

Clinicopathological study of 50 cases of Dengue

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Abstract

Aim and objective: The objective of this study was to correlate laboratory tests during the evolution of dengue fever, comparing frequencies between the different clinical forms in order to use test results to predict the severity of the disease. **Methods:** This is an observational, descriptive and retrospective study of 50 patients with clinical and serological diagnoses of dengue fever. The tests analyzed were blood count, platelet count, and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations. **Results:** Thrombocytopenia and elevated transaminases were observed in patients with classic dengue fever. The main laboratory abnormalities found in dengue hemorrhagic fever were thrombocytopenia, hemoconcentration and elevated transaminases, similar to severe dengue with the exception of hemoconcentration. Most laboratory abnormalities started on the 3rd day but were more evident on the 5th day with restoration of values by the 11th day; this was more prominent in under 15-year-olds and with the more severe clinical forms. **Conclusion:** These results are relevant in assessing the disease because they can be used as markers for more severe forms and can help by enabling the adaptation of the therapeutic conduct to the needs of individual patients.

Keywords: Dengue Hemorrhagic Fever, Dengue Shock Syndrome, Leucopenia, Thrombocytopenia

Introduction

Dengue is caused by one of the four serotypes of the dengue virus (DEN-1, DEN-2, DEN-3 and DEN-4) also referred to as an arbovirus (arthropod-borne viruses) that belongs to the genus *Flavivirus* of the family *Flaviviridae*. It is a disease with a wide clinical spectrum and a wide variety of presentations, ranging from asymptomatic to an undifferentiated fever (viral syndrome) to the more severe forms such as severe dengue (SD) or Dengue hemorrhagic fever (DHF).

Transmission to humans occurs by the bite of the female *Aedes aegypti* mosquito infected by one of four serotypes of the virus. The period of transmission from humans to mosquitoes begins one day before the start of fever up to the sixth day of illness corresponding to the viremia phase. In humans, the incubation period ranges from 3 to 15 days (intrinsic incubation) with an average of 5 days. The diagnosis of

dengue fever is carried out based on clinical, epidemiological and laboratory data.

Leucopenia is the most prominent hematological change, sometimes with counts of less than $2 \times 10^3/\mu\text{L}$. However, there are reports of mild leukocytosis at the onset of the disease, with neutrophilia. Lymphocytosis is a common finding, with the presence of atypical lymphocytes.

Of biochemical variables, the most frequent changes occur in liver function tests such as in serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), Gamma-glutamyl transpeptidase and alkaline phosphatase levels, and serum albumin concentrations.

In this context, the present study aimed to assess the biochemical and hematological dynamics of patients with dengue fever in order to increase the sensitivity of the screening by healthcare professionals in the most

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serious cases and to try to identify laboratory markers that may indicate this evolution.

Materials and Methods

Fifty clinically suspected cases of dengue were studied retrospectively. This case series was conducted in Bharati Medical college Sangli And Vaishnavi pathology laboratory Sangli, Maharashtra.

Inclusion criteria: All patients presenting with fever of less than 2 weeks duration, body rashes, flushing and bleeding manifestations were included.

Exclusion criteria: Patients with fever of more than 2 weeks duration, haematological disorders and malignancies, liver failure, peptic ulcer, chronic splenomegaly syndrome, enteric fever and autoimmune disorders, positive for malaria were excluded.

This is an observational, descriptive and retrospective study of 50 patients with clinical and serological diagnoses of dengue fever. The tests analyzed were

Results

Out of 50 cases seropositive for dengue 27(54%) were positive for NS1, 19 (38 %) for IgM and 4(8%) for IgG. Serological test sensitivity was 96-98.9% & specificity was 96.2-100%. Out of 50 patients 20 were females and 30 were males.

The clinical, hematological and biochemical features observed are as in Tables 1 to 7

Table 1 : Clinical features in Dengue fever

Clinical Features	NS1 N = 27	IgM N = 19	IgG N= 4
High Grade Fever	22 (81 %)	16 (83.2 %)	02 (50 %)
Fever With rash	13 (40.3 %)	10 (52 %)	00 (00)
Myalgia	19(70.3 %)	14 (72.8 %)	02 (50 %)
Arthralgia	10 (37 %)	04 (20.8 %)	01 (25 %)
Conjunctival congestion	11 (40.7 %)	08 (41.6 %)	01 (25 %)
Hepatomegaly	05 (18.5 %)	08 (41.6 %)	02 (50 %)
hepatosplenomegaly	02 (7.4 %)	02 (10.4 %)	01 (25 %)
Nausea	12 (44.4 %)	10 (50.2 %)	02 (50 %)
Vomiting	07 (25.9 %)	05 (26 %)	00 (00 %)
Headache	15 (55.5 %)	12 (62.4 %)	03 (75 %)
Swollen lymphnodes	10 (37 %)	07 (36.4 %)	01 (25 %)
Retroorbital pain	15 (55.5 %)	09 (46.8 %)	02 (50 %)

blood count, platelet count, and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations.

Fifty clinically suspected cases of dengue were studied. Case definition criteria for dengue fever were high fever, fever with rash, retro orbital pain, myalgia, arthralgia, and conjunctival congestion [1]. The criteria for dengue hemorrhagic fever (DHA)[1] 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.

Hematological examination included complete hemogram with Nihon Kohden 5 part differential cell counter and peripheral blood was evaluated. Hemoconcentration was seen as raised hemoglobin or RBC count. Leucopenia was defined as total WBC count less than 4000/cumm and thrombocytopenia as less than 1.0 lakh/cumm. Liver function tests were done on fully automated biochemistry analyzer.

Table 2 : Haemoglobin in Dengue fever

Haemoglobin	NS1 N = 27	IgM N = 19	IgG N= 4
9.0 -11.0	4 (14. 8 %)	05 (26 %)	00 (00 %)
11.0 -13.0	11 (40 .7 %)	07 (36.4 %)	02 (50 %)
13.0 -16.0	12 (44 .4 %)	07 36.4 %)	02 (50 %)

Table 3: Total leucocyte count in Dengue Fever

Total Leucocyte count	NS1 N = 27	IgM N = 19	IgG N= 4
7000- 10000	02 (7.4 %)	02 (10.4 %)	00 (00 %)
4000 – 7000	07 (25.9 %)	06 (31.2 %)	02 (50 %)
Less than 4000	18 (66.6 %)	11 (57.2 %)	02 (50 %)

Table 4: Platelet count in Dengue Fever

Platelet count	NS1 N = 27	IgM N = 19	IgG N= 4
More than 150000	01 (3.7 %)	02 (10.4 %)	01 (25 %)
150000 -100000	06 (22.2 %)	05 (26 %)	01 (25 %)
100000 -20000	20 (74 %)	12 (62.4 %)	02 (50 %)

Table 5: SGOT levels in Dengue fever

	NS1 N = 27	IgM N = 19	IgG N= 4
AST (SGOT) Increased	22 (81.4 %)	11 (57.2 %)	03 (75 %)
AST (SGOT)Normal	05 (18.6 %)	08 (41.6 %)	01 (25 %)

Table 6: SGPT Levels in Dengue Fever

	NS1 N = 27	IgM N = 19	IgG N= 4
ALT (SGPT) increased	17 (62.9 %)	12(63.4 %)	03 (75 %)
ALT (SGPT)normal	10 (37.1 %)	07 (36.6 %)	01 (25 %)

Table 7: Bilirubin levels in dengue fever

	NS1 N = 27	IgM N = 19	IgG N= 4
Bilirubin increased	02 (7.4 %)	03 (15.6 %)	00 (00 %)
Bilirubin normal	25 (92.5 %)	16 (83.2 %)	04 (100 %)

Hemoconcentration, Leucopenia, and Thrombocytopenia were chief hematological features of seropositive dengue cases. Peripheral blood smear showed activated lymphocytes .Aspartate aminotransferase and alanine aminotransferase were mildly elevated in significant number of cases. None had any bleeding manifestations

Discussion

Dengue fever is an infectious disease which is difficult to distinguish from other viruses as there are no specific markers that can diagnose the disease early. Because it is a disease that can evolve with serious consequences

and even be fatal, this study aimed at analyzing clinical and epidemiological data and laboratory dynamics in order to try to identify biomarkers that are predictive of severity.

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 [1,2,4]. Infection with one serotype confers immunity to only that serotype and hence a person may be infected upto four times [1,2,4]. Humans are the main reservoir of dengue virus [1,2,3]. Dengue presents as dengue fever, dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS).[1,2,3,4,5,].

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 [3,,5,,6,7,8]. In our study, none of the children had DHF/DSS. 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. [2,3,8,9,10]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 [1,2,11,12,13,]. Pervin et al [13] , 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, reported myalgia in 84.5% of patients. Hemoconcentration was seen in more than 50% of patients [13].

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 Nihon Kohden 5 part differential cell counter 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 [14], 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%.

Leucopenia was observed in 26% of patients by Ratagiri et al [14]. 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 [9,11,13]. 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 [14]. 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 [12]. The presence of IgM antibodies in the sera DHF cases that cross-reacted with platelets has been demonstrated [1,3,6,9]. These autoantibodies could be involved in the pathogenesis of the disease. IgM and IgM 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 [7,13,14]. 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) [8].

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 IgG early in the course of the disease is a criterion for secondary infection. The sensitivity of IgM 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.

According to World Health Organization (WHO), [1,2,3] PCR is a powerful method to be used for dengue diagnosis, but PCR is not widely available and it still needs to be better standardized

Conclusion

Dengue fever evolves with laboratory alterations starting on the 3rd day and becoming most evident on the 5th day with values restored to normal by the 11th day. The disease was more severe in individuals aged 15 years and older with a more pronounced and persistent presence of liver abnormalities (AST, ALT) and hemoconcentration. The study results are relevant in the characterization of biological markers in the evolution of the disease and can be used as markers for the most severe forms thereby enabling early help with the adaption of therapeutic conduct for specific patients

The results of this study have highlighted the importance of history, clinical examination and the triad of thrombocytopenia, raised hematocrit and elevated liver enzymes for the early diagnosis of DHF without waiting for dengue serology for diagnosis which takes 2-3 days. Therefore, it is recommended that the diagnosis of dengue fever should be made early on the basis of basic investigations. Timely diagnosis and early fluid and supportive therapy reduces the morbidity and mortality from DHF.

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