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Serological and virological features of dengue fever and dengue haemorrhagic fever in Thailand from 1999 to 2002

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SUMMARY

Serological and virological features of dengue fever (DF) and dengue haemorrhagic fever (DHF) in Thailand were analysed in 2715 patients from 1999 to 2002. The illness was caused by DEN-1 in 45%, DEN-2 in 32%, DEN-3 in 18% and DEN-4 in 5% of patients. Almost all of the DHF cases caused by DEN-2 and DEN-4 were in secondary infection, while approximately 20% of the DHF cases caused by DEN-1 and DEN-3 were in primary infection. Male:female ratio and age distribution were not different among four serotypes in primary and secondary infections. These results indicate that DEN-1 and DEN-3 induce DHF in both primary and secondary infections, and suggest that DEN-2 and DEN-4 in Thailand are less likely to cause DHF in primary infections.

INTRODUCTION

Dengue viruses belong to the family Flaviviridae, the genus *Flavivirus*, and are also classified as arboviruses [1, 2]. Dengue viruses include four serotypes: dengue virus type 1 (DEN-1), dengue virus type 2 (DEN-2), dengue virus type 3 (DEN-3), and dengue virus type 4 (DEN-4). Dengue viruses cause two clinically different manifestations, dengue fever (DF) and dengue haemorrhagic fever (DHF). DF is an acute self-limiting febrile illness with headache, bone or joint and muscular pains, rash and leucopenia as symptoms [3, 4]. DHF is a severe illness characterized by high fever, plasma leakage, haemorrhagic manifestations and hepatomegaly. Patients may develop hypovolaemic shock resulting from plasma leakage. This is called dengue shock syndrome (DSS) and can be fatal [5, 6]. DF/DHF is becoming an increasingly important public health problem in tropical and subtropical areas of the world, including Thailand [7, 8].

The incidence of DF/DHF has continuously increased since the first recognized outbreak in 1958 in Thailand [9–11]. Rates of DHF increased until 1987 when the largest epidemic ever with 174285 cases was recorded [12]; DF/DHF is now one of the most important public health problems in Thailand [13]. All the four regions in Thailand, north, northeast, central and south, have been equally affected [14]. In the present study, we analysed serological and virological features of DF and DHF cases in six hospitals located in the four regions from 1999 to 2002.

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METHODS

Patients

A total of 5980 and 4045 blood specimens were collected at acute and convalescent stages, respectively, from 5980 suspected dengue cases who visited Lampang Hospital in Lampang (north), Maharat Nakhon Ratchasima Hospital in Nakhon Ratchasima (northeast), Pathum Thani Hospital in Pathum Thani (central), Chareonkrung Pracharak Hospital in Bangkok, Ratchaburi Hospital in Ratchaburi (central) and Hadyai Hospital in Songkhla (south) from 1999 to 2002 (Fig.). Among the 5980 suspected cases, 3137 cases were determined to be dengue virus infections by serological and virological tests. Clinical diagnosis (DF or DHF and grades of DHF) and dengue virus types were determined for 2715 of the 3137 cases. These 2715 cases were analysed in the present study.

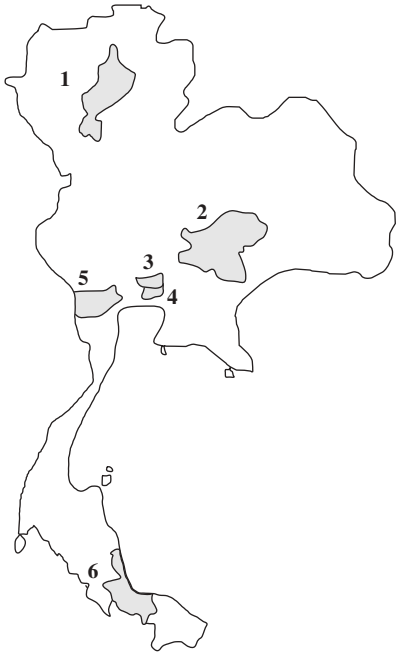

Fig. Location of the six provinces where the sentinel sites are located. 1, Lampang; 2, Nakhon Ratchasima; 3, Pathum Thani; 4, Bangkok; 5, Ratchaburi; 6, Songkhla.

Collection of blood specimens

Blood specimens were taken into tubes with EDTA anticoagulant, and centrifuged. Both buffy coat and plasma were obtained at the acute stage, but only plasma was obtained at the convalescent stage. Vials of buffy coat and plasma specimens were stored in a liquid nitrogen tank. All the specimens were transported in the tank to the Arbovirus Laboratory, National Institute of Health, Department of Medical Sciences, Nonthaburi, Thailand.

Virus isolation and determination of serotypes

Virus isolation and determination of serotypes were performed as previously reported [15]. Briefly, 10 μl of buffy coat samples collected at the acute stage were inoculated onto the monolayer of *C6/36* cells in a 24-well plate with rocking for 90 min and cultured in Leibovitz 15 medium (L-15; Gibco-BRL, Grand Island, NY, USA) containing 1% heat-inactivated fetal bovine serum. After 7 days of incubation at 28 °C, the cultured media were collected, the infected cells were stained by IFA, and dengue virus serotypes were determined [15, 16].

Detection of specific IgM and IgG

Dengue virus-specific IgM and IgG were measured in all plasma specimens by antibody-capture ELISA as described by Innis et al. with minor modifications [17]. For the specimens, 40 U of IgM and/or 80 U of IgG were considered to be the evidence of recent dengue virus infection. The IgM to IgG ratio of 1·8:1 or greater indicated a primary dengue infection. The ratio of less than 1·8:1 indicated a secondary dengue infection.

Reverse transcription–polymerase chain reaction (RT–PCR)

RT–PCR was performed as previously reported [18, 19]. Briefly, viral RNA was extracted from 100 μl of acute plasma with a QIAamp viral RNA mini kit (Qiagen GmbH, Hilden, Germany). RT–PCR was performed using One-step RT–PCR kit (Qiagen) and dengue-specific oligonucleotide primers. Negative and positive controls were included in each run. The second PCR products were analysed by agarose gel electrophoresis and then visualized by ethidium bromide staining.

Statistical analysis

χ^2 test was used for statistical analysis. The differences were considered to be significant when the P value was $<0{\cdot}05$.

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Table 1. *Ratio between DF and DHF cases caused by each of four dengue virus serotypes*

	DEN-1	DEN-2	DEN-3	DEN-4	
Diagnosis	No. (%)	No. (%)	No. (%)	No. (%)	Total
DF	404 (33)	294 (34)	242 (48)	46 (35)	986
DHF	808 (67)	568 (66)	266 (52)	87 (65)	1729
Grades 1, 2	642 (53)	427 (50)	225 (44)	69 (52)	1363
Grades 3, 4	166 (14)	141 (16)	41 (8)	18 (14)	366
Total	1212 (45)	862 (32)	508 (19)	133 (5)	2715

RESULTS

Number of DF and DHF cases caused by each of four dengue serotypes

Among 2715 confirmed dengue cases, 1212 cases (45%) were infected with DEN-1, 862 cases (32%) with DEN-2, 508 cases (19%) with DEN-3 and 133 cases (5%) with DEN-4 (Table 1). These included 986 DF cases and 1729 DHF cases: 1363 cases with grades 1 and 2 and 366 cases with grades 3 and 4 (DSS). The ratios between DF and DHF cases were approximately 1:2·0, 1:1·9, 1:1·1 and 1:1·9 in the patients infected with DEN-1, DEN-2, DEN-3 and DEN-4 respectively, in the present study (Table 1).

Ratios between primary and secondary infections in DF and DHF cases

The ratios between primary and secondary infections were calculated in DF and DHF cases (Table 2). In DF cases, the primary infection accounted for 26, 12, 30 and 23% in patients infected with DEN-1, DEN-2, DEN-3 and DEN-4 respectively. In DHF cases, the primary infection accounted for 20, 2, 21 and 3% in patients infected with DEN-1, DEN-2, DEN-3 and DEN-4 respectively. The percentages of primary infections in DHF cases were significantly lower in patients infected with DEN-2 and DEN-4 than in those infected with DEN-1 and DEN-3 (DEN-2 *vs.* DEN-1, $P<0{\cdot}001$; DEN-2 *vs.* DEN-3, $P<0{\cdot}001$; DEN-4 *vs.* DEN-1, $P<0{\cdot}001$; DEN-4 *vs.* DEN-3, $P<0{\cdot}001$). The results suggest that almost all of the DHF cases caused by DEN-2 and DEN-4 were secondary infection.

Ratios between DF and DHF cases in primary and secondary infections

The ratio between DF and DHF cases was calculated in primary and secondary infections with each of four serotypes. In primary infections, DHF cases accounted for 62% with DEN-1, 22% with DEN-2, 43% with DEN-3 and 18% with DEN-4. The percentages of DHF cases in primary infections were significantly lower in patients infected with DEN-2 and DEN-4 than in those infected with DEN-1 and DEN-3 [DEN-2 *vs.* DEN-1, $P<0{\cdot}001$; DEN-2 *vs.* DEN-3, $P<0{\cdot}05$; DEN-4 *vs.* DEN-1, $P<0{\cdot}01$; DEN-4 *vs.* DEN-3, $P<0{\cdot}02$ (not significant)]. The results suggests that DEN-2 and DEN-4 tend not to cause DHF in primary infections.

Table 2. *Ratio between primary and secondary infections among DF and DHF cases caused by each of four dengue virus serotypes*

	DEN-1	DEN-2	DEN-3	DEN-4
Diagnosis*	No. (%)	No. (%)	No. (%)	No. (%)
DF				
Primary	85 (26)	28 (12)	60 (30)	9 (23)
Secondary	248 (74)	210 (88)	137 (70)	30 (77)
Total	333	238	197	39
DHF				
Primary	134 (20)	8 (2)	46 (21)	2 (3)
Secondary	530 (80)	475 (98)	178 (79)	73 (97)
Total	664	483	224	75
DF + DHF				
Primary	219 (22)	36 (5)	106 (25)	11 (10)
Secondary	778 (78)	685 (95)	315 (75)	103 (90)
Total	997	721	421	114

* Information of clinical diagnosis and antibody response were not available for other 462 cases.

Sex and age distribution of DF and DHF cases

There were no significant differences between males and females in the percentage of DF and DHF by primary and secondary infections with each of four serotypes (data not shown). Age distribution of all the DF and DHF cases are shown in Table 3. Analyses based on serotypes demonstrated that the highest numbers of DF and DHF patients were in the 6–10 years age group in both primary and secondary infections by all the serotypes, except for DHF cases in the 11–15 years age group in secondary infections, by DEN-2 and DEN-3 (data not shown).

Age distribution in primary and secondary infections by each serotype was analysed. The highest numbers of cases were in the 6–10 years age group in primary and secondary infections among DF and

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Table 3. *Age distribution of all the DF and DHF cases*

Age* (years)	Primary				Secondary			
	DF		DHF		DF		DHF	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
<1	8	(4)	8	(4)	0	(0)	2	(0)
1–5	44	(24)	43	(23)	107	(17)	166	(13)
6–10	85	(47)	91	(48)	244	(39)	525	(42)
11–15	39	(22)	48	(25)	216	(35)	474	(38)
16–25	4	(2)	0	(0)	33	(5)	55	(4)
26–35	1	(1)	0	(0)	12	(2)	10	(1)
36–55	0	(0)	0	(0)	9	(1)	4	(0)
45–45	0	(0)	0	(0)	1	(0)	3	(0)
56–65	0	(0)	0	(0)	0	(0)	1	(0)
⩾66	0	(0)	0	(0)	0	(0)	0	(0)
Total	181		190		622		1240	

* Information of age and antibody response were not available for other 904 cases.

DHF cases by all the serotypes except for DHF by secondary DEN-2 and DEN-3 infections (data not shown).

DISCUSSION

In Thailand, all the four dengue serotypes circulate continuously, with one predominant serotype emerging as the cause of a periodic outbreak. Each dengue serotype has characteristics that affect the nature of dengue epidemic and disease severity. Analysis of dengue virus isolates at the Children's Hospital in Bangkok from 1970 to 1986 showed fluctuations in predominant dengue virus serotypes from year to year. Recently, Nisalak et al. reported the predominant dengue serotypes in Bangkok from 1973 to 1999. There were associations between DEN-1, DEN-2 and DEN-3 and the moderately severe dengue epidemic years 1984–1985, 1989–1990, 1997 respectively, and DEN-3 and the severe dengue epidemic years 1987 and 1998 [20]. Endy et al. also reported that all the four serotypes were detected from 1998 to 2000 in a well-defined cohort in Thailand and that there was marked spatial and temporal clustering of transmission of each serotype [21].

In the present study, we analysed serological and virological features of DF and DHF cases in six hospitals distributed throughout Thailand from 1999 to 2002. This study collected information of only the patients who visited the six hospitals. Nevertheless, this study reflects the relationship between primary and secondary infections, and DF and DHF caused by each of four serotypes in Thailand, and also general virological and immunological features in Thailand during this period.

Several earlier reports identified secondary dengue virus infections as a risk factor for severe dengue disease [20, 22, 23]. Our results in this study were consistent with the previous reports. The most interesting results of the present study were that almost all of the DHF caused by DEN-2 and DEN-4 were in secondary dengue virus infections, and one fifth of the DHF cases caused by DEN-1 and DEN-3 were in primary dengue virus infections. These results indicate that DEN-1 and DEN-3 induce DHF in both primary and secondary infections, and suggest that DEN-2 and DEN-4 in Thailand are less likely to cause DHF in primary infections.

The pathogenesis of DHF has been long studied, but as yet has not been fully elucidated. The reason why DEN-2 and DEN-4 can cause DHF only in secondary infection may be that DEN-2 and DEN-4 strains circulating in Thailand need enhancement of infection by enhancing antibodies to cause DHF. On the other hand, a good percentage of DHF cases were caused in primary infections by DEN-1 and DEN-3. Thus, DEN-1 and DEN-3 strains circulating in Thailand can proliferate sufficiently to cause DHF in primary infections. In that sense, DEN-1 and DEN-3 strains are more virulent than DEN-2 and DEN-4 in Thailand. Continued study is needed to further understand the serological and virological features of DF and DHF in Thailand and to elucidate the viral and immunological factors in the pathogenesis of DHF.

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