

**Determination of dengue antibody among the children
admitted to Mandalay Children Hospital**

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Myanmar belongs to the disease endemic countries (DEC) of dengue virus infection. In order to explore the dengue antibody status among the children, a hospital-based study was first introduced in Mandalay in the years 2006 and 2007 by the Virology Research Division, Department of Medical Research (Upper Myanmar) jointly with the Mandalay Children Hospital. Blood samples were collected from the patients who gave informed consent, and were sent to the Department of Medical Research (Upper Myanmar) for the virological studies. A total of 570 blood samples were obtained during dengue seasons of two consecutive years (from May 2006 to August 2007) from 484 clinically-diagnosed cases of dengue haemorrhagic fever (DHF), comprising 484 acute phase samples (S1) and 86 convalescent phase samples (S2). Paired sera were obtained from 86 patients (17.7% of patients from whom S1 was taken). A panel of samples (182 in number) was tested by PanBio Dengue Duo IgM and IgG ELISA tests. Among the tested samples, 153 samples showed positive result for dengue antibody (either IgM alone or both IgM and IgG) which in turn indicated 84% laboratory-confirmed cases of dengue infection. This study also paves the way to explore more about the seroepidemiology and molecular epidemiology of dengue viruses circulating in Mandalay and Upper Myanmar.

INTRODUCTION

Dengue is a continuing and growing global health issue especially in the tropical countries. Dengue virus infection is endemic in many parts of Asia, the Pacific and the Americas [1]. A significant proportion of the world dengue cases comes from South East Asia [over 38,000 cases in Indonesia in 2005 and up to 15,000 cases of dengue haemorrhagic fever (DHF) reported each year in Myanmar].

About 2.5 billion people are currently living in areas of risk and it is expected that this number will increase as transmission spreads to neighboring geographical regions. Symptomatic dengue infection is one of the leading

causes of hospitalization among children in endemic areas. Each year, estimated 100 million cases of dengue fever (DF) occur, and between 250,000 and 500,000 cases of DHF are reported to the WHO. The severe end of the disease spectrum, dengue shock syndrome (DSS), is associated with significant mortality with case fatality rates of between 1 and 5% [2]. As many as 2,000 patients with dengue virus infection are admitted to Mandalay Children Hospital (MCH) each year with very few deaths [3].

The first major epidemic of DHF occurred in Myanmar in 1970. Nowadays, DHF occurred throughout the country except in Chin State. Almost 80% of cases are reported from three

divisions (Yangon, Bago and Mandalay) and one state (Mon), with over 50% of cases recorded from Yangon Division only [4].

Major epidemics occurred in 1975, 1987, 1990, 1991, 1994, 1998, 2001, 2002, 2005 and 2007. The disease is spreading in rural areas, and morbidity trend is going up. Although mortality has declined, case fatality rate is still high. An extensive review on Dengue/DHF prevention and control programme in Myanmar has been conducted in June, 2003 by an International External Review Team. They recommended further improvement in disease surveillance, case management, laboratory services to support surveillance and case management, and multi-sectoral and community participation in dengue prevention and control.

In this connection, this study has been designed to collect the baseline data on dengue, and to explore the dengue antibody status among the children admitted to Mandalay Children Hospital who are diagnosed clinically as dengue virus infection. Mandalay Division is one of the divisions with the highest DHF endemicity in Myanmar, but only a few number of dengue serosurveys have been conducted in this area. Therefore, this study was conducted to establish the serological surveillance system for dengue/DHF in Mandalay Children Hospital, being the largest paediatric care centre of Upper Myanmar. It also served as the first serosurveillance on dengue/DHF in the Mandalay City area.

MATERIALS AND METHODS

Study design

Hospital and laboratory-based, cross-sectional descriptive study

Places of study

Mandalay Children Hospital, and Virology Research Division, DMR (Upper Myanmar)

Duration of study

March 2006-September 2007, covering the dengue seasons of two consecutive years

Subjects

Children admitted to Mandalay Children Hospital, for the complaint of fever of unknown origin, and being diagnosed as dengue virus infection according to the WHO criteria [5].

Inclusion criteria

- Children of both sexes
- Those presenting with an acute febrile illness with two or more of the following manifestations: rash, haemorrhagic manifestations, leukopenia, thrombocytopenia, evidence of plasma leakage, headache, retro-orbital pain, myalgia, arthralgia
- Whose parents/guardians give their written informed consent to participate their children in the study

Exclusion criteria

- Children with fever of known origin (apart from viral aetiology)

Procedures

Children with the complaint of fever of unknown origin who were admitted to the Paediatric Wards I, II and III of Mandalay Children Hospital (MCH) were clinically screened out to diagnose dengue virus infection according to the WHO criteria.

Those who met the inclusion criteria were enrolled into the study after explaining the nature of the study and procedures to parents/guardians and getting the written informed consents. Blood 3 ml was withdrawn from each child, and clinical data were obtained.

Sera were temporarily stored at 4°C for few hours in MCH, and subsequently transferred to the Virology Research Division of the Department of Medical Research (Upper Myanmar) situated in PyinOoLwin. These sera were separated by low-speed centrifugation, and stored at -20°C until further procedures. The second blood sample (convalescent sample) of the same volume was taken from the child, two weeks after the first sample, during the follow-up visit. A

panel of sera was tested for the presence of dengue antibodies (both IgM and IgG) by ELISA-based detection system, using the commercially available Dengue Duo IgM and IgG ELISA test kits (PanBio Pty Ltd, Brisbane, Australia), according to the manufacturer's instructions.

RESULTS

A total of 570 blood samples were obtained during dengue seasons of two consecutive years (from May 2006 to August 2007) from 484 clinically-diagnosed cases of DHF, comprising 484 acute phase samples (S1) and 86 convalescent phase samples (S2). Paired sera were obtained from 86 patients (17.7% of patients from whom S1 was taken). A panel of samples (182 in number) was tested by PanBio Dengue Duo IgM and IgG ELISA tests. Among the tested samples, 153 samples showed positive result for dengue antibody (either IgM alone or both IgM and IgG) which in turn indicated 84% laboratory-confirmed cases of dengue infection. Among 182 clinically-diagnosed dengue samples, 153 (84%) samples were found to be positive by dengue IgM ELISA, out of which 119 samples were also positive for dengue IgG capture ELISA. Four samples revealed IgM negative but IgG positive. Fifteen samples were negative for both IgM and IgG. Another 10 samples revealed equivocal results for either IgM or IgG.

Table 1 shows the clinical manifestations of 137 serologically-confirmed dengue cases in Mandalay Children Hospital. Fever was encountered in all patients. Other common features among the cases were hepatomegaly (98.54%), positive tourniquet test (84.84%), reduced blood pressure (76.64%), increased pulse rate (72.99%), vomiting (51.82%), bleeding (32.85%), increased packed cell volume (25.54%), haematemesis and malaena (24.82%), epistaxis (18.25%), thrombocytopenia (16.06%), skin rash (3.78%) and lymphadenopathy (mainly axillary glands) (2.27%). This scenario reflected the real image of dengue patients seeking medical treatment at the hospital as in-patients.

Table 1. Clinical manifestations of 137 serologically-confirmed DHF cases in Mandalay Children Hospital

Clinical manifestation	Cases	Percentage
Fever	137	100
Hepatomegaly	135	98.54
Tourniquet test positive	112	84.84
Hypotension	105	76.64
PR >115/min	100	72.99
Vomiting	71	51.82
Bleeding	45	32.85
PCV >42%	35	25.54
Haematemesis & malaena	34	24.82
Epistaxis	25	18.25
Thrombocytopenia	22	16.06
Skin rash	5	3.78
Lymphadenopathy	3	2.27

Fig. 1 reveals the days of fever on admission of dengue patients to Mandalay Children Hospital. Most of the patients (66 in number) were admitted on day 4 of fever.

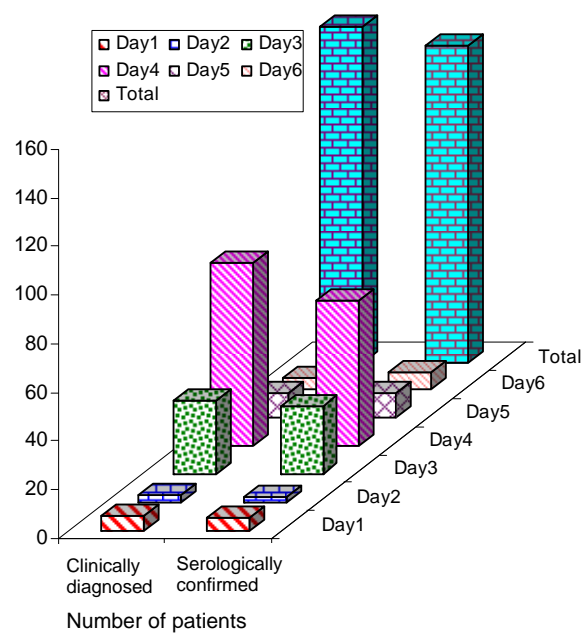


Fig 1. Days of fever on admission of dengue patients

According to the MCH Hospital Registry, there were total dengue admissions of 427 cases in year 1999, 130 cases in 2000, 1183 cases in 2001, 2118 cases in 2002, 326 cases in 2003, 406 cases in 2004, 2431 cases in 2005, 1764 cases in 2006, and 854 cases in 2007, respectively. Table 2 shows the dengue admissions to MCH from year 2002

to 2007, according to age groups, as well as the mortality and case fatality rate (CFR).

Table 2. Dengue admissions to Mandalay Children Hospital (2002-2007), according to age groups with mortality and case fatality rate (CFR)

Year	<1 Year	1-5 Years	>5 Years	Total	Death	CFR
2002	112	862	1144	2118	5	0.23%
2003	12	163	151	326	2	0.61%
2004	9	149	248	406	3	0.73%
2005	64	854	1513	2431	5	0.20%
2006	91	761	912	1764	20	1.13%
2007	21	355	478	854	5	0.58%

Table 3 shows the dengue admissions to MCH from year 2002 to 2007, according to disease severities (DHF grade I and II, and DSS).

Table 3. Dengue admissions to Mandalay Children Hospital (2002 to 2007), according to disease severities

Year	DHF G I & II	DSS	Total
2002	1947	171	2118
2003	249	77	326
2004	291	115	406
2005	1598	833	2431
2006	1267	497	1764
2007	660	194	854

There were 317 DHF grade I & II cases and 167 DSS (DHF grade III & IV) cases among 484 clinically-diagnosed dengue cases from whom the blood samples were collected. Twenty-two children were under 1 year of age (12 males and 10 females), 202 children were between 1 and 5 years of age (104 males and 98 females), and 260 children were above 5 years of age (113 males and 147 females), respectively. The youngest child was 5 months old, and the eldest was 12 years old.

Although the MCH was established in 1998, laboratory confirmation of the admitted dengue cases to MCH was made possible only in the year 2006 due to the sero-surveillance activities conducted by the Virology Research Division, Department of Medical Research (Upper Myanmar) with

the collaboration of the paediatricians and hospital staffs.

Total number of dengue admissions in year 2006 was 1764 patients (852 male and 912 female) with the peak of 383 patients in the month of August. The number of monthly admissions were 29 patients in January, 9 in February, 12 in March, 24 in April, 53 in May, 206 in June, 320 in July, 383 in August, 322 in September, 240 in October, 137 in November and 29 in December, respectively. There was a marked reduction in number of dengue admission in 2007 with a total of 854 patients. The patients of dengue admissions were 2 in January, 2 in March, 5 in April, 13 in May, 39 in June, 162 in July, 171 in August, 131 in September, 197 in October, 98 in November and 34 in December, respectively. No dengue admission in February 2007 was noted.

DISCUSSION

Although this study was entitled as "Determination of dengue antibody among the children admitted to Mandalay Children Hospital", this is, in fact, the first ever sero-epidemiological study on dengue virus infection which was conducted in Mandalay and Upper Myanmar in the hospital-based setting. Previous literatures on dengue virus studies in the areas other than Yangon city were mainly concerned with the outbreak investigations. The first serosurveillance study on dengue viruses outside Yangon city was conducted in Pyinmana, Central Myanmar, in the year 2004 for the whole year [6].

Present study provides the laboratory proven (evidence-based) baseline data on dengue virus infection prevailing in Mandalay and Upper Myanmar. In addition, by studying the molecular epidemiology of the new dengue virus strains isolated from this particular area in Myanmar, it can elucidate the changing epidemiology of dengue virus infection in Myanmar. It can also predict the future dengue outbreaks with its prevailing

serotype. This kind of genetic information may also be helpful in predicting the direction(s) of dengue epidemics not only in Myanmar but also in the neighboring countries at the regional level.

The periodicity and frequency of the dengue epidemics, in other words, the interval between two epidemics can also be estimated. With regards to the dengue epidemics in Mandalay, recent data showed that large-scale epidemics occurred for two consecutive years, e.g. in the years 2001 and 2002, as well as in years 2005 and 2006. This scenario of “coupling epidemics” or “sequential epidemics” warrants the immediate needs of interrupting dengue transmission cycle between the vector mosquitoes and the host men.

It is noted that small dengue outbreaks occurred in Sagaing Division in year 2006, particularly in Wet Let Township and Shwe-Bo Township, and a considerable number of dengue patients from these areas came to MCH for the necessary treatment. So also, pockets of cases were reported in PyinOo-Lwin Township in the year 2007.

With regard to the gender issue, the male to female ratio in Mandalay study in 2006 was 1:1.07 (852 vs 912). There was no significant difference. More or less similar finding was observed in the 1998 dengue outbreak in Taunggyi as the male to female ratio was 1:0.9 [9], also in the 2004 dengue outbreak in Pyinmana was 1:1.09 [6]. Dengue admissions to Mandalay Children Hospital under 1 year age group were 5.3% and 5.1%, 1 to 5 years age group were 40.7% and 43 %, above 5 years age group were 54% and 51.9% in 2002 and 2006, respectively. Therefore, no significant difference was found in all age groups. However, the admission data for above 5 years age group in Lashio in 1994, Mawlamyaing in 2001 and Pyinmana in 2004 shifted to the right during the respective dengue seasons [6, 7, 8, 10].

Concerning the disease severity among admitted dengue patients, total number of DHF grade I and II patients against DSS was

1947 vs 171 for the year 2002, 1598 vs 833 for the year 2005 and 1267 vs 497 for 2006. Hence, the pattern of disease severity directed towards the more severe forms in recent years. Based upon the above mentioned data, we can not underestimate the possibility of huge dengue outbreak in Mandalay area in the near future. The effects of increased urbanization, population migratory movement and water supply system may definitely play as key factors in future episodes.

In connection with the antibody study, a panel of serum samples was brought to the Institute of Tropical Medicine, Nagasaki University, Japan, and was undergone the molecular studies there. Dengue virus isolation attempts were made by inoculating the sera into C6/36 mosquito cell lines. Infected culture fluids were subjected to the dengue antigen ELISA tests, as well as the RT-PCR tests to detect the dengue virus genome. Among the tested samples, three new dengue virus strains from Upper Myanmar were successfully isolated for the first time. These strains included one strain of dengue serotype 3 and two strains of dengue serotype 4 [11]. Therefore, this study also paves the way to explore more about the seroepidemiology and molecular epidemiology of dengue viruses circulating in Mandalay and Upper Myanmar.

ACKNOWLEDGEMENT

The authors would like to acknowledge WHO-SEARO for the provision of funds for sample collection and for the purchase of ELISA test kits, through its APW activities. We would like to express our sincere thanks to Dr. Thein Tun, Director-General, DMR (Upper Myanmar), for his encouragement throughout the study period, and Dr. Myint Myint Thein, Medical Superintendent, for her kind permission to conduct this study in Mandalay Children Hospital. We wish to express our gratitude to Dr. Kyaw Moe, Director, and Dr. Hlaing Myat Thu, Deputy Director/Head, for their

kind permission to use the facilities of the Virology Research Division, DMR (Lower Myanmar). Thanks also go to Dr. Khin Maung Htun, Mandalay Divisional Health Director, Dr. Than Win, Deputy Director/Project Manager, Vector Borne Diseases Control (VBDC) Programme, Department of Health, and Dr. Thar Tun Kyaw, Mandalay Division and Northern Shan State VBDC Team Leader, for the provision of necessary data and encouragement.

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