EPIDEMIOLOGY, CLINICAL FEATURES, LABORATORY INVESTIGATIONS AND EARLY DIAGNOSIS OF DENGUE FEVER IN ADULTS: A DESCRIPTIVE STUDY IN SRI LANKA

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Abstract. A descriptive observational study was conducted to identify the epidemiology, clinical features, laboratory investigations and markers for early diagnosis of acute dengue virus infection in adults. We enrolled 404 patients over a period of two years, beginning from 2001, at the Teaching Hospital Peradeniya, Sri Lanka. Based on serology, 239 patients were grouped as: IgM 43 (18%), IgG and IgM 140 (58%), and IgG 28 (12%). The clinically diagnosed group without serology numbered 165 patients. Most of the parameters between groups showed a similar pattern: mean age of 30 years, mean duration of fever 7 days (range 1-19 days). Mean total white blood cell and platelet counts started to fall from the second day of fever, with the lowest counts on the 5th to 7th days. Packed cell volume (PCV) showed minimum fluctuation. One hundred and sixty (88%) patients showed elevated liver enzymes (ALT and AST), with 122 of them having a two-fold increase. Three patients died, and complications such as myocarditis, large effusions, encephalopathy, acute renal failure, acute liver failure and diarrhea were observed. These results suggest that a combination of clinical picture, thrombocytopenia, leukopenia and elevated liver enzymes could be used as markers for early diagnosis of dengue infection. Furthermore, evidence-based guidelines should be developed for managing dengue infection in adults.

INTRODUCTION

Dengue fever is an acute viral infection transmitted to humans by the bite of infected *Aedes* mosquitoes, which are closely associated with human habitation (Kalayanarooj, 1997; Rigau-Perez *et al*, 1998; WHO,1998). Dengue fever (DF) and dengue hemorrhagic fever (DHF) are caused by the four dengue virus types, DEN 1, 2, 3 and 4, which are closely related antigenically (Kalayanarooj *et al*, 1997; Rigau-Perez *et al*, 1998; WHO, 1998). The year 2001 witnessed unprecedented global dengue epidemic activity in the American hemisphere, the Pacific islands and continental Asia (Halstead, 2002). The prevalence of DF/DHF in Sri Lanka has been recognized since the early eighties, and currently it has become a major heath hazard with high morbidity (Vitarana, 1990; Messer *et al*, 2002). Furthermore, cases of DHF have dramatically increased since 1989 in Sri Lanka (Messer *et al*, 2002).

Early diagnosis of dengue infection is difficult, as it may mimic another viral fever. Therefore, detection of the virus by reverse transcriptase-polymerase chain reaction-based liquid hybridization (RT-PCR-LH) has been developed. However, this investigation is confined only to research laboratories in Sri Lanka (Gunasekare *et al*, 2003). Serological diagnosis is made by detection of antibodies using IgM capture (MAC) ELISA, IgG-ELISA and by hemagglutination inhibition assay (HIA), which take at least 5 to 7 days for seroconversion (Gunasekare *et al*, 2003). Diagnosis of early dengue infection should be based on clinical features and basic investigations (Kalayanarooj *et al*, 1997). Early diagnosis helps in the proper monitoring of the disease, admission to hospital, management of complications and reducing the case fatality rate.

Prospective studies have looked extensively into the clinical features of DF/DHF, particularly in children (Nimmannitya, 1987; Kalayanarooj *et*

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al, 1997). There have been increasing reports of dengue infection with newer manifestations, mainly with cerebral and hepatic symptoms in the recent past (Cam *et al*, 2001; Pancharoen *et al*, 2002). Currently, the adult population of Sri Lanka often succumbs to dengue infection and their clinical picture may not be similar to children.

We designed a descriptive study of adult dengue infection to describe the epidemiology and clinical picture, and to identify laboratory markers of early infection. This study classifiers the dengue cases on a serological basis and describes the clinical picture and laboratory investigations accordingly. Furthermore, we attempted to incorporate the WHO severity classification using the available data.

MATERIALS AND METHODS

Subject enrollment

The current study was conducted over a period of two years from January 2001 in Kandy in the Central Province of Sri Lanka, which is the second capital city of the island. Patients over 12 years of age on admission to the Professorial Medical Unit of the Teaching Hospital, Peradeniya were eligible for entry into the study if they met the following entry criteria: fever more than 2 days, axillary temperature ≥99.5ºF, erthymatous macular skin rash or generalized erythematous skin flush. Patients meeting these criteria were examined by a study physician for eligibility, and those who had an identifiable cause for fever, such as rickettsial infection, leptospirosis, typhoid fever, viral hepatitis and malaria were excluded.

These patients were managed according to the routine management protocol for DF/DHS at the unit. The management protocol included regular clinical and laboratory assessments of bleeding tendencies and hemoconcentration, monitoring vital parameters, replacement of fluids and management of complications. The current study did not influence or changed the routine management protocol of the unit.

Severity classification of dengue infection

WHO guidelines were followed to classify patients into severity groups (WHO, 1999). The tourniquet test was performed in all the patients and blood cultures were done in patients who had a WBC count below 3 x 10^9/l.

Serological diagnosis

Serology was derived from a single blood sample taken during the illness (WHO, 1998; Rigau-Perez *et al*, 2001; Messer *et al*, 2002). At the time of blood sampling all patients had fever for one week. A venous blood sample of 5ml was collected from each patient and placed in a sterile glass bottle and transported to the laboratory immediately. A commercially available strip test (Pan Bio®) was used to detect anti-dengue immunoglobulins IgG and IgM. The test results were expressed as positives or negatives for both IgG and IgM antibodies instead of particular titer levels. A working classification was devised based on the presence or absence of IgG and IgM antibodies. Accordingly, there were four categories as follows: 1) positive for IgM only, 2) positive for both IgM + IgG, 3) positive for IgG only, 4) negative for both IgG and IgM. There was a 5th category of patients with a provisional diagnosis of dengue infection without serology.

Follow-up

Patients who recovered were discharged from the hospital according to the WHO guidelines (WHO, 1999). The study physician followed them up at the clinic once in two weeks for two months. At each visit, patients were assessed clinically and by testing WEC/DC, PCV, platelet count, AST and ALT, and the data were recorded.

Data collection

At the time of enrollment, subjects were interviewed by a study doctor to collect demographic, clinical and laboratory data, and recorded using individual formatted data collection forms. Thereafter, patients were assessed on a daily basis. Clinical assessment included recording of presenting symptoms and their duration, symptoms of anticipated complications, such as bleeding into skin and mucous membranes, shock, and renal failure; known physical signs of dengue; vital parameters, such as pulse, blood pressure and respirations, bleeding tendencies, and urine output. In addition, basic demographic data, such as age, gender, resi-

dence, and information on the socioeconomical status of the patients were recorded. Investigations done in order to facilitate management of patients were: white blood cell count-differential count (WBC/DC), platelet count, packed cell volume (PCV), Hb%, alanine transaminase (ALT), aspartate transaminase (AST), blood urea and serum creatinine. Additionally, electrocardiogram (ECG), chest radiographs (CXR), blood cultures and investigations relevant to the individual patient were done.

Statistical analysis

The data were entered into a computer using the statistical software package SPSS version 10.0. A 10% sample of the entered data was rechecked for the correct data entry.

RESULTS

Out of 404 patients who fulfilled the inclusion criteria for dengue fever, serology was carried out in 239 patients. The distribution of serological categories were: 43 (18%) positive for IgM only, 140 (58%) positive for both IgG and IgM, 28 (12%) positive for IgG only, 28 (12%) negative for both IgG and IgM. The 5th category (provisional diagnosis only) included 165 patients.

Seasonal distribution showed the highest incidence in November to December, when the island receives the highest rainfall due to the northeast monsoon. Basic demographic and clinical data of subcategories were analyzed (Table 1). Positive contact history was elicited in 34 (16%) patients, and 171 (81%) patients were confined to their residences during the preceding month. The mean duration of fever was 7 days (range 1-19 days) and the degree of fever varied from low grade to high grade fever. The headache was more frontal and many patients had myalgia and nausea. Generalized erythematous skin flush was prominent in fairer skinned patients and 16 (7%) patients had islands of pallid areas mainly on the limbs. Some patients had a macular erythematous rash rather than a flush. The flush lasted beyond the febrile period and

Table 1
Demographic data and clinical features of different categories of patients.

		IgM	IgG+ IgM	Clinically diagnosed group
Gender	Males	21	87	110
	Females	22	53	55
	Ratio	1:1	1.6:1	1:2
	Total	43	140	165
Age (years)	Mean	28	30	30
	Range	14-50	13-68	13-85
Fever	<37	10 (23%)	54 (40%)	70 (42%)
Temperature ºC	37-38	24 (55%)	52 (38%)	56 (34%)
	>38	9 (21%)	30 (22%)	37 (22%)
Duration of fever (days)	Mean	7.37	7.11	6.22
	Range	4-17	3-19	2-16
Headache	%	36 (84%)	108 (77%)	129 (78%)
Duration of headache (days)	Mean	6.84	6.33	6.24
	Range	2-14	1-20	2-11
Myalgia	%	35 (81%)	101 (72%)	119 (72%)
Myalgia duration (days)	Mean	6.46	6.56	6.21
	Range	2-16	1-15	1-11
Vomiting		29 (67.5%)	85 (61%)	93 (56%)
Rash		6 (14%)	21 (15%)	30 (18.1)
Flush		20 (46%)	53 (38%)	59 (36%)

Table 2
Distribution of elevated liver enzyme levels two-fold rise (>80U/l) in ALT and AST among different serological groups.

	Number of patients 122 (%)	Mean liver enzyme level (range) (U/l)	
		ALT	AST
IgM group	8 (7)	330 (105-681)	443 (147-1,160)
IgM+IgG group	49 (40)	321 (80-1,824)	336 (80- 2,260)
IgG group	10 (8)	176 (85-396)	177 (80-380)
Sero negative group	9 (7)	208 (127-324)	129 (80-214)
Clinically diagnosed	46 (38)	308 (83-1,442)	339 (80-1,997)

Table 3
Summary of complications observed in dengue patients.

Complication	Serological groups			Total number	Number of deaths
	IgM	IgM+ IgG	IgG		
Thrombocytopenia < $100x10^9$/l	39 (91%)	128 (91%)	23 (82%)	190	1 (due to DSS)
Active bleeding[a]	6 (14%)	18 (13%)	7 (25%)	31	
Myocarditis		3	2	5	1 (arrhythmia)
Effusions (pleural or ascitis)		6		6	
Convulsions					
EEG changes		1	1	2	
Enteritis (diarrhea)		2		2	
Pneumonia	1	1	1	3	1 (due to ARDS)
Acute renal failure		2		2	
Jaundice and liver failure		2		2	

ARDS=Acute Respiratory Distress Syndrome, DSS = dengue shock syndrome
[a]includes bruises at venepuncture sites, purpura and mucosal bleeding

its fading was associated with pruritus and mild desquamation.

Laboratory investigations

The distribution of mean total white blood cell counts (WBC) showed a trend towards leukopenia with lowest count on the 5th and 6th days of fever (Fig 1). Similarly, daily observation of platelet counts revealed that there was a drop in the counts below the normal range of 140 x 10^9/l from the second day after the onset of fever (Fig 2). The drop was maximal on the 6th day and increased gradually to above 100 x 10^9/l by 9th day of fever. All the patients had persisting low platelet counts during the febrile period. The majority (88%) of patients showed a normalization of platelet counts between the 2nd and 6th day after defervescence. This was followed by a reciprocal thrombocytosis (platelet counts above 400 x 10^9/l) on the 14th day after defervescence and normalization thereafter. Distribution of packed cell volume (PCV) showed minimum fluctuation during the early fever period (Fig 3).

Liver enzymes, serum alanine transaminase (ALT) and serum aspartate transaminase (AST) were measured in 181 serology positive patients. A hundred and sixty (88%) patients showed elevated liver enzymes with 122 of them having a two-fold increase (Table 2). Normalization of ALT and AST took about 6 weeks after defervescence.

The tourniquet test was positive in 146 (36%) patients in the series. Similarly, blood cultures were done in 40 patients who had high fever and a WBC count below 3 x 10^9/l and all

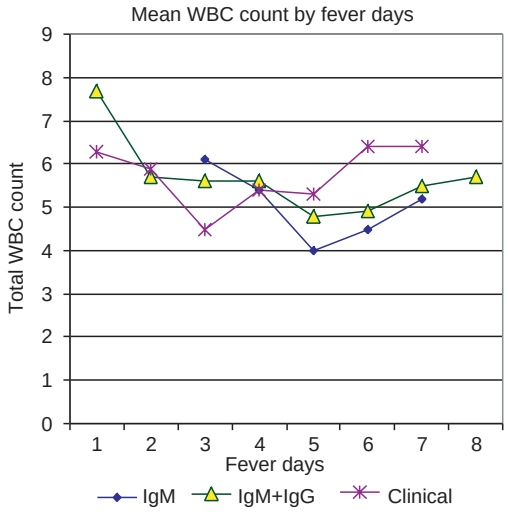

Fig 1–Mean total white count (WBC) x 10^9/l from the first day of fever (normal WBC 4-11 x 10^9/l).

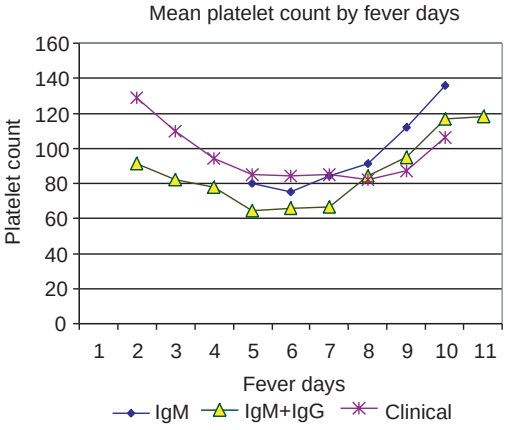

Fig 2–Distribution of mean platelet counts from the first day of fever (normal count 150-400 x 10^9/l)

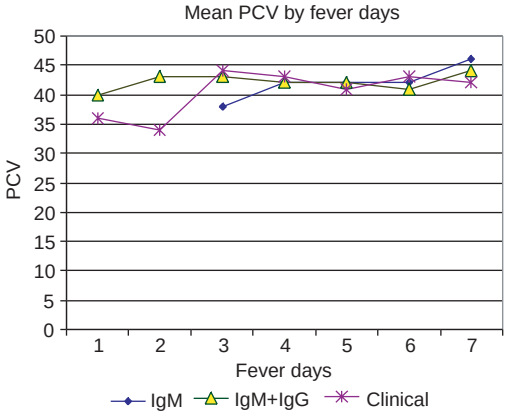

Fig 3–Distribution of mean packed cell volume (PCV) from the first day of the fever.

of them failed to give a positive result.

Complications and outcome (Table 3)

Three patients died, with a case fatality rate of 0.7%. Effusions (pleural and peritoneal) and myocarditis were the commonest complications; 2 patients had convulsions. These two patients had paroxysmal generalized spike-wave discharges on EEG, which disappeared two weeks after recovery. One of them had an alteration in the level of consciousness. One patient went into hepatic encephalopathy and improved after treatment. Dengue shock syndrome (DSS) was a rarity, except in one patient who was admitted in a state of shock and died within few hours despite resuscitation. A middle aged patient died due to sudden cardiac arrest. He had ECG evidence of acute myocardits. An adolescent girl developed acute respiratory distress (ARDS) with severe hypoxemia, died despite being on positive pressure ventilation. Congested solid lungs were found on autopsy and histology confirmed ARDS.

DISCUSSION

Dengue infection in Sri Lanka has had two phases. Before 1989, it was characterized by frequent transmission of all four serotypes but with a low incidence of dengue hemorrhagic fever (DHF). The emergence of DHF has been dramatic and persistent and it has become a major health issue in the country (Messer, 2002). There were 4,303 cases of dengue infection reported in Sri Lanka for the year 2001; more than 50% of them were in the adult age group (Ministry of Health, 2002). Hence, the current study in the adult population in Sri Lanka. We experienced difficulties in applying the WHO management guidelines to the adult age group.

Seasonal distribution showed a regular incidence of cases throughout the year which was highest during the rainy months. Persistence of viral transmission and inadequate control of the vector must be the reason. We used single sample serology (ELISA) on day 7 to classify the cases. Accordingly, 43 (20%) patients were positive for IgM, had primary infection, and the others [IgG 28 (13%) and IgM + IgG 140 (66%)] had secondary dengue infection. A study done in Co-

lombo showed a rate of primary infection of 30% in 1980 which increased to 62% in 1998 (Messer, 2002). Similarly, a study done in Thailand showed primary dengue rates of 29% and 13% among DF and DHF patients respectively (Krishnamuti *et al*, 2001). There were instances where the primary infection resulted in severe complications, such as shock syndrome (Scott *et al*, 1976; Kuberski *et al*, 1977). Similarly, we found significant thrombocytopenia and bleeding tendencies in the IgM group in the current study.

Most of the data in the serology groups and clinical groups had similar patterns (Table 1, Figs 1-3). Hence, early diagnosis of dengue infection based on clinical features and basic laboratory investigations is a reasonable method. The mean age of the study population and the individual groups were about 30 years, suggestive of a predilection for young adults to infection. Even though the mean duration of fever was about 7 days, it can last up to 19 days, suggesting a protracted nature. Generalized erythematous skin flush blanching with pressure was very characteristic, more than discrete rashes. However, in dark skinned patients the flush was not very obvious except in the lips, palms, soles and conjunctival. The recovery of skin flush took time and was usually associated with pruritus.

The white blood cell count (WBC) showed a significant leukopenia starting from the second day of illness with a nadir on the fifth day, irrespective of the serology (Fig 1). A question arose as to whether patients with severe leukopenia can contract secondary bacterial infections, leading to septicemia. This was excluded by blood cultures which gave negative results. However, clinically ill patients with respiratory signs and leukopenia were treated with broad-spectrum antibiotics. Thrombocytopenia was detected from the 2nd day of fever with nadir on the 5th to 7th days and normalization took about 14 days. This was followed by reciprocal thrombocytosis lasting about five days. The platelet, WBC and neutrophils were significantly lower in children with early dengue infection compared to other fevers in a study done in Thailand (Kalayanarooj *et al*, 1997). Hence, these parameters can be taken as markers for early diagnosis and follow-up of dengue infection. The tourniquet test is a simple clinical procedure that reflects vascular integrity has been found to be positive in a fewer number of patients. It has yielded positive results in children and has been taken as a parameter in the WHO severity classification (Kalayanarooj *et al*, 1997; WHO,1999; Krishnamuti *et al*, 2001).

Hematocrit or packed cell volume (PCV) has been given greater importance in the early detection and management of DHF and dengue shock syndrome (Kalayanarooj *et al*, 1997; WHO, 1998, 1999). In the current study, PCV showed minimum fluctuation in all the groups of patients. Hence, we were not able to classify the series according to the WHO grading, such as DF and DHF 1-4. Interestingly, patients who developed effusions did not show a rise in PCV. However, we observed a rising PCV towards the 7th day of illness that was the time of defervescence in all the dengue groups.

A significant elevation of liver enzymes (ALT and AST) was seen more in the secondary dengue group (Table 2). We were not able to correlate it with the severity of infection, however, it was higher in children who developed DHF in Thailand (Kalayanarooj *et al*, 1997). Furthermore, hepatic involvement has been well accepted in dengue and the serum albumin level is considered as a prognostic marker (Nimmannitya *et al*, 1987; Jose *et al*, 1998).

In the current series one death occurred due to over-enthusiastic management with overloading of intravenous fluid, plasma and platelet transfusion leading to pulmonary edema and ARDS. Myocarditis led to hypotension and circulatory failure in five patients. They all recovered, except for one patient who died due to sudden cardiac arrest. This scenario might be misinterpreted as DSS and excess fluid may have been given producing fatal acute heart failure. Cerebral involvement in dengue was not uncommon. In a series of 27 children in Thailand who had encephalopathy, the mortality rate was 22% (Cam *et al*, 2001). Two patients in the current study probably had encephalopathy. Two patients developed acute renal failure whilst having normal blood pressure and peripheral perfusion due to unknown causes. There was a report of acute renal failure in a patient with den-

gue where myoglobinuria was implicated as the cause (Gunasekara *at al*, 2000). Jaundice was not a feature despite elevated liver enzymes, but 2 patients developed icterus and hepatic failure leading to encephalopathy towards defervescence.

We presented a multifaceted picture of adult dengue infection in Sri Lanka. Early diagnosis is important for close monitoring of patients. The manifestations, such as thrombocytopenia and leukopenia developed early in the infection and elevation of liver enzymes was common. Some features of the clinical picture, such as erythematous flush, were present, though there is overlap with the symptoms and signs of other infections in the region. A summation of all these markers would be a help to make an early diagnosis of dengue infection. This study highlighted the changing severity of infections complications, and factors contributing to the deaths in adults. Clinical studies of this nature should go in parallel with molecular genetic studies of dengue viruses in Sri Lanka.

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