Original Article

Dengue:A Clinicohaematological Profile

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Abstract

Background: Fifty cases of fever, clinically suspected to be dengue were studied.
Methods: Complete clinical, haematological evaluation and IgM capture assay was done.
Result: 54% of patients clinically suspected to have dengue were positive for IgM antibodies by enzyme-linked immunosorbent assay (ELISA). The commonest clinical feature was fever with rash (85%). Thrombocytopenia was seen in 19 % of patients only. One patient died of dengue shock syndrome (DSS).
Conclusion: Out of the 27 cases of seropositive dengue there was one death due to dengue shock syndrome. Thrombocytopenia may not always be a feature of dengue.

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Key Words : Dengue haemorrhagic fever; Dengue shock syndrome; Thrombocytopenia

Introduction

Dengue fever is caused by a positive single stranded RNA virus of the flaviviridae family. The World Health Organization estimates that 50 to 100 million cases of dengue infection occur each year [1-3]. More than three lakh cases of dengue haemorrhagic fever are diagnosed each year. Dengue causes a fatality of 24,000 deaths per year. Dengue virus infection is transmitted from the bite of *Aedes aegypti* and *Aedes albopictus* mosquitoes [4-5]. We studied fifty cases of suspected dengue to correlate the clinical, haematological and serological features of the disease.

Material and Methods

Fifty clinically suspected cases of dengue were studied in a tertiary care hospital. None had any localizing signs. Patients of fever with slide positive malaria or immunochromatographic positivity for malaria, urinary tract infection, enteric fever, liver abscesses, pneumonitis were excluded. Case definition criteria for dengue fever were fever with rash, retro orbital headache, conjunctival congestion and myalgia. The criteria for dengue haemorrhagic fever (DHF) included a triad of haemorrhagic manifestations, platelet count of < 1, 00,000/ cmm and clinical signs of plasma leakage observed in the form of pleural effusion, ascites or hypoproteinemia. The case definition criteria for dengue shock syndrome (DSS) included features of shock in the form of rapid weak pulse and profound hypotension with systolic pressure of less than 90 mm Hg. The age group of patients was in the range of 4 - 62 years, of which 13 were females and 37 males. Clinical evaluation was done as per protocol given in Table 1. Haematological examination included haemoglobin, total leucocyte count, differential leucocyte count and platelet count assessment. Platelet count was done by sysmex KX-21 and also evaluated on peripheral blood smear simultaneously.

All the cases were serologically evaluated for dengue by IgM capture enzyme-linked immunosorbent assay (ELISA) manufactured by Panbio diagnostics. The standard protocol of collection of serum and storing at -20°C was adopted. Serum was collected from patients between three to five days of the onset of fever. The cut off value was obtained by calculating the average absorbance of triplicates of the cut off calibrator. An index value of each of the samples was calculated by dividing the sample absorbance by cut off value.

The interpretation of the ELISA was as follows :

Index value of sample	Result
< 0.9	Negative
0.9-1.1	Equivocal
> 1.1	Positive

The serological sensitivity of the kit for primary infection was 85.4- 98.9% and specificity was 95.7- 100%.

Results

The clinical and haematological features observed are in Tables 1-5. The platelet count ranged between 40,000 – 1,00,000/mm^3 in the patients with thrombocytopenia. None had any bleeding manifestations. The patient with dengue shock syndrome had a normal platelet count throughout the course of his illness. Three (11%) patients had normocytic normochromic anaemia with haemoglobin ranging between 6.5-9.5 g/dl. None of the patients had lymphocytosis or leucopenia.

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Table 1
Clinical profile

Clinical features	IgM +ve (n=27)	IgM-ve (n=23)	OR* (95% CI)
Fever with rash	23 (85%)	12 (52%)	5.27
Hepatomegaly	04 (15%)	08 (35%)	0.32
Hepatosplenomegaly	02 (7%)	01 (4%)	1.76
Conjunctival congestion	10 (37%)	10 (43%)	0.76
Fever > 7 days	10 (37%)	15 (65%)	0.31
Fever > 103°F	12 (44%)	11 (48%)	0.87
Retro orbital headache	17 (63%)	03 (13%)	11.3
Myalgia	22 (81%)	08 (35%)	8

*OR - Odds Ratio

Table 2
Haemoglobin

Haemoglobin g/dl	IgM +ve (n=27)	IgM-ve (n=23)	OR
6.5-9.5	03 (11%)	0	-
9.5-11.5	11 (41%)	11 (48 %)	0.75
11.5-14.0	13 (48%)	12 (52%)	0.85

Analysis of the serological results showed that 27 (54%) patients tested positive for IgM ELISA.

Discussion

Dengue is caused by a virus belonging to the flaviviridae family (single stranded, positive, nonsegmented RNA virus). It has four distinct serotypes DEN 1,DEN 2,DEN 3 and DEN 4 [6]. Infection with one serotype confers immunity to only that serotype and hence a person may be infected upto four times [7]. Humans are the main reservoir of dengue virus [8]. Dengue presents as dengue fever, dengue haemorrhagic fever or dengue shock syndrome.

Children are at a higher risk of DHF than adults. Studies have shown that age-specific DHF incidence was bimodal, with severe cases peaking at seven months of age and again at three to five years of age [7]. DHF or DSS occurred in infants who acquired maternal dengue antibody and subsequently experienced a dengue infection. In general, children less than one year of age were hospitalized almost exclusively during primary dengue infections. These infants were born to dengue immune mothers [9]. On the other hand, children three to five years of age have DHF during a secondary infection.

It has been suggested that baseline microvascular permeability in children is greater than that of adults and this could partly explain, why DHF is more frequentl in children [10]. In our study, none of the children had DHF/DSS. Two children had atypical features in the form of dengue encephalitis and dengue myositis.

In the present study there was one case of dengue

Table 3
Total leucocyte count

Total leucocyte count	IgM+ve (n=27)	IgM-ve (n=23)	OR
6.0- 10.0 x 10^3	22 (81%)	12 (52%)	4.0
4.4- 6.0 x 10^3	05 (19 %)	11 (48%)	0.24

Table 4
Percentage of lymphocytes

Lymphocytes (%)	IgM+ve (n=27)	IgM-ve (n=23)	OR
30-45	14 (52%)	12 (52%)	0.98
20-30	13 (48%)	11 (48%)	1.01

Table 5
Platelet profile

Platelet count	IgM+ve (n=27)	IgM-ve (n=23)
40,000-1,00,000	5 (19%)	0
1,50,000-3,00,000	22 (81%)	23

shock syndrome who succumbed to his illness. He was a 38 year old male who presented with history of fever of eight days duration, body ache, subconjunctival haemorrhage, erythematous rash and hypotension. The patient had normal haematological and biochemical parameters. He was positive for dengue IgM antibodies. The patient had upper gastrointestinal bleed inspite of a normal platelet count. This can be explained by the fact that haemorrhage is due to secondary infection with another serotype [11]. Cross reactive anti dengue antibodies from the previous infection bind to the new infecting serotype and enhance viral uptake by monocytes and macrophages. This antibody dependant mechanism results in an amplified cascade of cytokines and complement activation causing endothelial dysfunction and consumption of coagulation factors leading to plasma leakage and haemorrhagic manifestations. The severity of the disease depends on the strain and serotype of the virus, age of the patient and degree of viremia. IgG ELISA assay was not done hence cross reactivity could not be proved in this case.

It has been demonstrated that memory dengue T lymphocyte response after a primary infection includes both serotype-specific and serotype-cross reactive T lymphocytes [12]. NS3 protein seems to be the major target for CD4+ and CD8+ T cells, although some T cell epitopes have been recognized in other proteins such as envelope and capsid [13,14].The magnitude of proliferation to heterologous dengue serotypes is variable depending on different factors such as the serotype causing the primary infection and the ethnicity of the individual [15]. These findings support the possibility that

during a secondary infection T cells become activated due to interactions with infected monocytes. Recent observations suggest a massive T-cell activation during DHF, which could partly explain the mechanism of plasma leakage through cytokine production and infected cell lysis by CD4+ and CD8+ dengue-specific T lymphocyte. Cytokines could be released either directly from monocytes/macrophages as a result of infection or after interactions between infected and immune cells or both [15,16]. Cytokines that may induce plasma leakage such as interferon γ, interleukin (IL) 2 and tumor necrosis factor TNF-α are increased in DHF cases [16, 17]. Interferon γ enhances uptake of dengue particles by target cells through increasing Fc cell receptors[17]. Other cytokines such as IL-6, IL-8 and IL-10 are also increased. A protein of 22–25 kDa responsible for increased capillary permeability has been detected in sera of DHF patients [18]. Besides secondary infection, chronic diseases such as bronchial asthma and diabetes predispose to a higher risk of developing DHF. Dengue 2 virus is known to replicate to higher concentration in the blood cells of whites [19].

The most common clinical feature of dengue in our study was fever with rash seen in 85% of patients. The rash was typically macular or maculopapular, often becoming confluent and sparing small islands of normal skin. The rash was not associated with scaling or pruritus [20]. Pervin et al [21], reported occurrence of rash in 33 % of patients. Hepatomegaly was observed in 15% of our patients. Hepatomegaly is more common in patients with secondary infection and some of these may be associated with a increase in liver transaminases. Myalgia was observed in 22 (81%) of patients. Pervin et al [21], reported myalgia in 84.5% of patients.

Normocytic normochromic anaemia (Hb 6.5-9.5 g/dl) was observed in 11% patients. One patient with Hb of 6.5 g/dl also had a concomitant infection with MT malaria. The other two patients were females with Hb of 9.0 & 9.5g/dl, due to nutritional deficiency.

Thrombocytopenia (platelets <1,00,000/ cmm) was seen in 19% of patients. The platelet count in these patients ranged between 44,000 – 1, 00,000/cmm. None had any bleeding manifestations. Platelet count was evaluated by Sysmex KX-21 as well as on peripheral blood smear. The counter gives a false low reading when large platelets are present. Such cases were obviated by assessment of platelets on smear. Ratagiri et al [22], reported thrombocytopenia in 82%, DHF in 60 %, DSS in 22% and DF in 18% of patients. Our study on the other hand reflected DF in 96% and DSS in 4% patients. This can be explained by the fact that these patients probably had primary infection with a serotype other than the one mentioned in the preceding study.

Leucopenia was observed in 26% of patients by Ratagiri et al [22]. Leucopenia was not observed in our study. This can be explained by the fact that infection in our study was caused by a less virulent serotype.

Development of antibodies potentially cross-reactive to plasminogen (due to a similarity in 20 aminoacid sequence of dengue E glycoprotein and a family of clotting factors) could have a role in causing haemorrhage in DHF [23]. The increased destruction or decreased production of platelets could result in thrombocytopenia. Virus-antibody complexes have been detected on the platelet surface of DHF patients suggesting a role for immune-mediated destruction of platelets [24]. The release of high levels of platelet-activating factor by monocytes with heterologous secondary infection may explain the haemorrhage, given that platelet-activating factor may induce platelet consumption and augment adhesiveness of vascular endothelial cells resulting in thrombocytopenia [25]. The presence of IgM antibodies in the sera of DHF cases that cross-reacted with platelets has been demonstrated [26]. These autoantibodies could be involved in the pathogenesis of the disease.

IgG and IgM antibodies assay by ELISA is the commonest diagnostic test. The test based on an increase in the Ig G titre by a factor of four is difficult in routine clinical care because a second blood sample is required at the convalescent stage. Cross reactions with other flaviviruses interfere with serologic testing, particularly the ELISA for IgG and this affects the interpretation of test results in travellers exposed to other flavivirus infections, including those previously vaccinated against flavivirus infections, such as yellow fever and Japanese encephalitis [27]. Rheumatoid factor may lead to an IgM capture assay that is false positive for dengue and like many other flavivirus infections (albeit lesser than with dengue IgG assays) [28] .

Primary infections are characterized by an increase in dengue-specific IgM antibodies four to five days after the onset of fever and by an increase in IgG antibodies only after seven to ten days. IgM antibodies are detectable for three to six months, whereas IgG antibodies remain detectable for life. In secondary infections, the level of IgM antibodies is lower than in primary infections and the antibodies are sometimes absent, whereas levels of IgG antibodies rise rapidly in secondary infections, even during the acute phase. Thus, the presence of high titers of IgG early in the course of the disease is a criterion for secondary infection. The sensitivity of IgM ELISA ranges from 90- 97% as compared with the gold standard haemagglutination-inhibition test. Some false positive reactions can be observed in less than 2% of cases and a low or negative IgM reaction in secondary infections.

This study shows that DSS is an uncommon manifestation of dengue virus infection. Dengue virus infection is generally self limiting. Thrombocytopenia is seen in approximately 19 % of patients. Patients with bleeding manifestations may have a normal platelet count. Leucopenia and relative lymphocytosis as a feature of dengue infection was not seen in our study. The non specific presentation underscores the importance of laboratory testing and a high index of suspicion to reduce the morbidity and mortality due to this disease.

Conflicts of Interest

None identified

Intellectual Contribution of Author

Study Concept : Lt Col M Banerjee
Drafting & Manuscript Revision : Lt Col M Banerjee, Lt Col T Chatterjee
Study Supervision : Col V Srinivas, Brig VK Kataria, Lt Col GS Choudhary

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