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CLINICAL INVESTIGATION

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An epidemic of acute haemorrhagic conjunctivitis caused by enterovirus 70 in Okinawa, Japan, in 1994

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Abstract ● Background: Although enterovirus 70 (EV70) has been identified as the major aetiological agent of acute haemorrhagic conjunctivities (ACH),no EV70 strain has been isolated by cell culture method since 1988. Therefore, recent clinical and epidemiological characteristics of AHC caused by EV70 have not been clarified.
● Methods: Clinical and serological studies were carried out on patients during the AHC epidemic in Okinawa, Japan, in 1994 in which 7509 cases were reported by national epidemiological surveillance. EV70 was confirmed as the causative agent by reverse-transcription polymerase chain reaction. ● Results: The 11–15 years age group contained the highest number of cases (62% of the total). Conjunctival hyperaemia was present in all patients, and subconjunctival haemorrhage, superficial punctate keratitis and preauricular lymphadenopathy were present in 24.0%, 11.7% and 9.3% of AHC cases, respectively. No neurological complication was observed in this epidemic. Out of 31 paired serum samples, 10 pairs showed a fourfold rise in antibody level to EV70. None of the paired serum samples showed a fourfold rise in antibody level to Coxsackie A24 variant virus.
● Conclusion: These findings demonstrate that the clinical features of AHC observed in this study were milder than those reported previously, in contrast to the high transmission rate during an epidemic. Changes in clinical features of AHC, such as a low incidence of subconjunctival haemorrhage and disappearance of neurological complications, might be due to biological transformation of EV70. It should be noted that EV70 is still an important aetiological agent of exploxive epidemics of AHC.

Introduction

Epidemic acute haemorrhagic conjunctivities (AHC) first occurred during June 1969 in a suburb of Accra, Ghana [9]. Since then, the disease has spread to many parts of the world, affecting millions of people, and has gained recognition as a major international public health problem [29]. Enterovirus 70 (EV70) has been identified as the major aetiolgoical agent of AHC [3–7, 11–13, 16, 17, 21, 24, 25]. In Japan, the first epidemic occurred in 1971 and then outbreaks were locally recognized every year until 1985, but a large-scale epidemic has not occurred since 1986 [19]. Commonly used diagnosed tests for enterovirus infection are based on virus isolation in cell culture, followed by identification of serotypes with neutralizing antisera, or on serological tests. Although no EV70 strain has been isolated by cell culture method since 1988 [24], a reverse-transcription polymerase chain reaction (RT-PCR) [10], which is more sensitive than immunofluorescence [22], enzyme-linked immunosorbent assay [1], or electron-microscopic methods [32], has been applied to detect epidemic EV70 that could not be isolated by cell culture. We have previously reported that 12

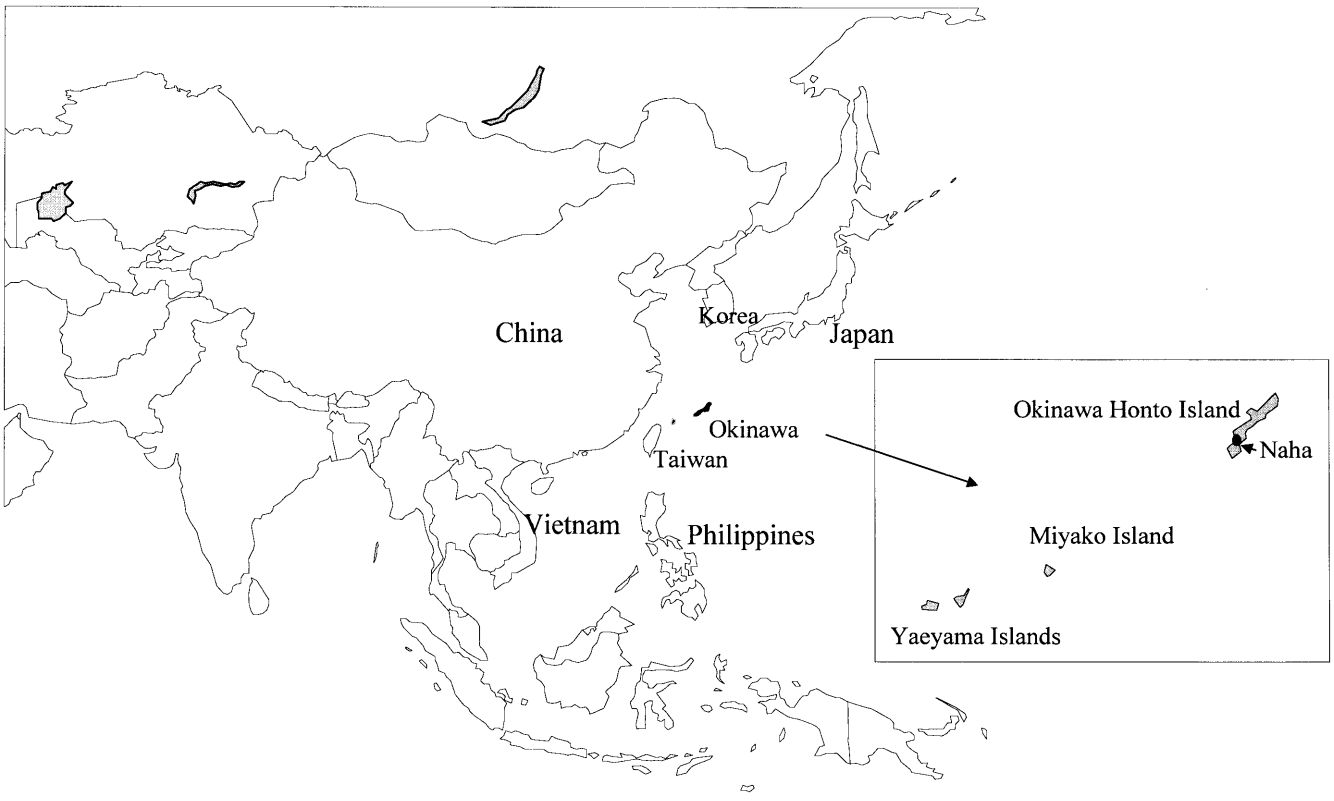

Fig. 1 Location of Okinawa prefecture, Japan

samples out of 27 culture-negative specimens from conjunctival swabs of patients among the population of an AHC epidemic in Okinawa, Japan, in 1994 were positive by RT-PCR [27]. This method has been applied for phylogenetic analysis of EV70 using specimens from patients among a community outbreak of AHC in Israel [26].

Due to the inability of isolate EV70 from patients with AHC, recent clinical and epidemiological characteristics of AHC caused by EV70 have not been clarified. Most epidemiological studies on AHC outbreaks caused by EV70 have been carried out in tropical countries [3, 4, 6, 7, 11–13, 16–18, 21, 24, 25]. Although Okinawa is located in the subtropical region, the most northern area of a recent AHC epidemic was in the nothern hemisphere, suggesting a characteristic epidemiological feature based on the geographical situation.

In the present study, the epidemiological features and the clinical findings of the 1994 AHC epidemic in Okinawa, Japan, in which the aetiological agent was identified as EV70 by RT-PCR, will be described.

Materials and methods

Okinawa prefecture comprises 48 islands and constitutes the southern half of the Nansei Islands located between Kyushu, Japan and Taiwan (Fig. 1). It is located at latitude 24–28° north and longitude 122–133° east. Climatologically the area is subtropical with an average annual rainfall of 2100 mm; the lowest temperature is 16.0 °C, in January, and the highest 28.1 °C, in July. The prefecture covers 2250 km^2 in area and the population numbered 1.28 million in 1994. Ninety per cent of the inhabitants live on Okinawa Honto Island, where all of the sentinel eye clinics are located. Several United States military bases are located on the island and there is official and military traffic to and from the US mainland, Southeast Asia and the Pacific islands. There are also busy international, domestic and inter-island flights and maritime traffic.

Collaborating sentinel eye clinics under the program conducted by the Ministry of Health and Welfare, Japan, report clinically diagnosed cases of AHC to the Surveillance Center on a weekly basis. Three hundred and fifteen eye clinics in all 47 prefectures of Japan and 10 designated cities serve as sentinel stations for ocular diseases. Okinawa prefecture has five sentinel eye clinics.

After obtaining informed consent, blood was collected by venipuncture from 31 patients in Naha and Itoman cities at first presentation and from 44 patients, including the above-mentioned 31, at 3–4 weeks after the onset (convalenscent phase). Serum was separated from these samples on the same day and kept frozen at −80 °C. Serum samples were titrated by microneutralization in HeLa cells. The serum-virus mixtures were incubated for an hour at 37 °C and then overnight at 4 °C before inoculation. The viruses were J670/71 strain of EV70 (National Institue of Health, Tokyo, Japan) and EH24/70 strain of Coxsackie A24 variant virus (CA24v) (National Institute of Health). The neutralization levels were determined as described previously [2]. The serum samples which showed 1:8 or greater of the neutralization antibody level against EV70 or CA24v were considered as antibody-positive samples.

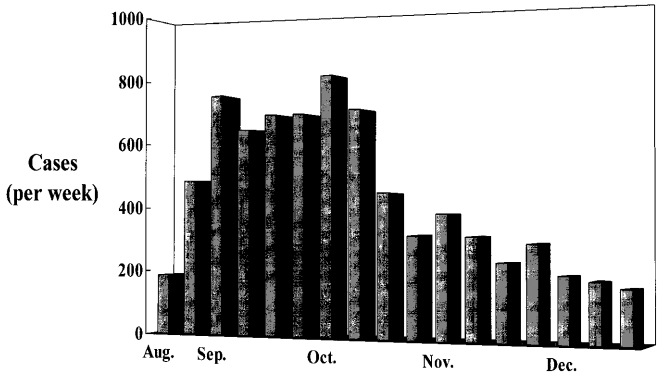

Fig. 2 Time course of the acute haemorrhagic conjunctivitis (AHC) epidemic: weekly reported number of AHC patients in Okinawa prefecture from 26 August 1994 to 30 December 1994 (data based on National Epidemiological Surveillance of Infectious Diseases)

Table 1 Age distribution of AHC patients reported by sentinel eye clinics

Age group (years)	Male (%)	Female (%)	Total (%)
0–5	2 (0.5)	2 (0.6)	4 (0.5)
6–10	24 (5.5)	17 (5.2)	41 (5.4)
11–15	275 (63.6)	196 (60.0)	471 (62.0)
16–20	73 (16.9)	50 (15.3)	123 (16.2)
21–30	15 (3.5)	19 (5.8)	34 (4.5)
31–40	24 (5.6)	22 (6.7)	46 (6.1)
41–50	13 (3.0)	11 (3.4)	24 (3.2)
51–	6 (1.4)	10 (3.0)	16 (2.1)
Total	432	327	759

Table 2 Source in infection of 759 patients with AHC, 1994

Source of infection	Number of patients (%)
School	535 (70.5)
Home	95 (12.5)
Neighbours	13 (1.7)
Workplace	10 (1.3)
Others	3 (0.4)
Unknown	103 (13.6)
Total	759

Results

The weekly incidence of AHC reported by five sentinel clinics in Okinawa from September to December in 1994 under the national epidemiological surveillance of infectious diseases is presented in Fig. 2. The epidemic started in the last week of August, 1994 and spread explosively, with a peak incidence in late October, then subsided slowly towards December. The number of reported cases from the five sentinel clinics was 7509. The age distribution of cases reported from three sentinel eye clinics is presented in Table 1. There were 432 male (56.9%) and 327 female (43.1%) patients. Their ages varied from 3 to 79 years, mean 17.3 years. The age group comprising the largest proportion of patients was junior high school children (11–15 years; 62.0%). The sources of infection in 759 outpatients seen at three sentinel clinics are presented in Table 2. Most of the patients studied (70.5%) contracted the infectio at their school. The second larges proportion of patients (12.5%) contracted the infection within their family. The following clinical findings were obtained from 201 patients (367 eyes) who attended one sentinel clinic. Both eyes were infected in 166 cases (82.5%) and one eye in 35 (17.5%). In 131 (78.9%) of the bilateral cases, the second eye developed infection within 24 h of the first. For the rest, it varied from 2 to 6 days. The main symptoms and clinical signs are shown in Table 3. Of the 367 eyes, conjunctival hyperaemia was present in all patients, and 88 eyes (24.0%), 43 eyes (11.7%) and 34 eyes (9.3%) had subconjunctival haemorrhage, superficial punctate keratitis and preauricular lymphadenopathy, respectively. No neurological complication was observed in this epidemic.

The neutralizing antibody level to EV70 in the 44 convalescent serum samples ranged from <1:4 to 1:128 (Table 4). Eleven out of 44 (25%) convalescent sera showed

Table 3 Clinical features of 201 cases of AHC in Okinawa, 1994

	Number (%)
Symptoms (no. of patients)	
Discharge	192 (95.5)
Itchy eyes	157 (78.1)
Foreign body sensation	134 (66.7)
Eye pain	87 (43.3)
Blurred vision	26 (12.9)
Others	8 (4.0)
Signs (no. of eyes)	
Bulbar hyperaemia	367 (100)
Subconjunctival haemorrhage	88 (24.0)
Superficial punctate keratitis	43 (11.7)
Preauricular lymphadenopathy	34 (9.3)
Gross lid oedema	18 (4.9)
Gross chemosis	8 (2.2)
Iritis	0 (0)

Table 4 Antibody levels to EV70 and CA24v in serum samples collected from 44 patients in the convalescent phase

Antibody level	Number of patients	
	EV70 (J670/71)	CA24v (EH24/70)
<1:4	21	39
1:4	7	5
1:8	5	0
1:16	3	0
1:32	3	0
1:64	3	0
1:128	2	0

Table 5 Rise of antibody levels to EV70 in 31 paired serum samples

Rise in Ab level	No. of patients
None	17
Twofold	4
Fourfold	10
Total	31

a neutralizing antibody level of 1:16 or greater. In contrast, no samples had a neutralizing antibody level to CA24v of more than 1:8. Ten pairs of sera (32%) showed a fourfold rise, whereas four pairs of sera (13%) showed a twofold rise in level to EV70 (Table 5). The rest of the pairs (55%) showed no rise in antibody level. None of the paired serum samples showed a fourfold rise in antibody level to CA24v.

Discussion

In Okinawa, Japan, we experienced an unusually extensive AHC epidemic in the summer of 1985 [20]. Many strains of CA24v were isolated from the patients, and wide dissemination of the virus was confirmed seroepidemiologically. The outbreaks reappeared in 1986. The prefectural surveillance centre was notified of 9952 cases in 1985 and 6096 cases in 1986. The epidemic of AHC in the present study was the largest epidemic in Japan since the 1985–1986 epidemic in Okinawa. In Japan, EV70 had been the only causative agent of AHC after introduction of the disease in 1971 prior to the large epidemic in 1985–1986 in Okinawa. After the large outbreak in 1985–1986, no large outbreak of AHC due to CA24v has occurred. Therefore, although most islands were involved, the 2-year epidemic due to CA24v was confined to Okinawa prefecture and the virus did not spread to the mainland of Japan [20]. There was an 8-year interval between the two AHC epidemics, 1985–1986 and 1994, in Okinawa. It is reported that there is no cross-reaction of neutralizing antibodies between EV70 and CA24v [11]. Moreover, in spite of the repeated outbreaks caused by CA24v and EV70, antibody to both picornaviruses was not demonstrated in acute sera [11]. Neutralizing antibody to both CA24v and EV70 does not appear to last long, despite exposure to the viruses from recurring epidemics [31]. It has also been reported that neutralizing antibody levels to EV70 after an outbreak markedly decrease every year, and only 8% of the subjects showed a 1:8 titre 7 years later [2]. It thus seems reasonable to consider that a high porportion of the population in Okinawa was susceptible to both viruses at the beginning of this AHC epidemin in 1994.

The fact that a large proportion of AHC patients comprised junior high school students indicates that AHC spread rapidly owing to the unhygienic habits of the students. Person-to-person transmission among school-aged children and their family members seemed to occur and accounted for the widespread epidemic in Okinawa Honto island (Table 3). In the previous AHC epidemic due to CA24v in Okinawa, in age-specific attack rate was highest in the 10–14 years age group (20%) and lowest in adults (6–8%) [20]. The most common place of infection was reported to be schools (50%), followed by homes (46%) [20], indicating a similar source of infection to that in the present epidemic. While in the past school exclusion may actually have curtailed an epidemic [16, 21], school exclusion was carried out and was reported to be effective in the control of the AHC epidemic in 1994 (unpublished data). Since AHC epidemics can be explosive and no effective treatment is available, it is reassuring to note the epidemiological aspects of AHC documented here and that public health measures should be taken to prevent or control outbreaks. These aspects include the association of AHC transmission with intra-family personal hygienic factors [23, 28]. Climatologically, summer and autumn seem to have favoured this AHC epidemic. However, it was not explained why EV70 spread to suddenly and widely only on the Okinawa Islands. It is conceivable that the climate of the Japanese mainland may be unfavourable for dissemination of EV70 [20].

Serological studies, especially tests for neutralizing antibodies, have been used to investigate the role of EV70 as the causative agent of AHC. The tests are still particularly useful where facilities for EV70 RT-PCR do not exist. Thirty-one paired and 13 single convalescent serum samples were available for serological studies. Ten pairs (32%) of sera showed a fourfold rise in antibody level to EV70, while 11 of 44 (25%) convalescent sera showed a neutralizing antibody level to EV70 of 1:16 or greater. A neutralizing antibody level to EV70 of 1:16 or greater in convalescent serum has been considered a critical diagnostic level [15]. Taken together, with the fact that no samples had neutralizing antibody level to CA24v of more than 1:8 and none of the paired serum samples showed a fourfold rise in antibody level to CA24v, it can be concluded serologically that the causative agent of this epidemic AHC was EV70.

The presence of subconjunctival haemorrhage and superficial punctate keratitis was noted in 10–70% and 0–53%, respectively, of patients in the past reports. Preauricular lymphadenopathy has been reported to be found in 4–40% of AHC cases. The clinical features and severity of AHC observed in our patients were apparently milder than reported elsewhere [4–6, 11, 13]. During the short follow-up in our patients, we did not see any neurological manifestation. Neurological complications, such as poliolike motor paralysis, have been reported during epidemics of AHC, particularly in India and Thailand [8, 14]. Changes in clinical features of AHC, such as a low incidence of subconjunctival haemorrhage and corneal complications or disappearance of neurological complica-

tions, might be due to biological transformation of EV70. Phylogenetic changes of recent EV70 strains are reported [26, 30]. For this purpose, a comparative phylogenetic study of viral protein 1 (VP1) and VP2 regions of EV70 among strains from AHC epidemics has been initiated.

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