DENGUE: A CLINICO-PATHOLOGICAL STUDY OF 50 CASES

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ABSTRACT: Hundred cases of fever, clinically suspected to be dengue were studied. Complete clinical, laboratory evaluation and immunochromatographic test for NS1, IgM and IgG was done. Fifty percent of patients were positive for either NS1, IgM or IgG. Seropositive dengue cases were correlated with hematological and biochemical findings. The commonest clinical feature was high fever. Leucopenia and thrombocytopenia were most common hematological features. **CONCLUSION:** Out of 50 cases of seropositive dengue there were two deaths due to dengue shock syndrome. Leucopenia and thrombocytopenia were almost always a feature of dengue.

KEYWORDS: Dengue hemorrhagic fever, Dengue shock syndrome, Leucopenia, Thrombocytopenia.

INTRODUCTION: Dengue fever is caused by single stranded RNA virus of flavi viridae family. The World Health Organization estimates that 50 to 100 million cases of dengue infection occur each year.^{1,2,3} More than three lakh cases of dengue hemorrhagic fever are diagnosed each year. Dengue causes a fatality of 24,000 deaths per year. Dengue virus infection is transmitted by the bite of *Aedes aegypti* and *Aedes albopictus* mosquitoes.^{4,5} We studied hundred cases of suspected dengue to correlate clinical, hematological, biochemical and serological features of disease.

MATERIALS AND METHODS: Hundred clinically suspected cases of dengue were studied. None had any localizing signs. Patients of fever with slide positive malaria or immunochromatographic positivity for malaria, enteric fever, urinary tract infection, pneumonitis were excluded. Case definition criteria for dengue fever were high fever, fever with rash, retro orbital pain, myalgia, arthralgia, and conjunctival congestion. The criteria for dengue hemorrhagic fever(DHA) included a triad of hemorrhagic manifestations, platelet count of less than 1.0 lakh/cumm and clinical signs of plasma leakage observed in the form of pleural effusion or ascites. 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. Out of 50 cases seropositive for dengue 26(52%) were positive for NS1, 21(42%)for IgM and 3(6%) for IgG. Serological test sensitivity was 95-98.9% & specificity was 96.2-100%. The age group of patients was in the range of 2-56 years, of which 17 were females and 33 were males. Clinical evaluation was done as per protocol given in Table 1.

Hematological examination included complete hemogram with Sysmex KX21 and peripheral blood was evaluated. Hemoconcentration was seen as raised hemoglobin or RBC count. Leucopenia was defined as less than 4000/cumm and Thrombocytopenia as less than 1.0 lakh/cumm. Liver function tests were done as a part of fever profile.

RESULTS: The clinical, hematological and biochemical features observed are as in Tables 1 to 6.

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Clinical feature	NS1 (n=26)	IgM (n=21)	IgG (n=03)
High Fever	20(76%)	19(90%)	2(65%)
Fever with rash	10(40%)	12(56%)	0(0)
Hepatomegaly	08(32%)	10(47%)	2(65%)
Hepatosplenomegaly	02(8%)	03(14%)	1(35%)
Myalgia	18(72%)	16(75%)	2(65%)
Arthralgia	08(32%)	05(23%)	1(35%)
Fever 7 days	05(20%)	04(18%)	2(65%)
Conjunctival congestion	10(40%)	09(42%)	2(65%)
Retro- orbital pain	17(68%)	15(70%)	2(65%)

Table 1: Showing clinical features

Hemoglobin(g /dl)	NS1 (n=26)	IgM (n=21)	IgG (n=03)
9.0-11.5	3(11%)	4(19%)	00(0)
11.5-14.0	10(38%)	08(39%)	02(65%)
14.0-18.0	13(50%)	09(42%)	01(35%)

Table 2: Showing Hemoconcentration in Dengue fever

Total leucocyte count/cumm	NS1 (n=26)	IgM (n=21)	IgG (n=03)
6-10 x 10 ³	3(11%)	02(10%)	1(35%)
4-6 x 10 ³	07(27%)	05(23%)	1(35%)
< 4 x 10 ³	16(62%)	14(66%)	1(35%)

Table 3: Showing Leucopenia in majority of patients

Platelet count	NS1 (n=26)	IgM (n=21)	IgG (n=03)
20000 - 1.0 lakh	21(80%)	14(67%)	02(65%)
1.0 – 1.5 lakh	04(15%)	03(14%)	01(35%)
>1.5 lakh	01(5%)	04(19%)	00(0)

Table 4: Showing Thrombocytopenia in majority of patients

	NS1 (n=26)	IgM (n=21)	IgG (n=03)
AST elevated	20(76%)	12(57%)	03(100%)
AST normal	06(24%)	09(43%)	00(0)

Table 5: Showing aspartate aminotransferase levels

	NS1 (n=26)	IgM (n=21)	IgG (n=03)
ALT elevated	15(58%)	11(53%)	03(100%)
ALT normal	11(42%)	10(47%)	00(0)

Table 6: Showing alanine aminotransferase levels

Hemoconcentration, Leucopenia, and Thrombocytopenia were chief hematological features of seropositive dengue cases. Peripheral blood smear showed plasmacytoid lymphocytes with eccentric nuclei and deep blue cytoplasm. Aspartate aminotransferase and alanine aminotransferase were mildly elevated in significant number of cases. None had any bleeding manifestations. The 2 patients with dengue shock syndrome had the above features throughout the course of their illness, and death occurred due to dengue shock syndrome(DSS).

DISCUSSION: Dengue is caused by a virus belonging to the flavi viridae 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 (DHF) or dengue shock syndrome (DSS).

Children are at high 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 two 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 frequent 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 were two cases of dengue shock syndrome who succumbed to their illness. They were 21 and 38 year old male patients who presented with history of fever of eight days duration, body ache, subconjunctival haemorrhage, erythematous rash and hypotension. The patients had leucopenia,thrombocytopenia and raised AST,ALT levels.

They were positive for dengue IgM antibodies. The patient had upper gastrointestinal bleed. This can be explained by the fact that hemorrhage is due to secondary infection with another serotype [11]. Cross reactive anti dengue antibodies from 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.

The most common clinical feature of dengue in our study was high fever of patients. The fever 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 more than 30% 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 72% of patients. Pervin et al [21], reported myalgia in 84.5% of patients. Hemoconcentration was seen in more than 50% of patients.

One patient with Hb of 9.5 g/dl also had a concomitant infection with MT malaria. The other two patients were females with Hb of 9.0 & 9.5 g/dl, due to nutritional deficiency.

Thrombocytopenia (platelets < 1,00,000/cmm) was seen in 80% of patients. The platelet count in these patients ranged between 4,000-1,00,000/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 observed in more than 60% of patients in our study.

Development of antibodies potentially cross-reactive to plasminogen (due to a similarity in 20 amino acid 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 DHF cases that cross-reacted with platelets has been demonstrated [26]. These autoantibodies could be involved in the pathogenesis of the disease. IgM and IgG antibodies assay by ELISA is the commonest diagnostic test. The test based on an increase in the IgG 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 characterised by an increase in dengue-specific NS1 antigen and 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 1gG early in the course of the disease is a criterion for secondary infection. The sensitivity of 1gM 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 infection is generally self limiting. Patients with bleeding manifestations usually have decreased platelet count and leucocyte count.

Hemoconcentration, leucopenia, thrombocytopenia, raised AST, raised ALT and plasmacytoid lymphocytes in peripheral blood smear shall give enough clue to test for Dengue serology so as to reduce the morbidity and mortality due to this disease.

REFERENCES:

1. World Health Organization: Dengue Haemorrhagic Fever: Diagnosis, treatment, prevention and control, 2nd edn. Geneva: WHO 1997.
2. Monath TP. Dengue: The risk to developed and developing countries. *Proct Natl acad Sci USA* 1994;91:2395-400.
3. Gubler DJ. Dengue and Dengue Haemorrhagic Fever. *Clin Microbiol Rev* 1998; 11:480-96.
4. Gubler DJ, Meltzer M. Impact of Dengue and Dengue Haemorrhagic Fever on the developing world. *Adv Virus Research* 1999; 53:35-70.
5. Platt KB, Linthicum KJ, Myint KS, Innis BL, Lerdthusnee K, Vaughn DW. Impact of dengue virus infection on feeding behavior of *Aedes aegypti*. *Am J Trop Med Hyg* 1997; 57:119-25.
6. Gubler DJ. The global emergence/resurgence of Arboviral diseases as public health problems. *Arch Med Res* 2002;33:30-42.
7. Guzman MG, Kouri G. Dengue: An update. *Lancet Infect Dis* 2002;2:33-42.
8. Gibbons RV, Vaughn DW. Dengue: An escalating problem. *BMJ* 2002;324:1563-6.
9. Kliks SC, Nimmanitya S, Nisalak A, Burke DS. Evidence that maternal Dengue antibodies are important in the development of Dengue haemorrhagic fever in adults. *Am J Trop Med Hyg* 1988; 38:411-9.
10. Gamble J, Bethell D, Day NPJ, et al. Age-related changes in microvascular permeability: A significant factor in the susceptibility of children to shock? *Clinical Science* 2000; 98:211-6.
11. Gubler DJ. The global pandemic of dengue/dengue haemorrhagic fever: current status and prospects for the future. *Ann Acad Med Singapore* 1998; 27:227-34.
12. Kurane I, Ennis FA. Cytokines in dengue virus infections : Role of cytokines in the pathogenesis of dengue hemorrhagic fever. *Semin Virology* 1994; 5:443-8.
13. Kurane I, Zeng L, Brinton MA, Ennis FA. Definition of an epitope on NS3 recognized by human CD4+ cytotoxic T lymphocyte clones cross-reactive for dengue types 2, 3 and 4. *Virology* 1998; 240:169-74.
14. Spaulding AC, Kurane I, Ennis FA, Rothman AL. Analysis of murine CD8+ T-cell clones specific for the dengue virus NS3 protein: flavivirus cross-reactivity and influence of infecting serotype. *J Virol* 1999; 73:398-403.

15. Rothman AL, Ennis FA. Toga/Flaviviruses: immunopathology In: Cunningham MW, Fujinami RS, Editors. Effects of microbes on the immune system. Philadelphia: Lippincott Williams & Wilkins, 1999; 473-87.
16. Hober D, Nguyen TL, Shen L, et al. Tumor necrosis factor alpha levels in plasma and whole-blood culture in dengue infected patients: relationship between virus detection and pre-existing specific antibodies. J Med Virol 1998; 54:210-8.
17. Kontny U, Kurane I, Ennis FA. Gamma interferon augments FC receptor – mediated dengue virus infection of human monocytic cells. J Virol 1988; 62:3928-33
18. Mukerjee R, Chatuverdi UC, Dhawan R. Dengue virus-induced human cytotoxic factor: Production by peripheral blood leucocytes in vitro. Clin Exp Immunol 1995; 102:262-7.
19. Guzman MG, Alvarez M, Rodriguez R, et al. Fatal dengue haemorrhagic fever in Cuba, 1997, Int J Infect Dis 1999; 3:130-5.
20. Waterman SH, Gubla DJ. Dengue fever. Clin Dermatol 1989; 7:117-22.
21. Pervin M., Tabassum S, Ali M, Kazi M. Dengue Bulletin 2004; 28.
22. Rategiri h, Shepur TA, Wari Pk, Chavan SC. Clinical profile and outcome of dengue fever cases. Ind J of Paediatrics 2005; 72:705-6.
23. Chungue E, Poli L, Roche C, Gestas P, Glaziou P, Markoff LJ. Correlation between detection of plasminogen cross-reactive antibodies and hemorrhage in dengue virus infection. J Infect Dis 1994; 170:1304-7.
24. Wang S, He R, Patarapotikul J, Innis BL, Anderson R. Antibody-enhanced binding of dengue – 2 virus to human platelets. Virology 1995; 213:254-7.
25. Yang KD, Wang CL, Shaio MF. Production of cytokines and platelet activating factor in secondary dengue virus infections. J Infect Dis 1995; 172:604.
26. Lin CF, Lei HY, Liu CC, et al. Generation of IgM anti-platelet autoantibody in dengue patients. J Med Virol 2001; 63:143-9.
27. Schwartz E, Mileguir F, Grossman Z, Mendelson E. Evaluation of ELISA based serodiagnosis of Dengue fever in travelers. J Clin Virol 2000; 19:169-73.
28. Jelinek T, Wasthuber J, Proll S, Schtatterkcher m, Loscher T. Influence of Rheumatoid factor on the specificity of a rapid immunochromatographic test for diagnosing Dengue infection. Euro J Clin Microbiol Infect Dis 2000; 19:555-6.

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