Original Article

Dengue in Malaysia: An epidemiological perspective study

Muhammad Hasyim Chew[1], Md. Mostafizur Rahman[2], Sharifah Azura Salleh[3]

ABSTRACT

Objectives: Dengue is a mosquito-transmitted infection and is an important health problem in Asia for its fatality. The objective of the study was to determine some epidemiological parameters relating to age, gender, community, and prevalence nature, serology and disease severity.

Methodology: One hundred forty nine dengue suspected sera samples were obtained from suspected patients presenting with dengue symptoms. The samples collected were analyzed by serological detection of Immunoglobulin-M (IgM), Immunoglobulin G (IgG), virus culture in cell-line C36/36 and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).

Results: The studies showed that out of 149 sera samples, 48 (32.2%) were detected dengue virus by cell culture and RT-PCR and 108 (72.5%) positive by serology. Of the 108 cases, 40 (26.8%) were positive to IgM, 61 (41%) were positive to both IgG and IgM, and 7(4.7%) cases were positive to IgG only. Prevalence of dengue was higher in age group 20-29 years and infection was more in male and in Malay population. Epidemic intensity was the highest in the month of June.

Conclusions: Dengue is highly endemic in Malaysia and age group 20-29 was vulnerable to infection, male infected more than female and infection was higher in Malay community. Effective preventive and control measures may be strengthened to reduce the infections.

KEY WORDS: Dengue virus, Serology IgM, IgG, Epidemology, RT-PCR.

Pak J Med Sci July - September 2012 Vol. 28 No. 4 643-647

How to cite this article:

Chew MH, Rahman Md.M, Salleh SA. Dengue in Malaysia: An epidemiological perspective study. Pak J Med Sci 2012;28(4):643-647

1. Muhammad Hasyim Chew,
Public Health Laboratory Kota Kinabalu,
Ministry of Health,
Kota Kinabalu, 88850,
Sabah, Malaysia.
2. Md. Mostafizur Rahman,
3. Sharifah Azura Salleh,
2, 3: Department of Medical Microbiology & Immunology,
Faculty of Medicine,
The National University of Malaysia,
Cheras, 56000 Kuala Lumpur, Malaysia.

Correspondence:

Dr. Md. Mostafizur Rahman,
Professor, Department of Medical Microbiology & Immunology,
Faculty of Medicine,
The National University of Malaysia,
Cheras, 56000 Kuala Lumpur, Malaysia.
E-mail: mostabau@yahoo.com
mmr@ppukm.ukm.my

* Received for Publication: February 13, 2012
* 1st Revision Received: March 19, 2012
* 2nd Revision Received: May 30, 2012
* Final Revision Accepted: May 31, 2012

INTRODUCTION

Dengue (DEN) is a virus under the family Flavirividae, consists of four serotypes: DEN-1, DEN-2, DEN-3 and DEN-4. All the serotypes are able to infect humans and develop dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[1] Infecting with one serotype does not confer immunity to the other serotype but instead may lead to serious disease, such as DHF / DSS due to immune-pathological Antibody-Dependent Enhancement (ADE).[2] World Health Organization (WHO, 2009) estimates there may be 50 million dengue infections worldwide every year.

Until now 1,020,333 cases reported from Malaysia, Cambodia, Philippines, and Viet Nam and the highest numbers of cases and deaths reported from four countries in the Western Pacific Region

during 2001 and 2008.[3] In Malaysia dengue virus vector *A. aegypti* is found in urban areas where as *Ae. albopctus* is more in peri-urban setting. *A. aegypti* is commonly associated with DF / DHF in urban areas, however, there is no convincing evidence of the association of *A. albopictus* with severe dengue.[4] DF and DHF has become a major health problem in Malaysia as it recorded the several outbreaks before.[5]

Previously numerous DF and DHF outbreaks were observed in Malaysia.[6-8] Malaysia experienced the worst DF / DHF outbreaks in the year 1982. Fang et al, 1984 reported a total of 3,005 cases and 35 deaths of DF/ DHF and majority of cases occurred between July to October. The epidemiological pattern of dengue has been changing in Malaysia as a result the number of cases increased dramatically from year 1988 to 1998. In a comprehensive report of the Ministry of Health Malaysia (1998, 1999), mentioned that 26,240 cases of dengue fever and 1,141 cases of dengue hemorrhagic fever at the time of 1998 outbreak. To date, incidence rate has increased to 63.6 per 100 000 population.[9] Extensive development and urbanization favored higher incidence of dengue viral infections due to the creation of more breeding areas of mosquitoes. Kobayashi et al[10] reported based on sequence analysis that dengue virus infection caused in Malaysia during 1993-1994 was due to the introduction of DEN- 3 which was previously endemic in Thailand.

The present study was aimed to determine dengue infection in different ages, genders, communities, and months and disease severity in the reported cases of University Kebangsaan Malaysia Medical Centre (UKMMC), Kuala Lumpur, Malaysia.

METHODOLOGY

Study area and population: The present study was performed at UKMMC, Kuala Lumpur from June to November 2011. The patients presented with the symptoms of dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) on the basis of Ministry of Heath (MOH), Malaysia clinical case definition[11] were selected for this study.

Ethical consent: The study was agreed and funded by Research Committee of the University Kebangsaan Malaysia Medical Centre.

Sample collection: A total 149 blood samples were collected from different age groups of patients reported to UKMMC. Age, gender, communities, time and disease severity were recorded. A 3-5ml of venous blood was drawn from each patient presenting with dengue symptoms. Blood was allowed to clot for 1hr at room temperature, centrifuged at 3500 rpm for 5 minutes, and then serum collected and stored at -21°C until further use.

Serology assay: Dengue antibodies were analyzed by SD BIOLINE Dengue IgM and IgG Rapid test commercial kit (SD, Korean). Manufacturer's method was followed in the test protocol and result was interpreted according to change of color as observed by naked eye. The kits detected IgM, IgG and antibodies of four serotypes of dengue virus.

Virus Culture: Cell culture was performed as per the procedure of Chew et al[12], briefly cell-line C6/36 clone of *Aedes Albopictus* cells were grown in Roswell Park Memorial Institute (RPMI) 1641 media 16 (Gibco, USA) with heat-inactivated 10%fetal calf serum (Gibco, USA) in 25 cm2 cell culture flask. After 10 days of incubation, the infected cells were harvested, pelleted by centrifugation and resuspended in 1ml of phosphate-buffer saline (PBS), pH 7.2. An amount of 10ul of cell suspension was added to Teflon coated slide for preparing antigen detection. Then, the slides were dried on hotplate at 45°C, kept to fix in cold acetone (-20°C) for 15 minutes, heat dried and stained immediately or stored at -81° until use.

Virus Identification: Indirect immunoflourescene assay was performed using serotype specific polyclonal and monoclonal antibodies to identify DENV serotype as reported by Chew et al.[12] Briefly, slides were prepared using 10ul of infected cell suspension from each tube for viral antigen detection. The slides were incubated in humidified chamber at 37°C for 30 min. Initially the slides were screened by group specific flavivirus antibodies, later on tested by commercially monoclonal mouse anti- dengue type 1 (Hawaiian), mouse anti dengue type 2 (New

Table-I: Primer sequence generated by C-prM

No	*Primer*	*Primer Sequence*	*Specifity*	*Serotype*	*Primer set*	*Product size (bp)*
1	mD1	TCAATATGCTGAAACGCGAGAGAAACCG	DENV (all)			
2	D2	TTGCACCAACAGTCAATGTCTTCAGGTTC	DENV (all)		mD1-D2	511
3	rTS1	CCCGTAACACTTTGATCGCT	DENV-1	DENV-1	mD1-rTS1	208
4	mTS2	CGCCACAAGGGCCATGAACAGTTT	DENV-2	DENV-2	mD1-mTS2	119
5	TS3	TAACATCATCATGAGACAGAGC	DENV-3	DENV-3	mD1-TS3	288
6	rTS4	TTCTCCCGTTCAGGATGTTC	DENV-4	DENV-4	mD1-rTS4	260

Guinea C), mouse dengue type 3 (H87) and mouse anti -dengue type 4 (H241) from Milipore, USA. Subsequently the slides were washed twice with PBS, pH 7.2, and then incubated with conjugated flourescein-labeled goat anti-mouse IgG at at 37°C for 30 minutes. Slides were rinsed twice in PBS and allowed to dry on the warm plate. Then slides were mounted with aqueous mounting medium (glycerol saline, pH 8.5, Milipore, USA) and covered with cover slip. Slides were then observed under fluorescent microscope.

Viral RNA extraction: Using a QIAmp viral RNA kit (QIAGEN, Inc., Germany) total RNA was extracted from 140ul of virus-infected tissue culture fluid according to the manufacturer's protocol**.** Primer selection: The C-prM amplimers were initially reported by Lanciotti et al 1992, and later on redesigned by Chien et al.[13] The primers listed in Table-I were used in this study.

Molecular detection: Methods of Chew et al[12] was followed, briefly - One-step RT- PCR was used to identify all four serotypes of dengue viruses. This protocol was performed by two amplifications. The 1st RT-PCR assay: 25ul total reaction mixture that contains 5ul of RNA + 25 pmol of the mD1 + D2 primers + contents of one-step RT-PCR kit (QIAGEN). The following were the steps:

One step RT-PCR amplification

Steps	*Temp.*	*Time*	*No. of Cycle*
Preheated thermal cycle	50°C		
Reverse Transcription :	50°C	30 min	1
Initial PCR activation step :			
- Denaturation	95°C	15min	1
- Annealing	55°C	15second	1
- Extension	72°C	30 second	1
Denaturation :	95°C	15 second	34
Annealing :	55°C	15 second	34
Extension :	72°C	30 second	34
Final Extension	72°C	10min	1
Hold	4°C		

HotStartTaq master mix kit (QIAGEN) was used in semi nested PCR with the same amount of the total reaction mixture as above taking 5ul of RT-PCR product and primers (mD1, rTS1, mTS2, TS3, and rTS4). The following were the steps:

Semi-nested PCR amplification by HotStart Taq Polymerase

Steps	*Temp.*	*Time*	*No. of Cycle*
Activation Taq polymerase	95°C	15min	1
Denaturation	95°C	15second	25
Annealing	55°C	15 second	25
Extension	72°C	30 second	25
Final Extension	72°C	30 second	1
Hold	4°C		

Gel electrophoresis: Gel electrophoresis was used for visualization of the PCR product. After gel electrophoresis the PCR product was stained with gel red (Biotium, USA) and then observed under ultraviolet radiation. RT-PCR products sizes of DEN-1, DEN-2, DEN-3 and DEN-4 were 208bp, 119bp, 288bp, and 260bp, respectively (Chew et al.[12])

RESULTS

Results of the present study have been presented in Table-II. It is revealed from the table that out of 149 sera samples examined, 48 (32.2%) were dengue virus positive by cell culture and RT-PCR and 108 (72.5%) were positive by serology. Of the 108 cases, 40 (26.8%) were positive to IgM, 61 (41%) were positive to both IgG and IgM, and 7(4.7%) cases were positive to IgG only. In respect to age group the prevalence of dengue was higher in age group 20-29 years, in relation to genders, infection was more in male, considering communities living in Malaysia, the prevalence was more in Malay. In relation epidemic intensity of the dengue infection in different months during study period starting from June to October, 2011, it revealed that the highest infection recorded in the month of June and lowest in the month of August.

Distribution of dengue infection as determined by serology in respect to gender has been shown in Fig.1. It was observed that male was at the higher exposure to dengue infection compared to female. The data showed that infection in male and female was 63% (68) and 37% (40), respectively. Detection of primary and secondary dengue cases observed in the study based on serology revealed (Fig.1) that 28 were primary dengue, 7 were secondary dengue and 61 were positive for both primary and secondary

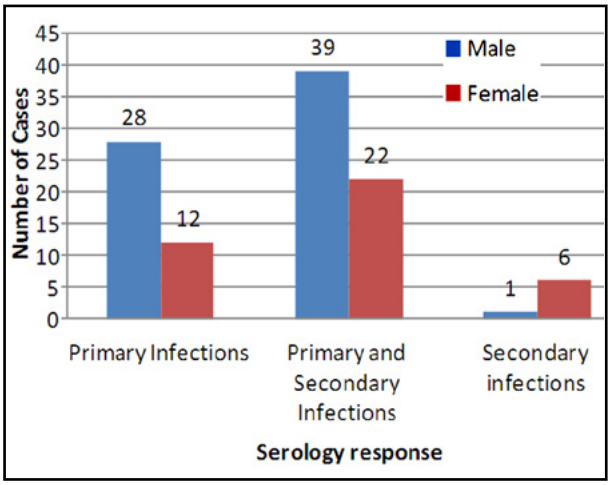

Fig.1: Detection of primary and secondary dengue in male and female based on serology.

Table-II: Detection of dengue virus by cell culture, RT-PCR and serology in relation to age group at UKMMC.

Age Group (years)	*Culture and RT-PCR positive*	*Serology Assay**			*Total (%)*
		IgM Positive	*IgM and IgG positive*	*IgG positive*	
0-9	1	2	1	0	4(2.6)
10-19	16	10	14	0	40(25.6)
20-29	15	20	16	3	53(34.6)
30-39	6	5	11	2	24(15.4)
40-49	6	0	8	0	14(9.0)
50-59	4	3	4	1	12(7.7)
60-69	0	0	5	0	5(3.2)
70-79	0	0	2	1	3(1.9)
Total	48	40	61	7	155+(100)

Lengend: * Dengue IgM and IgG rapid test (SD BIOLINE, Korea)
+Though the total number samples were 149, however, 6 samples showed positive results in serology both IgG & IgM

dengue, it means these patients' sera gave positive results for both IgM and IgG. Among the primary dengue infections 28 were male and 12 were female. In secondary dengue infection one was male and six were female. In primary-secondary mixed reactions out of 66 patients 39 were male and 22 were female (Fig.1)

Distribution record (Fig.2) of dengue infection in different communities living in Malaysia observed that 81 Malay, 37 Chinese, 4 Indian and 12 foreigners suffered from dengue fever (DF). Eight Malay and 3 Chinese suffered from Dengue hemorrhagic fever (DHF). Three Malay and only one Chinese suffered from dengue shock syndrome (DSS).

During the study period it was observed (Fig.3) that the highest number of cases (32) documented in June then September (22) and October (22). The lowest number of cases (6) were in August.

DISCUSSION

In the present study we could classify the dengue serological results by primary, secondary and primary-secondary mixed dengue infections. From the available literature no such classified study has so far been conducted in Malaysia. In our results overall dengue seroprevalence which includes primary, secondary and primary-secondary mixed dengue infections were 71.8%. On the contrary, similar studies conducted by numerous authors and observed prevalence of dengue based on serology were 45% (Singapore), 66% (Iquitos, Peru), 69% (Salvador, Brazil), and 78% (Delhi, India).[14-17]

The present communication provides the sero prevalence of 71.8% which is next higher to that of India. In respect to age group the prevalence of dengue was higher in age group 20-29 years, in relation to genders, infection was more in male, considering communities living in Malaysia, the prevalence was more in Malay. In relation epidemic intensity of the dengue infection in different months during study period starting from June to October, 2011, it revealed that the highest infection recorded in the month of June and lowest in the month of August. The higher prevalence of dengue in the age group of 20-29 might be due to more outdoor activities of this young to adult group which allowed them to be exposed to Aedes mosquitoes.

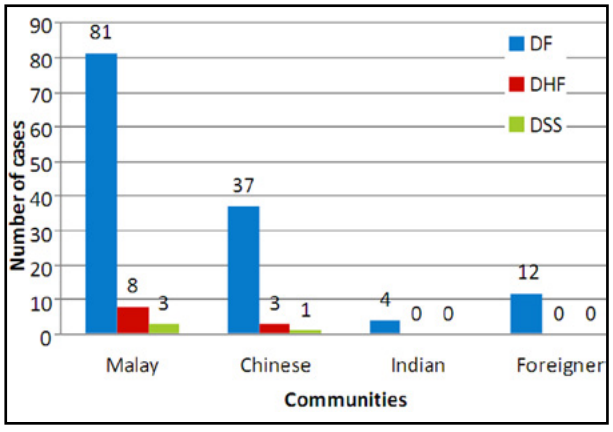

Fig.2: Distribution of dengue infection in different communities.

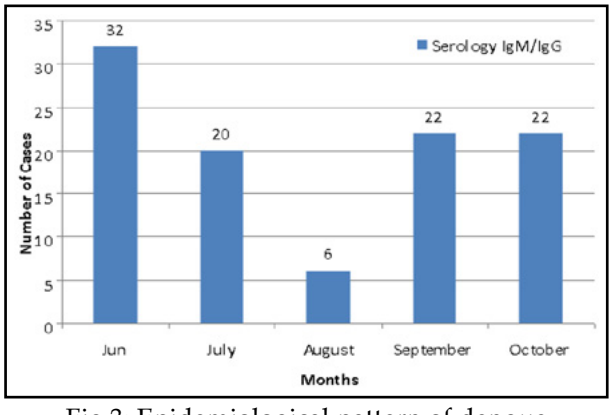

Fig.3: Epidemiological pattern of dengue infections in different months during 2011.

Similar results obtained from the study of Ang and Sigh[18] who mentioned that most of the reported cases were from the young and middle age groups and described similar possibilities of getting higher infections. Others age groups 40-49, 50-59 and 70-79 showed low infection rate of dengue which could be due to development of immunity to them from previous exposure. The present data showed that male and female were 63% (68) and 37% (40), respectively. It may be due to that males have the tendency to travel and do more outside works than the female favors them to be exposed by Aedes mosquitoes.

Goh[19] also reported higher seropositive of dengue in men compared to women. Dengue infection in different communities living in Malaysia observed that 81 Malay, 37 Chinese, 4 Indian and 12 foreigners suffered from dengue fever (DF). Eight Malay and 3 Chinese suffered from Dengue hemorrhagic fever (DHF). Three Malay and only one Chinese suffered from dengue shock syndrome (DSS).In the present study, dengue infection was higher in Malay population. This is probably due to the population density in Kuala Lumpur area where Malay communities reside more. Epidemiological data in terms of different months during study period, it was observed (Fig.3) that the highest number of cases (32) were in June then September (22) and October (22).

The lowest numbers of cases (6) were in August. It is difficult to explain the highest prevalence of dengue infection in June, however, it may be predicted that more raining is observed in the month that might favors to allow stagnancy of water in different locations in city areas, act as breeding site of Aedes. Dengue infection is related to season's due to higher activity after monsoon.[20] Dengue is still an important public health problem in Malaysia like other countries in this region. In this study, it is observed that children, younger an adult population were the high risk to infection. Strengthening of present dengue control program undertaken by the Malaysian Government and development of suitable tetravalent vaccines against 4 circulating serotypes of dengue virus could solve the prevailing problem.

ACKNOWLEDGEMENTS

Authors' acknowledge the Ministry of Health for providing financial support during postgraduate study of the 1st author. Authors' also acknowledge the authority of the National University of Malaysia Medical Centre for providing all cooperation and financial support of the present study.

REFERENCES

1. Gubler DJ, Meltzer M. Impact of dengue/dengue hemorrhagic fever on the developing world. Adv Virus Res 1999;53:35-70.
2. Shu PY, Huang JH. Current advances in dengue diagnosis. Clin Diag Lab Immunol 2004;11:642-650.
3. WHO, Dengue and dengue haemorrhagic fever. Fact Sheet No.117. Available from: http://www.who.int/mediacentre/factsheets/fs117/en/index.html. 2009.
4. Lam SK, Two Decades of Dengue in Malaysia. J Trop Med 1993;35(4):195-200.
5. Wallace HG, Lim TW, Rudnick A, Knudsen AB, Cheong WH, Chew V. Dengue hemorrhagic fever in Malaysia: the 1973 epidemic. Southeast Asian J Trop Med Public Health 1980;11:1-13.
6. Fang R, Lo E, Lim TW. The 1982 dengue epidemic in Malaysia: epidemiological, serological and virological aspects. Southeast Asian J Trop Med Public Health 1984;15:51-58.
7. Lim TW, Wallace HG, Rudnick A. The 1973 epidemic of dengue hemorrhagic fever in Malaysia: (a preliminary report). Southeast Asian J Trop Med Public Health 1974;5:453-454.
8. Ministry of Health, Malaysia. Annual report 1974, 1980, 1984, 1998, 1999, 2000.
9. Ministry of Health Malaysia. Press Release of Dengue Fever Situation In Malaysia Week 48 (27 November - 3 December 2011), 2011. http://moh.gov.my/press_releases/127.
10. Kobayashi N, Thayan R, Sugimoto C. Type-3 dengue viruses responsible for the dengue epidemic in Malaysia during 1993-1994. Am J Trop Med Hyg 1999;99;60:904-909.
11. Ministry of Health Malaysia. Clinical Practice Guidelines on Management of Dengue Infection in adults. Revised 2nd Edition; 2010.
12. Chew MH, Rahman MM, Jelip J, Hassan MR, Isahak I. All Serotypes of Dengue Viruses Circulating in Kuala Lumpur, Malaysia. Curr Res J Biologic Sci 2012;4(2):229-234.
13. Chien, LJ, Liao TL, Shu PY, Huang JH, Gubler DJ, Chang GJ. Development of real-time reverse transcriptase PCR assay to detect and serotype dengue viruses. J Clin Microbial 2006;44:1295-1304.
14. Wilder-Smith A, Foo W, Earnest A, Sremulanathan S, Paton NI. Seroepidemiology of dengue in the adult population of Singapore. Trop Med Int Health 2004;9(2):305-308.
15. Reiskind MH, Baisley KJ, Calampa C, Sharp TW, Watts DM, Wilson ML. Epidemiological and ecological characteristics of past dengue virus infection in Santa Clara, Peru. Trop Med Int Health 2001;6:212–218.
16. Teixeira Mda G, Barreto ML, Costa Mda C, Ferreira LD, Vasconcelos PF, Cairncross S. Dynamics of dengue virus circulation: a silent epidemic in a complex urban area. Trop Med Int Health 2002;7(9):757-762.
17. Kurukumbi M, Wali JP, Broor S. Seroepidemiology and active surveillance of dengue fever/dengue haemorrhagic fever in Delhi. Indian J Med Sci 2001;55:149–156.
18. Ang KT and Singh S. Epidemiology and New Initiatives in the Prevention and control of dengue in Malaysia. Dengue Bulletin 2001; Vol:25.
19. Goh K. Dengue – a re-emerging infectious disease in Singapore. In: Dengue in Singapore, Vol. 2 (ed. K Goh), Institute of Environmental Epidemiology, Ministry of Environment. Technical Monograph series, Singapore, 1998: 33–49.
20. Gupta E, Dar L, Kapoor G, Broor S. The changing epidemiology of dengue in Delhi, India. Virology J 2006;3:92.