



HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN RELATION TO IMMUNOCHROMATOGRAPHIC TEST POSITIVE DENGUE CASES

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ABSTRACT

Introduction: Dengue Fever is the most common arboviral disease in the world and it is considered a major global health threat by the World Health Organization. Dengue infection presents with varied clinical conditions ranging from simple fever to shock. The aim of the study is to identify the hematological and biochemical indicators that can predict and correlate serological phases of dengue infection with clinical outcome. **Material And Methods:** Haematological, biochemical and serological investigations were studied in suspected patients. Blood was drawn for complete hemogram and for serological investigations. The blood counts were performed on the fully automated hematology analyzer. The peripheral smears were stained with Leishman stain and the biochemical investigations were performed on automated analyser. **Results:** A total of 137 serologically positive patients were included in the present study. Of them 66.4% had dengue fever and 33.5% had dengue haemorrhagic fever (DHF). Dengue shock syndrome was present in 1.4% of patients. Dengue fever (DF) as well as DHF commonly occurred in age group of 15-30yrs. Males (53.3%) were more commonly affected than females (46.7%) with the M:F ratio of 1.1:1. NS1 positive cases constituted 48.1% and IgG positive cases accounted for 25.8% cases. Thrombocytopenia was observed in 67.8% and its association with NS1 antigen was significant. Lymphocytopenia was seen in 31.3% and neutrophilia of 27% supporting the fact that most of the cases were in acute phase. Hemoconcentration was seen in (27.7%) of the cases. Raise in serum AST concentration (86.9%) was observed among DHF. Majority of the cases showed normal levels of serum urea and creatinine. **Conclusion:** A total of 137 cases with dengue infection were analysed. The result shows that dengue infection is common among young adults. The result also shows that association of thrombocytopenia with NS1 antigen positivity was significant. Early diagnosis of the patient with the help of complete blood examination and serological dengue tests can prevent the fatality of the disease.

KEYWORDS : Dengue, Dengue haemorrhagic fever, Thrombocytopenia**INTRODUCTION**

Dengue Fever is the most common arboviral disease in the world and it is considered a major global health threat by the World Health Organisation (WHO). According to the WHO there are about 390 million cases of dengue fever worldwide.

Dengue virus is transmitted by female mosquitoes mainly of the species *Aedes aegypti* and to a lesser extent *Aedes albopictus*. There are 4 distinct but closely related serotypes of the virus that cause dengue (DEN-1, DEN-2, DEN-3 and DEN-4)(Bonilla-Aldana et al., 2024). Dengue infection presents with varied clinical conditions ranging from simple fever to shock. Dengue infection can lead to severe bleeding, shock and death (Tejo et al., 2024). Hence early and rapid laboratory diagnosis of dengue is crucial.

Dengue can be diagnosed by isolation of the virus, by serological tests, or by molecular methods. Serological test like NS1, IgG and IgM antigen-antibody assay are widely used (Hegde & Bhat, 2022). PCR is also commonly used for confirmation.

Hematological and biochemical parameters are also an important entity which indicates and assess the severity of the disease. Some of the hematological parameters which are commonly altered in dengue infection are platelet count (thrombocytopenia), increase in hemoconcentration, decrease in total count (leukopenia) and lymphocytosis (Rajbhandari et al., 2022). The hepatic enzymes mainly alanine transaminase, aspartate transaminase and alkaline phosphatase are the biochemical parameters which are commonly raised in dengue infection. Other parameters like urea and creatinine are also increased but in late phase of the disease (Swamy et al., 2021).

To assess the course of the disease it is important to follow up the patient and monitor for first 24 hour. Some of the commonly followed laboratory examination are complete blood count, prothrombin time, activated partial thromboplastin time, serum urea and creatinine.

The commonest complication of dengue infection is haemorrhagic manifestation followed by shock (Wang et al., 2020). Thus, the

primary target is to maintain the platelet counts and fluid balance. Most of the patient recover by fluid therapy alone but if required platelet transfusion must be given. Therefore, this study aimed to correlate the hematological and biochemical findings in immunochromatographic positive dengue cases.

AIMS OF THE STUDY

1. To study the age, sex and prevalence among the spectrum of dengue.
2. To correlate the haematological, and biochemical findings in relation with various antigens in dengue infection.

MATERIALS AND METHODS

This study was conducted in Department of Pathology, Sree Balaji Medical College and Hospital Chrompet between Dec 2014 – Sept 2016.

Inclusion Criteria:

All patients with immunochromatographic positive for dengue infections were included in the study. Their clinical history for fever, headache, myalgia, arthralgia and bleeding manifestation, laboratory data and outcome were recorded.

Exclusion Criteria

Patients with malaria, enteric fever, pneumonia, urinary tract infection are excluded by doing appropriate investigations.

Haematological, biochemical and serological investigations were studied in suspected patients. 5ml of blood was drawn, 2 ml of blood in EDTA vacutainers for complete hemogram and 3ml of blood in plain vacutainers for serological investigations. The blood counts were performed on the fully automated hematology analyzer. The peripheral smears were stained with Leishman stain and studied. The biochemical investigations were performed on automated biochemistry analyser.

I. Immunochromatographic test

The serological test for dengue was done using a rapid solid phase immune-chromatographic test (SD dengue duo) for the qualitative detection of NS1 antigen and differential detection of IgM and IgG

antibodies to dengue virus in human serum and confirmed by ELISA.

II. Hematological parameters

Complete blood count includes peripheral smear, RBC, WBC, hemoglobin, platelet counts, red cell indices and hematocrit. Hemoconcentration, defined as a 20 percent rise in hematocrit from the baseline (as per WHO guidelines), is difficult to ascertain with a single hematocrit value. As most of our study population was OPD based, we have calculated the hemoconcentration by hematocrit hemoglobin ratio (Rigau-Pérez & Laufer, 2006).

III. Biochemical parameters

Liver function test-ALT, AST, ALP, Direct/ indirect protein, globulin, AG ratio, GGT, Urea and Creatinine

OBSERVATION AND RESULTS

A total of 137 cases were studied, based on positive dengue test (Rapid card test), and were divided into 3 groups based on WHO classification, out of 137 cases 92 (67.2%) patients were of Dengue fever, 43 (31.3%) were of Dengue haemorrhagic fever and 2 (1.4%) were Dengue shock syndrome. There was no fatality. Dengue infection was observed commonly in the age group between 11 to 30 years, with the mean age of 26.02 years. Seventy-three (53.30%) were male and 64 (46.7%) were female out of 137 cases. The male and female ratio was about 1.1:1

CLINICAL FEATURES

Out of 137 patients fever was present in all the patients. Ninety two percent of patients presented with continuous and high-grade fever. Myalgia, arthralgia and retro orbital pain was present in 109 (79.5%), 72 (52.5%) and 24 (17.5%) patients respectively. Abdominal pain was present in 16 (11.6%) patients and 98 (71.5%) patients complain of lethargy.

Table 1- Serological Distribution Of Antigen And Antibodies In Spectrum Of Dengue Infection

Spectrum of Dengue	NSI	IgM	IgG	NSI+IgM	NSI+IgG
DF (n=92)	45	14	24	8	2
DHF (n=43)	21	4	10	5	2
DSS (n=2)	0	0	1	1	0
Total	66	18	35	14	4

The most common antigen observed was NSI presenting in 66 (48.1%) cases followed by IgG in 35 (25.5%) cases. The positivity of NSI among DF cases were 45 (48.9%) out of 91 cases and IgG was positive in 24(26.1%) cases.

Among 43 cases of DHF, NSI was positive in 21(48.8%) cases whereas IgG positivity was observed in 10(23.2%) cases (Table 1).

Table 2- Total Count Distribution

Parameters	DF	DHF	DSS	Total
Leukopenia <4000	0	12	2	14
Leukocytosis >11000	2	0	0	2

Leukopenia was observed in 14 (10.2%) cases out of 137 cases and leukocytosis was seen in 2(1.4%) cases. Leukopenia was encountered more among the DHF (Table 2).

Table 3 - Distribution Of Differential Count

Parameters	DF	DHF	DSS	Total
Lymphocytopenia	38	5	0	43
Lymphocytosis	1	5	2	8
Neutrophilia	13	24	0	37

Neutrophilia was observed in 37 (27%) cases and lymphocytopenia was seen in 43 (31.3 %) cases out of 137 cases studied. Lymphocytopenia was observed more among the DF (38 cases) as compared to DHF and DSS (Table 3).

Table 4- Platelet Counts Distribution

Platelet count	NSI	IgG	IgM	NSI+IgG	NSI+IgM
<20000	0	3	10	0	2
5 0000-1 lakh	48	17	5	1	7
Total	48	19	15	1	9

Thrombocytopenia (< 1 lakh/ml) was observed in 93 (67.8%) out of 137 cases. Fifteen (10.9%) cases were below 20000/ml.

There were 48 (72.2%) cases of thrombocytopenia among 92 NSI positive cases and 19 (52.2%) cases among 35 IgG positive cases (Table 4).

Table 5: Hemoconcentration Distribution By Hematocrit Hemoglobin Ratio

Hemoconcentration	DF	DHF	DSS	Total
>= 3.5	13	24	2	39

Hemoconcentration=Hematocrit (%) / Hemoglobin (g/dl) (Rigau-Pérez & Laufer, 2006).

Ratio	Interpretation
<= 3.19	Normal
>= 3.5	Hemoconcentration

Out of 137 patients studied 39 (28.4%) shows raise in hemoconcentration (Table 5).

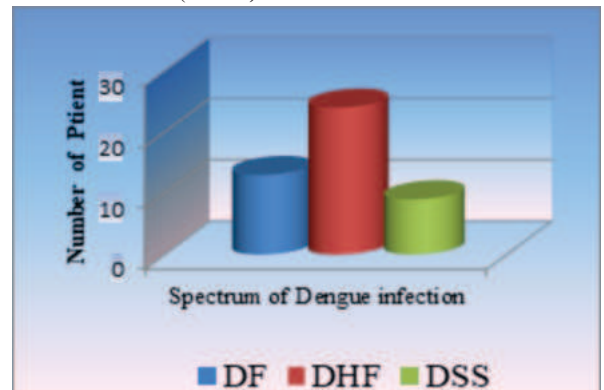


Figure 1– Distribution Of Hemoconcentration In Relation With Spectrum Of Dengue

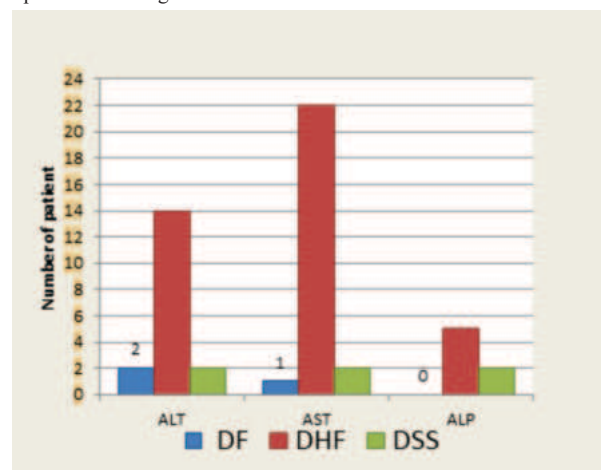


Figure 2- Hepatic Enzyme Distribution

There was raise in hepatic enzyme concentration in about 50 (36.4%) cases. Serum ALT was raised in 18 (13.1%) cases and AST in 25 (18.2%) cases. Among DHF cases there was raised in ALT, AST and ALP concentration in about 14(32.5%), 22 (51.1%) and 5 (11.6%) cases respectively. Out of 2 cases of DSS, all the hepatic enzyme was raised (Figure 2).

Urea and Creatinine

Serum urea was normal in 97.8% (134) of cases and serum creatinine were normal in 96.3% (132) cases.

DISCUSSION

A rise in dengue cases has been observed in recent years, which can be linked to rapid, unplanned urban development and inadequate sanitation systems that create ideal breeding grounds for mosquitoes. In 2014 a total of 40571 cases of dengue infection was reported in India out of which 137 were fatal and in 2015, 99913 cases were reported which is more than two-fold increase in the occurrence of the disease (Krishna, 2017). Tamil Nadu has reported 7339 cases of dengue infection and 15 fatalities between 2014-15, which constitute about 5.2% of the total case reported in India between 2014-15.

A total of 137 cases were studied, based on the serological positivity of dengue test (SD duo, and were divided into 3 groups based on WHO classification. Of the 137 cases 92 (67.2%) patients were of Dengue fever, 43 (31.3%) were of Dengue haemorrhagic fever and 2 (1.4%) were Dengue shock syndrome (**Figure 2**).

The mean age of dengue infection in the present study was 26.2 years with range from 4-83 years and male to female ratio was about 1.1:1. Present study correlated with the study done by Malavige *et al* where the mean age was 26.6 years (Malavige *et al.*, 2006). The present study has shown a male preponderance (53.3%) as compared to the females (46.7%) with the male: female ratio being 1.1:1. Similar results were observed by Su DH *et al* who gave the male: female ratio as being 1.5:1 (Su *et al.*, 2007).

The commonest antigen detected in the present study was NS1 66 (48.1%) cases followed by IgG 35 (25.5%) cases out of 137 cases which has similar findings with the study done by Gargi Ghosh *et al* where NS1 antigen accounted for 51.5% of the cases (Ghosh *et al.*, 2013). But cases showing IgG positivity (25.5%) were more in the present study as compared to other studies and this may be explained by the presence of a greater number of secondary infections in the present study population.

In the present study thrombocytopenia (< 1 lakh) was observed in 93 (67.8%) cases out of 137 cases, among 93 cases 15 (10.9%) cases was presented with platelet count < 20000.

The present study correlated with the study done by RD Kulkarni *et al* and Parameswarappa Jyothi *et al* where they have observed thrombocytopenia in 68.8% and 61.6% of cases respectively (Jyothi & Metri, 2015; Kulkarni *et al.*, 2011).

There were 48 (72.2%) cases of thrombocytopenia among 92 NS1 positive cases in the present study. In the study conducted by Santosh Shivajirao Tathe *et al* out of 56 NS1 positive cases, thrombocytopenia was evident in 50 (89 %) (Tathe *et al.*, 2013). Similar study of association of thrombocytopenia with NS1 antigen was observed by RD Kulkarni *et al* where in 130 cases positive for NS1, thrombocytopenia was evident in 103 (79.2%) cases, which correlated with the present study (Kulkarni *et al.*, 2011).

In the present study the hemocentration was calculated by hematocrit hemoglobin ratio (Rigau-Pérez & Laufer, 2006). Out of 137 patients studied we have observed 28.4% (39) cases with raise in hemoconcentration (**Figure 1**). A study conducted by Malavige *et al* has evidence that out of 108 serologically positive cases 26% show increase in hemoconcentration (Malavige *et al.*, 2006). Similar study conducted by Janak Kishore *et al* where out of 100 cases studied 33.8% of the patients show raise in hemoconcentration which is higher than our study (Kishore *et al.*, 2006).

In the present study, maximum levels of hepatic enzymes recorded were 190 U/L, 205 U/L and 194 U/L in ALT, AST and ALP respectively. Increase in serum ALT and AST concentration was observed in about 30 (21.9%) and 29 (21.2%) cases respectively.

There was increase in liver enzymes specially ALT and AST in females with age less than 20 years of age in the present study. Similar finding was evident by Souza *et al* where he stated that association of liver injury in dengue infection were observed more among females (Souza *et al.*, 2004).

In the present study hepatic injury was greater in patients with DHF than DF. Among DHF cases there was raise in ALT, AST and ALP concentration in about 14(32.5%), 22 (51.1%) and 5 (11.6%) cases respectively. In the study conducted by Wahid SF *et al* which comprised of 50 serologically confirmed cases (25 cases of DF and 25 of DHF) noted that the serum levels of ALT and ALP were significantly greater among patients with the DHF (Wahid *et al.*, 2000).

Total Count Distribution In Dengue Infection In Comparison With Present Study

Leukopenia was observed in 14 (10.2%) cases out of 137 cases and leukocytosis was seen in 2 (1.4%) cases in the present study.

In the study conducted by Deparis B.P *et al* out of 196 cases leukopenia was observed in 53% cases and leukocytosis in 2.5 cases (Deparis *et al.*, 1998). Less number of leukopenia cases encountered in the present

study may be due to early phase presentation of the disease in the study population.

Distribution of Neutrophils and Lymphocytes in dengue infection

In the present study neutrophilia was observed in about 37 (27%) patients and lymphocytopenia was seen in 43 (31.3 %) cases out of 137 cases. The increase in neutrophil counts may be due to presentation in acute phase of the disease in the study population.

In the study conducted by Deparis B.P *et al* out of 196 cases lymphocytopenia was observed in 63.5% cases which is higher than the present study (Deparis *et al.*, 1998). In another study conducted by Azin *et al* lymphocytosis was observed in all forms of dengue (Azin *et al.*, 2012).

Urea and Creatinine

In the present study out of 137 patients 95.6% cases presented with normal concentration of serum urea and creatinine. A study conducted by Futrakul *et al.* has reported that there is mild elevation in serum creatinine in 43% out of 24 DHF cases (Futrakul *et al.*, 1973). In another study conducted by Riffat Mehboob *et al* has concluded that urea was raised in dengue patients while creatinine levels were low (Mehboob *et al.*, 2012).

CONCLUSION:

A total of 137 cases with dengue infection were analysed. The result shows that dengue infection is common among young adults. The result also shows that association of thrombocytopenia with NS1 antigen positivity was significant. Early diagnosis of the patient with the help of complete blood examination and serological dengue tests can prevent the fatality of the disease.

Limitations

This research included dengue patients who underwent complete blood count upon arrival. The differing times of presentation have led to inconsistencies in the patients' hematological parameters. Furthermore, the lack of hematological and biochemical results for over half of the patients represented a limitation in this study

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