



Outbreak, Surveillance and Investigation Reports

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Clinical Profile and Circulating Dengue Virus Serotype among Adults Admitted to Yangon General Hospital during the 2015 Dengue Outbreak

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Abstract

During the 2015 dengue season in Myanmar, there was an unusual increase in occurrence of adult dengue cases. To identify circulating serotypes and clinical profiles of adult dengue during the outbreak, blood samples were collected from clinically suspected dengue patients admitted to Yangon General Hospital during July to September 2015. Among 75 samples tested for NS1Ag and immunoglobulins IgG/IgM, 33 (44.0%) were serologically confirmed, including 11 (33.3%) primary and 22 (66.7%) secondary infection. The mean age was 20.8 years (range 13-49 years). There were 77.3% (17/22) of secondary infection and 45.5% (5/11) of primary infection developed into severe types of dengue infection. Bleeding manifestations occurred in 13 (39.4%) patients, with gastrointestinal bleeding as the most common form. Out of the 33 samples serologically confirmed, dengue virus was detected in six (18.2%) and all were serotype 1 which has been the predominant serotype in Myanmar since 2009. These findings contributed information on the recent adult dengue outbreak and aided to bridge the knowledge gap concerning adult dengue in Myanmar. Further molecular research should be conducted on serotype negative samples.

Keywords: dengue outbreak, adult dengue, dengue virus serotypes, Myanmar

Introduction

Dengue is the most important arthropod-borne viral disease of public health significance.¹ In 2012, approximately 390 million dengue infections occurred annually worldwide and about four billion people which was 55% of the world's population were living in 128 dengue endemic countries.² Among dengue infections around the world, nearly two million cases developed into severe dengue hemorrhagic fever (DHF), resulting in 21,000 deaths.³

Although dengue is typically acknowledged to be a childhood disease, there is evidence of a changing epidemiology of the disease among older age groups.⁴ An increasing occurrence of adult dengue infections have been reported from Latin America since the early 1980s and also from Asian countries such as Singapore, Indonesia, Bangladesh and Sri Lanka.⁵⁻⁸

Myanmar is a dengue endemic country as well and epidemic peaks occur every 2-3 years. During 2009-2015, the number of reported dengue cases increased

from 24,285 to 42,913, an increase of 77% over six years. The most common affected age group was 5-9 years (50-60%), and serotypes 2 and 3 were found to be more associated with severe dengue.⁸⁻⁹ As most studies on dengue in Myanmar have focused on children, epidemiological and serotype data concerning adult dengue is relatively rare.¹⁰ A few studies reported an increase in the number of adult dengue infections in 1994, 2007 and 2009, most presenting with bleeding manifestations.¹¹⁻¹²

In July 2015, the admission number of adult dengue cases in Yangon General Hospital was increased when compared to the data from previous months. Identification of the dengue serotypes that caused this outbreak is important and would help to fill the knowledge gap on adult dengue epidemiology. This study was, therefore, conducted, aiming to describe the clinical profile of adults diagnosed with dengue during the 2015 outbreak and identify the circulating dengue serotypes.

Methods

Study Setting

A cross-sectional descriptive study was carried out at Yangon General Hospital.

Selection Criteria

We recruited all patients aged more than 12 years and admitted with any of clinical diagnoses of dengue, namely dengue fever (DF), DHF and dengue shock syndrome (DSS). Patients who were admitted to intensive care unit with severe shock or had fever lasting for more than five days were excluded.

Case Definition

In hospital settings, the dengue case definition of World Health Organization in 1997 was applied for clinical diagnosis and for grading the severity of dengue¹³.

Probable dengue was defined as an acute febrile illness with two or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations and leucopenia. A DF case was a probable case confirmed with supportive serology. A DHF case was defined if the following signs or symptoms were clinically observed: high fever of acute onset, and hemorrhagic manifestations with at least positive tourniquet test and hepatomegaly; plus one of the following laboratory findings: thrombocytopenia ($\leq 100,000$ cells per mm^3) or hemoconcentration (hematocrit $>45\%$).

In addition, DHF was classified into four grades, depending on clinical presentation and severity. DHF Grade I included those with fever accompanied by non-specific constitutional symptoms, positive tourniquet test and/or easy bruising. In Grade II, there was spontaneous bleeding usually in the forms of skin or other hemorrhages. Grade III patients were those exhibited circulatory failure (rapid and weak pulse, and narrowing of pulse pressure $<20\text{mmHg}$), or hypotension (presence of cold and clammy skin, and restlessness). Grade IV was deemed when profound shock occurred with undetectable blood pressure or pulse. Grade III and IV were considered to be DSS.¹³

Sample Collection and Serological Confirmation

Approximately 3 ml of blood was collected from each patient under aseptic conditions on the first day of admission after proper history taking and clinical examination. Blood collection was carried out between Monday and Friday. The blood samples were labeled and transported to the Virology Research Division, Department of Medical Research, Ministry of Health and Sports, Myanmar. The sera were tested

by standard diagnostics BIOLINE Dengue Duo NS1 Ag and IgG/IgM test kit (SD, Korea) on the same day of blood collection. The presence of immunoglobulin M (IgM) line was regarded as primary dengue infection while observation of either immunoglobulin G (IgG) line alone or both IgM and IgG lines were regarded as secondary infection. The positive samples were stored at -20°C until serotype identification was done.

Serotyping

RNA was extracted from the seropositive samples using QIAamp viral RNA extraction columns (Qiagen) as per manufacturer's instruction. The extracted RNA was transcribed to cDNA which was used for reverse transcription polymerase chain reaction (RT-PCR) with sense primer Seah DV1 and anti-sense primer (DSP1, DSP2, DSP3 and DSP4). PCR products were then checked for specific target by gel electrophoresis.¹⁴

Ethical Issue

Written informed consent was obtained from each study participant. The study was approved by the Ethics Review Committee, Department of Medical Research.

Data Analysis

For descriptive analyses, frequencies and percentages were used for categorical variables, and means and standard deviations for continuous variables. Chi-square tests were used to determine statistically significant differences between groups and p-value of less than 0.05 was considered as significant.

Results

The clinically suspected dengue cases admitted to Yangon General Hospital mostly in July and August 2015 (Figure 1). A total of 75 suspected dengue patients (30 in July, 30 in August and 15 in September) were enrolled in the study. Among the participants, 33 (44.0%) were serologically confirmed to have dengue infection, including one (3.0%) DF case and 32 (97.0%) DHF cases.

Of 33 confirmed cases, there were 11 (33.3%) primary and 22 (66.7%) secondary dengue infections. The mean age was 20.8 years (standard deviation 7.6 years, range 13-49 years). Majority (85.0%) of the cases are 15-24 years old (Figure 2). There were 23 males and 10 females, with a male to female ratio of 2.3:1.

All cases presented with fever; 11 (33%) and 22 (67%) cases were admitted on onset days 1-3 and days 4-5 of fever respectively. Most common presentations were tourniquet test positive (84.8%) and headache (60.6%).

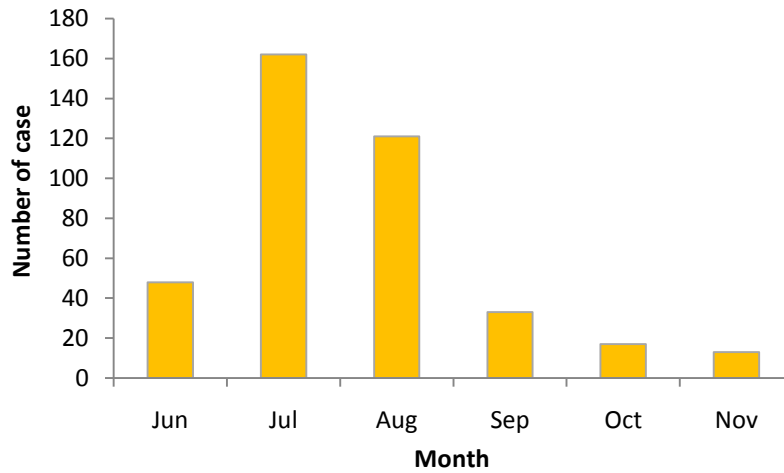


Figure 1. Clinically diagnosed dengue cases admitted to Yangon General Hospital, Myanmar, June to November 2015

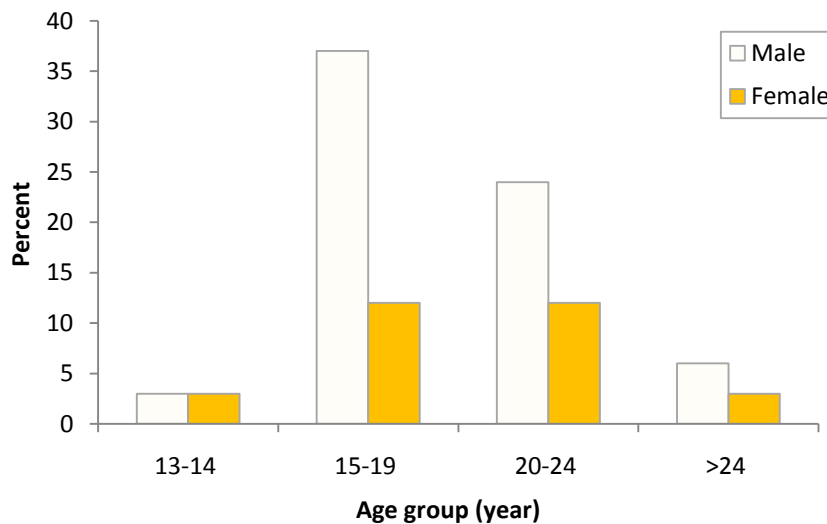


Figure 2. Age and gender distribution of adult dengue cases admitted to Yangon General Hospital, Myanmar, July to September 2015 (n=33)

Bleeding manifestations in one or more forms occurred in 13 (39.4%) patients. Thrombocytopenia (platelet count $<100,000/\text{mm}^3$) was found in 24 (72.7%) and mean platelet count was $61 \times 10^9/\text{l}$. Regarding to platelet counts, 13 (54%) were $50-100 \times 10^9/\text{l}$, 10 (42%) were $20-50 \times 10^9/\text{l}$, and one (4%) was less than $20 \times 10^9/\text{l}$. Hemoconcentration was present in 15 (45.5%) patients. Among 13 DHF cases that presented with bleeding manifestations, there were thrombocytopenia in nine (69.2%) and hemoconcentration in seven (53.8%) patients.

Out of 11 primary dengue infections, 1 (9.1%) developed into DF 5 (45.5%) while five (45.5%) each were progressed to DHF I and DHF II (Figure 3). About 77.3% (17/22) of secondary infection and 45.5% (5/11) of primary infection developed into severe dengue. There was no significant association between severity of dengue infection and type of infection (primary or secondary).

Table 1. Clinical manifestation and laboratory results of serologically confirmed adult dengue cases in Yangon General Hospital, Myanmar, July to September 2015 (n=33)

Clinical manifestation	Number (%)
Fever	33 (100)
Tourniquet test positive	28 (84.8)
Headache	20 (60.6)
Vomiting	12 (36.4)
Skin rash	11 (33.3)
Drowsiness	10 (30.3)
Muscle and joint pain	9 (27.3)
Abdominal pain	8 (24.2)
Hematemesis	8 (24.2)
Melena	6 (18.2)
Epistaxis	4 (12.1)
Bleeding gum	2 (6.1)
Hemoptysis	1 (3.0)
Hepatomegaly	12 (36.4)
Platelet count $<100,000/\text{mm}^3$	24 (72.7)
Hematocrit $>45\%$	15 (45.5)

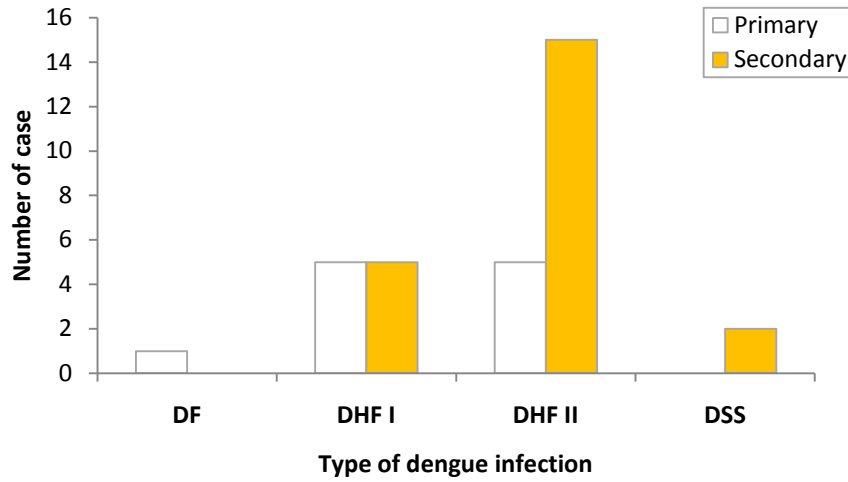
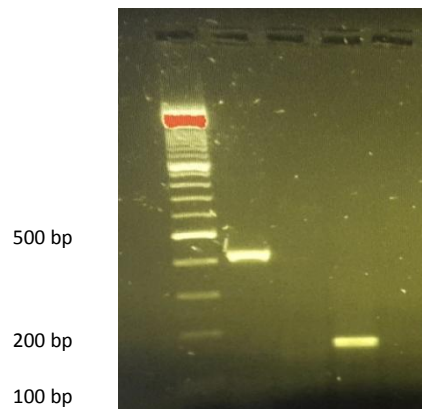


Figure 3. Distribution of primary and secondary dengue infections among dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) cases in Yangon General Hospital, Myanmar, July to September 2015 (n=33)

Among 33 samples analyzed for dengue serotype by RT-PCR, dengue antigen was detected in six (18.2%) samples and all were of serotype 1 (DENV 1) (Figure 4). The characteristics of PCR positive cases are shown in Table (2).

Discussion

The year 2015 was characterized by large dengue outbreaks worldwide with 3.2 million reported cases globally.¹⁵ According to reports from the Vector Borne Disease Control Programme, Ministry of Health and Sports, Myanmar, the number of reported dengue cases more than doubled from 20,255 cases in 2013 to 42,913 cases in 2015 while the affected rate among those aged more than 15 years increased from 2.5% to 5.0%. In Yangon General Hospital, total 392 suspected dengue cases were admitted during June to November 2015, with peaks in July and August, the period of the rainy season and the months when dengue is known to be in highest transmission.¹¹



Lane 1 100 bp DNA ladder
 Lane 2 Positive control (DENV4 - 426 bp)
 Lane 3 Sample (Serotype not detected)
 Lane 4 Sample (DENV1 - 169 bp)
 Lane 5 Negative control

Figure 4. Gel image of polymerase chain reaction products, showing DENV 1 among adult dengue cases admitted to Yangon General Hospital, Myanmar, July to September 2015

Table 2. Information of adult dengue cases admitted to Yangon General Hospital, Myanmar, July to September 2015

ID	Age (year)	Gender	Diagnosis	Type of infection	Serotype	Hemorrhagic manifestation		Platelet count (per mm ³)	Hematocrit (%)/ Evidence of plasma leakage
						Tourniquet test	Symptom		
AD 1	18	M	DHF I	Primary	DENV 1	Positive	-	78,000	53.3%
AD 2	16	M	DHF I	Primary	DENV 1	Positive	-	87,000	41.7%
AD 4	18	F	DHF II	Secondary	DENV 1	Positive	Hematamesis	98,000	44% Hepatomegaly
AD 5	14	F	DHF II	Primary	DENV 1	Positive	Epistaxis, melena	64,000	39.2%
AD 6	15	F	DHF II	Primary	DENV 1	Positive	Hematamesis	100,000	43%
AD 20	24	M	DF	Primary	DENV 1	Negative	-	171,000	43.2%

Based on serology, secondary infections accounted for nearly two-thirds of all cases. In dengue endemic countries like Myanmar where all four dengue serotypes are co-circulating and the vector *Aedes aegypti* are abundant year round, most of the population might have been infected at least once by a dengue virus in their childhood. A higher proportion of secondary dengue infection in adults had also been reported from other dengue endemic countries such as Thailand and Sri Lanka.^{8,16}

In this study, the age of confirmed dengue cases ranged from 13-49 years and the majority (85%) was in 15-24 year age group. However, another study from Yangon General Hospital between 2000 and 2008¹² and one from Pyin Oo Lwin in 2009¹⁷ revealed lower attack rates in 15-24 year age group. Therefore, this study indicated that an increasing number of dengue infection occurred in economically productive young adults, a fact that might have significant adverse financial effects on the community. A previous study from 12 countries in Southeast Asia, using available data from 2001-2010, showed an aggregate annual economic burden of dengue reaching USD 950 million among the studied nations, with approximately 52% of these costs coming from productivity loss.¹⁸

Fever, headache, vomiting and skin rash were the most common presentations found in this study with hematamesis (24.2%) and melena (18.2%). Similarly, the study from Pyin Oo Lwin revealed that fever and vomiting were the most common clinical presentations in adults while hematamesis and melena was found in 33% and 41% of patients respectively.¹² Therefore, physicians should be aware that gastrointestinal bleeding may be a common hemorrhagic manifestation in adults diagnosed with dengue. Thrombocytopenia is not an uncommon presentation among adults hospitalized with dengue infection. Our thrombocytopenia of 72.7% was comparable to a study in Sri Lanka which reported a rate of 79%.⁸

Evidence of an association between sequential dengue infection and increased risk of more serious disease has long been reported.¹⁹ In this study, 77.3% of secondary infection developed severe dengue types of infection, DHF II and DSS, while only 45.5% of severe dengue was observed among primary infection. Moreover, 97.0% of cases in this study were DHF and all DSS cases were also secondary infection.

DENV-1 has been the predominant serotype in Myanmar since 2009 although the other serotypes have been identified,²⁰ which was also predominant during recent years in other countries such as Thailand, Nepal and Singapore.²¹⁻²³ Intensive

virological surveillance should be continued to detect changes of serotypes.

Limitations

The findings of this study might not be fully representative of the current adult dengue outbreak due to small number of cases enrolled. Analysis of the association between severity and serotype was not possible as only six samples were identified as being DENV-1. Additional molecular analysis of PCR negative samples was recommended to ensure that these were truly negative as different primer sequences and thermal cycling conditions or further nucleotide sequencing methods could detect similar nucleotide sequence of the target virus.

Acknowledgements

We would like to thank Director General and Board of Directors from the Department of Medical Research for their permission to conduct this study. We also appreciate the Medical Superintendent and staff of Yangon General Hospital for their kind permission to review the data and recruit the patients, and Dr. John Aaskov from the Queensland University of Technology for providing dengue PCR primers.

Suggested Citation

Myat TW, Thu HM, Win HM, Than KS, Tun ZT, Aye KM, et al. Clinical profile and circulating dengue virus serotype among adults admitted to Yangon General Hospital during the 2015 dengue outbreak. OSIR. 2017 Jun;10(2):8-13.

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