

**Identification of Dengue Serotypes in Children with Dengue Infection at Yangon Children's Hospital in 2015**

*Win Kay Khine<sup>1\*</sup>, Theingi Win Myat<sup>1</sup>, Hlaing Myat Thu<sup>1</sup>, Wah Win Htike<sup>2</sup>, Khine Mar Oo<sup>2</sup>, Khin Mar Aye<sup>1</sup>, Mo Mo Win<sup>1</sup>, Ye Myint Kyaw<sup>3</sup> & Kyaw Zin Thant<sup>1</sup>*

<sup>1</sup>Department of Medical Research

<sup>2</sup>University of Medicine 1 (Yangon)

<sup>3</sup>Yangon Children's Hospital

Dengue is the most important arthropod-borne viral infection of humans. All four dengue virus serotypes (DENV1-DENV4) were circulating in Myanmar and DENV-1 was the most frequently isolated one starting in 2001. To identify dengue serotype, a total of 234 paired serum samples were collected from patients with clinically suspected dengue infection attending Medical Wards of Yangon Children's Hospital from January to July 2015. The convalescent serum samples (S2) were tested by Immunochromatographic tests. The proportion of serologically confirmed cases was 76% (178 out of 234). Out of these, 178 seropositive cases, 46(26%) were diagnosed as primary and 132(74%) as secondary dengue infection. Among 46 cases of primary infection, 24(52%) were DHF grade I, 10(22%) were grade II, grade III and grade IV were 7(15%) and 5(11%), respectively. Among 132 cases of secondary infection, 72(55%) were DHF grade I, 24(18%) were grade II, 19(14%) were grade III and 17(13%) were grade IV. DHF GI and DHF GII (mild form of dengue) were the most common in both infections. The corresponding acute phase serum (S1) of the ICT positive (S2) samples with <5 fever days were selected for virus isolation by tissue culture in C6/36 mosquito cell lines and further typed with serotype specific monoclonal antibodies to four dengue viruses by using Indirect Immunofluorescence Assay. Out of 103, dengue virus was isolated from 20 samples accounting for 19% isolation rate. Among isolated dengue viruses, 14(70%) were DENV-1, 2(10%) were DENV-2, 1(5%) was DENV-3 and 3(15%) were found to be of concurrent infections (1 case of DENV-1+DENV-2 and 2 cases of DENV-1+DENV-4). In Myanmar, since 2001 except in 2007, the predominant one was serotype 1. The present study showed that it has not changed. Concurrent infections were also detected in 2015 but they did not associate with severe form of disease.

*Key words:* Serotype, Primary infection, Secondary infection, Dengue

**INTRODUCTION**

Dengue is a mosquito-borne viral disease that has rapidly spread in all regions of WHO in recent years. The global incidence of dengue has grown dramatically in recent decades. About half of the world's population is now at risk. Dengue is found in tropical and sub-tropical climates worldwide, mostly in urban and semi-urban areas. One recent estimate indicates 390 million dengue infections per year, of which 96 million manifest clinically.<sup>1</sup>

Dengue virus is a small single-stranded RNA virus comprising four distinct serotypes (DENV1-DENV4). These closely related serotypes of the dengue virus belong to the genus *Flavivirus* of the family *Flaviviridae*.<sup>2</sup> In National Health Plan (2006-2011), DHF is ranked as seventh priority disease. Epidemic dengue is a major public health problem in Myanmar where

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\*To whom correspondence should be addressed.

Tel: +95-9254019860

E-mail: drwinkaykhine@gmail.com

dengue is a leading cause of hospitalization and death in children.<sup>3</sup>

The majority of dengue virus infections result in self-limiting undifferentiated fever, dengue fever. In some patients, however, infection evolves to severe disease, dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS), characterized by coagulopathy, increased vascular fragility, and loss of fluid due to capillary permeability that may progress to hypovolemic shock and complications.<sup>4</sup>

Morbidity from dengue virus infection can be attributed to any serotype, with some reports indicating that multiple infections with different serotypes may contribute to increased disease severity.<sup>5</sup> Each serotype of dengue virus may have a different genetic component associated with virulence but there has been no solid confirmation. Another possibility is that antibody-dependent enhancement (ADE) may be associated with severity in that primary infection induces insufficient protection and assists entry of virus into cells, resulting in higher peak viral titres.<sup>6</sup>

As Myanmar is one of the countries that reported dengue virus infections, enhancing our knowledge on viral dissemination patterns would be beneficial. Therefore, continuous and comprehensive disease surveillance is essential for identification of dengue virus serotypes because a change in serotype and strain is particularly important to bring about a surge in dengue fever/DHF cases as well as the severity of disease. In this study, clinically suspected dengue infections were confirmed by serological testing, isolation of the dengue virus and detection of isolated viruses by indirect immunofluorescence assay was done for monitoring of circulating dengue serotypes in 2015.

## MATERIALS AND METHODS

This study was a cross-sectional descriptive study carried out from January to July, 2015. After getting informed consent, a total

of 234 paired serum samples were collected from patients with clinically suspected dengue infection attending the medical wards of Yangon Children's Hospital. Acute phase serum sample (S1) was collected as soon as possible after admission and the convalescent (S2) was collected 7 days after the first sample. Serological confirmation was done by Immunochromatographic tests (SD BIOLINE Duo NS1 Ag and IgM/IgG test kit) on S2 samples. The corresponding acute phase serum (S1) of the ICT positive (S2) samples with <5 fever days were selected for virus isolation by tissue culture in C6/36 mosquito cell lines and further typed with serotype specific monoclonal antibodies to the four dengue viruses by using Indirect Immunofluorescence Assay (IFA).

## RESULTS

Among 234 samples, 178 cases (76%) showed seropositive results and 56 cases (24%) showed seronegative results.

### *Percentage of primary and secondary dengue infections among confirmed dengue cases*

In 178 seropositive cases, 132 patients (74%) were classified as having secondary dengue infection and 46(26%) as having primary infection.

### *Monthly distribution of confirmed dengue cases admitted to hospital*

In the period of specimen collection, most DHF cases were found in May, June and July with peak number in June and July. Among the 178 seropositive DHF cases, 7(4%) in January, 4(2%) in February, 3(2%) in March, 5(3%) in April, 34 cases (19%) in May, 64(36%) in June and 61(34%) in July were detected in the present study (Fig. 1).

### *Age distribution of DHF cases*

Out of 178 confirmed dengue cases, 9 cases (5%) were <1 year age group, 46(26%) in 1-4 years age group, 78(44%) in 5-8 years age group, 37(21%) in 9-12 years age group and 8(4%) in 12-14 years age group. In this study, dengue virus infection was most prevalent in 5-8 years age group (Fig. 2).

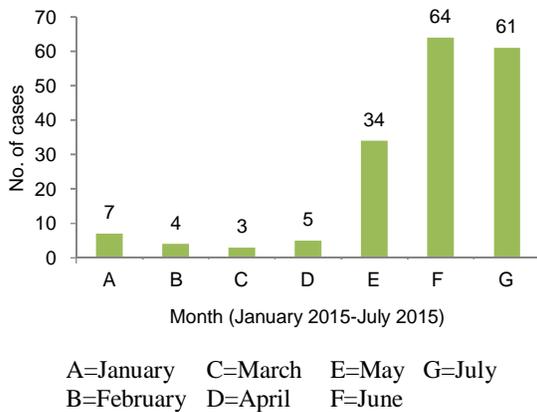


Fig. 1. Monthly distribution of confirmed dengue cases admitted to the hospital

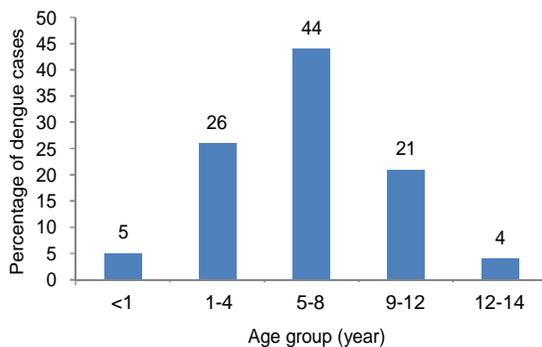


Fig. 2. Age distribution of DHF cases

Table 1. Association between DHF grading and cell culture positive cases

Clinical grading	DHF (%)				Total (%)
	GI	GII	GIII	GIV	
DENV-1	10(50)	1(5)	1(5)	2(10)	14(70)
DENV-2	0(0)	0(0)	2(10)	0(0)	2(10)
DENV-3	1(5)	0(0)	0(0)	0(0)	1(5)
DENV-1+ DENV-2	1(5)	0(0)	0(0)	0(0)	1(5)
DENV-1+ DENV-4	1(5)	1(5)	0(0)	0(0)	2(10)
<b>Total</b>	<b>13(65)</b>	<b>2(10)</b>	<b>3(15)</b>	<b>2(10)</b>	<b>20(100)</b>

#### Association between DHF grading and different serotypes of dengue virus

Among isolated dengue viruses, 13 cases (65%) were clinically diagnosed as DHF GI, 2(10%) as DHF GII, 3(15%) were observed as DHF GIII and 2(10%) as DHF GIV. Out of 13 cases of DHF GI, 10(77%) were found to be caused by DENV-1 and 3 were caused by DENV-3, DENV-1+ DENV-2

and DENV-1+DENV-4 respectively. In two cases of DHF GII patients, one DENV-1 and one concurrent infection of DENV-1+DENV-4 were detected. Among 3 cases of DHF GIII, one was DENV-1 and two were DENV-2. In the 2 cases of DHF GIV, DENV-1 was identified. Therefore, it was found that the predominant serotype was DENV-1 (70%) and it was responsible for 73% of mild dengue infection (11 out of 15 DHF GI and DHF GII).

## DISCUSSION

In this study, the convalescent serum samples from 234 patients with clinically suspected dengue infection who admitted to the Yangon Children's Hospital were tested by SD BIOLINE Dengue Duo NS Ag and IgG/IgM test kit for serological confirmation. Among 234 samples, 178(76%) showed seropositive result and 56(24%) showed seronegative. A study in Bangladesh, showed that 126(62%) were seropositive and 75(38%) were sero-negative.<sup>7</sup> Another study, 727(64%) showed seropositive and 406(36%) showed seronegative.<sup>8</sup>

In seropositive results, 132(74%) were of secondary dengue infection and 46(26%) were primary infection which is consistent with the data from Myanmar.<sup>9</sup> A study done in Karachi showed that 16.74% were primary infection and 83.26% were of secondary infection.<sup>10</sup> The majority of serologically confirmed cases had secondary dengue. So, it has been suggested that if a person infected with one dengue virus type is re-infected with other different serotypes, he or she may develop DSS. For this phenomenon, the postulated pathogenesis is antibody dependent enhancement of viral infection.

Regarding this study, dengue cases were found in every month from January to July with peak occurrence in June and July. It was consistent with the finding of a study in which reported that the dengue cases were on the rise in May, June and July with the peak numbers in June.<sup>11</sup> One study reported

that DHF outbreaks observed mainly in the rainy season (Monsoon).<sup>12</sup> Dengue in Bangkok peaks during the monsoon months of June to October from 1973 to 1999.<sup>13</sup> In tropical countries like Myanmar, although dengue occurred throughout the year, it usually peaks in rainy season. It is because of transmission intensifies during the rainy season. The time of this increased intensity of infected mosquito was coincident with the school opening season in Myanmar so that the children were exposed to the mosquitoes at school during the day time and this might explain the peak occurrences of dengue in these months among school-going children.

In the present study, among the 178 serologically confirmed DHF cases, the most common age groups were detected in 5-8 years old children (44%) followed by 1-4 years old children (26%), then 9-12 years old children (21%). In <1 years and 12-14 years age group showed 5% and 4%, respectively. Similar findings were observed by a study which reported that the highest proportion of dengue infections occurred in 5-8 years old children (43%) followed by 1-4 years old children (27%).<sup>8</sup> In the study done during 1991-1998, the most affected age group was 3-8 years.<sup>14</sup> A study done in 2009 showed the most vulnerable age group was 5-8 years (41%).<sup>11</sup> One study reported that 6-12 years was the most commonly affected age group (45.8%).<sup>15</sup> Age as an important modulator of clinical dengue attack poses a paradoxical problem of an increase in adult patients resulting from a decline in the force of infection, which may be caused by various factors including time-dependent variations in epidemiological, ecological and demographic dynamics.

In this study, virus isolation in C6/36 cell line and identification of serotypes by indirect IFA technique were also performed. Out of 103, the corresponding acute phase serum samples (S1) of the ICT positive (S2) subjected to virus isolation, dengue virus was isolated from 20 samples, accounting for (19.4%) of isolated rate. In a preliminary

study of dengue infection in Brazil from 2004 to 2006, dengue virus was isolated from 78 out of 160 samples (48.75% isolation rate).<sup>16</sup>

In the present study, serotyping of 20 isolated dengue viruses by IFA using monoclonal antibodies showed that isolated dengue serotypes were DENV-1 in 14 cases (70%), DENV-2 in 2(10%), DENV-3 in 1(5%) and concurrent infections (1 case of DENV-1+DENV-2 and 2 cases of DENV-1+DENV-4) in 3 cases (15%).

Among isolated dengue viruses, 13(65%) were clinically diagnosed as DHF GI, 2(10%) as DHF GII, 3(15%) were observed as DHF GIII and 2(10%) as DHF GIV. Out of 13 of DHF GI, 10 cases were serotype 1 and 3 (serotype 1, serotype 1+2, serotype 1+4) were detected. One case of DENV-1 and another case of concurrent infection (serotype 1+4) were observed in DHF GII cases. Among 3 cases of DHF GIII, one was serotype 1 and two were serotype 2. Only 2 cases of DHF GIV were serotype 1. Regarding association of dengue serotypes and DHF grades, 15/20(75%) of virus isolates were from DHF grade I and II cases and 11 isolates (73%) were identified as DENV-1. Therefore, dengue virus serotype 1 (DENV-1) was found to be more commonly presenting with mild form of DHF in this study.

A study in 2009 also reported that the predominant serotype was DENV-1 (84%).<sup>11</sup> A dramatic increase of dengue cases in 2012-2013 was also caused by DENV-1, accounting 94% of all viruses isolated.<sup>8</sup> A study in Saudi Arabia from January 2006 to February 2008 showed that the main isolated serotype was DENV-1(65/70, 92.8%), followed by DENV-3 (4 of 70 positive samples, 5.7%) and DENV-2 (1 of 70 positive samples, 1.4%), DENV-4 was not.<sup>17</sup> A study in Nicaragua reported that DENV-1 was predominated (87% of identified serotypes).<sup>18</sup>

Concurrent infections with more than one dengue viruses were also detected in this study. The first documented case of

concurrent infections with more than one serotype was reported in Puerto Rico in 1982. Since then, several cases have been reported in New Caledonia, Thailand, Somalia, Mexico and China.<sup>19</sup> The reason for dual infection was explained by the unique feeding behavior of *Aedes aegypti* mosquitoes.<sup>20</sup>

However, a study reported that concurrent infection with serotype 1 and 2 in a patient traveled to India and Singapore was not found an association between concurrent infection and disease severity.<sup>20</sup> This is consistent with the present study. Similar finding observed in a study done in Brazil reported that this was the first documented case of simultaneous infection with two dengue serotypes. The clinical picture of this patient was not serious in that study.<sup>19</sup>

### Conclusion

Virological surveillance system is to detect newly introduced virus serotypes and existing dengue serotypes which can be identified. Therefore, studies like this where virological surveillance is done to detect serotype currently prevalent is an important aspect of dengue surveillance especially in a country where paediatric morbidity and mortality due to dengue viruses are high and thus essential to implement effective measures in prevention of DHF outbreak.

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