hence, complete safety and efficacy data are lacking. Under these circumstances strategies should not only be repurposed antiviral or other medicines but also herbal drugs based on traditional knowledge for the treatment of COVID-19 that may be made available. Various countries from Asia and Africa have been looking forward to developing herbal formulation to fight against COVID-19.

In India, the Ministry of AYUSH has issued the advisory and guidelines for the prevention and progression of the COVID-19. Some of these recommendations include consumption of indigenous *Ayurveda* immune boosters, like *Chyawanprash*, and a decoction made from *Ocimum sanctum, Cinnamomum zeylanicum, Piper nigrum*, and *Zingiber officinale*. Other plants suggested are *Syzygium aromaticum*, *Vitis vinifera*, jaggery/lemon juice, and hot milk containing turmeric (https://www.ayush.gov.in/index.html#courses). A large number of clinical trials got started. As per the Clinical Trials Registry of India (CTRI), there were 842 trials for COVID-19 with 232 based on Ayurveda (142), Unani (08), Siddha (24), homeopathy (26), phytopharmaceuticals (03), and miscellaneous (29), i.e., yoga, naturopathy, and nutraceutical system of medicine (http://ctri.nic.in/Clinicaltrials/pubview.php). The most common plants in the formulations under clinical trials are *Tinospora cordifolia, Glycyrrhiza glabra, Piper longum, Zingiber officinale*, and *Terminalia chebula*. Furthermore, a clinical trial was permitted for the first phytopharmaceutical or plant-based drug AQCH containing the purified aqueous extract of *Cocculus hirsutus*. This was initially developed for dengue. Moreover, only *Solanum nigrum* exhibited all the properties, viz. antiviral, antitussive, anti-inflammatory, and immunomodulatory properties out of several plants.

An inverse correlation was found between the case fatality rate and complementary alternative medicine (CAM) in various countries across the world. Similar to modern drugs, initial strategies concerning CAM were to repurpose the available treatments in various systems of medicines. The main focus in the CAM has been to increase the immunity in the body rather than provide antiviral activities and provide the treatment for associated cardiovascular and respiratory complications. In countries like Australia, New Zealand, and Japan the consumption of complementary medicine and medicinal practices like acupuncture, massage therapy, homeopathy, and naturopathy were applied for prevention of COVID-19 (Goyal et al. 2021, Unpublished data). In this chapter, we have described some successful herbal formulations that have been approved and used in China, Africa, and India.

25.2 Pathophysiology and Clinical Consequences of COVID-19

SARS-CoV-2 infection can occur through direct, indirect, or close contact with infected people, with or without symptoms, through their saliva or respiratory droplets. SARS-CoV-2 is a single-stranded RNA-enveloped virus that targets the angiotensin-converting enzyme-2 (ACE2) through its spike protein. It enters the body after endosome formation through transmembrane protease serine 2 (TMPRSS2) that facilitates cell entry via the S-protein. Once the virus has entered inside the cell, viral polyproteins are synthesized that encode for the replicase-transcriptase complex. The virus then synthesizes RNA via its RNA-dependent RNA polymerase (RdRp). Structural proteins are synthesized, and after assembly, new viruses are formed, and viral particles are released (Fig. 25.1) (Wan et al. 2020). Subsequently, because of high virus titer, strong cytokine surge and inflammatory response are induced in lungs and other organs where ACE2 is highly expressed,

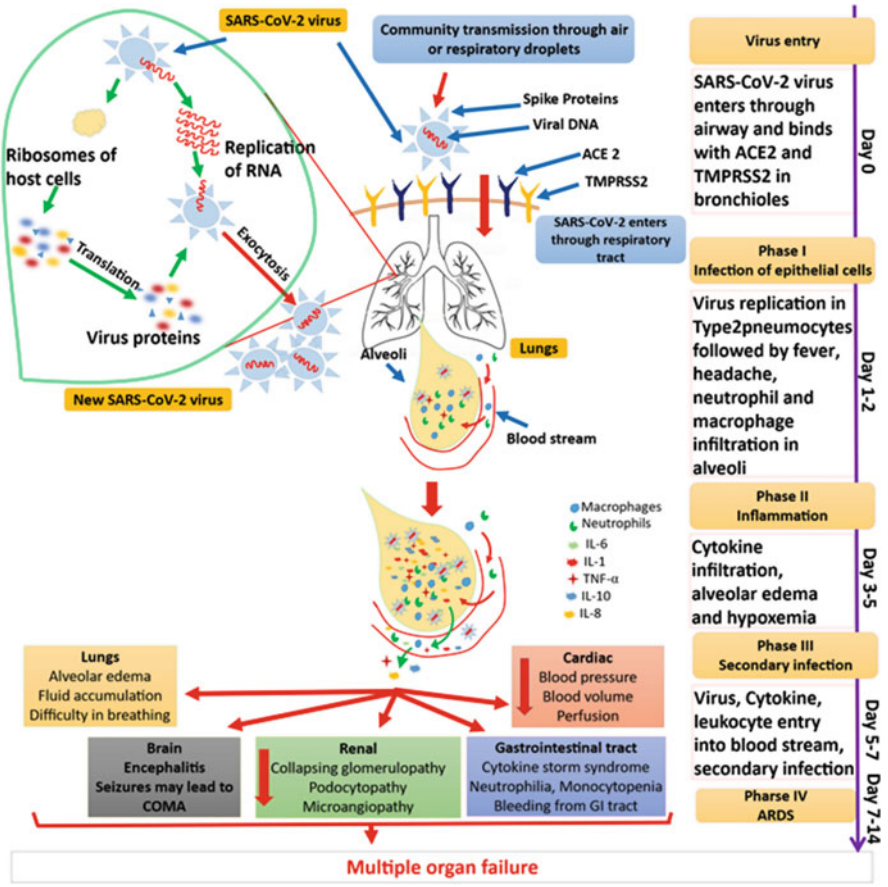

Fig. 25.1 Pathogenesis and various stages of SARS-CoV-2 infection

leading to high morbidity and mortality. The cytokines and neutrophils get into the bloodstream along with the SARS-CoV-2 virus. The alveoli infected with SARS-CoV-2 produce severe endothelial injury associated with disrupted vascular membranes and thrombotic microangiopathy. Severe endothelial injury increases vascular permeability and pulmonary edema, finally leading to the abolishment of hypoxic pulmonary vasoconstriction (vasoplegia) together with fluid-filled alveoli that finally cause acute respiratory distress (ARD) (Gattinoni et al. 2020). There are also secondary complications in cardiac, brain, kidney, and gastrointestinal tract leading to multiple-organ failure (Fig. 25.1).

Transmission of SARS-CoV-2 virus in host body is via respiratory tract through the air or respiratory droplets; SARS-CoV-2 virus binds to ACE2 and TMPRSS2 on the epithelial cells of bronchioles in lungs, specifically type 2 pneumocytes. The spike proteins help in binding to the receptor sites and further undergo ribosomal translation. While viral DNA is replicated through RNA and along with virus proteins, new cells are formed and exit the host cell through exocytosis. This triggers a host response to the virus through the infiltration of macrophages, neutrophils, and inflammatory cytokines leading to fever and headache as primary symptoms. Subsequently, alveolar edema is observed, causing difficulty in breathing and hypoxemia; also, the cytokines and neutrophils get into the bloodstream along with the SARS-CoV-2 virus. This causes secondary infection in the brain, lungs, kidney, and gastrointestinal tract and decreases cardiac function. Finally, there occurs acute respiratory distress syndrome (ARDS) leading to multiple-organ failure. ACE2, angiotensin-converting enzyme 2; IL-6, interleukin 6; IL-1, interleukin 1; IL-8, interleukin 8; IL-10, interleukin 10; TMPRSS2, transmembrane protease serine 2; TNF-α, tumor necrosis factor α

25.3 Strategies for Clinical Management with Drugs

In the beginning, there were no WHO- or FDA-approved medicines or vaccines available for COVID-19. The first strategy for drugs for the patients of COVID-19 was “repurposing of drugs” available for other viral diseases. In the month of April 2020 for COVID-19, there were 282 clinical trials initiated with “drug repurposing” as the strategy in India. Chloroquine, hydroxychloroquine, various monoclonal antibodies, corticosteroids, antibiotics, and antiviral drugs like arbidol, remdesivir, favipiravir, lopinavir, ritonavir, and oseltamivir were tested in clinical trials in various countries. WHO also came up with an initiative known as multicountry “Solidarity Trial” for developing a potential drug or therapy against COVID-19.

Considering previous coronavirus infections Middle East respiratory syndrome (MERS-CoV) and severe acute respiratory syndrome (SARS-CoV), and the epidemics that have occurred in the past decade, the first set of trials for the discovery of new drugs for COVID-19 was to repurpose antiviral drugs used in various retroviruses, i.e., for MERS, SARS, dengue, and AIDS. In addition, antiretrovirals, antimalarial drugs, antibiotics, antiparasitic, nonspecific anti-inflammatory, and immunosuppressive drugs, kinase inhibitors, monoclonal antibodies, and other

miscellaneous drugs are utilized for the design of new drugs and vaccines against coronavirus disease. **Remdesivir**, a nucleotide analog, is a broad-spectrum antiviral prodrug with effective *in vitro* antiviral activity against different RNA viruses such as SARS-CoV, MERS-CoV, Hendra virus, Nipah virus (NiV), Marburg, Ebola virus (EBOV), and respiratory syncytial virus (RSV) that provided success to a great extent. But it was again not a clear drug. Similar was the case with **favipiravir**, chemically known as 6-fluoro-3-hydroxy-2-pyrazine carboxamide, a pyrazine derivative that is used as an antiviral agent for the treatment of influenza in Japan. Other drugs like **ribavirin, arbidol** (**umifenovir), oseltamivir, bromhexine**, a transmembrane protease serine (TMPRSS2) inhibitor, and **fingolimod** (Gilenya), a sphingosine-1-phosphate receptor (S1P receptor) could not come up as real as an efficacious and safe drug for COVID-19.

Chloroquine and hydroxychloroquine are well-known antimalarial drugs with some immunomodulatory effects. It was later shown that they possess antiviral action, occurring at ACE2 cell entry level by inhibiting glycosylation of host receptors, proteolytic processing, and endosomal acidification. When hydroxychloroquine was reported to be potent against SARS-CoV-2, other antimalarial drugs like mefloquine and amodiaquine were tried and were not found effective against SARS-COV-2. Soon excessive immune mechanism involvement of COVID-19 surfaced, triggering repurposing research of many immunomodulators. Baricitinib, an inhibitory action on Janus kinase (JAK)1/JAK2, approved for rheumatoid arthritis, imatinib mesylate and dasatinib, the inhibitors of the kinase signaling pathway; trametinib and selumetinib, the potent inhibitors of MEK, and many other immunomodulators came into the race. Tocilizumab, however, got a greater success among them. Surprisingly, simple dexamethasone proved highly useful as per the reports from Italy.

Apart from respiratory failure and immune or cytokine surges, other clinical reports showed that in COVID-19 patients who are critically ill, patients have high incidences of thromboembolism. Hemostatic abnormalities include mild thrombocytopenia and increased D-dimer levels leading to death. The use of anticoagulant drugs in COVID-19 patients became yet another strategy but only to the patients having higher D-dimer levels. Based on the clinical symptoms reported from time to time and then clarity of the pathophysiological consequences of COVID-19, the use of nonconventional antibiotics like azithromycin, steroids like dexamethasone and monoclonal antibodies for immunomodulation, antithrombotics or thrombolytics, and certain food supplements were recommended by the statutory bodies in different countries.

In lieu of these, major targets that have emerged for the treatment include antiviral drugs that inhibit viral TMPRSS2, 3-chymotrypsin-like protease, and RdRp and immune-modulatory agents that prevent or attenuate cytokine surge during COVID-19. In addition, the majority of the drugs are used to treat symptoms like fever, dry cough, malaise, respiratory distress, and neurological complications like anosmia. Table 25.1 lists drugs that have emerged as most commonly used worldwide and a comparison of some herbal medicines that have emerged in India.

Table 25.1 Comparison of successful allopathic and equivalent herbal drugs from India

Sr. No.	Stage of disease	Recommendations as per NIH/ICMR (Modern Medicine)	Herbal medicines based on Ayurvedic literature/Ayurvedic Pharmacopeia of India
1	Virus entry (day 0)	None in particular for prevention or asymptomatic patients	*Tinospora cordifolia, Piper longum, Terminalia chebula, Withania somnifera, Glycyrrhiza glabra, Solanum nigrum*
2	Phase I Infection of epithelial cells (days 1–2)	Remdesivir, favipiravir, ivermectin, doxycycline	*Ocimum sanctum, Tinospora cordifolia, Glycyrrhiza glabra, Piper longum, Piper nigrum, Terminalia chebula, Zingiber officinale, Solanum nigrum, Adhatoda vasica*
3	Phase II Inflammation (days 3–5)	Prednisolone, dexamethasone, enoxaparin	*Terminalia chebula, Withania somnifera, Solanum nigrum*
4	Phase III Secondary infection (days 5–7)	Prednisolone, dexamethasone, enoxaparin, remdesivir	*Solanum nigrum*[a], *Glycyrrhiza glabra, Cinnamomum zeylanicum*
5	Acute respiratory distress syndrome	Prednisolone, dexamethasone, enoxaparin, tocilizumab, itolizumab	*Solanum nigrum*[a]

The treatment using modern medicine as recommended by NIH/ICMR is provided. Based on Ayurvedic literature/Ayurvedic Pharmacopeia of India, herbal medicines which can be used in different stages as adjuvant to modern medicine are also provided

[a]Herbal medicines' recommendation based on studies in our laboratory

25.4 Expectations from Traditional Medicine(s)

World Health Organization (WHO) has recognized that traditional, complementary, or alternative medicines have many benefits. Various Asian countries and Africa have a long history of traditional medicine and practitioners that play an essential role in providing care to populations (World Health Organization 2020). Traditional medicine, unlike allopathic medicine, resorts to a holistic approach to the treatment of a given disease and mainly uses plants that are available and affordable to the general population. Modern scientists often call traditional medicine quacks and criticize variation from batch-to-batch samples and toxicities of some herbal medicinal preparations. Despite these criticisms, traditional Chinese medicine (TCM) and Indian traditional medicinal systems like Ayurveda, Unani, Siddha, and homeopathy have been present for over a thousand years and are still being used in many countries. These medicines cannot treat COVID-19 and are usually not a single entity since these systems are based on the principles of symptom-oriented holistic treatment. The objective of herbal based traditional medicines has always been to provide a cure and not just to treat the symptoms and thereby help the patient regain

strength and boost up the immune system and mental confidence to deal with the virus and the disease.

25.5 Some Potential Formulation of Traditional Chinese Medicine Used for Prevention/Treatment of COVID-19

From the beginning of the COVID-19 outbreak around December 2019, herbal medicines in TCM were widely used across China to slow down the surge of COVID-19 cases. A clinical report was published in March 2020, which showed that a herbal formulation recommended by the National Health Commission of China effectively attenuates ARDS syndrome in a mild COVID-19 patient (Xu and Zhang 2020). It was the first of its kind to report the beneficial role of plants in the treatment of COVID-19 infection. Several reports systemically summarized the traditional medicines frequently used in China during the COVID-19 pandemic and performed a meta-analysis, proving the therapeutic outcome of herbal medicine.

Approximately 91.5% of confirmed patients in China were treated with TCM formulae, and the total effective rate has reached about 90%. In one of the hospitals in Wuhan, 564 COVID-19 patients received both the treatment, i.e., TCM and modern medicine, and none of them developed into severe conditions. The addition of TCM significantly reduced the course of hospitalization (Ren et al. 2020). The efficacy of herbal medicine in alleviating ARDS caused by SARS-CoV-2 has been endorsed by a regulatory agency of China and the frontline healthcare workers.

Huang et al. reviewed all the herbal products used for COVID-19 and approved them by China's regulatory agency, which has assigned patent numbers. The licensed Chinese herbal formulations which have been used for acute respiratory infection are Lian-Hua-Qing-Wen Capsule, Huo-Xiang-Zheng-Qi, Jin-Hua-Qing-Gan Granule, Shu-Feng-Jie-Du, Su-He-Xiang, An-Gong-Niu-Huang, Xi-Yan-Ping, Xue-Bi-Jing, Re-Du-Ning, Tan-Re-Qing, Xing-Nao-Jing, Shen-Fu, Sheng-Mai, Pu-Di-Lan, Yin-Qiao, Yu-Ping-Feng-San, Sang-Ju, Shuang-Huang-Lian, Ma-Xing-Shi-Gan, Bai-He-Gu-Jin, and Ren-Shen-Bai-Du. The most common plants identified in the licensed Chinese herbal formulations are *Glycyrrhiza inflata* Batalin, *Forsythia suspensa* (Thunb.) Vahl, *Lonicera Japonica* Thunb., *Scutellaria baicalensis* Georgi, *Platycodon grandiflora* (Jacq.) A. DC., *Mentha canadensis*, *Gardenia jasminoides* J. Ellis, Gypsum Fibrosum, and *Moschus anhuiensis* (Huang et al. 2020).

Out of those abovementioned 21 licensed Chinese herbal formulations, Lian-Hua-Qing-Wen Capsule and Jin-Hua-Qing-Gan Granule have been recommended by China Food and Drug Administration for the treatment of COVID-19 and have played a vital role in the prevention of a variety of viral infections (Huang et al. 2020).

Lian-Hua-Qing-Wen Capsule and Jin-Hua-Qing-Gan Granule belong to “Three-Drugs, Three-Prescriptions”, official prescriptions of TCM used to fight against SARS CoV-2 infection in China. Lian-Hua-Qing-Wen Capsule, is composed of

plants *Forsythia suspensa* (Thunb.) Vahl, *Ephedra sinica* Stapf, *Lonicera japonica* Thunb., *Isatis tinctoria* L, *Mentha canadensis* L., *Dryopteris crassirhizoma* Nakai, *Rhodiola crenulata* (Hook.f. and Thomson) H. Ohba, Gypsum Fibrosum, *Pogostemon cablin* (Blanco) Benth., *Rheum palmatum* L., *Houttuynia cordata* Thunb., *Glycyrrhiza glabra* L., and *Prunus sibirica* L. This Lian-Hua-Qing-Wen Capsule Chinese patent medicine is an innovative formulation and has been approved during the SARS epidemics in 2003. Jin-Hua-Qing-Gan Granule is another patented Chinese medicine composed of plants Forsythia *suspensa* (Thunb.) Vahl, *Lonicera japonica* Thunb., *Ephedra sinica* Stapf, *Prunus sibirica* L., *Glycyrrhiza glabra* L., *Scutellaria baicalensis* Georgi, *Fritillaria thunbergii* Miq., *Anemarrhena asphodeloides* Bunge, *Arctium lappa* L., *Artemisia annua* L., and *Mentha canadensis* L. Jin-Hua-Qing-Gan Granule formulation has been approved for the treatment of H1N1 influenza virus infection since 2009 (Shi et al. 2021). Table 25.2 describes the major plants/herbs present in the various formulations in TCM and Indian system of medicine, which are approved/under clinical trial/under investigation and frequently used to prevent and treat COVID-19 infection.

25.5.1 Some Potential Leads from African Plants

The majority of the Southern African population depends primarily on traditional medicine as a source of health care. In traditional African medicine systems, the use of a plant is noted for the treatment of a specific symptom, rather than a particular disease or infectious organism.

Some of the potential Southern African plants like *Artemisia afra* Jacq. ex Willd., *Aspalathus linearis* (Burm.f.) R. Dahlgren, *Camellia sinensis* (L.) Kuntze, *Chondropetalum mucronatum* (Nees) Pillans, *Cyclopia latifolia* DC., *Dodonaea viscosa* (L.) Jacq., *Glycyrrhiza glabra* L., *Helichrysum* spp., *Pelargonium sidoides* DC., *Prunus africana* (Hook.f) Kalkman., *Rauvolfia caffra* Sond., and *Ziziphus mucronata* Willd. are traditionally used in the treatment of coughs, fevers, colds, and influenza, which can be considered for investigation as the potential inhibitors for the SARS-CoV-2 and related targets. These plants were reported to possess activity against human coronaviruses or similar viruses, and the list of medicinal plants has been compiled from the Medicinal Plant of South Africa (Van et al. 2009). Only a few African plant species have been tested for scientific studies, especially for antiviral activity. Therefore extensive toxicity studies and in vivo testing are necessary to investigate the pharmacological use of these plants.

For the treatment of COVID-19 symptoms, in June 2020, *Artemisia afra* was in high demand in South Africa. People of African countries have used this bitter plant for centuries to treat illnesses arising from colds to intestinal infections. With the increase in deaths in South Africa due to COVID-19 first wave, people have turned to use *Artemisia afra* and other traditional medicines, including *Cannabis*. Furthermore, *Artemisia afra* has not been tested for its inhibitory potential against coronaviruses; however, a closely related species, *A. annua*, could inhibit

Table 25.2 Some frequently used formulations used in India, China, and Africa and their composition and therapeutic effects repurposed (or under investigation) for the prevention/treatment of COVID-19

Name of the formulation	Composition (major plants of various formulations)	Therapeutics effect	References
Indian system of medicine			
AYUSH-64[a]	*Alstonia scholaris* (L.) R. Br. (Apocynaceae), *Picrorhiza kurroa* Royle ex Benth (Plantaginaceae), *Swertia chirayita* (Roxb.) Buch.-Ham. ex C. B. Clarke, orth. var. (Gentianaceae), *Caesalpinia crista* L. (Fabaceae)	**Anti-inflammatory**: Downregulation of inflammatory cells and inflammatory cytokines, viz. TNF-α, IL-1β, IL-6, and IL-8, via inhibition of NF-κB signaling **Immunomodulatory**: Enhanced proliferation of lymphocytes, improvement in phagocytic index and CD4/CD8 population, and thymocyte activation	Gundeti et al. (2020)
Sanshamani Vati	*Tinospora cordifolia* (Willd.) Hook. f. & Thomson (Menispermaceae)	Inhibition of SARS-CoV-2 surface glycoprotein, receptor-binding domain preventing attachment of the virus to host cell, inhibition of RNA-dependent RNA polymerase (RdRp) and protease, thereby preventing replication and multiplication of virus[b]	Shree et al. (2020), Sagar and Kumar (2020), Chowdhury (2020)
AYUSH Kwath	*Ocimum sanctum* L. (Lamiaceae), *Cinnamomum zeylanicum* Blume (Lauraceae), *Zingiber officinale* Roscoe (Zingiberaceae), *Piper nigrum* L. (Piperaceae)	**Anti-inflammatory**: Inhibition of NF-κB, PI3K, and PDK1 signaling; activation of CD42 and CD29; upregulation of CD69, CD80, CR3, and TRL2; reduction in inflammatory cytokines, viz. IL-1β, IFN-γ **Immunomodulatory**: Increased sheep RBC agglutinin titers, decrease in activity of lipoxygenase (LOX)-5 and cyclo-oxygenase (CoX)-2, increase in T-helper and natural killer cells	Gautam et al. (2020)
Unani Joshanda	*Cydonia oblonga* Mill. (Rosaceae), *Ziziphus jujuba* Mill. (Rhamnaceae), *Cordia myxa* L. (Cordiaceae)	Decrease in TNF-α and IL-8 via inhibition of NF-κB, p38MAPK, and Akt signaling	Essafi-Benkhadir et al. (2012), Zhan et al. (2018), Tran et al. (2019), Hong et al. (2015)

(continued)

Table 25.2 (continued)

Name of the formulation	Composition (major plants of various formulations)	Therapeutics effect	References
Khamira Marwareed	*Mytilus margaritiferus, Bambusa arundinacea* (Retz.) Willd. (Poaceae), *Santalum album* L. (Santalaceae), *Ambra grisea, Rosa damascena* Herrm. (Rosaceae), *Salix caprea* L. (Salicaceae)	**Immunomodulatory**: Elevated levels of IgG2a and IgG2b, increase in the delayed-type hypersensitivity (DTH) response	Khan et al. (2009)
Kabasura kudineer[a]	*Zingiber officinale* Roscoe (Zingiberaceae), *Piper longum* L. (Piperaceae), *Syzygium aromaticum* (L.) Merr. & L. M. Perry (Myrtaceae), *Tragia involucrata* L. (Euphorbiaceae), *Anacyclus pyrethrum* (L.) Lag. (Asteraceae), *Andrographis paniculata* (Burm. f.) Wall. ex Nees (Acanthaceae), *Hygrophila auriculata* (Schumach.) Heine (Acanthaceae), *Terminalia chebula* Retz. (Combretaceae), *Justicia adhatoda* L. (Acanthaceae), *Plectranthus amboinicus* (Lour.) Spreng. (Lamiaceae), *Costus speciosus* (J. Koenig) Sm. (Costaceae), *Tinospora cordifolia* (Willd.) Hook. f. & Thomson (Menispermaceae), *Clerodendrum serratum* (L.) Moon (Lamiaceae), *Sida acuta* Burm. f. (Malvaceae), *Cyperus rotundus* L. (Cyperaceae)	Binding and inhibition of SARS-CoV-2 spike protein, thereby prevention of entry of virus; inhibition of SARS-CoV-2-RNA-dependent RNA polymerase (RdRp) preventing replication and multiplication of virus[b]	Pitchiah Kumar et al. (2020), Sivaraman and Pradeep (2020)
Arsenicum album 30C	Not applicable (it is dilute aqueous arsenic trioxide solution)	Repair of DNA damage in arsenic toxicity	Kundu et al. (2000), Mathie et al. (2013)
Traditional Chinese Medicine			
Lian-Hua-Qing-Wen Capsule[c]	*Forsythia suspensa* (Thunb.) Vahl (Oleaceae), *Ephedra sinica* Stapf (Ephedraceae), *Lonicera japonica* Thunb. (Caprifoliaceae), *Isatis indigotica* Fortune (Brassicaceae), *Mentha haplocalyx* Briq. (Lamiaceae), *Dryopteris crassirhizoma* Nakai (Dryopteridaceae), *Rhodiola rosea* L. (Crassulaceae),	Direct virucidal activity; inhibit viral entry; inhibit viral replication and release; regulate host immune responses and inflammation; immunomodulatory effects (with respect to SARS coronavirus): stimulate mouse splenic lymphocytes; proliferation and increasing of the proportion of CD4 (+) and CD8 (+)	Shi et al. (2021)

	Gypsum Fibrosum, *Pogostemon cablin* (Blanco) Benth. (Lamiaceae), *Rheum palmatum* L. (Polygonaceae), *Houttuynia cordata* Thunb. (Saururaceae), *Glycyrrhiza uralensis* Fisch. ex DC. (Fabaceae), *Armeniaca sibirica* (L.) Lam. (Rosaceae)	T cells, increase secretion of IL-2 and IL-10 by mouse splenic lymphocytes; regulate redox homeostasis *Isatis tinctoria* L and *Rheum palmatum* L being the predominate viral inhibitors also	
Jin-Hua-Qing-Gan Granule[c]	*Forsythia suspensa* (Thunb.) Vahl (Oleaceae), *Lonicera japonica* Thunb. (Caprifoliaceae), *Ephedra sinica* Stapf (Ephedraceae), *Prunus sibirica* L. (Rosaceae), *Glycyrrhiza glabra* L. (Fabaceae), *Scutellaria baicalensis* Georgi (Lamiaceae), *Fritillaria thunbergii* Miq. (Liliaceae), *Anemarrhena asphodeloides* Bunge (Asparagaceae), *Arctium lappa* L. (Asteraceae) and *Artemisia annua* L. (Asteraceae), *Mentha canadensis* L. (Lamiaceae)	Virucidal activity; inhibit viral entry; inhibit viral replication and release; regulate host immune responses and inflammation; immunomodulatory effects (with respect to SARS coronavirus): stimulating mouse splenic lymphocytes; proliferation and increase of the proportion of CD4 (+) and CD8 (+) T cells; increase secretion of IL-2 and IL-10 by mouse splenic lymphocytes; regulate redox homeostasis. *Scutellaria baicalensis* Georgi and subsequently *Lonicera japonica* Thunb are the most important virucidal herbs	Shi et al. (2021)

[a]Frequently (recommended) used traditional medicine for the prevention/treatment of mild-to-moderate cases of COVID-19
[b]These are in silico-based mechanism of action; AYUSH-64 approved by the Ministry of AYUSH for the treatment of mild-to-moderate case of COVID-19 in India
[c]Recommended by China Food and Drug Administration for the treatment of COVID-19

SARS-CoV BJ-001 viral replication in Vero cells with an EC50 value of 34.5 ± 2.6 mg/mL. Although these are two different species, there has been similarity in many constituents within the *Artemisia* genus (Abad et al. 2012); closely related medicinal plant species may also produce similar or chemically similar compounds responsible for their biological activity. *Ziziphus mucronata*, another Southern African species, has not been studied for antiviral activity; however, cyclopeptide alkaloids isolated from *Ziziphus jujuba* showed inhibition of a porcine-related coronavirus (Kang et al. 2015). Likewise, helichrysetin, *β*-sitosterol, and reserpine obtained from *Helichrysum* species, *Dodonaea viscosa*/*Prunus africana*, and *Rauvolfia caffra*, respectively, have been studied against the human coronavirus. The further testing of these plant species could identify a lead candidate from African plants to treat COVID-19.

25.6 Indian System of Medicine for the Prevention and Treatment of COVID-19

Ministry of AYUSH, Government of India, has recommended many herbal medications as therapeutic approaches and some guidelines for COVID-19. They suggested using *Andrographis paniculata, Ziziphus jujuba, Tinospora cordifolia, Cordia myxa, Arsenicum album 30,* and *Cydonia oblonga* as a preventive and prophylactic treatment. AYUSH-64, Anu Taila, Agastya haritaki, *Bryonia alba, Adathodai manapagu, Rhus toxicodendron, Bignonia sempervirens, Atropa belladonna,* and *Eupatorium perfoliatum* are considered as symptomatic management interventions and Kabasura Kudineer and Vishasura Kudineer as add-on interventions in COVID-19 (Shree et al. 2020; Yadav et al. 2005).

Research for testing the safety and adequacy of various interventions for COVID-19 is going on at a remarkable rate. The fast spread of COVID-19 in India and the boundless dread associated with the disease make it a high priority to investigate the potential interventions to control the infection flare-up. Since the outbreak of COVID-19, the number of clinical trials for COVID-19 enrolled on different trial registry platforms has quickly expanded.

More than half of the registered trials (59.91%) of AYUSH system belong to ayurvedic intervention indicating a keen interest in pharmaceutical industries and the Ministry of AYUSH in promoting Ayurveda in a more prominent line of action against COVID-19. Interventions like AYUSH-64, Ayurcov, Zingivir-H, Ayush Kwath, Vyaghradi Kashay Tablet, and Virunil capsules are common interventions used in trials that showed immunomodulatory, anti-inflammatory, and antiviral actions. AYUSH-64, a polyherbal ayurvedic formulation, was found to be the most common ayurvedic formulation under trial which the Ministry of AYUSH has recently approved to treat mild-to-moderate cases of COVID-19 (Table 25.2).

Critical analysis of phytochemical constituents of these interventions showed the presence of medicinal plants *Tinospora cordifolia, Withania somnifera, Ocimum sanctum, Piper longum, Zingiber officinale, Swertia chirayita, Justicia Adhatoda, Nigella sativa, Glycyrrhiza Glabra, Curcuma longa, Phyllanthus emblica,*

Terminalia chebula, Aloe vera, Azadirachta Indica, Asparagus racemosus, Rhus toxicodendron, Andrographis paniculata, and *Artemisia annua* as common among these formulations.

Worldwide due to COVID-19 in patients with comorbidities, including hypertension, diabetes, and renal failure, both renin-angiotensin system (RAS) and angiotensin-converting enzyme-2 (ACE2) emerged to play a central role in COVID-19. Based on the clinical reports and the prognosis of COVID-19 globally, SARS-CoV-2 binding to the host ACE2 receptor binding is a promising target for developing new drugs. Interaction of ACE2 receptor with the receptor-binding domain (RBD) of the viral spike (S) protein leads to viral replication, total derangement of RAS and immune response, inflammation, and cytokine surge (increase in TNFα, IL1, IL6, IL10, etc.). Ultimately it leads to inflammation in the alveoli, cell death in alveoli, and acute respiratory distress or syndrome. In addition, thrombotic events and embolism and binding with ORF8 proteins lead to dissociation of iron from the 1-beta chain of hemoglobin getting attached to the surface glycoprotein porphyrin considered (Goyal et al. 2020).

In the light of the clinical picture of the COVID-19, we described it as the ***"virus-induced cardiovascular pulmonary disease."*** Delhi Pharmaceutical Sciences and Research University (DPSRU) is the first pharmacy university in India. It has been working for the last 1 year with team members from pharmacognosy and pharmaceutical chemistry departments. In addition, we partnered with Remedium Therapeutics to develop a formulation targeting ACE2 and conducted clinical trials using this formulation. The formulation was expected to be an innovative therapy from herbal resource treatment of COVID-19.

It is now well known that membrane-anchored ACE2 is well expressed in the epithelial cells of the lung, intestine, kidney, heart, and blood vessels and is also present in the oral and nasal mucosa, skin, lymph nodes, thymus, bone marrow, spleen, liver, and brain. Due to its expression in a wide range of tissues and organs, ACE2 regulates vasculature and inflammation, oxidative stress, fibrosis, and proliferation. ACE2 regulates Ang II action by hydrolyzing Ang II to Ang1-7. Ang1-7 exerts anti-inflammatory, antithrombotic, antihypertensive, antiarrhythmic, and cardioprotective action through G-protein-coupled Mas receptors (MasR). On the other hand, Ang II acting via AT1 receptors causes vasoconstriction, inflammation, cytokine secretion, thrombosis, and endothelial and myocardial dysfunction. It also acts on AT2 receptors to reduce inflammation and oxidative stress. Thus, ACE and ACE2 arms of RAS counterbalance their actions fine-tuning many physiological functions. Angiotensin receptor blockers (ARBs) and ACE inhibitors were investigated for the treatment of COVID-19, but it was a failure.

Various parts of the world have revealed that cardiovascular disturbances, including hypertension and thrombotic events, diabetes, and acute respiratory distress, are emerging comorbidities. Principal molecular targets for COVID-19 are the host ACE2, viral RdRp, and open reading frame (ORF8) proteins. Viral replication, cytokine surge, inflammation, apoptosis of type 1 and 2 cells in alveoli, and dissociation of iron from hemoglobin involving porphyrin, and thereby the failure of internal respiration, turned out to be the therapeutic targets for the management of COVID-19.

25.7 Novel Herbal Approach for the Treatment of COVID-19

Various herbal phytoconstituents (baicalin, glycyrrhizin, scutellarin, and hesperetin) are considered for COVID-19 due to their potential action on ACE2. The significant advantage of using herbs in viral respiratory infections is due to their potent anti-inflammatory and immunostimulatory action. Various edible plants have been reported to have a potential for the treatment of COVID-19 disease. Considering the pathogenesis of COVID-19, we identified some Indian medicinal plants with potent anti-inflammatory, immunomodulatory, and antiviral activities for developing potential treatment of COVID-19 (Patel et al. 2021). We investigated potential chemical constituents from plants (phytoconstituents).

Several *Rasayana* botanicals described in Ayurveda are used in clinical practice for strengthening immunity. Among various *Rasayana,* we found that *Solanum nigrum (Makoya) and Euphorbia prostrate* were found to be the most appropriate plants that can be used for the treatment of COVID-19. Both are reported to possess antiviral, immune modulator, and anti-inflammatory activities. In addition, they also have other desirable effects that may be due to ACE2 or ORF. *Solanum nigrum* L. (Fam. Solanaceae), commonly called black nightshade in English and *Makoya* in Hindi, grows in temperate climate zones and is found throughout the country in dry parts. The drug "Kakamachi (whole plant)" of *Ayurveda* is used in *Sotha* (inflammation), *Hrdroga* (heart disease), *Jvara* (fever), *Prameha* (diabetes), and *Hikka* (hiccups). It is described as *Kaphahara, Pittahara, Vatahara*, *and Rasayana*. *Solanum nigrum* possesses various compounds that are responsible for diverse activities. *Euphorbia prostrata* (Euphorbiaceae) is a small, prostrate, more or less pubescent annual herb found throughout India as a naturalized weed. It is commonly called *Dudhi* in Hindi and Svaduparni ksirni in Sanskrit. The Ayurvedic drug, *Dugdhika*, consists of the whole plant and is mainly used for *Svasa* (dyspnea, bronchial asthma), *Prameha* (Diabetes), *Raktapitta* (bleeding disorders), and *Raktarsa* (bleeding piles). In Ayurveda, it has been described as *Kaphahara*, *Murtala* (diuretic), *Hrdya* (heart disease), *Vistambhini* (anti-carminative), *Grahi* (anti-diarrhea), Malastambhaka, etc. *Solanum nigrum* and *Euphorbia prostrata* possess various compounds that are responsible for diverse activities. Among various compounds reported, we found *Solanine to have a docking score of −9.424 with ACE2, and also Rutin to have a docking score of −10.871.* Similarly, from compounds from *Euphorbia prostrata also had higher docking score* (apigenin: −6.204; apigenin-7-glucoside: −8.06 and luteolin 7-O-glucoside: −9.297) with ACE2. Interestingly some compounds from *Euphorbia prostrata showed very high binding with ORF 8* (apigenin: −9.033, apigenin-7-glucoside: −12.603, and luteolin 7-O-glucoside: −13.06). Clinical reports of the patients and mortality cause analysis of the deceased patients from various parts of the world have revealed that cardiovascular disturbances including hypertension and thrombotic events, diabetes, and acute respiratory distress are emerging comorbidities. Principal molecular links of these morbidities and COVID-19 prognosis that emerged are angiotensin-converting enzyme-2 (ACE2) and open reading frame (ORF8). Viral multiplication, cytokine surge, inflammation and apoptosis of type 1 and 2 cells in alveoli along with

dissociation of iron from the 1-beta chain of hemoglobin, getting attached to the surface glycoprotein porphyrin leads to the failure of internal respiration. Preliminary work was carried out on different plants reported in Ayurveda as the *Rasayana*, out of which two plants, *Solanum nigrum* and *Euphorbia prostrata,* found in Aravalli Hills Biodiversity of Delhi as well as available in Andaman and Nicobar Islands were found to be effective against ACE2.

Based on the extensive work on certain plants reported in Ayurveda as the *Rasayana*, we found that *Solanum nigrum* L. might be the most effective in COVID-19. Its phytoconstituents may have antiviral activity against SARS-CoV-2. Also, it may be effective against associated comorbidities, including respiratory failure and cardiovascular complications. The results were correlated with published pharmacological studies. Since certain formulations of exclusive trials were available in India and this plant is an eatable vegetable in certain parts of the world, it was given to some patients as an add-on nutraceutical. They are now looking into the development of a phytopharmaceutical product containing single-plant bioactive compounds that are quantitatively and qualitatively defined.

Out of various plants identified, we short-listed *Solanum nigrum, Euphorbia prostrata, Acalypha indica, Lantana camara,* and *Ricinus communis* as potential plants (Goyal et al. 2020). A literature search to identify the phytoconstituents of these plants revealed that they contain flavonoids, flavonoid glycosides, triterpenoids, steroidal glycoalkaloids, and other compounds of different classes. Flavonoids, in particular, luteolin, quercetin, apigenin, amentoflavone, epigallocatechin (EGC), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), and kaempferol, have been reported to inhibit the proteolytic activity of SARS-CoV 3CLpro and 3a channel protein of coronavirus. We docked certain compounds for the ACE2 activity 10. Results of docking studies showed that many compounds from *Solanum nigrum* like rutin, verbascoside, and hesperidin bound tightly at the active site of ACE2. The compound rutin (docking score: −11.5 kcal/mol) well occupied the receptor cavity through hydrogen bonding with **Ala348, Asp350, Phe390,** Ser44, Ser47 Arg393, Asn394, and verbascoside (docking score: −10.2 kcal/mol) and showed hydrogen bonding interaction with **Ala348, Asp350, Asp382, Phe390**, Glu375, His37, Asn394, and Glu402, whereas hesperidin (docking score: −9.5 kcal/mol) showed hydrogen bonding interaction with **Ala348, Asp350,** Trp69, and Tyr385.

We developed a phytopharmaceutical formulation and validated its pharmacological activities, including antiviral, anti-inflammatory, and immunomodulatory actions. A purified extract of *Solanum nigrum* with four active phytoconstituents (solanine, solamargine, rutin, and luteolin) was used for pharmacological evaluation and validation of the beneficial activities on ACE2 with its receptors AT1 and AT2 as well as ORF8 as the principal targets based on our preliminary docking studies. The extract with phytoconstituents was tested in in vitro viral cell lines at the University of Missouri, USA.

Most patients turned COVID negative (as per the qRT-PCR), and there has been a distinct improvement of the WHO Ordinal scale by 2+. It is expected that the herbal formulation will soon be released after due approvals from the government bodies.

25.8 Conclusions

The treatment for COVID-19 is still based on the drugs either being repurposed or given "Emergency Authorization." They should be used with care applying emerging scientific evidences of pathology, biotechnology, pharmacology, and pharmacy. Herbal medicines like other repurposed medicines must be used if the allopathy fails not only because one has a trust on traditional medicines but also if there is scientific basis for targets of COVID and evidences through clinical trials.

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Covid-19, Nutrition, Immunity, and Diet

26

Neelam Khetarpaul

Abstract

The coronavirus COVID-19 pandemic is the defining global health crisis of our time and the greatest challenge we have faced since World War II. The coronavirus disease 19 (COVID-19) is a highly transmittable and pathogenic viral infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in Wuhan, China, and spread around the world. Coronavirus is one of the major pathogens that mainly target the human respiratory system. This pandemic is posing severe threats to international health and the economy. Undernourished people have weaker immune systems, and may be at greater risk of severe illness due to the virus. At the same time, poor metabolic health, including obesity and diabetes, is strongly linked to worse Covid-19 outcomes, including risk of hospitalization and death. Symptoms include fever, cough, shortness of breath, trouble of breathing, fatigue, body aches, headache, sore throat, loss of taste and smell, nausea, diarrhea, etc. Globally, there have been 151,803,822 confirmed cases of COVID-19 in 213 countries, including 3,186,538 deaths till date (May 2021), as reported to WHO. As of 28 April 2021, a total of 1,011,457,859 vaccine doses have been administered. In addition to the well-known personal hygiene and preventive measures against the new coronavirus (COVID-19), we can also follow some simple recommendations regarding our nutrition that strengthen our immune system and could better prepare us for an epidemic in which the virus rapidly spreads to many people within a short period of time. One thing we can do is to eat as healthily as possible. Although no food could prevent or treat coronavirus transmission alone, a balanced diet, including all food groups, supports an effective immune system

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and may provide protection against infections. To preserve organism defense mechanisms, adequate nutritional status should be maintained with appropriate intakes of energy, vitamins, minerals, and water that should be continuously provided by a healthy diet. Hence, the common denominator that drives most of the nutrition and dietary recommendations to combat viral infections, including COVID-19, lies within the link between diet and immunity. Foods rich in vitamins A, D, E, C, dietary fibre, Zn, Se, iron, etc. should be taken. The person should keep himself or herself well hydrated. The responsibility of the individuals during the COVID-19 pandemic lies in making an effort to choose a healthy lifestyle, eat diets high in fruits and vegetables, exercise during free time, try to maintain a healthy weight, and get an adequate amount of sleep. In addition to taking care of one's dietary intake, the collective responsibility of individuals is to avoid the spread of misinformation related to nutrition and dietary intake, and the COVID-19. Since the outbreak, networks of social media were flooded by messages of single foods/herbs promising cure or prevention of the infection. The effects of such unfounded claims could lead to negative implications ranging from giving a false sense of protection against the infection to toxicity.

Keywords

COVID-19 · Immunity · Nutrition · Protective foods · Balanced diet · Physical activity · Carbohydrates · Protein · Vitamin C, A, D, E · Zinc · Nutritional status

26.1 Introduction

COVID-19 (also commonly called coronavirus), a disease caused by a novel coronavirus, became a major global human threat that has turned into a pandemic. The coronavirus disease 19 (COVID-19) is a highly transmittable and pathogenic viral infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in Wuhan, China, and spread around the world. Coronavirus is one of the major pathogens that mainly target the human respiratory system. In late December 2019, clusters of patients were admitted to hospitals with an initial diagnosis of pneumonia of an unknown etiology. Firstly, coronavirus (2019-nCoV) was isolated from Wuhan market, China, on 7 January 2020 (Fig. 26.1).

A virus infects the body by entering healthy cells. There, the invader makes copies of itself and multiplies throughout the body. The new coronavirus latches its spiky surface proteins to receptors on healthy cells, especially those in lungs. Specifically, the viral proteins bust into cells through ACE2 receptors. Once inside, the coronavirus hijacks healthy cells and takes command. Eventually, it kills some of the healthy cells. There is no clinically approved antiviral drug or vaccine available to be used against COVID-19. This pandemic is posing severe threats to international health and the economy.

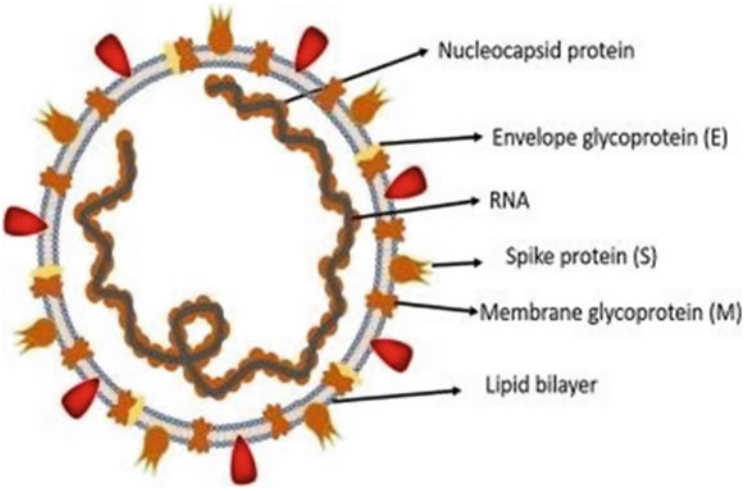

Fig. 26.1 Structure of respiratory syndrome causing human coronavirus

26.2 Symptoms

COVID-19, the illness caused by the coronavirus, starts with droplets from an infected person's cough, sneeze, or breath. They could be in the air or on a surface that one touches before touching his or her eyes, nose, or mouth. That gives the virus a passage to the mucous membranes in the throat. Within 2–14 days, immune system of the infected person may respond with symptoms including:

- Fever
- A cough
- Shortness of breath
- Trouble breathing
- Fatigue
- Chills, sometimes with shaking
- Body aches
- Headache
- A sore throat
- Loss of taste
- Loss of smell
- Nausea
- Diarrhea

The virus moves down the respiratory tract. That is the airway that includes mouth, nose, throat, and lungs. The lower airways have more ACE2 receptors than the rest of the respiratory tract. So COVID-19 is more likely to go deeper than

viruses like the common cold. The lungs might become inflamed, making it tough for the infected person to breathe. This can lead to pneumonia, an infection of the tiny air sacs (called alveoli) inside lungs where your blood exchanges oxygen and carbon dioxide. For most people, the symptoms end with a cough and a fever. More than 8 in 10 cases are mild. But for some, the infection gets more severe. About 5–8 days after symptoms begin, they have shortness of breath (known as dyspnea). Acute respiratory distress syndrome (ARDS) begins a few days later. Many people who get ARDS need help in breathing from a machine called a ventilator. As fluid collects in the lungs, they carry less oxygen to blood. That means blood may not supply the organs with enough oxygen to survive. This can cause your kidneys, lungs, and liver to shut down and stop working. Not everyone who has COVID-19 has these serious complications. And not everyone needs medical care. However, if symptoms include trouble breathing, get medical help right away.

Acute respiratory complications that require intensive care unit (ICU) management are a major cause of morbidity and mortality in COVID-19 patients. Patients with worst outcomes and higher mortality are reported to include immunocompromised subjects, namely older adults and polymorbid individuals and malnourished people in general.

26.3 Persons More Prone to COVID-19

About 80% of people recover fully from COVID-19 without medical treatment. But COPD puts persons at higher risk to get seriously sick if they:

- Are over 60
- Are undernourished people
- Have high blood pressure
- Have diabetes
- Have heart disease
- Have cancer
- Have a weakened immune system
- Have smoke
- Have chronic kidney disease
- Have chronic obstructive pulmonary disease (COPD), like emphysema
- Are people with lower immune health because of a solid organ transplant
- Have obesity—those with a BMI greater than 30
- Have sickle cell disease
- Have asthma
- Have dementia
- Have cerebrovascular diseases, such as stroke
- Have cystic fibrosis
- Have pregnancy
- Have liver disease

- Have scarring in the lungs (pulmonary fibrosis)
- Have thalassemia (a blood disorder)

Therefore, COVID-19 does not treat us equally. Undernourished people have weaker immune systems, and may be at greater risk of severe illness due to the virus. At the same time, poor metabolic health, including obesity and diabetes, is strongly linked to worse COVID-19 outcomes, including risk of hospitalization and death. People who already suffer as a consequence of inequities including the poor, women, and children; those living in fragile or conflict-affected states; minorities; refugees; and unsheltered are particularly affected by both the virus and the impact of containment measures. It is essential that they are protected, especially when responses are implemented.

The ongoing epidemic has been declared by the World Health Organization (WHO) as a global public health emergency.

26.4 Impact of COVID-19 on Food Security and Health System

COVID-19 has exposed the vulnerability and weaknesses of our already fragile food systems. Containing the virus has caused food and nutrition shortages and driven governments to reduce social services, such as midday meal programs and distribution of food to vulnerable groups through ICDS, that the most marginalized children and pregnant women rely upon. In the context of food and nutrition shortages, accessibility and affordability of healthy, sustainably produced food become even more challenging. Access to staple food distribution and local food markets is at risk. Millions of households in formerly food-secure regions of the world have fallen into severe food insecurity. Levels of hunger and malnutrition could double within the space of just a few weeks.

As measures to slow the spread of COVID-19 are enacted around the world, we must ensure that there is enough nutritious food, distributed fairly, to cover basic nutrition needs, especially for the most vulnerable. Food systems everywhere must become equitable, nutritious, efficient, and inclusive. National Institute of Nutrition, ICMR, has also recommended the amount of food to be distributed as free ration for the economically deprived, daily-wage earners, and migrant workers during the ongoing lockdown to keep them food and nutrition secure during these tough times.

Healthcare system should also be enhanced to address challenges faced by specific populations, especially older people and those with preexisting conditions, such as weakened immune systems and poor metabolic health. They should specifically pay attention to women and children, especially to their nutritional well-being and healthcare. Yet even the strongest health systems are struggling with high healthcare costs and a shortage of medical personnel, equipment, and facilities.

There is a need to integrate nutrition into universal health coverage as an indispensable prerequisite for improving diets, saving lives, and reducing healthcare spending while ensuring that no one is left behind. Reversing the obesity epidemic would also lessen the burden on our healthcare systems, as obesity is not only one of

the costliest health conditions but also a major risk of COVID-19 hospitalizations and complications.

26.5 Prevalence

The coronavirus COVID-19 pandemic is the defining global health crisis of our time and the greatest challenge we have faced since World War II. Since its emergence in Asia late last year, the virus has spread to every continent except Antarctica. Cases are rising daily in Asia, Africa, the USA, Canada, and Europe. Globally, there have been 151,803,822 confirmed cases of COVID-19 in 213 countries, including 3,186,538 deaths till date (May 2021), as reported to WHO. As of 28 April 2021, a total of 1,011,457,859 vaccine doses have been administered.

26.6 Prevention and Control

The COVID-19 pandemic is causing many changes in the daily lives of people around the world. The coronavirus presents many uncertainties, and none of us can completely eliminate our risk of getting infected with COVID-19. However, there are things that can be done to maintain a healthy lifestyle in these difficult times. First and foremost, everyone is encouraged to follow World Health Organization (WHO) and governmental advice to protect against COVID-19 infection and transmission. Physical distancing and good hygiene are the best protection for yourself and others against COVID-19.

In addition to the well-known personal hygiene and preventive measures against the new coronavirus (COVID-19), we can also follow some simple recommendations regarding our nutrition that strengthen our immune system and could better prepare us for an epidemic in which the virus rapidly spreads to many people within a short period of time. One thing we can do is to eat as healthily as possible.

According to National Institute of Nutrition, ICMR, a balanced diet, comprising nutrient-rich vegetables, fruits, pulses, cereals, and curd coupled with a healthy lifestyle, is the key to boost the immune system, a focal point in the fight against coronavirus.

26.7 Interaction Among Coronavirus, Immunity, and Nutrition

Good nutrition is an essential part of an individual's defense against COVID-19. Nutritional resilience is a key element of a society's readiness to combat the threat. Focusing on nutritional well-being provides opportunities for establishing synergies between public health and equity, in line with the 2030 Agenda for Sustainable Development.

As highlighted recently by the World Health Organization, a healthy lifestyle makes all bodily functions work better, including immunity. Although no food could prevent or treat coronavirus transmission alone, a healthy and balanced diet has been proven to strengthen the immune system along with physical activity and healthy sleeping habits. Good nutrition supports the body throughout the life course, from birth to old age. A balanced diet, including all food groups, supports an effective immune system and may provide protection against infections, cancers, and other diseases. Hence, having a healthy diet especially, including lots of fruits and vegetables in the daily diets, is a key component of a healthy lifestyle and plays a vital role in supporting a well-functioning and effective immune system to help protect against infection and other diseases.

26.7.1 How Does the Immune System Work

The immune system is involved in the protection of host against environmental agents such as pathogenic microorganisms (bacteria, fungi, and viruses) and chemicals, thereby preserving the integrity of the body. The immune system is one of the most complex bodily systems, made up of a network of cells, molecules, tissues, and organs all working together to protect the body. This complexity means that it cannot be modified acutely by a specific nutritional intervention. Rather, adhering to a healthy diet provides ongoing support to the immune system and may even delay the process of immunosenescence (the natural gradual deterioration of the immune system as we get older).

The emergence of new infectious diseases with new pathogenic properties constitutes a serious health issue worldwide. Severe acute respiratory syndrome (SARS) represents one of the most recent emerging infectious diseases, caused by a novel coronavirus member called (SARS-CoV-2), identified in Wuhan, Hubei, China, in December 2019, and recognized as a pandemic by the World Health Organization (WHO). The nutritional status of each COVID-19-infected patient should be assessed prior to undertaking treatments. Nutritional support should be the basis of management of any infected individual. Good nutrition is key to build immunity, protect against illness and infection, and support recovery of COVID-19 patients. Not a cure for COVID-19 but healthy, balanced diets are key for boosting immunity, improving immunometabolism, and preventing noncommunicable diseases that are risk factors for higher COVID-19 morbidity and mortality. However, prevention measures should remain the first priority and strategy to develop throughout proper hygiene, healthy diet, and staying home.

To preserve organism defense mechanisms, adequate nutritional status should be maintained with appropriate intakes of energy, vitamins, minerals, and water that should be continuously provided by a healthy diet. Hence, the common denominator that drives most of the nutrition and dietary recommendations to combat viral infections, including COVID-19, lies within the link between diet and immunity. In fact, existing evidence highlights that diet has a profound effect on people's immune system and disease susceptibility. No specific one food or single

supplement alone can prevent from catching COVID-19/coronavirus. Besides following social distancing, good hygiene practices, and wearing mask, there are many nutrients that are involved with the normal functioning of the immune system, which is why maintaining a healthy balanced diet is also the best way to support immune function. Hence, no one food is recommended over another but eating a variety of foods will help to maintain a healthy balanced diet.

It has been demonstrated that specific nutrients or nutrient combinations through a variety of foods in the balanced diet may affect the immune system through the activation of cells, modification in the production of signaling molecules, and gene expression. Furthermore, dietary ingredients are significant determinants of gut microbial composition and consequently can shape the characteristics of immune responses in the body. Nutritional deficiencies of energy, protein, and specific micronutrients are associated with depressed immune function and increased susceptibility to infection. An adequate intake of iron, zinc, and vitamins A, E, B_6, and B_{12} is predominantly vital for the maintenance of immune function. Therefore, the key to maintaining an effective immune system is to avoid deficiencies of the nutrients that play an essential role in immune cell triggering, interaction, differentiation, or functional expression.

Creation of a prooxidant environment through generation of damaging reactive oxygen species is one element of innate immunity; the host needs protection against these through classic antioxidant vitamins (vitamins C and E) and antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase); the latter require manganese, copper, zinc, iron, and selenium. Thus, the roles for nutrients in supporting the function of the immune system are many and varied and it is easy to appreciate that an adequate and balanced supply of these is essential if an appropriate immune response is to be mounted.

Important nutrients for effective immune function are:

- Copper
- Folate
- Iron
- Selenium
- Zinc
- Dietary fibre
- Vitamins A, B_6, B_{12}, C, E, and D
- Essential fatty acids

It is well known that vitamin C supports immune functions and protects against infection caused by coronavirus. The COVID-19 has been reported to infect the lower respiratory tract, and vitamin C could be one of the effective alternatives to treat COVID-19. It was reported throughout some controlled trials that vitamin C-supplemented patients presented, under certain conditions, had lower incidence of pneumonia. However, supplying patients with high-dose vitamin C has not been received any evidence-based approval.

Some nutrients, such as vitamins A and D, and their metabolites are direct regulators of gene expression in immune cells and play a key role in the maturation, differentiation, and responsiveness of immune cells. Vitamin A acts in the proliferation of T lymphocytes and production of immune-reactive cytokines and natural killer cells. Vitamins D and E regulate the immunological system. Vitamin D is known to play a role by stimulating maturation of several cells including immune ones. Vitamin D reduces the risk of acute respiratory infections. A significant number of healthy individuals have been found to be with decreased levels of vitamin D, particularly at the end of winter season that coincides with COVID-19 discovery in the winter of 2019.

There is evidence from animal models showing the relationship between diets deficient in vitamins A, E, and D, and compromised immune response in respiratory infections and transmission caused by other coronaviruses. These deficient diets have also decreased the effectiveness of bovine coronavirus-inactivated vaccines, which has made animals more susceptible to infectious diseases. Thus, these studies highlight the importance of consuming food sources of these nutrients especially during the pandemic.

Selenium, zinc, and iron supplementing COVID-19-affected patients with selenium could be an effective intervention for the treatment of this novel virus. COVID-19-related symptoms such as diarrhea and lower respiratory tract infection could be improved by zinc supplementation. It has been shown that iron deficiency constitutes a risk factor for the development of recurrent acute respiratory tract infections.

The European Food Safety Authority permits claims of "maintenance of functions of the immune system" for vitamins A, B_6, B_{12}, C, and D and folate (vitamin B_9) and for the trace elements zinc, iron, selenium, and copper. Zinc and selenium act by reducing the symptoms of colds. Essential fatty acids help control inflammation and infections, by presenting an intrinsic relationship with the production of hormones and contributing to the production of antibodies. Dietary fibers promote a change in the microbiota with a positive effect on the immune system.

In essence, good nutrition creates an environment in which the immune system is able to respond appropriately to challenge, irrespective of the nature of the challenge. Conversely, poor nutrition creates an environment in which the immune system cannot respond well. This is amply illustrated in conditions of nutrient deficiency (either "real life" or experimentally induced) which are accompanied by impairments of both innate and acquired immunity and increased susceptibility to, and severity of, infections. Both the immune impairments and the susceptibility to infection can be reversed by correcting the deficiency(ies) showing a causal relationship between availability of specific nutrients and immune defenses.

26.7.2 Effect of Coronavirus Infection on General Eating Pattern and Nutrient Intake of Masses

The COVID-19 pandemic caused by the SARS-CoV-2 virus brought several individual and collective protection measures to contain the expansion of its

transmission, such as social distancing and lockdown. Although extremely necessary, these measures restricted the activities in commerce, restaurants, and street markets and even closed borders. Thus, for many individuals, usual shopping routines and eating have been entirely upended. In addition to providing a framework of economic and social instability, the lockdown could affect the food supply chain and generated a situation of food and nutritional insecurity in some regions of the world. Therefore, undernutrition and obesity might have increased, due to limited access to food, concern of running out of staple items, limited culinary ability, more sedentary lifestyle, and change in food purchasing, eating behaviors, and perceptions of food safety. The higher consumption of processed foods with higher caloric content, saturated fats, sugars, and refined carbohydrates; greater durability; and easier access and use, especially in children, could have contributed to increase the prevalence of obesity in the times of COVID-19.

During the lockdown period, everybody had nothing to do but was busy in cooking and eating all the time at home especially in those families where all the family members including children were together after a long period. On social media also, people were giving demonstrations on new dishes and some were earning money through it too.

Still, staying home was a great opportunity to promote healthy eating and prevent obesity, when people are cooking more at home, are buying more fresh and unpackaged foods than before the pandemic, and are more prone to change in eating habits. Some studies demonstrated that consuming homemade foods and having cooking skills are important to the ingestion of a healthy diet and the maintenance of proper nutritional status. For children, cooking may positively change intake and preference for vegetables and fruits, by involvement and exposing them to healthier food in a fun, family, and ludic environment.

As recently discussed by the European Society for Clinical Nutrition and Metabolism (ESPEN) (Barazzoni et al. 2020. ESPEN expert statements and practical guidance for nutritional management of individuals with SARS-CoV-2 infection. Clin Nutr 2020; S0261-5614(20)30140-0. DOI: 10.1016/j.clnu.2020.03.022), the obesity condition is dangerous to the severity of COVID-19 and has emerged as one of the most prominent risk factors increasing the disease mortality. In this sense, nutritional status and diets might influence the individual risk for the progression of SARS-CoV-2, but information on the impact of nutrition on COVID-19 is still arising.

Adequate dietary intake may be essential to protect against an excessive inflammatory response to SARS-CoV-2 infection, preventing the evolution of the infection to severe disease or even during COVID-19, improving its outcome. Some nutrients have been reported as potentially relevant to the prevention and COVID-19 treatment. Among them are vitamins A, D, and E; minerals zinc and selenium; fiber; and essential fatty acids, as they promote an effect on the immune system. Thus, inadequate intake and status of these nutrients might lead to a decrease in the resistance to infections and an increase in disease burden.

Because of sedentary lifestyle during the lockdown period, energy intake should be less than the recommended intake for that age group. Protein intake and intake of vitamins and minerals should be as per recommendations.

- **Energy requirement:** Because of the impossibility of using indirect calorimeter, the daily requirement must be estimated through predictive equations based on body weight, such as 27–30 kcal\kg\day, adapted on the personal nutritional status, level of physical activities, clinical status, and comorbidities.
- **Protein requirement:** In case of the absence of chronic renal insufficiency, the protein intake is >1 g\kg\day (up to 1.5 g\kg\day). This must be adapted to the aforementioned features as for energy intake, in order to prevent weight loss, reduce the risk of complications, and promote a global recovery.
- **Carbohydrate and lipid requirements:** It must refer to the nonprotein energy requirement, with a lipid/carbohydrate ratio ranging from 30:70 (patients without respiratory insufficiency) to 50:50 (patients with respiratory insufficiency). Low glycemic index carbohydrates have to be considered. Omega-3 and omega-6 PUFAs predominantly promote anti-inflammatory and pro-inflammatory effects. Being precursors of resolvins/protectins and prostaglandins/leukotrienes, respectively, omega-3 including protectin D1, serving as a novel antiviral drug, could be considered for one of the potential interventions of the novel virus COVID-19.
- **Micronutrients (vitamins and minerals):** Deficiencies of trace elements such as iron, selenium, copper, and zinc and vitamins A, B_6, B_{12}, folic acid, C, D, and E are associated with immune dysfunction. A healthy balanced diet has the opportunity to furnish most of the essential micronutrients to exert modulatory effects on immune function, including zinc, iron, magnesium, manganese, selenium, and copper that contribute to immune cells and function sustainment and modulation. Several epidemiologic and clinical studies suggest that the risk of infection is favored by nutritional deficiencies besides poor personal hygiene, sanitation, or contaminated food and water.
- **Water requirement:** Adequate hydration must be maintained according to remote (heart or renal failure) and recent clinical history (diarrhea, vomiting, and electrolyte imbalances).

Subjects with malnutrition should ensure sufficient supplementation with vitamins and minerals. In COVID-19 non-intubated ICU patients not reaching the energy target with an oral diet, oral nutritional supplements (ONS) should be considered first and then enteral nutrition treatment. If there are limitations for the enteral route, it could be advised to prescribe peripheral parenteral nutrition in the population not reaching energy-protein target by oral or enteral nutrition.

Reducing of infectious risk is achieved best by quarantine at home, which is heavily recommended during moderate disease course. However, prolonged home stay may lead to increased sedentary behaviors, such as spending excessive amounts of time sitting, reclining, or lying down for screening activities (playing games, watching television, using mobile devices), reducing regular physical activity and hence lowering energy expenditure. Thus, quarantine can lead to an increased risk

for and potential worsening of chronic health conditions, weight gain, loss of skeletal muscle mass and strength, and possibly also loss of immune competence since several studies have reported positive impact of aerobic exercise activities on immune function. Every day >30-min or every second day >1-h exercise is recommended to maintain fitness, mental health, muscle mass, and thus energy expenditure and body composition.

26.8 Nutritional Recommendations at Individual, Community, National, and Global Levels Amid COVID-19 Pandemic

There is currently no treatment for infection with SARS-CoV-2 or for COVID-19. Current strategies aim to limit the spread of the virus by preventing contact between people. The search for vaccines to offer immune protection against SARS-CoV-2 and for pharmacological treatments to prevent the virus from replicating is underway. In the meantime, approaches to ensure that individuals' immune systems are well supported should be taken. Nutrition should be at the forefront of these approaches.

The nutritional status of individuals has for long been considered as an indicator of resilience against destabilization. The ecology of adversity and resilience demonstrates that substantial stressors, such as inadequate nutrition, can lead to long-lasting effects that are linked to health. In fact, poor diet quality has been associated not only with physical but also mental health. Optimal nutrition and dietary intake are resources that transcend the individual and the community to reach global influence (Fig. 26.2).

A multilevel framework to support nutrition and food security during the COVID-19 pandemic, using the various levels of the ecological health model: individual, community, national, and global.

i. **At the individual level:** The COVID-19 pandemic caused by the SARS-CoV-2 virus brought several individual and collective protection measures to contain the expansion of its transmission, such as social distancing and lockdown all over the world. Hence, COVID-19 world pandemic imposed a new set of challenges for the individual to maintain a healthy diet. First, the state of lockdown announced in many countries around the globe led all public and private sector institutions, with the exception of healthcare facilities and a limited number of essential services, to close down and, if possible, carry its operations remotely (without face-to-face interactions). Individuals were asked to stay home and avoid contact with other people. Such measures of self-isolation and social distancing are known to be crucial in limiting the spread of the virus, flattening the curve of incidence rate, and ultimately disease containment. These measures have severe repercussions on both food access and utilization.
   Food access, however, is dependent on factors that could reach beyond the individual and are more directly related to actions and policies at the community, national, as well as global levels. That said, the individual remains capable of

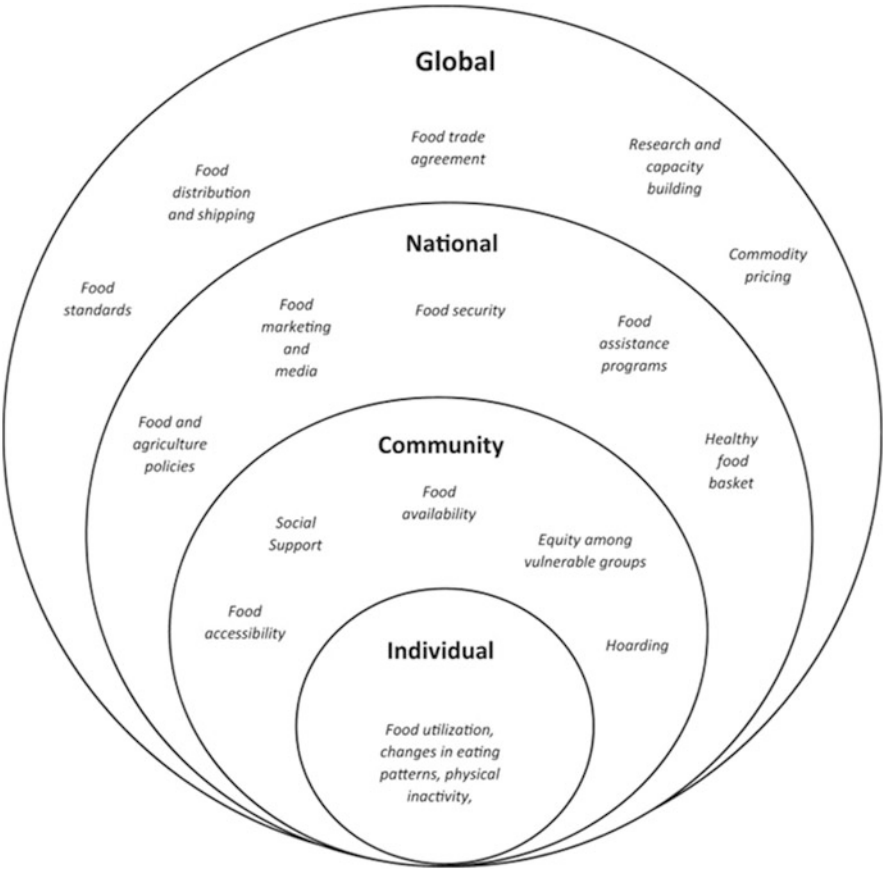

Fig. 26.2 A multilevel framework of action to support nutrition during the COVID-19 pandemic. Source: Naja, F. and Hamadeh, R. 2020. Nutrition amid the COVID-19 pandemic: a multilevel framework for action. Eur. J. Clin. Nutr. 2020 (April, 20): 1–5

making a few choices related to food utilization. The confinement to one's home has direct effects on one's lifestyle, including dietary habits, eating, and physical activity patterns. Confinement increases sedentary behaviors that involve activities with very low energy expenditure, performed mainly in a sitting or supine position. The low physical activity levels, even for short periods, could negatively affect physical and mental health. During the lockdown period, everybody had nothing to do but was busy in cooking and eating all the time at home especially in those families where all the family members including children were together after a long period and they wanted to eat something good to eat all the time to cope up with the mental stress. On social media also, people were giving demonstrations on new dishes and some were earning money through it too. All this promoted overconsumption of food in most of the well-off families. The state of lockdown and confinement could also lead to irregular

eating patterns and frequent snacking, both of which are associated with higher caloric intake and increased risk of obesity. The higher consumption of processed foods with higher caloric content, saturated fats, sugars, and refined carbohydrates; greater durability; and easier access and use, especially in children, could have contributed to increase the prevalence of obesity in the times of COVID-19.

The changes in dietary patterns during the outbreak of COVID-19 could also be driven by the fear and anxiety many people around the globe are experiencing. Compelling evidence showed that dietary habits are affected by conditions of stress, distress, and emotional disturbance, whereby elevated distress levels are associated with unhealthy dietary patterns and poor quality of the diet. Furthermore, emotions like fear and sadness are associated with less desire or motivation to eat and with lessened enjoyment during eating.

More recently, in explicating a five-way model of emotions and diet, it was found that changes in food intake may be the "natural" response to stress and heightened emotional states through both psychological and physiological mechanisms.

Therefore, the responsibility of the individuals during the COVID-19 pandemic lies in making an effort to choose a healthy lifestyle, eat diets high in fruits and vegetables, exercise during free time, try to maintain a healthy weight, and get an adequate amount of sleep. In addition to taking care of one's dietary intake, the collective responsibility of individuals is to avoid the spread of misinformation related to nutrition and dietary intake, and the COVID-19. Since the outbreak, networks of social media were flooded by messages of single foods/herbs promising cure or prevention of the infection. The effects of such unfounded claims could lead to negative implications ranging from giving a false sense of protection against the infection to toxicity.

Thus, for many individuals, usual shopping routines and eating have been entirely upended. In addition to providing a framework of economic and social instability, the lockdown could affect the food supply chain and generated a situation of food and nutritional insecurity in some regions of the world. Therefore, undernutrition and obesity might have increased, due to limited access to food, concern of running out of staple items, limited culinary ability, more sedentary lifestyle, and change in food purchasing, eating behaviors, and perceptions of food safety.

ii. **At the community level:** At the community level, food access and availability are particularly vulnerable to the implications of the COVID-19 outbreak, primarily because of difficulties in transportation, distribution, and delivery. This situation has led in many instances to "hoarding." One way that a pandemic would indirectly impact the food supply chain is by changing consumer behavior. Pandemics create uncertainty and volatility in consumer demand, making it particularly challenging to maintain food inventories in a just-in-time economy. In a study of the effect of an outbreak on behavior, the most recurrent response is to stockpile supplies, food, and water. Those who can afford extra food may hoard more than they need and pose devastating consequences on at-risk

populations. Hoarding could lead to extreme shortages in markets, leading to rapidly rising prices. Therefore, at a community level, it is crucial to spread awareness against "panic-buy." Furthermore, during the COVID-19 pandemic, older adults and patients with chronic diseases became particularly vulnerable and most at risk to nutrition imbalance. Firstly, available research indicated that adults 60 years and older and patients with preexisting medical conditions, especially heart disease, lung disease, diabetes, or cancer, are more likely to have severe even deadly coronavirus infection than other groups. Second, the recommendations to stay home and abide by social distancing targeted these groups specifically, given their vulnerability. Third, the elderly and patients with chronic diseases may already be susceptible to malnutrition given their compromised health and limited purchasing ability. Therefore, at the level of the community, it is crucial to identify these vulnerable groups and extend assistance in food access and availability through a structured and reliable support system.

During this period, laborers were affected a lot. Because they are daily-wage earners, they had no money to buy food articles during the lockdown period. However some of the social organizations tried to provide food to such laborers. But still many of them had a great sense of food insecurity and moved towards their villages in other states. This class of our society suffered the maximum during the lockdown period because of no work and no food.

iii. **At the national level:** While governments of countries around the globe are dealing with the burden of the COVID-19 and its enormous strains on the healthcare system, they are also battling a destabilization in their economies and a rising threat of food insecurity. In light of these challenges to provide adequate and nutritious food at times of pandemics, each country is urged to define, finance, and distribute a food basket of a least-cost diet that supports the health needs of the population; ensure the use of the local agricultural produce of the country; and minimize reliance on food imports. Significant planning is needed at the national level to increase the nation's preparedness, including the formulation of policies to support the production, distribution, and access of this food basket to different communities. Among these policies are those related to mobilization of resources in order to finance food purchases and provisions, tax waiving for staple foods and commodities, and support for agricultural and food production industries. Given the effect of the COVID-19 pandemic on the demand and supply dynamics of food, price hikes became prevalent reaching at times uncontrollable levels, a situation that requires national efforts to closely monitor and inspect food prices and markets.

The COVID-19 pandemic imposed a paradigm shift on governments, whereby it became imperative to build networks with the private sector, international agencies, and local communities. It is only through a coordinated effort of these different entities that securing of essential nutritious food stocks becomes possible.

At the global level: While border protection is legitimate in safeguarding the health of citizens from external threats, it can severely disrupt travel, trade, and

tourism, as well as infringe civil liberties. Countries that depend heavily on imported food to meet demand might face inconsistent risk from supply chain failures, especially in the face of border crossing closures. Therefore, it is essential to ensure the smooth flow of global trade and make full use of the international markets as a vital tool to secure food supply across the globe to prevent food insecurity. An important lesson of the COVID-19 relates to its global nature whereby no country is immune to its spread and infliction: a global threat requires global action. The protectionist strategies that each country is implementing should be complemented by global cooperation, solidarity, and coordination among countries to ensure that humanity emerges from this pandemic with the least possible losses (Table 26.1).

26.9 General Dietary and Lifestyle Recommendations

26.9.1 Healthy Eating Is Important in Quarantine Applications

Since the clearest known practice for COVID-19 is social isolation, where all kinds of contacts are minimized, many global healthcare providers recommend keeping at least 2 weeks of medicine and food for everyone as part of quarantine practices. The food needs to be nutritious, durable with a long shelf life. Balanced nutrition is highly important during these times with a diet that is rich in proteins, fibers, vitamins, minerals, and antioxidants. A healthy meal plate is recommended, in which one-quarter of the plate per main meal would comprise vegetables, the other quarter is from whole-grain products, and the remaining half is from fruits, high-protein foods (dried legumes, meat, eggs, fish, chicken, oily seeds, etc.), and dairy products (milk, yogurt, cheese, etc.) in equal three pieces. Some amount of fats and oils should be used in cooking meals. Besides, it is recommended to ensure adequate water consumption.

Daily adequate intake of vitamin and mineral should be maintained. Finally, even if adequate intake of vitamins and minerals should be mainly provided from foods such as vegetables, fruits, whole grains, and seeds, for people who are not able to consume according to the "Healthy Food Plate," it may be recommendable to take vitamin and mineral supplements cautiously as an alternative in this special temporary period.

One should follow the following lifestyle practices as recommended by NIN to protect from this infection:

- Maintain ideal body weight.
- Be physically active.
- Follow right eating pattern.
- Eat a variety of foods from each food group and prefer to eat whole grains, fruits, vegetables, and beans.
- Eat fresh fruits and vegetables daily.
- Avoid fruit juices and carbonated drinks.

Table 26.1 Recommendations to mitigate the impact of COVID-19 on nutrition and food security at the individual, community, national, and global levels

	Nutrition recommendations during COVID-19 pandemic
Individual level	• Try to eat balanced meals, avoid irregular snacking. • Choose foods rich in vitamins A, C, E, B_6, and B_{12}, iron, zinc such as citrus fruits, dark green leafy vegetables, nuts, and dairy products. • Consumption of highly processed foods should be limited and fruit juices and carbonated drinks should be avoided. • Consuming meat, poultry, and eggs was not risky in present circumstances, but hand-wash hygiene must be followed after handling raw meat, eggs, or even vegetables. Thoroughly cooked meat and poultry products may be included in diets in moderation. • Avoid too much fat (no more than 30 g per person per day—preferably more than two varieties of oils) and salt (no more than 5 g per person a day). Sugar is just calories with no nutrients; hence, keep it to a bare minimum. • Maintain ideal body weight, keep the body hydrated with adequate water intake, and take up moderate physical activity including yoga to reduce stress and build immunity. • Avoid smoking, alcohol, and drugs. • Most infections can be prevented by practising good personal hygiene such as washing hands before preparing or eating food, washing hands after cleaning vegetables or meat, and covering mouth with a tissue or cloth while coughing or sneezing. • Stay physically active during quarantine. • Refrain from spreading misinformation related to nutrition and dietary intake and COVID-19.
Community	• Spread awareness regarding the devastating consequences of hoarding and panic-buy. • Identify and support populations at risk of malnutrition within the community especially the elderly and patients with chronic diseases. • Create a structured and reliable support system to ensure availability, access, and affordability of essential food commodities to all members of the community.
National	• Define, finance, and distribute a food basket of a least-cost diet that addresses the health needs of the population, ensures the use of local agricultural produce of the country, and minimizes reliance of food imports. • Mobilize resources in order to finance food purchases and provisions. • Waive taxation for staple foods and commodities. • Support agricultural and food production industries. • Closely monitor and inspect food prices and markets. • Build networks with the private sector, international agencies, and local communities. • Maintain high level of transparency, critical to build trust, support, and compliance.
Global	• Assure continuous flow of global trade; avoiding any trade restrictions would be beneficial to keep food and feed supplies, as well as those of agricultural inputs, from worsening local conditions already strained by COVID-19 response measures. • Reduce import tariffs and other restrictions on food commodities.

- Limit fast foods. Avoid junk foods/dairy/refined wheat flour/non-veg/deep-fried/package foods as much as possible.
- Limit red and processed meat.
- Limit sugar-sweetened drinks.
- Limit foods containing excessive salt.
- Do not rely on supplements.
- Limit consumption of highly processed foods.
- No risk in consuming meat, poultry, and eggs in present circumstances.
- Follow hand-wash hygiene after handling raw meat, eggs, or even vegetables.
- Cook meat and poultry products thoroughly and include these foods in moderation in your diet.
- Avoid too much fat (no more than 30 g per person per day) and salt (no more than 5 g per person a day).
- Keep sugar intake to a bare minimum as it is just calories with no nutrients.
- Consume enough dietary fibre.
- Keep the body hydrated with adequate water intake.
- Take up moderate physical activity including yoga to reduce stress and build immunity.
- Avoid smoking and alcohol consumption. Limit alcohol consumption as it has an adverse effect on vitamin and mineral absorption and is associated with sleep problems. There is no evidence that alcohol consumption prevents coronavirus infection. Additionally, the consumption of adulterated beverages containing methyl alcohol instead of ethyl alcohol can cause very serious poisoning reactions.
- Practise good personal hygiene such as washing hands before and after preparing or eating food.

26.9.2 Immunity-Boosting Foods with Possible Antiviral Properties

There has been much talk about one's immunity and the corona survival. Hence, the dietary management of COVID-19 should be considered in terms of improving immunity and utilizing antiviral properties of few nutrients. Eating a low-fat, plant-based vegetarian diet may boost the immune system. Vegetarians have been shown in a few studies to have more effective white blood cells compared to nonvegetarians, because of a higher intake of vitamins and lower intake of fat (Davison et al. 2016).

There are many traditional food items, which can increase the immunity with an additional benefit of some antiviral properties. Some of the foods that increase immunity and with possible antiviral properties have been reported as below which must be included in the daily diets:

26.9.2.1 Citrus Fruits

Citrus fruit is one of the nature's best and easily available sources of vitamin C, a key nutrient in supporting our immune system. Citrus fruits are known to have other

benefits like antioxidant, antitumor, cardioprotective, and neuroprotective effects. They have additional fibre content also. But what makes them significant is their immune-boosting potential.

26.9.2.2 Red Capsicum

Vitamin C present in red capsicum is three times higher than the vitamin C present in an orange. It can be included in salad every day before meals.

26.9.2.3 Other Fruits

Fruits like papaya, guava, apple, grapes, mango, tangerines, lemons, sweet lime, and gooseberries; all seasonal vegetables, including green leafy; and spices, legumes, millets, flesh foods, and fish are among those with rich sources of nutrients. Even though almost every fruit is good for health and human immunity, it has been proved that apple, pumpkin, and papaya have got antiviral effects against specific viruses (Konowalchuk and Speirs 1978; Suchitra and Parthasarathy 2015). Even though extrapolation to coronavirus is unscientific, the antiviral and immune-boosting properties of the above said fruits are established.

26.9.2.4 Nuts and Seeds

Many nuts and seeds including almonds, pumpkin seeds, peanuts, and groundnuts have high vitamin E levels. Vitamin E is a lipid-soluble antioxidant commonly present in the membrane of all cells including immune cells. This is supposed to prevent stress-induced damage to cells. Three to four spoons of pumpkin seeds daily can provide substantial quantities of Mg, zinc, and healthy fats that are essential for immune functions. Almonds have been recently used to treat common flu symptoms. It has been suggested that almonds exhibit some antiviral actions. The peanut skin has also significant antiviral activities according to recent research (Makau et al. 2018).

26.9.2.5 Curd

Curd is a source of many nutrients and it also improves gut health by regulating gut bacteria, aids immune function, and reduces inflammation.

26.9.2.6 Green Tea

Green tea botanically termed as *Camellia sinensis* contains a group of flavonoids called catechins. These chemicals appear to inhibit viral infections by blocking the enzymes that are important in replication. Green tea has shown to be effective in inhibiting HIV, hepatitis B, and herpesviruses (Chacko et al. 2010).

26.9.2.7 Vegetables

Broccoli and other cruciferous vegetables have been proven to help boost immunity. Researchers claim that sulforaphane, a chemical found in this vegetable, switches on the antioxidant genes and enzymes in specific immune cells. This effect combats free radicals in our body and prevents the disease from getting worsened. Broccoli has

also been found to have antiviral properties against influenza viruses (Antonenko et al. 2013).

26.9.2.8 Garlic

Garlic has been known to have antioxidant, cardioprotective, and antitumor effects. Allicin (chemically allyl 2-propenethiosulfinate) is the primary bioactive chemical which is present in the aqueous extract of garlic. This chemical is also found even in the raw garlic homogenate. When garlic is chopped, the enzyme alliinase is activated to produce allicin. Many studies have noted the antiviral activity of garlic extracts against HIV, herpesvirus, cytomegalovirus, and flu viruses (Bayan et al. 2014). The exact mechanism is unknown.

26.9.2.9 Turmeric

Turmeric is a herbaceous perennial plant (botanical name: *Curcuma longa*) belonging to the ginger family. The medicinal properties of turmeric, the source of bioactive compound curcumin, has been known for centuries; its exact mechanism of action and bioactive components are still not completely understood. The compound is known to have antioxidant, antibacterial, antiviral, cardioprotective, and immune-stimulating properties. The bioavailability of curcumin is increased by the addition of black pepper. In a study, researchers have found that the inflammatory cytokines like the mean serum IL-1β and the vascular endothelial growth factor were found to be significantly reduced by curcumin therapy (Hewlings and Kalman 2017). This assumes significance in the wake of corona epidemic where the cytokine surge is worsening patients rather than the virus replication.

26.9.2.10 Ginger

Ginger and its products are being used to raise the function of the immune systems. The extracts of ginger have anti-inflammatory, digestive, and antitumor effects. Fresh ginger but not a dried one has been shown to have antiviral activity against human respiratory syncytial virus in a human respiratory tract cell line study. Hence, to extrapolate for flu and a trial of such nutrient as an additive in our diet can prove useful. The ginger extract actually has been reported to stimulate the production of TNF-alpha expression by the immune system. Researchers also studied ginger along with other natural compounds in combination for inhibiting H1N1 influenza A (Chang et al. 2013; Mashhadi et al. 2013) and demonstrated the inhibition of viral replication.

26.9.2.11 Miscellaneous Foods

Beta-carotene is a powerful antioxidant that can reduce inflammation and boost immune function by increasing leucocytes in the body. Excellent sources of beta-carotene include sweet potatoes, carrots, and green leafy vegetables. Coconut water is rich in vitamins like riboflavin, niacin, thiamine, and folates and it possesses antiviral and antibacterial properties that can help increase our body's immune system and increase our capacity to fight viral infections like flu.

Onions contain organosulfur compounds like quercetin and allicin which are associated with the inhibition of viral infection. These bioactive compounds can hinder virus attachment to the host cell. They can alter transcription and translation of viral genome inside the host cell and hence affect the viral assembly. Inhibition of viral entry into the cell and inhibition of RNA polymerase have also been postulated as mechanisms of antiviral actions of this vegetable. Tamarind leaves, fruits, and seeds with a multitude of uses have also been demonstrated to have antiviral properties. Regular intake of probiotics allows their intimate interaction with the gut mucosa and mucosal immune system. Probiotics can modulate immune and inflammatory response in the human gut through their interaction with gut epithelial cells. The curd is a simple nutrient supplement for probiotics.

Sesame is a simple nutrient food with enough zinc as its content. Zinc has always been noted for its antibacterial and antiviral properties. Zinc also has a positive effect on body's defense mechanisms. Extracts of the plants and leaves of the mint family have shown antiviral effects.

26.10 COVID-19 and Physical Activity

With the rapid coronavirus spread, the general population has been highly advised, for safety and prevention, to reduce moving and traveling and stay at home aiming to limit the COVID-19 transmission. Unfortunately, such restrictions against regular physical activities will unavoidably affect individuals' routine daily activities, psychosocial status, and well-being and may increase sedentary behavior with lowering energy expenditure, favoring screening activities such as watching television, using mobile devices, and playing games. Hence, regular physical activity; routine exercise, e.g., walking and push-up; at least 30 min of moderate physical activity every day; and/or at least 20 min of vigorous physical activity every other day in a safe home environment should be maintained to avoid stress, anxiety, and depression and this constitutes a strategy for healthy living during the coronavirus crisis.

26.11 General Guidelines

- Avoid handshakes.
- Cover your mouth with a tissue or cloth while coughing or sneezing.
- Wear mask.
- Avoid crowded places and quarantine yourself.
- Avoid going out.
- Avoid public transport and unnecessary travel.
- Do not panic.
- Maintain social distancing.

Reference

Barazzoni R, Bischoff SC, Breda J et al (2020) ESPEN expert statements and practical guidance for nutritional management of individuals with SARS-CoV-2 infection. Clin Nutr:S0261-5614(20)30140-0. https://doi.org/10.1016/j.clnu.2020.03.022

Challenges Faced in Treating COVID Patients and Lessons Learnt

27

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Abstract

Treatment of COVID-19 patients is an immense administrative as well as clinical challenge. The setting up of a dedicated COVID-19 care centre within a short time span, adequate manpower deployment, healthcare worker education and training, provision of facilities for donning and doffing and waste disposal were some of the unique administrative problems. On the other hand, as clinicians we faced enormous hurdles in attempting to treat a disease on which there was no established knowledge and no defined, well-proven treatment protocols and which could strike anyone, anywhere in myriad ways. Coupled with this difficulty in diagnosis and treatment was the challenge of serving COVID-19 patients of every age and clinical requirement, under one roof. Healthcare workers faced a tough time, handling physical discomfort while working for long hours in PPE, along with the fear and apprehension of contracting the infection in the line of duty, and carrying it back home. Mental health issues abounded, both amongst the patients and their caregivers, due to heightened fear, anxiety and loneliness. We share our experience in dealing with the pandemic, the administrative and clinical challenges we faced and some of the ways we overcame them. We further share some of the insights we gleaned from this experience, which may help in better preparation for the future.

Keywords

COVID-19 · Challenges · Administration · Treatment · Healthcare workers · Education and training · Personal protective equipment (PPE) · Mental health

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27.1 Introduction

The year 2020 was unlike any other in recallable history, both for citizens the world over and for the medical fraternity in particular. The healthcare systems of the world were totally unprepared for a health crisis of such magnitude as we have witnessed. Whole cities and countries were brought to their knees and social structures totally upturned. The medical fraternity faced several difficulties and challenges unique to this pandemic, which this chapter will attempt to address. The chapter has been divided into sections. The first part will deal with the administrative and logistic challenges, like putting infrastructure in place, managing healthcare workers, and system protocols. The second part will deal with the issues we faced as clinicians, from dealing with a large lacuna in knowledge to dealing with psychosocial issues in patients. Finally, we will share our experience at the All India Institute of Medical Sciences, Delhi, India, in combating this pandemic, the challenges we faced on the ground, as well as the lessons we learnt for the future.

27.2 Section 1: Administrative Challenges

27.2.1 Setting Up Dedicated Facility

A dedicated centre separate from the main hospital area earmarked for COVID-19 patients needed to be identified. If this was not feasible, a special wing or section of the hospital was to be dedicated to the care of COVID-19 patients (Centers for Disease Control and Prevention, 2021; National Centre for Disease Control, 2021). This section or centre needed to be envisioned as a stand-alone, independent section equipped with all necessary facilities (National Centre for Disease Control, 2021). These included wards, ICUs, operating rooms, radiology suites, dialysis facilities, laboratory areas and blood bank, amongst others. Separate entry and exit points for staff and patients needed to be identified and strictly maintained (National Centre for Disease Control, 2021). Within the COVID section, corridors, staircases, elevators and common areas had to be clearly demarcated as COVID-infected or non-COVID, and labelled as such[1]

27.2.2 Demarcating Suspect Areas

There were bound to be patients who were suspected to have COVID, based on their symptoms or clinical condition, but had either not tested positive or were awaiting their test result. Reserving a holding area for such patients, as well as ward and ICU areas if they deteriorated in the meantime, also became necessary. Protocols for shifting patients into and out of these areas needed to be made and followed (Centers for Disease Control and Prevention, 2021).

27.2.3 Donning and Doffing

'Donning' refers to the procedure of changing from civil clothes to complete personal protective equipment (PPE), ready for entering COVID areas. This starts from changing into hospital scrubs and footwear, removing all jewellery and accessories, and then systematically wearing the entire PPE equipment, including a coverall, shoe cover, N95 respirator, goggles, face shield and double gloves, under the supervision of a dedicated staff. Similarly, 'doffing' refers to the process of step-by-step removal of every article of the PPE under close supervision of a 'buddy', followed by a complete head-to-toe shower, change of scrubs and then final changing back into civil clothes and footwear (National Centre for Disease Control, 2021). Setting up a 'donning area' and 'doffing area' required a separate wing, which could cater to dozens of people donning at a time, along with dedicated infection control nurses to supervise and assist in the procedure (Pandey et al., 2020). These areas had to be equipped with multiple changing rooms, separate for male and female healthcare workers, along with shower cubicles and washrooms (National Centre for Disease Control, 2021). Since the workers could not carry any article inside the COVID areas, arrangements were necessary for providing towels, shower gel and soap in these areas (National Centre for Disease Control, 2021).

27.2.4 Healthcare Worker Education

The medical fraternity was unprepared for such a pandemic. Thus, all workers involved in the care of COVID-19 patients, from sanitation workers to nurses to doctors, had to be collectively and repeatedly sensitised and educated on all issues pertaining to their work in such special circumstances. This included education on infection control and hygiene practices, proper techniques for donning and doffing, following institutional protocols on movement within the hospital, patient care practices and latest diagnostic and treatment recommendations (Centers for Disease Control and Prevention, 2021). The recruitment of doctors and nurses from several specialities into the pool of those involved in COVID-19 care (Centers for Disease Control and Prevention, 2021) necessitated re-education and training in the aspects of oxygen therapy, mechanical ventilation and respiratory monitoring that they were unaccustomed to (Pandey et al., 2020; Peiffer-Smadja et al., 2020).

27.2.5 Fear and Apprehension Amongst Healthcare Workers

The terror and stigma associated with the COVID-19 disease in society, especially in the early days, were very strong. Healthcare workers were no exception. Fear of catching the disease at their workplace, apprehension about the severity of the disease and untimely disability or death, as well as fears of transmitting the disease to the family members, were widespread amongst the healthcare fraternity (Peiffer-Smadja et al., 2020). There was immense reluctance to work in the COVID-19 setup.

Proper counselling and education to allay these fears, assurance of proper PPE and other protective measures at the hospital (Centers for Disease Control and Prevention, 2021), provision of accommodation near the workplace and provision of transport facility for hassle-free travel between the workplace and the home were some of the ways that this issue was addressed.

27.2.6 Government and Central Administrative Support

Complete upheaval and upgradation of existing healthcare facilities along with a parallel setup dedicated to COVID-19 care required immense financial and logistical support from the government agencies. Prompt administrative support, delegation of decision-making and allocation of adequate funds helped in achieving various targets in an efficient and time-bound manner. Most importantly, the imposition of a nationwide lockdown in late March 2020 brought a sudden halt to the sudden spread of cases; this gave us the much-needed few weeks to get various facilities and resources ready, train healthcare workers and get all systems in place before the surge of cases began (Gupta et al., 2021).

27.2.7 Area-Specific Administrative Requirements

Since SARS-CoV-2 was believed to spread by airborne droplets and aerosols, the existing air-conditioning and ventilation systems in the various areas of the hospital needed to be reimagined (Pandey et al., 2020). Normally, operating rooms and ICUs are often positive-pressure areas, i.e., areas where air-conditioning units throw air into the room. In COVID areas this could have been a danger because the virus in the exhaled air of the patients may get further spread by the airflow. Negative-pressure operating rooms and ICUs needed to be set up, so that the infected air could be channelled away safely. Air-conditioning vents needed to be fitted with ultraviolet filters to treat the air exiting from the COVID areas and air-handling units reset to allow complete air change within the ICU 12 times every hour (National Centre for Disease Control, 2021).

27.2.8 Leadership and Motivation

The COVID-19 pandemic has been described as a crisis of epic proportions the world over, and the response to this ‘warlike’ situation similarly needed to have a battle-like urgency and military precision. The situation demanded robust and decisive leadership, which was responsive and sensitive to on-ground realities (Pandey et al., 2020). Specific national agencies, including the AIIMS, Delhi; Indian Council of Medical Resources; and National Institute of Virology, were identified by the government to carry the responsibility of framing national

guidelines and protocols for testing and treatment, which were followed across the country.

27.2.9 Disposal and Processing of Waste, Old Records, Articles

Processing and disposal of waste of the COVID patients was a unique challenge. Normally, hospital waste is classified into household or uninfected waste and infected waste (Department of Health Research, Ministry of Health and Family Welfare, Government of India, n.d.). However, all waste arising out of COVID areas needed to be treated as potentially infected waste, needed to undergo proper processing and needed to be disposed off separately in dedicated COVID waste disposal facility (Centers for Disease Control and Prevention, 2021; National Centre for Disease Control, 2021). Further, all records, files, patient record charts, radiographs and CT scan films were treated as infected waste and were not sent to the medical records section as is routine in our centre, but scanned electronically as soft copies and stored in the computer facility.

27.3 Section 2: Clinical Challenges

27.3.1 Nature of the Virus

Till early 2020, few people in the world had heard of SARS-CoV-2, an enveloped RNA virus, belonging to the Coronavirinae family and widely found in humans and other mammals. Its exponential spread has led experts from every field trying to understand the characteristics. Till date, the world is grappling with this ever-changing virus, with new mutations and more virulent strains emerging in various parts of the world every few months (Naqvi et al., 2020). The constantly changing nature of the virus, its pathogenicity, its infectiousness and clinical manifestations have made it difficult not just to understand and treat, but also to design effective vaccines and offer appropriate clinical advice (Naqvi et al., 2020).

27.3.2 High Contagiousness

SARS-CoV-2 is extremely contagious spreading via droplets, direct contact and aerosols (Setti et al., 2020). The extent of contagiousness is unlike any other rapidly spreading disease the world has witnessed. This has made even normal patient care, like history taking, examination, simple procedures like blood sampling and wound dressing, challenging due to the risk of infection posed to the healthcare workers (Zhang et al., 2020). All contact with patients necessitated PPE and distancing protocols. Also, the risk of contact between infected and uninfected patients in common hospital areas was so serious that all areas had to be strictly segregated

(Centers for Disease Control and Prevention, 2021; National Centre for Disease Control, 2021).

27.3.3 Long Asymptomatic Phase

Patients infected with SARS-CoV-2 often stay asymptomatic for up to a week (Sakurai et al., 2020). This has a twofold negative impact. Firstly, the patient presents to the healthcare facility at a later date, which drastically reduces the effectiveness of several treatment options. Secondly the patient, being unaware of his or her infection, is likely to go about his or her daily activities normally, thus spreading the virus in society.

27.3.4 No Treatment Guidelines

The medical fraternity was unprepared for a viral pandemic of such epic proportions. A few antiviral drugs, like remdesivir and ritonavir based on previous experience with the MERS and H1N1 epidemics, were considered the mainstay of treatment (World Health Organization, n.d.). Hydroxychloroquine, an antimalarial drug (World Health Organization, n.d.), and antibiotics like doxycycline and ivermectin, earlier reserved for specific atypical microbial infections, were all considered to combat the virus. Apart from medications targeting the virus, other therapeutic approaches were also tried. These included strong immunosuppressants to contain the uncontrolled immune storm (University of Oxford, n.d.), anticoagulants, supplementation with vitamins C and D, and micronutrients like zinc amongst a host of other drugs (Shakoor et al., 2021). Treatment with convalescent plasma, i.e. plasma of recovered COVID patients, gained much prominence (University of Oxford, n.d.). However, no single therapeutic approach was conclusively shown to improve patient outcome significantly (World Health Organization, n.d.; University of Oxford, n.d.). A number of trials and studies were launched by WHO and various international agencies, but till date, strong evidence-backed guidelines recommending a particular set of drugs is lacking (World Health Organization, n.d.; University of Oxford, n.d.). This has led several institutes to develop their own customised institutional protocols.

27.3.5 Systemic Manifestations of Disease

COVID-19 illness was earlier thought to affect primarily the respiratory system, but our expanding understanding of the disease has demonstrated that almost any organ system of the body can be involved. From the respiratory to gastrointestinal manifestations, and from the haematological to neurological pathologies, the spectrum of manifestations of COVID-19 disease is very wide (Gupta et al., 2020). Atypical or non-respiratory manifestations are particularly common in paediatric,

pregnant and geriatric age groups (Liu et al., 2020). This makes the disease behaviour very unpredictable, making timely accurate diagnosis and appropriate treatment challenging.

27.3.6 Fear of Blood Samples

For a long time, it was unclear whether the blood of COVID-19 patients can transmit the infection. This made the routine sampling and processing of blood samples difficult. Usually, in the ICU, multiple blood samples are sent daily or twice a day to monitor clinical progress or deterioration. Transport and processing of such blood samples became a problem as there was fear of handling and dealing with the blood. Eventually, this issue was addressed by setting up a dedicated laboratory area within the COVID area, and ensuring full PPE for all staff handling these samples.

27.3.7 Psychological Issues Amongst Patients

Patients with COVID-19 illness had to be strictly kept away from all uninfected individuals. Often patients had to be in isolation, away from family and friends. Further, the social stigma associated with COVID-19 illness incites feelings of persecution and harassment. Thus, at a time when patients needed the most support and motivation, they were often alone and cut off from the world and their loved ones. In addition, healthcare workers in COVID-19 areas were always kept to a minimum in order to avoid unnecessary exposure; furthermore, these workers were in full PPE, making any speech, facial recognition and nonverbal communication difficult (Parush et al., 2020). Thus, the rapport and trust between the patient and his or her doctors and nurses suffered. The usual psychological support and motivation of the treating clinical team were not as effective. Thus, these patients were lonely, anxious and depressed (Luo et al., 2020). They often lost motivation to recover, which made them less cooperative and compliant to treatment. Borderline or latent mental health issues in several patients surfaced, making them aggressive, combative and wilfully uncooperative (Luo et al., 2020). These issues posed a challenge for the treating clinical team as well as the patient's well-being.

27.3.8 Catering to Patients Needing Special Care

COVID-19 hospital catered to every confirmed COVID-19 patient in the hospital. These included patients of every age group from newborn and underweight babies to elderly patients over 90 years of age, pregnant and post-partum mothers, accident victims, patients awaiting surgery and several other patient subtypes. This diversity in patient profile posed a challenge, because every specific patient subtype had a unique set of requirements. For instance, newborn babies needed specialised incubators and nurses well versed in baby feeding techniques, while the presence

of a pregnant woman in the ICU meant that radiology could not be performed as freely as is usually done. Further, no clinician could be proficient in the clinical management of such a wide array of illnesses. This challenge was addressed by composing a team consisting of doctors and nurses from every specialty, and trying to cluster patients specialty-wise.

27.3.9 Challenges of Working in PPE

One who has never donned and worked in a level 3 PPE cannot truly understand the experience of doing so, and one who has donned and worked in level 3 PPE cannot forget the experience of doing so! Working for hours in complete PPE was a challenge of a different magnitude. Heat exhaustion due to profuse sweating and dehydration leads to fatigue and feeling of light-headedness (Davey et al., 2021). Fogging within the goggles and face shields leads to extreme vision difficulty, making fine procedures very challenging (Parush et al., 2020). The tight-fitting N-95 mask, necessary for personal protection, also leads to nasal stuffiness and feelings of suffocation. Presence of double gloves, in addition to sterile gloves if necessary for a procedure, compromises manual dexterity. Difficulty in speech and hearing, along with the inability to read non-verbal cues like facial expression and body language, makes communication amongst the team members extremely challenging (Parush et al., 2020). Worst of all, perhaps, is the inability to eat or drink, or use the washroom while in PPE. Taking just a 5-min break, for a cup of tea or coffee, or even a few sips of water, allows one to recover one's energy during a demanding work shift. Inability to do so further worsened the dehydration and weakness experienced in PPE, making even a 6-h shift feel as exhausting and draining as one thrice as long.

27.4 Section 3: Our Experience at the All India Institute of Medical Sciences, Delhi

In the midst of this unprecedented crisis, the nation looked towards an institution of undisputable national repute to lead the way in terms of management of the COVID situation. Thus AIIMS, Delhi, took upon itself the responsibility of acting as the lead player for the healthcare systems of the country at this time.

27.4.1 Administrative

In March 2020, preparation began for the oncoming surge of cases. A dedicated centre, the AIIMS Trauma Centre, which is separate from the main hospital campus, was converted to a dedicated COVID-19 centre. Entry and exit points and all hospital areas, including lifts and staircases, were clearly labelled as 'COVID' or 'non-COVID' and compliance was enforced. The basement of the centre was

converted into a donning area, complete with locker facility and multiple changing rooms, while two doffing areas were set up with multiple shower and bathroom facilities. Dedicated nurses were posted round the clock in both areas to supervise and help workers. All departments in the hospital contributed a set proportion of their doctors and nurses to a common pool of COVID-19 workers. These staff were then rostered according to their core training and duration of experience, to the ward, ICU or other clinical areas. Duty hours were limited to 6-h duration to allow for the challenges of working in PPE. Shift timings of nurses, sanitation and other workers, and doctors, were staggered to avoid overcrowding of donning and doffing areas. In the early days of the pandemic, when transport facilities were scarce, accommodation close to the hospital was offered to those interested, and dedicated bus and transport facilities were arranged for those commuting from their homes.

27.4.2 Clinical

To ensure standardised treatment across all treating teams, an institutional treatment protocol was made based on latest recommendations of various world health agencies and aggregated evidence from ongoing trials and scientific literature. This was constantly updated based on evolving evidence. Regular clinical meetings were held to discuss challenging patients and logistical problems if any, and to analyse mortalities, for quality improvement. In addition, in light of the role of AIIMS as national leader for COVID management protocols, regular clinical grand rounds and seminars were held at a national level, and broadcast across the country to share our experience and expertise. This allowed a platform for knowledge-sharing with clinicians in every corner of the country, from the most far-flung and remote areas to the heart of large tertiary care centres in big cities. A dedicated communication team was constituted (Centers for Disease Control and Prevention, 2021), consisting of consultants from multiple specialties, who was tasked with the responsibility of communicating with the patient's family and relatives. Every day, a member of the team would telephonically call up the patient's family and inform the current status of the patient, share the ongoing medical plan and answer queries of the relatives. In case of any change in plan or new development, additional phone calls were made to keep the relatives apprised of the status. Further, video conferencing facility was made available for all relatives and a special team made to facilitate video calling at specified times, which helped motivate and support the patient for rapid recovery. In order to avoid unnecessary visits and creation of paperwork, consent for procedures was digitised and electronic or soft copies were accepted.

27.5 Section 4: Lessons Learnt for the Future

The past year, although unprecedented and challenging on several fronts, also provided a unique learning experience for all involved.

A pandemic, much like a war, can strike anytime without warning. It is essential to recognise an oncoming healthcare problem at the earliest, and immediately start setting in place the management programmes for the same. Hospitals should have earmarked areas for such infectious disease disasters along with a blueprint for setting up and management of wards, ICUs and operating rooms, in addition to staff recruitment and reassignment. Interdepartmental cooperation, along with a robust and responsive leadership, is necessary to create large teams of responders. Proper, inclusive training and education of all workers is important. Infection control protocols, as well as education on PPE, donning and doffing, should be made part of medical, nursing and paramedical training. Standardised institutional protocols need to be made for logistics, administration, transport as well as clinical aspects like diagnosis, monitoring and treatment. These protocols needed to be clearly communicated to all teams and adherence enforced. Finally, the psychological aspects involved in such a pandemic deserve emphasis. Psychological support of patients is given with regular counselling and regular communication with family members whenever feasible. Similarly, the well-being and satisfaction of the healthcare workers, the backbone of the pandemic response across the globe, need utmost and urgent addressal. Fears and apprehensions need to be dealt with, ease of working needs to be facilitated, regular breaks should be given and overall satisfaction should be ensured. Hopefully, the coming generations will be spared from horrors similar to those experienced during the COVID-19 pandemic, but should the need arise, we hope that the experience gleaned will help in better management of future healthcare crises.

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Fears, Challenges and Opportunities During COVID-19: A Frontliner's Perspective in Healthcare System

28

Goverdhan Dutt Puri and Divya Jain

Abstract

The current coronavirus (COVID-19) pandemic has challenged the healthcare systems worldwide. In India, when the whole country went into lockdown, the medical fraternity geared up for the battle against the invisible enemy, which was knocking at the doors. The major hurdles faced by doctors as frontline workers in this battle were the fear and anxiety of contracting the disease; logistics and resource crunch—both trained manpower and equipment—and lack of knowledge about this new disease. Despite all the snags, the dedication, zeal and commitment shown by the medical fraternity have been commendable. The doctors especially the resident staff, nursing and technical staff have been working tirelessly overcoming their own fears. Fighting this battle has brought the best out of the healthcare workers in the form of leadership qualities and teamwork. Within a short span of time, well-equipped COVID units were created. This period saw a surge in research and innovation worldwide. In this chapter, we share our experience amid the COVID crises. This is an ode to all the frontline warriors who started this battle with some fears, gradually acclimatized to the new scenario and put their best foot forward.

Keywords

COVID-19 · Teamwork · COVID set-up · Training · Vaccination

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28.1 Introduction

On March 24, when the whole country went into lockdown, the medical fraternity geared up for the battle against the invisible enemy knocking at the doors.

We had examples from various nations in the form of success and failure stories. Lessons needed to be learnt from their experiences to form a strategy to deal with the upcoming catastrophe. The hardest hit European countries like Italy witnessed an overwhelming of the healthcare facilities despite having the best of healthcare set-up, owing to the sudden surge in the number of infected patients (Grasselli et al. 2020). On the other hand, Singapore, a small nation which initially saw huge numbers, could curb the numbers early by strategic planning and preparation to ensure adequate health-system resource availability (Lee et al. 2020).

The major impediment in developing nations like India was the humongous population and limited resources to deal with it. Despite all the snags, the dedication, zeal and commitment shown by the medical fraternity have been commendable. The doctors especially the resident staff, the nursing and technical staff have been working tirelessly in the battle.

This chapter is an ode to all the frontline warriors who started this battle with some fears, gradually acclimatized to the new scenario and brought the best out of themselves.

28.1.1 Hurdles in the Path

28.1.1.1 Fear or Attitude

A study assessing the anxiety among the healthcare workers pointed out the fear of developing infection, fear of failing to provide adequate care for patients with given limited resources, fear of carrying the virus home and infecting family and friends and fear of stigmatization as valid reasons for concern during the pandemic (Jiang 2020).

(a) Fear of getting infected and becoming carriers: Working in high-risk areas, the fear of getting infected and transmitting the infection to their family members lingered in the minds of all the frontline warriors. There were elderly people and small children at home who were more vulnerable to infection.
Interestingly, frontline healthcare workers (HCWs) caring for COVID-19 patients had less fear about becoming infected than HCWs in other units. This counterintuitive finding may be related to less direct education and communication with the lower risk group, thereby failing to allay their concerns. Fear was also noted to be greater at the peak of the SARS epidemic among lower risk HCWs, which aligns with potential increased belief in self-perceived risk.
We at our institution too faced the consequences of this fear, with the reluctance among the HCW to work in COVID-dedicated areas.

(b) Fear of stigmatization: Then there was a fear of stigmatization. Corona warriors who were fighting the battle for humanity against an invisible enemy had to fight

a whole new front in the COVID-19 battle: stigma. There were cases of doctors, nurses and other HCWs on the frontline of the battle being shunned by others for the fear of being infected. This included the threat of being evicted from their own apartments, general ostracism and even police assaults (Urooj et al. 2020). A cross-sectional study undertaken among the HCPs working in Delhi during the period of May 2020 to July 2020 using a semi-structured online survey showed that 70% of the participants perceived some kind of stigma. 50% of study participants perceived some form of stigma in their residential colony, and 46% observed change in the behaviour of their neighbours. Around 20% of participants experienced stigma, most commonly being rude behaviour or harassment from neighbour/landlord (Grover et al. 2020).

COVID-19 misinformation played an important role in shaping such beliefs and behaviours across the world. The false information about the disease exacerbated the fear of contagion, misconceptions and myths about the virus.

In this critical situation, healthcare workers who were directly involved in the diagnosis, treatment and care of patients with COVID-19 were at risk of developing psychological distress and other mental health symptoms (Yadav et al. 2020).

28.1.1.2 Assets

The crises of resources were felt globally. Developed nations faced the crunch due to sudden surge in the number of patients infected with the contagion, exhausting the resources. Developing nations like India faced a bigger challenge where healthcare was already crippled. Building a dedicated COVID hospital in a short span of days, equipped with trained staff and equipment like ventilators and adequate PPE, was a major challenge.

Healthcare resources that were scarce included test kits for diagnosis of severe acute respiratory coronavirus virus 2 (SARS-CoV-2), personal protective equipment (PPE), hospital equipment (ventilators), hospital capacity and healthcare workers (HCWs), particularly those trained to care for the critically ill.

(a) Manpower: Anaesthetist along with the chest physicians and internal medicine formed the core team involved in the management of COVID patients. Anaesthesiologist with the knowledge about the critical care formed the backbone of this team. There were hardly 12,000 anaesthetists for a population of 1.3 billion, roughly 1 for every 50,000.
This crunch was witnessed at each and every hospital designated as COVID centre.

One of the major difficulties while setting up the critical care areas in the COVID hospital was the shortage of trained staff and their reluctance to work continuously in these high-risk areas due to the fear of infection. Furthermore, the guidelines framed in the beginning of the pandemic that necessitated that the healthcare workers should work for 7–10 days at a stretch followed by 1–2 weeks' duty off called for a huge requirement of trained manpower, both

Fig. 28.1 Dedicated 250-bedded COVID hospital

doctors and nursing staff, which could be changed every week in the COVID hospital.

To add to the problem during the progressing pandemic, there were anticipated staffing shortage due to exposure and illness. To deal with these anticipated resource/manpower crunch, contingency strategies had to be devised.

(b) Material: Looking at the basic statistics, in March 2020 when the lockdown was declared, India had 9500 ICU beds, 8000 ventilators and roughly 37,600 isolation beds.

India was completely dependent upon import from other countries for its supplies of PPE and other critical care equipment, especially sophisticated ventilators for managing the patients with COVID lungs. We had traditionally imported ventilators primarily from Europe and China to meet our requirements. With the lockdown and complete shutdown of the internation movement, there was a looming fear of gross shortage of all the equipment.

In February, there were only eight basic ventilator manufacturers in the country and recognizing the importance of ventilators in the near future, the Indian Government banned the export of ventilators the day before the country started a 21-day lockdown on 25 March 2020.

Setting up COVID centres dedicated to provide care to COVID patients while segregating them from the rest of the patients in a hospital catering to a vast number of patients required huge infrastructure and resources.

At our institution, a recently built partially inhabited 250-bedded hospital block was earmarked for the COVID patients (Fig. 28.1). It was a mammoth task to equip the facility with adequate resources for taking care of critically ill COVID patients. A group of clinicians and administrators were given the task to equip and run this COVID block.

Another committee of doctors was constituted to look into the requirement of personal protective gear in not only the COVID areas but also non-COVID working areas. Mass procurement of PPE kits along with other equipment like ventilators, patient monitoring systems, X-ray machines, dialysis machines and ultrasound machines had to be done in a short time span.

Special committee of doctors was constituted to look into the quality of the equipment being procured and also to fast track the procurement of these equipment needed for patient care.

28.1.1.3 Awareness

Epidemics and pandemics are a periodic phenomenon. People in the community face several challenges during such periods. Lack of awareness often leads to an unconcerned attitude, which may adversely affect the preparedness to meet these challenges.

India, a country of 1.3 billion, is home to the largest population of 287 million illiterate adults. The major challenge was to create awareness about the disease.

There was flooding of information about the new disease in the social media. The ubiquity of social media made it easier to spread and create a COVID-19 falsehood, making the work of public health officials harder.

28.1.2 Overcoming Challenges

Winston Churchill had once said, "Never let a good crisis go waste" and what better opportunity did we have than this pandemic. Turning challenges and fears into opportunities requires a major change. Any change is triggered by a sense of urgency and what can be more urgent than the question of life or death!!

The COVID-19 crisis triggered a wave of change.

28.1.2.1 Elements of the Change

(a) Leadership: To navigate through the crises and bring about a change, leadership among the healthcare workers was the need of the hour. COVID-19 crisis was a challenge for which there was no pre-existing solution. Therefore, the HCWs had to take the role of change agents who envision, lead and implement a plan to prevent the disease and save lives. The role of HCWs to plan the strategy to curb the spread of virus at the community level was crucial. Regular war-room meetings of the heads of the COVID centres with the administration were held every week to formulate the action plan. These plans were conveyed to the various executive committees to ensure its implementation.

(b) Teamwork: The war against COVID-19 had to be fought at many fronts. The disease was fast spreading in the community; therefore to segregate the carriers of the disease, testing had to be intensified. The patients had varied presentations, which called for a team of doctors from all specialities to communicate, interact and plan the management.

Fig. 28.2 24 Surveillance of doffing by nursing staff

The clinical management team worked cohesively throughout this pandemic. Right from the day one, from planning of clinical protocols to carrying out the clinical care of the patient, the team of doctors worked to achieve the common goal of best patient care and outcome. Nowhere in the history of my 40 years of experience I have seen daily, 7 days a week, combined rounds of intensivists, pulmonologists, internists, endocrinologists, radiologists, psychiatrists, surgeons and many other specialists sitting together discussing threadbare each and every patient admitted in the COVID block/hospital—and this resulted in best clinical management planning. There was no delay in getting the specialists' opinion for complex cases whether in the ICU or in the high-dependency units or in the isolation ward of the COVID block.

These combined rounds lasted for 2–5 h depending upon the number of patients admitted in the hospital. Such interactions/consultations resulting in best patient management to the critically ill patients have been possible only because of harmonious teamwork by the treating physicians and surgeons.

Some of the departments which had never collaborated or actively worked on the same platforms as the clinicians came together for the common goal, each one playing his or her bit in the team activities. Nursing institute students and teachers were also roped in to give their bit in training the nursing officers as well as monitoring the nursing care and remote doffing areas through CCTV (Fig. 28.2). Infection control team ensured the best infection control practices followed closely by remote CCTV monitoring of each and every activity of HCWs (Teixeira da Silva et al. 2021).

(c) Research: There was a tremendous outflow of information in the form of research publications coming over. To disseminate the research information at a faster rate, there had been acceleration and fast tracking for the evaluation and authorization of the clinical trials related to the management of the pandemic. As a result there has been no shortage of research since the beginning of the

pandemic. A study estimated 23,638 original research papers published on Web of Science and Scopus in a span of just 6 months from January 2020 to June 2020 (Singh et al. 2020).

Ours, being one of the premier research institutes in the country, can also boast of a multitude of research publications from various fields of medicine on the management of COVID patients over a span of 8 months.

Based on the research and experience, clinical management protocols were modified and the knowledge was disseminated not only to the rest of the other hospitals in the region but also to the clinicians taking care of the patients all over the world through weekly webinars. This resulted in quick bilateral exchange of knowledge.

(d) Innovations: The pandemic has offered us a glimpse into a better healthcare system powered by innovation. The abrupt nature of the pandemic knocked down many long-standing obstacles—opening up the opportunity for more rapid change. In a matter of weeks, patients have moved to a "virtual visit first" approach in a watershed moment for telemedicine. New technology in biology is being applied to diagnostics, therapeutics and vaccines. Automation and artificial intelligence within healthcare are allowing for more individualized patient journeys and more efficient use of health resources.

The two spheres which have witnessed a significant change are:

(a) Telemedicine and Digital Therapeutics: There has been a spurt of growth in the field of telemedicine. In our institution, telemedicine is being used as a safe and effective alternative to take care of patients with chronic diseases such as bronchial asthma, hypertension and diabetes when physical appointments are not a possibility. We have been using it for providing psychological support to patients and their family members without getting them exposed to infection.
(b) Growth in Biotechnology: The need for rapid, point-of-care, accurate and low-cost diagnostics has spurned innovation in new and existing methods, promising to produce new platforms for diagnosing diseases. There have been advances in the development of vaccines utilizing novel methods.

28.1.2.2 Consequences

(a) **Positive Mindset:** The major hurdle was overcoming the fear and changing the mindset. The first step was to use science, data and factual information only and getting rid of the crazy, baseless, misinformation that was causing anxiety, panic and negativity.

Fear is a reaction and courage is the decision to trust tested infection prevention practices to provide the highest standard of care, in the safest environment that we can, for as long as we can. **The warriors chose courage!!**

Implementation of effective infection prevention practices was paramount to both ensuring safety and combating fear of contracting the infection. Protocols and guidelines were developed not only to protect frontline staff but also to prevent the spread of the disease to the family members, neighbours and other near and dear ones.

The guidelines and SOPs were established in all working areas of the hospital to minimize the spread of infection. The high-risk category of HCWs which included elderly and immune compromised was exempted from duties. Extensive training of frontline warriors for donning and doffing the PPE was undertaken at all COVID-dedicated hospitals to ensure the safety for all the frontliners. All the staff including the doctors, nurses, paramedical and sanitation were trained on how to wear and dispose the safety gears, follow hand hygiene and pursue mask wearing.

Quarantine facilities were established for the frontliners after the COVID duty exposure to prevent the exposure to the family members. Regular testing of the HCWs after the COVID duties were undertaken not only to detect any subclinical infection but also to build confidence in the HCWs.

Overcoming the Mental Stress: Long working hours in unfavourable environment compounded with the stress of staying away from the family and loved ones were mentally stressful and exhausting. Sharing of authentic information by sharing succinct messages and regularly interacting and counselling with their HCWs by the team leaders or the authorities helped in allaying uncertainties and fear among the HCWs.

(b) **Building the Assets**: The basic elements which drove this change like team leaders and a surge in innovation played a vital role in building the assets, both the manpower and material.

Manpower: In the beginning management of the trained manpower at the COVID centre ensuring the continuity of patient care despite weekly change of the duties seemed a herculean task. In our institution, doctors who had the critical care skills or were willing to take up this job were posted on a rotation basis, as the guidelines directed 7 days' quarantine after 7 days of posting in a COVID centre.

Owing to a huge requirement of nursing staff, a general principle of rotating every nursing officer of the institute fit to work in the COVID area had to be followed.

There was a big challenge of not only training these nursing officers with critical care skills but also acquainting them with infection control policies which included donning and doffing of the PPE kits and safe disposal of patient waste just to mention a few (Fig. 28.3).

But this also gave us the opportunity to train the whole of the nursing officers of this institute as well as other healthcare workers in the critical care of sick COVID patients in the ICU as well as high-dependency units.

Material: Manufacturers and entrepreneurs responded efficiently to the COVID-19 pandemic by fast tracking innovation, revamping assembly lines and expediting manufacturing of everything from N95 masks and personal protective equipment (PPE) to diagnostic kits and ventilators in record time. Remarkably, from producing almost no ventilators domestically, India indigenously manufactured 60,000 ventilators in just 3 months. In ventilators, for instance, almost a whole new domestic industry was created in these times of turmoil. Government hospitals had an estimated 8432 ventilators in March and

Fig. 28.3 Training of the frontline healthcare workers

by May, India could boast of 19,398 ventilators countrywide. This was possible because domestic production of ventilators increased from 2500 in February to 5500–5750 in March.

In our hospital in a short span, the dedicated COVID centre was equipped with ventilators, high-flow nasal cannulas, X-ray machines, dialysis machines and monitoring devices to ensure complete clinical management of COVID patients under one roof.

While the platform for these huge innovations was being set at one side, the pandemic also witnessed a surge in the local innovations or what we call as "Jugad" in our local terminology, completely driven by the need to protect oneself. These rough and ready innovations included face shields made from overhead transparent sheets, intubation boxes to limit the spread of contagion during aerosol-generating procedures and robots to deliver food and medicines to the COVID patients (Fig. 28.4).

(c) **Creating a Wave of Information**: To get rid of the fake news and reduce the psychological impact of misinformation/rumour on the mental health, an authentic and updated public health information was created like the COVID-19 portal and Covid Dashboard. In our hospital, a COVID portal has been created at the hospital website to provide day-to-day information about the number of patients getting admitted and discharged. Additionally, government and many academic institutes like ours have tried to address the mental health needs of the patients as well as publicly release resource materials, conduct webinars and set up helpline numbers.

Fig. 28.4 Innovative ideas for protection from aerosol generated by COVID patient

28.1.2.3 The Final Exit Strategy: Vaccination Drive

The immense efforts of the scientists globally involved in finding an ultimate solution from deadly virus bore fruits when the vaccine against the SARS-CoV-2 virus was launched. Vaccination came as the final exit strategy from the pandemic. Driven by the need and advancement in medical technology, we have witnessed a significant acceleration in the development of vaccine.

However, the vaccination drive in a country like India is definitely not going to be a cake walk. Fraught with both manpower and resource crunch, vaccinating 1.35 billion population is not an easy task. Various challenges lay in the execution of this mammoth task.

Building the resources: Specialized training programs were organized at all the vaccination sites to train the HCWs for the vaccination programme. They had to be oriented with the management of the adverse events which could arise from vaccination.

Fig. 28.5 Vaccination site at PGIMER, Chandigarh

All over India approximately 2 lakh HCWs received training for this biggest vaccination drive.

Logistics challenges: The whole population cannot be vaccinated in a single go. Therefore, a strategic priority-based vaccination policy was formulated. The HCWs were the first to be covered followed by the other frontliners including the police, sanitation workers, etc.

Resistance hurdle: The culprit here was the infodemic. Concerns and information being circulated about the vaccination in the social media increased the anxiety among the general population. The HCWs emerged as role models, being the first recipients in the vaccination drive, setting an example for the general population.

Overcoming these hurdles, on 16th January 2021, India launched the biggest vaccination drive in the human history. Till today, as of 7th February, almost 57.75 lakh beneficiaries have received the vaccine.

Postgraduate Institute of Medical Education and Research, Chandigarh, has been actively involved in vaccination programme from its commencement by being partners in the initial vaccination trials being conducted on human volunteers along with 12 other hospitals in India.

In preparation for the mass vaccination drive, special committee of doctors was constituted. Two hundred HCWs in our institute received training for vaccination and management of the adverse events. Special vaccination sites, equipped with staff and resources, have been set up (Fig. 28.5). Almost 100 HCWs are being vaccinated each day at our institution.

The aftermath of this pandemic will be felt till long. We need to prepare ourselves to handle the long-term effects of this deadly disease.

At PGIMER, from the first admitted patient of COVID-19 on 24th March 2020 to the first recipient of COVID-19 vaccine on 16th January 2021, we have seen it all.

The end war has begun and we will fight till the very end!!

28.2 Summary

In the crucial times of COVID-19 pandemic the HCWs as frontliners are fighting the battle from various fronts. It has not been just the management of the patients with crunched resources but a mental war also to overcome the fear of contracting the disease themselves and being stigmatized by the society.

As Kurt Hans said, "**Plus et envois**." There is much more in us. "*You have to do everything you can, you have to work hardest, and if you do, if you stay positive, you have a shot at a silver lining.*"

Fighting this battle has exhibited the best of the HCWs from leadership qualities to innovation. From managing the first COVID patients, we have now seen the first recipients of the COVID vaccine. With this armamentarium of hope and perseverance . . . *We shall overcome this one day!!*

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