

TYPING OF DENGUE VIRUSES IN CLINICAL SPECIMENS FROM CENTRAL THAILAND

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Abstract. Typing of dengue viruses was done for clinical specimens from a total of 136 patients (children under 15 years old) suspected of having dengue virus infections and who had been admitted to Pathum Thani Provincial Hospital during the period May 1999 to April 2000. Altogether, 44 strains were isolated (isolation rate: 32.4%), consisting of 18 DEN-1, 18 DEN-2, 7 DEN-3 and 1 DEN-4. The isolation rate decreased according to the number of days after the onset of disease, from day 4 to day 8.

INTRODUCTION

Dengue virus infection is a very old disease (Nimmanitty, 1996; Gubler, 1998a) that had a widespread distribution in the tropics and subtropics, with all four dengue serotypes co-circulating epidemic in the large urban centers of most countries (Igarashi, 1997; Gubler, 1998b; 2002). Dengue fever (DF) is caused by four distinct serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) of dengue virus, which are transmitted to humans by the domestic mosquitos *Aedes aegypti* and *Aedes albopictus*. Dengue hemorrhagic fever (DHF) is one of the leading causes of hospitalization and death, and was known as secondary infection by the other serotypes of dengue virus (Halstead, 1970). During the final years of the 20th century in Thailand, cases of dengue virus infection were reported from almost every province, with high morbidity and mortality each year (Division of Epidemiology, 1991-2001).

Pathum Thani Provincial Hospital is located in one of the endemic areas of central Thailand that has high numbers of DF and DHF cases admitted every year; and the trend of dengue morbidity is increasing. Since this area had a high prevalence of dengue cases and greater distribution of distinct dengue serotypes, indicating a higher risk of developing DHF. It is

therefore interesting to make a serotype study in this area.

MATERIALS AND METHODS

This is a cross-sectional study. Data and blood specimens were collected from a total of 136 patients suspected of dengue virus infection and admitted to Pathum Thani Provincial Hospital, Thailand, during the period May 1999 to April 2000. All were children under 15 years old. Approximately 58.1% of them were in the age group 7-12 years old. The numbers of males and females were similar with a ratio of 1.1:1.

All of the patients had fever. Over half of the samples (55.9%) were collected in the rainy season in Thailand, June to August 1999; 70.6% were diagnosed as DF and DHF cases, that were classified into 4 grades according to the guidelines of the World Health Organization (1997) (Table 1).

Blood specimens were taken by venipuncture within the first day of admission; 72.8% of the blood specimens were collected between 3 to 7 days after onset. Sera were separated from blood specimens and kept at -70°C until used.

Cell culture

Ae. albopictus C6/36 cells (*ie* provided by AFRIMS, US Component, Bangkok, Thailand) were grown for cell line stock in growth medium at 28°C for a few days until the cells became a monolayer.

Growth medium

The clone C6/36 cells were grown in Eagle's minimal essential medium (E-MEM) (Gibco BRL,

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Table 1
Number of patients according to their clinical diagnosis and days after clinical onset before the collection of blood samples.

Clinical diagnosis	Days after clinical onset								Total
	1	2	3	4	5	6	7	8	
DF	0	1	1	1	0	0	0	0	3
DHF grade I	7	9	10	12	7	3	2	0	50
DHF grade II	1	1	5	7	10	2	2	0	28
DHF grade III	0	0	4	1	1	3	0	2	11
DHF grade IV	0	0	1	1	1	0	0	1	4
Others	7	7	4	5	8	5	3	1	40
Total	15	18	25	27	27	13	7	4	136

Grand Island, NY) containing 1% L-glutamine, 1% non-essential amino acid, 200U of penicillin-streptomycin per ml and 10% fetal bovine serum (FBS).

Maintenance medium

The same as growth medium but FBS was reduced to 5%.

Virus isolation

Five hundred microliters of each serum specimen were inoculated to the clone C6/36 in each flask. After absorption, by tilting tissue culture flasks on a thermal rocker (Lab-line) at 28°C for two hours, the clone C6/36 in each flask was covered with 2ml/flask of the maintenance medium. The flasks were incubated at 28°C for seven days, then 1ml of infected maintenance medium fluid from each flask was used for the second passage by inoculation onto the new cell culture in each of the new flasks and absorbed as per the previous procedure. This process was repeated once again in the third passage. After seven days, the infected maintenance medium fluid in each third passage flask was harvested into a small vial and kept at -70°C until used for identification.

Dengue virus serotype identification

The method was performed by cell culture supernatant using a modification of the monoclonal antibody capture ELISA, described by Kuno *et al* (1985).

RESULTS

One hundred and thirty-six patients were enrolled

in this study in one full year. The isolation rate was 32.4%, consisting of 18 DEN-1 (40.9%), 18 DEN-2 (40.9%), 7 DEN-3 (15.9%) and 1 DEN-4 (2.3%), detected from 136 serum specimens of patients suspected of having dengue virus infection (Fig 1).

According to the duration of onset, the isolated cases were highest on the fourth day after onset (increased from the first day to the fourth day, then declined to day 8) (Table 2 and Fig 2).

DISCUSSION

This study found that all four dengue serotypes of DEN-1 and DEN-2 combined were the highest, so there was a high risk of secondary dengue virus infection, by first being infected with DEN-1 or DEN-2, followed

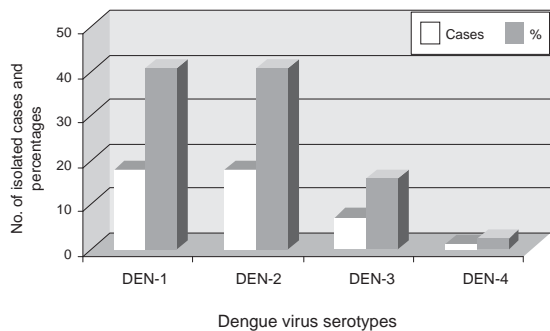


Fig 1- Numbers and percentages of isolated dengue serotypes in the sera.

Table 2

Number of isolated cases and percentages of dengue virus infection according to the days after disease onset.

	Days after clinical onset								Total
	1	2	3	4	5	6	7	8	
Suspected dengue cases	15	18	25	27	27	13	7	4	136
Number of isolated cases	4	6	9	13	8	2	1	1	44
Percentages of isolated cases	9.1	13.6	20.5	29.5	18.2	4.5	2.3	2.3	100

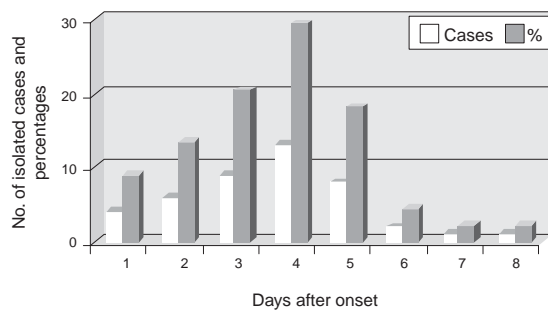


Fig 2- Numbers of isolated cases and percentages of dengue virus infection, according to the days after onset of the disease.

by a secondary infection with DEN-2 or DEN-1.

Thirteen strains of dengue virus were isolated from 27 serum samples (29.5%) on fever day 4, which is the highest, followed by day 3 (20.5%), day 5 (18.2%), day 2 (13.6%), day 1 (9.1%), day 6 (4.2%), and day 7 or >day 7 (2.3%), respectively (Table 2). Dengue viremia should be found most during day 1 to day 7 and be high during day 3 to day 4.

Dengue and dengue hemorrhagic fever (DF and DHF) remain one of the serious public health problems (especially in children) in many tropical and subtropical countries in the world (Rodhain, 1996; Pinheiro and Corber, 1997; Gubler, 1998a; Jacob, 2000; Guzman and Kori 2002). Dengue virus has been isolated from the specimens of patients in many tropical and subtropical areas and were identified during epidemics, as follows: Okuno *et al* (1983), indicated that the virus strains isolated in Osaka District in 1943 were DEN-1; Ha *et al* (1994), studied the epidemiology of DHF in Ho Chi Minh City in Vietnam in the 1987 outbreak and concluded that the predominant type was DEN-2, but in 1990, DEN-1 was continuing to increase; Thanh and Giao (1999) isolated dengue virus during the large outbreaks in South Vietnam and found

30 cases (2 DEN-1, 27 DEN-2 and 1 DEN-3) in 1987, and found 13 cases (7 DEN-1, 5 DEN-2 and 1 DEN-4) in 1990; Qiu *et al* (1993), studied the first epidemic of dengue in China and found the epidemic due to DEN-4 took place in Shiwan Town, Foshan City, Guangdong Province, in 1987, and a local outbreak of dengue due to DEN-1 occurred in Shiqi Town, Zhongshan County, Guangdong Province, in 1979; Chungue *et al* (1992) reported an epidemic of DEN-1 in French Polynesia in 1988-1989; Fagbami *et al* (1995) reported an epidemic of DEN-1 in Fiji in 1989-1990; Caraballo and Hernandez (1991) reported an outbreak of dengue in San Jose of Guaribe, Venezuela, in 1990, caused by DEN-2; Nogueira *et al* (1993) reported a co-circulating epidemic of DEN-1 and DEN-2 in the State of Rio de Janeiro, Brazil, in 1990-1991; Rodier *et al* (1996), reported an epidemic of DEN-2 in the city of Djibouti in 1991-1992; Diet *et al* (1994) used RT-PCR to determine the serotypes of dengue virus isolated in North Vietnam, in 1991-1993, and found 20 cases (10 DEN-1, 9 DEN-2 and 1 DEN-4); Makino *et al* (1994) reported that human sera, since 1993, which had been obtained from Khammuane Provincial Hospital and from Sok Yai Village in Vientiane Municipality, Lao PDR, assayed by NT; which the positive rates to DEN-1, DEN-2 and DEN-3 reached high (over 90%) by 11-15 years old and kept high thereafter; Richards *et al* (1997) reported an outbreak of DHF in Irian Jaya, Indonesia, in 1993-1994 and found 2 DEN-1, 1 DEN-2 and 6 DEN-3; ter Meulen *et al* (2000) isolated dengue virus from 12 patients with DF or DHF in Mindanao, Republic of the Philippines, in 1995, and found 7 DEN-2 and 2 DEN-4; Deparis *et al* (1998) reported specific epidemiological surveillance and confirmed that DEN-2 was circulating in French Polynesia, in 1996; in Thailand, in 1980-1984, Sangkawibha *et al* (1984), studied in Rayong Province by isolating dengue virus from 360 patients, and found 23 DEN-2, 16 DEN-1, 5 DEN-3 and 6 DEN-4; in 1993, Chanyasanha *et al* (1995) isolated and identified dengue virus by RT-PCR,

in 162 patients suspected of dengue virus infection, who were admitted to the Children's Hospital, Bangkok, and found 2 DEN-1, 2 DEN-3 and 9 DEN-4; in 1999, Meesiri *et al* (2001) isolated dengue virus and identified it by RT-PCR in 181 patients in Nakhon Nayok Province in central Thailand, and found 19 DEN-1, 5 DEN-2, 4 DEN-3 and 1 DEN-4; in 1994-1996, Vaughn *et al* (2001) conducted a study at 2 hospitals in Thailand (Children's Hospital and Kamphaeng Phet Provincial Hospital) and isolated dengue virus in 165 patients (46 DEN-1, 47 DEN-2, 47 DEN-3 and 25 DEN-4); the study by Endy *et al* (2000), in Kamphaeng Phet Province, Thailand, found 8 DEN-1, 3 DEN-2 and 39 DEN-3, in 1998, and found 17 DEN-1, 24 DEN-2, 6 DEN-3 and 1 DEN-4 in 1999; from prior studies and this one may indicate that the annual pattern of co-circulating dengue serotypes varies from year to year. DEN-2 was the predominating serotype, followed by DEN-1, while DEN-3 and DEN-4 have appeared during alternate years.

Since the method of isolating dengue virus from the serum specimens of patients with DF or DHF is not easy, has low sensitivity, and consumes a lot of time, the method described here may not suitably contribute to the diagnosis of DF or DHF in many tropical and subtropical areas.

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