COXSACKIE B VIRUS INFECTION AND MYOPERICARDITIS IN THAILAND, 1987-1989

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Abstract. Serum specimens of 363 myopericarditis patients from the hospital all over the country were examined for coxsackie B virus antibody during 1987-1989 by means of microneutralization test in order to assess association between myopericarditis and coxsackie B virus infection. The data established that certain virus infection rates were 24.3%, 19.4% and 23.6% respectively, no differences in incidence were found between sex ($p > 0.05$) and the incidence rate between age groups below 15 years and 15 years and older was significantly different ($p < 0.05$). It was found that the epidemic happened throughout the year and the most common serotype in 1987 and 1989 was B4 whereas in 1988 it was B3.

INTRODUCTION

A member of the genus Enterovirus, coxsackie B virus consists of six serotypes (B1-B6) that are antigenically distinct and commonly infectious to humans. Contagion is from person to person by the fecal-oral route, but sometimes the spread occurs by the airborne route. Transplacental spread from mother to fetus has been observed (Modlin and Crumpacker, 1982). Incubation periods range from 1 day to 3 weeks. Entering alimentary tract via mouth, the viruses replicate in lymphoid tissue of pharynx and gut. In some cases viremia occurs leading to involvement of target organs such as spinal cord, heart and liver (Grist, 1988). The consequences of infection are either asymptomatic virus shedding or a wide spectrum of clinical manifestation ranging from mild respiratory illness to severe central nervous system diseases (Cherry, 1987), for example meningitis, encephalitis, myopericarditis, hepatitis, acute renal failure, myocardial infarction, postpolio syndrome and, more recently, insulin-dependent diabetes mellitus (IDDM) (Aronson, 1975; Lansky et al, 1979; Banatvata, 1985; King, 1983; Vella et al, 1992; Griffiths et al, 1980; Muir, 1992; Bowles et al, 1986; Sharief, 1991).

Coxsackie B viruses have long been known to be probably the most common cause of myopericarditis (EL-Hagrassy et al, 1980). In Thailand, heart disease predominates and mortality is high. To establish whether myopericarditis caused by coxsackie B virus infection, we therefore examined type-specific neutralizing antibody (Ab) to coxsackie B viruses in 363 patients with suspected myopericarditis during 1987-1989. The epidemiological features of coxsackie B virus infection associated with myopericarditis are described herein.

MATERIALS AND METHODS

Viruses and antisera: Prototype strains of coxsackie B virus serotype B1-B6 (Conn-5 strain, Ohio-1 strain, Nancy strain, JVB strain, Faulkner strain and Schmitt strain respectively) and reference coxsackie B virus antisera were kindly donated by National Institute of Health, Japan.

Cells: Human heteroploid Hep-2 cells were cultured in Eagle's Minimum Essential Medium (MEM) supplemented with 5% fetal calf serum.

Collection of specimens: Paired sera of 363 patients presenting clinical signs of myopericarditis were submitted from hospitals in most provinces in Thailand during 1987-1989. Sera were collected in the onset of symptom (acute serum) and 2-3 weeks later (convalescent serum). These sera had been inactivated at 56°C for 30 minutes and stored at -20°C until testing.

Microneutralization test: Sera from the patients were tested for neutralizing Ab against coxsackie-virus B1-B6 (Melnick, 1979). Serial two fold dilution of inactivated sera (from 1:4 through 1:256) were tested with 100 $TCID_{50}$/0.025 ml of each of the six reference strains of B1-B6. Hep-2 cell sus-

pension was added to each well (3×10^5 cells/ml). The neutralizing Ab titer was expressed as the highest serum dilution that completely inhibited the cytopathic effect. Titer ≥ 4 fold rising in microplate neutralization tests indicates coxsackie B virus infection.

RESULTS AND DISCUSSIONS

Serosurveillance of coxsackie B virus infection in suspected myopericarditis patients during 1987-1989 indicated that incidence was 19-24% and did not demonstrate seasonality. The incidence occurred throughout the year (Fig 1) and accounted for 24.3% (19 of 78), 19.4% (20 of 103) and 23.6% (43 of 182) respectively of the year (Table 1). The results of the study show that coxsackie B viruses can cause myopericarditis and remain a public health problem in tropical countries as reported by other researchers elsewhere (Sainani *et al*, 1975; Melnick, 1982). The general vehicle for transmission, water, results in the ease of infection. Transmission depends on the hygienic levels, sanitation conditions, crowding and opportunities for fecal contamination. Close human contact, particularly asymptomatic, appears to be the primary route of spread and the most frequent being the fecal-oral route.
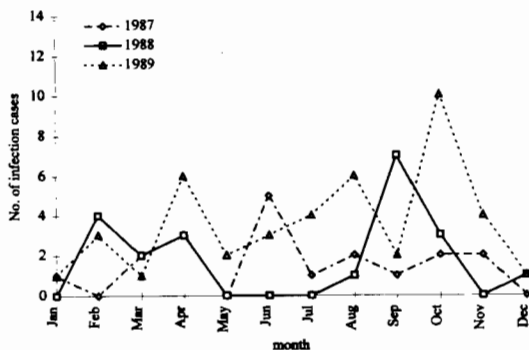

Fig 1–Distribution of myopericarditis with coxsackie B virus infection in Thailand, by the month of the onset of illness, 1987-1989.

The ratio of coxsackie B virus infection in males to females was 1:1.29, 1:1.06 and 1:1.05 from 1987 to 1989 as shown in Table 1. The ratio was not significantly different (Z-test = 0.65, 0.04 and 0.76 respectively, p > 0.05). Gelfand and Holgvin (1962) reported a predominance of coxsackie B virus infection in males among healthy children. On the other hand, Frisk *et al* (1985) found more females than males with IDDM caused by coxsackie B viruses.

For frequency of serotype (Table 1), coxsackie virus B4 was found to be the most common serotype in 1987 and 1989, whereas B3 was dominant in 1988. The results of the investigation differed from the earlier finding in 1968-1975 (B2) reported by Sankawibha (1976). The data illustrated a shift in the most common serotype.

Over a 3-year period from 1987-1989, the percentage incidence was significantly higher in the age group < 15 years (26.8%) in comparison to 17.4% in the age group ≥ 15 years (p = 0.016), as established in Table 2. Our findings concur with data from previous studies in 1975 (Sankawibha *et al*, 1975), since most investigators agree that children are more likely to be infected than adults (Artenstein *et al*, 1965; Grist *et al*, 1978; CDC, 1981).

The regional distribution of coxsackie B virus infection is given in Fig 2. For 1987, the incidence was high in the northern region (38.4%, 5 of 13), followed by the central region (25%, 2 of 48), the north-eastern region (0%, 0 of 2), there was no report in the southern region. For 1988, 33.3% (13 of 39) was reported in the northern region, 8.9% (4 of 45) in the central region and 0% (0 of 3 and 1 respectively) in the northern region and the southern region. While in 1989, the north-eastern region account for 40% (2 of 5), followed by the central region with 21.6% (16 of 74), the northern region with 18.9% (14 of 74) and the southern region with 0% (0 of 5). Some variation appeared in regional distribution, partly because of variation in the number of received specimens and partly because of true variation in the rate of infection. During the entire 3 years, the highest incidence seemed to be found in the northern (in 1987 and 1988) and northeastern regions (in 1989). Furthermore, the most common serotype in the entire country was often similar to the most common serotype for a region. In 1987 and 1988, the most common serotype was also the most common serotype for a region (B4 and B3 respectively), except in 1989 when the most common serotype in the central region (B2) was

Table 1

Cases of myopericarditis associated with coxsackie B virus infection serotype B1-B6 in Thailand, by sex, 1987-1989.

Year	Sex	No. exam	No. positive (%)	Male/female	Coxsackie virus					
					B1	B2	B3	B4	B5	B6
1987	M	50	11 (22)		-	3	4	4	-	-
	F	28	8 (28.5)	1/1.29	-	1	2	5	-	1
	Total	78	19 (24.3)		-	4	6	9	-	1
1988	M	53	10 (18.8)		1	3	5	5	1	-
	F	50	10 (20)	1/1.06	2	4	5	3	3	-
	Total	103	20 (19.4)		3	7	10	8	4	-
1989	M	98	21 (21.4)		6	7	3	8	1	2
	F	84	22 (26)	1/1.05	1	8	5	10	6	2
	Total	182	43 (23.6)		7	15	8	18	7	4

Table 2

No. of myopericarditis with coxsackie B virus infection in Thailand, by age group, for 1987-1989.

Age group (year)	No. exam	No. positive (%)
< 15	216	58 (26.8)[a]
≥ 15	138	24 (17.4)
Total	354	82 (22.6)

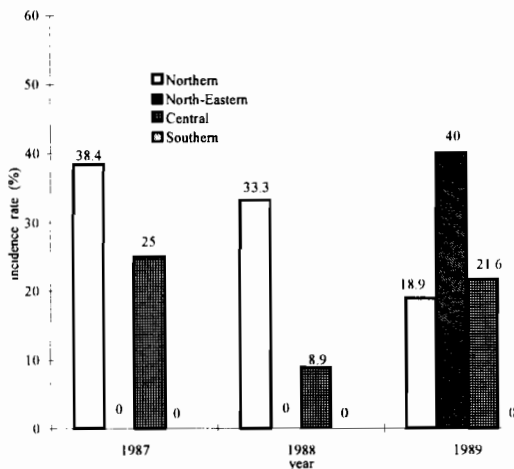[a] significantly (p < 0.05) higher than age ≥ 15

Fig 2–The percentage distribution of myopericarditis associated with coxsackie B virus infection in Thailand, by geographic region, 1987-1989.

different from the most common serotype in the year (B4) (data not shown). However, additional epidemiological investigation will be required to complete these data.

Coxsackie B viruses are now a more important cause of morbidity in economically developed countries because they can cause a broad spectrum of disease, from mild to moderate to life threatening. In Thailand little is known concerning these viruses due to very few surveillance data. With the sharp decline in poliomyelitis and Japanese encephalitis resulting from the WHO eradication program, coxsackie B viruses may play a role with increasing trend. Laboratory surveillance encompassing these agents should be strengthened as in other countries (Nelson et al, 1979; CDC, 1981); first, to confirm clinical diagnosis; second, to provide an epidemiological data for public health monitoring and disease control. A more complete picture of coxsackie B viruses involved in these diseases may emerge.

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