Epidemiological and Clinical Features of Dengue Versus other Acute Febrile Illnesses Amongst Patients Seen at Government Polyclinics

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SUMMARY:

Classical dengue fever is characterized by the clinical features of fever, headache, severe myalgia and occasionally rash, which can also be caused by a number of other viral and bacterial infections. Five hundred and fifty eight patients who fulfilled the criteria of clinical diagnosis of acute dengue from 4 government outpatient polyclinics were recruited in this prospective field study. Of the 558 patients, 190 patients were categorized as acute dengue fever, 86 as recent dengue and 282 as non-dengue febrile illnesses based on the results of a number of laboratory tests.

Epidemiological features of febrile patients showed that the mean age of patients in the dengue fever group was significantly younger in comparison with patients in the non-dengue group. There was no significant difference between the two groups with respect to gender but there was significant ethnic difference with foreign workers representing a higher proportion in the dengue fever group. Patients with acute dengue fever were more likely to have patient-reported rash and a history of dengue in family or neighbourhood but less likely to have respiratory symptoms, sore-throat and jaundice in comparison to patients with non-dengue febrile illnesses. As with patients with dengue fever, patients in the recent dengue group were more likely to have history of patient-reported rash and a history of dengue contact and less likely to have respiratory symptoms in comparison to patients with non-dengue febrile illnesses. In contrast to patients with dengue fever, patients in the recent dengue group were more likely to have abdominal pain and jaundice in comparison to non-dengue febrile patients. The finding strongly suggests that a proportion of patients in the recent dengue group may actually represent a subset of patients with acute dengue fever at the late stage of illness.

KEY WORDS:
Dengue, epidemiological feature, clinical feature.

INTRODUCTION

Dengue virus is an envelope positive-sense single stranded RNA virus that belongs to the genus Flavivirus, under the family Flaviviridae. There are four serotypes of dengue viruses (dengue serotype-1, 2, 3, and 4).[1-3] All four serotypes of dengue viruses are known to be able to infect humans and also have been documented to cause severe fatal diseases. Infection with one serotype does not confer cross-protection against the other serotypes but instead may lead to serious disease, such as dengue haemorrhagic fever/dengue shock syndrome, through immunepathological enhancement of the disease. However, infection with one serotype results in production of cross-reactive antibodies (both IgM and IgG) against other serotypes which contributes to the problem of laboratory diagnosis of acute dengue based on serological platform .[4-6]

Dengue virus infection in humans causes a spectrum of illnesses ranging from clinically asymptomatic or transient nonspecific febrile illness to classic dengue fever (DF), and dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS).[1-3] The usual presenting clinical features of patients with classical dengue fever (DF) and early DHF/DSS are fever, headache, rash, bone and muscular pains with or without abdominal pain. These presenting features are broad and non-specific which can also be caused by a number of other viral and bacterial infections or even non-infective cause of systemic inflammatory process occurring in the early phase of acute illnesses such as autoimmune diseases. In an effort to improve clinical diagnosis of acute dengue virus infection for early correct management of patients with dengue and institution of effective public health control measures of dengue outbreaks, a number of prospective studies were carried out previously to evaluate the correlation of each of these presenting clinical features in differentiating acute dengue from other febrile illnesses.[7-14]

In 2006, the National Public Health Laboratory (NPHL) Malaysia undertook a retrospective laboratory study to evaluate the sensitivity of a commercial dengue NS1 antigencapture ELISA kit for early laboratory confirmation of acute dengue.[15] The NPHL was subsequently requested to carry out a prospective evaluation of the same commercial kit with the view of its application in national wide clinical laboratories for early laboratory confirmation of acute dengue. In addition to the intended evaluation as the main focus of this prospective study, the epidemiological and clinical features of patients recruited for this study were simultaneously captured and analysed to identify features that may have potential values in differentiating acute dengue from other febrile illnesses with the prospect of improving clinical diagnosis of acute dengue in outpatient polyclinics.

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MATERIALS AND METHODS

This prospective study was carried out under the directive and funding support of the Ministry of Health Malaysia. The study protocol was designed in close collaboration with the Institute for Medical Research Malaysia. A presentation on clinical, epidemiological and laboratory aspects of dengue, together with the study protocol was presented to the health-care providers (doctors, nurses and laboratory technologists) of each of the respective collaborating
primary health-care centres (Poliklinik Seksyen 7, Shah Alam; Poliklinik Kelana Jaya, Petaling Jaya; Poliklinik Sg. Buloh; Poliklinik Jinjang) in the Klang valley at the beginning of the study.

The recruitment of patients for this study was based on the clinical judgment/diagnosis of doctors treating the patients at the selected primary health-care polyclinics but the doctors were advised to use the WHO clinical criteria of acute dengue diagnosis (fever with headache and/or myalgia and/or rash) as the guideline. Patients who presented with obvious respiratory illness (rhinitis, cough) or had a clinically known cause of fever were not included in the study. Upon consent, patients' demographic, epidemiological and presenting clinical features were collected on a standardized data entry form. The date of onset of fever and the date of blood sample collection were also recorded in the data entry form for calculation of sample age. Sample age was defined as the interval in days between the date of onset of illness and the date of collection of blood sample. It was considered as Day 0 if the blood samples were collected on the same day of the date of fever onset and Day 1 if the samples were collected on the following day. Following physical examination, five milliliters of venous blood was collected from each patient as part of the workout for laboratory diagnosis of acute dengue infection. The venous blood samples were allowed to clot at room temperature in the laboratories of primary care health centres. As soon as the blood had clotted, serum samples were separated and stored in a 4 0C refrigerator. Serum samples together with the completed standardized data entry forms were collected and transported to the National Public Health Laboratory in the following morning. Six types of laboratory tests were performed on each acute serum sample and excess sera were stored at -80 0C for future reference and further confirmatory or other tests when needed. A second (convalescent) blood sample was requested and collected from the patient whenever his or her acute serum sample was tested positive by dengue NS1 antigen-capture ELISA but negative by virus isolation or any of the molecular diagnostics. Serological tests were subsequently carried out on the pair serum samples from this category of patients to check for sero-conversion or a four fold or greater rise in antibodies against dengue virus.

Serological assays for the detection of anti-dengue IgM and anti-dengue IgG present in acute serum samples were carried out using a commercial IgM-capture ELISA kit and a dengue IgG Capture ELISA kit respectively (Panbio Diagnostics, Brisbane, Australia). Test procedures and interpretation of results were in accordance with the manufacturer's instructions. For the anti-dengue IgG assay, the results were interpreted as primary dengue (negative) for Panbio units (P.U.) of 22 or less and secondary dengue (positive) for Panbio unit (P.U.) >22.

The ribonucleic acids (RNA) of dengue virus were extracted from acute serum samples using High Pure Viral Nucleic Acid Kit (Roche Diagnostic, Mannheim, Germany) according to the manufacturer's protocol. Molecular detection of dengue virus RNA by conventional reversetranscriptase polymerase chain reaction (RT-PCR) was performed using a set of pan-dengue generic oligonucleotide primers as described in Lanciotti *et al*.[16] Molecular detection of dengue virus RNA by real-time RT-PCR (rRT-PCR) was carried out in accordance with a published method by Chutinimitkul *et al*.[17]

A mosquito cell-line, C6/36 (ATCC CRL-1660), was used to isolate dengue virus from patients' serum samples. Detection for the presence of dengue virus infected C6/36 cells at the end of the 10th post-inoculation day was by an indirect immunofluorescence assay using a commercial anti-dengue monoclonal antibody complex covering all serotypes of dengue viruses (Chemicon Int. Inc. USA; Cat. No. MAB8705). The procedures for dengue virus isolation and identification were previously described in Chua et al.[18]

Immunological assay for dengue NS1 antigen was carried out using a commercial dengue NS1antigen-capture ELISA kit, PLATELIA™ DENGUE NS1 AG (Bio-Rad Corporate HQ, Hercules, USA). The assay system is based on a one-step sandwich format microplate enzyme immunoassay for detection of dengue virus NS1 antigen in human serum or plasma. The assay uses murine monoclonal antibody (MAb) for capture and revelation. An immune-complex MAb-NS1-MAb/peroxidase will be formed if NS1 antigen is present in the sample. In this study, the test was performed strictly according to the assay procedure of the commercial Platelia Dengue NS1 antigen capture ELISA kit.

The demographic, epidemiological and clinical features together with laboratory data were tabulated in appropriate worksheets using the Microsoft Excel program and evaluated by Chi-square test using the Epi Info 6 (Center for Disease Control and Prevention, Atlanta) free computer program, for any statistically significant association. A probability (p) value of 0.05 or less was taken as the level of significant association for each ordinal variable with the relevant adjusting variables. The patients were categorised into 3 groups based on the results of the laboratory tests. Group A consisted of patients who were laboratory confirmed as acute dengue based on positive result by any or a combination of the 4 methods (RT-PCR, real-time RT-PCR, virus isolation, detection of dengue NS1 protein) for laboratory confirmation of acute dengue virus infection. Group B consisted of patients classified as recent dengue based on positive detection of anti-dengue IgM but negative by any of the 4 methods for laboratory confirmation of acute dengue. Group C consisted of patients classified as non dengue febrile illnesses based on negative detection of anti-dengue IgM and any of the 4 methods for laboratory confirmation of acute dengue.

RESULTS

This prospective field study involved 4 outpatient polyclinics of government health centres (Poliklinik Seksyen 7, Shah Alam; Poliklinik Kelana Jaya, Poliklinik Petaling Jaya; Poliklinik Sg. Buloh; Poliklinik Jinjang) situated in the Klang valley of peninsular Malaysia. The period of the study was from mid-August 2006 to March 2009. During the period, 558 patients who fulfilled the criteria of clinical diagnosis of acute dengue were recruited and a total of 589 serum samples, consisting of 558 acute serum samples and 31 convalescent (2nd) serum samples, were collected. Of the 558 patients, 344 were males and 214 were females with a male to female ratio of 1.6:1. Three

hundred and forty seven patients were Malays, 80 patients were Chinese, 97 patients were Indians and 34 other patients were foreign workers. The age of the patients ranged from 1.5 to 76 years old with a mean of 26 and standard deviation of 13.5 years.

Of the 558 acute serum samples, the number of samples tested positive by each respective type of laboratory tests is shown in Table I. Group A consisted of 190 patients whose acute serum samples were tested positive by anyone or a combination of the 4 methods (RT-PCR, real-time RT-PCR, virus isolation, detection of dengue NS1 protein) that is able to give laboratory confirmation of acute dengue virus infection. Of the 190 patients confirmed to have acute dengue, anti-dengue specific IgM was also detected in the acute serum samples of 120. Thus, Group B, under the category of recent dengue, consisted of 86 (206 – 120) patients and Group C, under the category of non-dengue febrile illnesses, consisted of 282 (558 – 190 – 86) patients. The total number of patients recruited by each of the respective health centres and the number and proportion of patients which was subsequently laboratory confirmed as having acute dengue, recent dengue and non-dengue is shown in Table II. The proportion of patients that was laboratory confirmed to have acute dengue by each respective polyclinic varied from 23.1% to 55.6%. There is a significant difference in the ability of doctors in different polyclinics to diagnose acute dengue correctly ($\chi2 = 68.77$, df = 6, p = 0.0000). The doctors in Sungai Buloh and Shah Alam polyclinics were able to achieve a respective 54.3% and 55.6% correct diagnosis of acute dengue which is higher than the overall correct diagnosis of acute dengue (34.1%) in this study (Table II).

The epidemiological features of patients at the time of presentation at the outpatient polyclinics amongst the three different categorized disease groups are shown in Table III. There is a significant difference in mean age of patients among the three disease groups (Kruskal-Wallis H = 11.62, df = 2, p = 0.0030) with the acute dengue group (Group A) having the lowest mean age. All three disease groups had a higher proportion of male patients and there is no significant difference in gender composition among the three disease groups ($\chi2 = 0.66$, p = 0.7203). There is a significant difference in the ethnic composition among the three disease groups ($\chi2 = 22.92$, df = 6, p = 0.0008) and the statistical difference occurred between Group A and Group C ($\chi2 = 19.77$, df = 3, p = 0.0002) with Group A having a higher proportion of foreign workers and a lower proportion of Indians. There is no statistical difference in ethnic composition between Group A and Group B ($\chi2 = 6.46$, df = 3, p = 0.0914) and similarly between Group B and Group C ($\chi2 = 3.25$, df = 3, p = 0.3550).

A comparison of selected clinical features at entry into the study among patients who were eventually classified into Groups A, B and C based on laboratory test results is shown in Table IV, with the results of statistical analysis shown in Table V. The clinical features that showed statistical significant differences among the three disease groups were rash, abdominal pain, respiratory symptoms, sore-throat, jaundice and a history of recent dengue affected member(s) in the family or neighbourhood (Column 3, Table V). Patients with acute dengue were more likely to have rash and a history of dengue in the family or neighbourhood and less likely to have respiratory illnesses, sore-throat and jaundice. History of dengue contact, abdominal pain and jaundice were significantly more likely to occur in patients in the recent dengue group (Group B) than those in the non-dengue group (Group C) (Column 7, Table V). Clinical features such as headache, myalgia, diarrhoea and history of taken oral anti-pyretic had no significant value in differentiating acute dengue from non-dengue and even differentiating recent dengue from non-dengue.

DISCUSSION

Most dengue virus infections, especially in children, are asymptomatic or subclinical with minimal symptom that cannot be easily distinguished clinically from other viral infections.19-20 Classical dengue fever is characterized by the clinical features of fever, headache, severe myalgia and occasionally rash, which last from 4 to 7 days. However, these presenting features can also be caused by a number of other viral and bacterial infections. Thus, clinical diagnosis of acute dengue fever, especially in the early stage of illness can never be 100% correct and affirmative diagnosis still requires appropriate laboratory test(s). As exemplified in this study that the attending doctors in the polyclinics were able to achieve an overall correct clinical diagnosis of dengue fever in about a third (34.1%) of the total cases enrolled. The proportion of correct clinical diagnosis achieved by doctors in this study is compatible with the achievable level of correct clinical diagnosis achieved by physicians in other studies.[10,14,21] The achievable level of correct clinical diagnosis achieved by attending doctors in this study varied significantly between different polyclinics which ranged from a low percentage of 23.1% to as high as 55.6%. The ability to achieve a higher level of correct clinical diagnosis may reflect the clinical competency of the doctors in the respective polyclinics and/or there was simultaneous occurrence of dengue outbreak with high dengue transmission affecting population in the
localities served by the polyclinics.[10,14]

Epidemiological features of febrile patients recruited in this study showed that the mean age of patients in the dengue fever group was significantly younger in comparison with patients in the non-dengue group. The finding supports other studies and WHO findings that dengue virus infections tend to affect children and young adults in countries of South-east Asia region with the exception of Singapore.[1,10,14,21] Previous studies demonstrated that there was no significant epidemiological difference between patients in the dengue fever group and non-dengue fever group with respect to gender and ethnicity. Although there was no significant difference between the two groups with respect to gender in this study, there was significant ethnic difference between the two groups, with foreign workers representing a higher proportion in the dengue fever group. This may reflect a higher dengue vector density with a higher rate of dengue virus transmission in certain localities housing the foreign workers.

Previous studies showed that certain clinical features such as rash, myalgia, headache and positive tourniquet test are more likely to be present in patients with dengue fever than those of other febrile illnesses, and thus can lead to an improvement in the accuracy of clinical diagnosis.[9-14,21] This study supports the findings of other previous studies and shows that patients with acute dengue fever were more likely to have patient-reported rash and a history of dengue in family or neighbourhood. Headache and myalgia, which were reported to be more likely to occur in patients with dengue fever in other studies, were found to be insignificant in this study. As in other studies,

patients with acute dengue fever were less likely to have respiratory symptoms, sore-throat (pharyngitis) and jaundice. However, it is important to note that these clinical features were not exclusive for each group as there were a substantial proportion of patients with confirmed dengue fever who also had respiratory symptoms and sore-throat and vice-versa. In this study, though diarrhoea was a fairly common presenting clinical feature which occurred in about a quarter of patients in all the three groups, it had no differentiating value.

An interesting finding in this study is the spectrum of clinical features present in patients under the recent dengue group. The patients in this group had clinical features at times similar to those in the dengue fever group but in other occasions shared similar clinical features with patients in the non-dengue group or having unique feature on its own. As with patients with dengue fever, patients in the recent dengue group were more likely to have history of patient-reported rash and a history of dengue contact and less likely to have respiratory symptoms in comparison to those patients with non-dengue febrile illnesses. In contrast to patients with dengue fever, patients in the recent dengue group were more likely to have abdominal pain and jaundice in comparison to non-dengue febrile patients. This finding strongly suggests that a proportion of patients in the recent dengue group may actually represent a subset of patients with acute dengue fever but presented to doctors at the polyclinics at a late stage of illness in which their blood samples may by then; not be tested positive by any of the 4 methods for laboratory confirmation of acute dengue fever. In fact, this subset of patients showed clinical features compatible with acute liver involvement, which is the late stage manifestation of dengue virus infection with sign and symptom suggestive of early dengue haemorrhagic fever/ dengue shock syndrome. The setback of this study is the failure and difficulty of getting paired convalescent blood samples for serological confirmation of acute dengue virus infections especially for this subset of patients.

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Table I: The number of acute serum samples from 558 patients with clinical diagnosis of dengue tested positive by each of the laboratory test methods.

Diagnostic Test Method	Number of samples tested positive (%)
NS1 Ag-capture ELISA	174 (31.2)
Real-time RT-PCR	112 (20.1)
RT-PCR	92 (16.5)
Virus Isolation	77 (13.8)
Anti-dengue IgM	206 (36.9)
*Anti-dengue IgG (>22 P.U.)	109 (19.5)

* The positive (Panbio unit >22 P.U.) result for anti-dengue IgG is equivalent to a value above the cut-off point for secondary dengue.

Table II: The number and percentage of patients that was recruited by each of the respective polyclinics and its distribution amongst the three disease groups.

		Disease Group		
Polyclinic	Number (%)	Acute Dengue	Recent Dengue	Non-Dengue
Kelana Jaya	186 (33.3)	43 (23.1%)	21 (11.3%)	122 (65.6%)
Sungai Buloh	46 (8.2)	25 (54.3%)	8 (17.4%)	13 (28.3%)
Shah Alam	135 (24.2)	75 (55.6%)	24 (17.8%)	36 (26.7%)
Jinjang	191 (24.6)	47 (24.6%)	33 (17.2%)	111 (58.1%)
Total	558 (100)	190 (34.1%)	86 (15.4%)	282 (50.5%)

Table III: Epidemiological features and demographics of patients in the study population.

		Disease Group (%)			*p* - value
		Acute Dengue (n = 190)	**Recent Dengue** (n = 86)	**Non-Dengue** (n = 282)	
Age (year)	Mean	22.8	25.6 28.3	0.0030	
	Range	5 - 75	3 - 59	1.5 - 76	
	Standard deviation 1	0.2 12.7	15.1		
Gender	Male	114 (60.0)	56 (65.1)	174 (61.7)	0.7203
	Female	76 (40.0)	30 (34.9)	108 (38.3)	
	Male : Female	1.5 : 1	1.9 : 1	1.6 : 1	
Ethnicity	Malay	126 (66.3)	51 (59.3)	170 (60.3)	0.0008
	Chinese	25 (13.2)	17 (19.8)	38 (13.5)	
	Indian	19 (10.0)	14 (16.3)	64 (22.7)	
	Others	20 (10.5)	4 (4.7)	10 (3.5	

Table IV: Frequency distribution of clinical features of patients in the study population.

	Disease Group (%)								
	Acute Dengue (n = 190)			**Recent Dengue** (n = 86)			**Non-Dengue** (n = 282)		
Clinical Feature	Present	Absent	NA	Present	Absent	NA	Present	Absent	NA
Headache	130 (68)	56 (29)	4	64 (74)	18 (21)	4	195 (69)	82 (29)	5
Myalgia	141 (74)	44 (23)	5	64 (74)	20 (23)	2	186 (66)	89 (32)	7
Rash	63 (33)	114 (60)	13	30 (35)	45 (52)	11	61 (22)	200 (71)	21
Abdominal Pain	64 (34)	123 (65)	3	36 (42)	46 (53)	4	82 (29)	196 (70)	4
Diarrhoea	53 (28)	134 (71)	3	28 (33)	55 (64)	3	64 (23)	213 (76)	5
*Respiratory Sy	57 (30)	121 (64)	12	32 (37)	47 (55)	7	177 (63)	97 (34)	8
Sore-throat	47 (25)	129 (68)	14	29 (34)	50 (58)	7	125 (44)	149 (53)	8
Jaundice	2 (1)	161 (85)	27	5 (6)	69 (80)	12	3 (1)	228 (81)	51
#Dengue Contact	94 (49)	76 (40)	20	36 (42)	36 (42)	14	70 (25)	180 (64)	32
Oral Anti-pyretic	145 (76)	4 (13)	21	62 (72)	13 (15)	11	21 (78)	46 (16)	15

*Respiratory Sy = Respiratory symptom such nasal blockage, runny nose or cough.
#Dengue Contact = History of recent dengue affected member(s) in the family or neighbourhood.
NA = Not available.

Table V: Statistical analysis of the clinical features of patients amongst the three disease groups (Gp A = Acute Dengue, Gp B = Recent Dengue, Gp C = Non-Dengue) in the study population.

	Gp A vs Gp B vs Gp C		Gp A vs Gp C		Gp B vs Gp C		Gp A vs Gp B	
Clinical Feature	Chi-square	*p*- value	Chi-square	*p*- value	Chi-square	*p*- value	Chi-square	*p*- value
Headache	2.12	0.3469	0.01	0.9073	1.48	0.2234	1.51	0.2194
Myalgia	4.94	0.0800	3.95	**0.0468**	1.84	0.1749	3.55	0.0594
Rash	11.66	**0.0029**	7.17	**0.0074**	7.34	**0.0068**	0.27	0.6030
Abdominal Pain	6.04	**0.0489**	0.95	0.3294	5.33	**0.0209**	1.89	0.1691
Diarrhoea	4.20	0.1228	1.36	0.2439	3.26	0.0712	0.56	0.4543
*Respiratory Sy	49.22	**0.0000**	44.56	**0.0000**	13.76	**0.0002**	1.39	0.2392
Sore-throat	16.34	**0.0003**	15.45	**0.0001**	1.63	0.2011	2.15	0.1424
Jaundice	8.97	**0.0113**	Fisher	1.0000	Fisher	**0.0225**	Fisher	**0.0321**
#Dengue Contact	34.30	**0.0000**	0.54	**0.0000**	11.28	**0.0008**	0.38	0.5391
Oral Anti-pyretic	0.77	0.6791	0.50	0.4808	0.02	0.8794	0.19	0.6629

*Respiratory Sy = Respiratory symptom such nasal blockage, runny nose or cough.
#Dengue Contact = History of recent dengue affected member(s) in the family or neighbourhood.
NA = Not available.

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