Research Article

Clinical and hematological profile in dengue

Prabhavati J Patil[1], Deepak R Kanabur[2]

[1]Department of Pathology, Terna Medical College, Navi Mumbai, Maharashtra, India, [2]Department of Pathology, SDM College of Medical Sciences and Hospital, Dharwad, Karnataka, India

Correspondence to: Prabhavati J Patil, E-mail: drprabhasanjay@gmail.com

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ABSTRACT

Background: Dengue is the most rapidly spreading mosquito-borne viral disease. The spectrum ranges from a non-specific febrile illness to severe disease, i.e., dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) with the development of hematological complications. Since the death in these patients is due to hematological complications, their study would have a substantial impact on reducing the mortality and morbidity associated with dengue. **Objective:** The objective of this study was to evaluate the hematological changes in serologically positive dengue patients and to correlate the same with different stages of serology and clinical outcome. **Materials and Methods:** Evaluation of the hematological parameters and peripheral smear study in clinically suspected cases of dengue with serological confirmation was carried out. Clinical data along with the outcome were collected from medical records. Chi-square test, somatosensory evoked potential, and logistic regression analysis were applied to analyze the results. **Results:** Three hundred and forty-eight serologically positive dengue cases were analyzed. The disease manifested as dengue fever (DF) in 83.5% of cases, DHF in 10.6% of cases, and DSS in 4.9% of cases. Thrombocytopenia was the most common hematological finding followed by anemia, leukopenia, leukocytosis, and increased hematocrit. Peripheral smears showed atypical lymphocytes, neutrophilic toxic granules, giant platelets, and granulocytic shift to left. About 94.26% of patients recovered and 3.45% had fatal outcome. **Conclusion:** Platelet count and hematocrit play a crucial role in predicting prognosis of DHF and DSS. Thrombocytopenia was associated more with DHF and DSS than DF. There was a significant association between decreased platelet count and mortality rate.

KEY WORDS: Dengue Virus; Dengue Fever; Dengue Hemorrhagic Fever; Dengue Shock Syndrome; Thrombocytopenia

INTRODUCTION

Dengue is an acute, rapidly spreading, self-limited disease (typically lasting 5–7 days), characterized by fever, prostration, headache, myalgia, rash, lymphadenopathy, and leukopenia, and caused by four antigenically related, but distinct types of dengue viruses transmitted by the bite of infected mosquitoes of the genus *Aedes*, especially *Aedes aegypti*, *Aedes albopictus,* and *Aedes polynesiensis*. It occurs epidemically and sporadically in India, Japan, West Africa, and Southeast Asia.[1]

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An estimated 50 million dengue infections occur worldwide annually and approximately 2.5 billion people, i.e., two-fifth of world's population in tropical and subtropical countries are at risk. The reported case fatality rate in India is 3–5%.[2,3] Most developing countries have epidemics of febrile illnesses including typhoid, measles, leptospirosis, and severe acute respiratory distress syndrome that can be confused with dengue due to similar clinical features.[4] 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.[2] In view of this, the laboratory support has become a critical component of diagnosis

and management of dengue.[5] Severe forms of dengue virus infections are associated with major hematological complications such as bleeding tendency, thrombocytopenia, and plasma leakage.[4]

Since the death in these patients is due to hematological complications, the study of the same would have a substantial impact on reducing morbidity and mortality associated with dengue.

Objectives

The objectives of this study were as follows:

- To evaluate the hematological changes in patients suffering from clinical manifestations of dengue with serological confirmation
- To correlate the hematological findings with different stages of serology and clinical outcome.

MATERIALS AND METHODS

The study was conducted in SDM College of Medical Sciences and Hospital, Dharwad, which is a tertiary care hospital from September 2010 to September 2011. Venous blood samples were collected from clinically suspected cases of dengue which were admitted to the hospital during this period. A commercially available immunochromatography based kit, "advantage dengue NS1 Ag and Ab combi card" supplied by J. Mitra and Co. Pvt. Ltd., New Delhi, was used to detect NS1 antigen and immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies.

Evaluation of the hematological parameters and peripheral smear study was carried out in cases which were positive for either NS1 antigen assay and/or IgM and/or IgG antibody assay. The hematological analysis was done by "automated hematology analyzer 1800i" by Sysmex Corporation, Japan. Peripheral smears were stained with Leishman's stain. The clinical data of each patient along with the outcome were collected from medical records.

Cases were distributed into dengue fever (DF), severe DF, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) based on the WHO criteria.[3,6,13]

The ethical clearance was obtained from the institutional ethical committee for the study.

RESULTS

Of the 3394 suspected cases, a total of 365 were positive for either one or more of the markers. Clinicohematological changes were studied in 348 cases as other 17 cases had coexistent malaria and/or enteric fever which were excluded from the study. Males were affected more commonly than females (1.87:1). Age ranged from birth to 88 years with most common being 21–30 years. Cases were more during rainy season [Figure 1]. Analysis of various symptoms showed fever in all the patients followed by vomiting in 51.9% and headache in 48.8%. Other symptoms are listed in Table 1 with percentage of cases showing each symptom.

DF was seen in 255 cases, severe DF in 39 cases, DHF in 37 cases, and DSS in 17 cases.

Hematology

Hemoglobin level ranged from 4.5% g to 20.1% g with the mean of 12.6% g. One hundred and eighty-eight (55%)

Table 1: Case distribution based on the symptoms

Symptoms	Cases	Percentage
Fever	348	100
Rash	39	11.2
Retro-orbital pain	161	46.2
Headache	170	48.8
Vomiting	181	51.94
Myalgia	160	45.9
Arthralgia	69	19.8
Pain abdomen	77	22
Bleeding manifestations	56	16
Hepatomegaly	129	37
Splenomegaly	79	22.7
Lymphadenopathy	12	3.4
Central nervous system manifestations	22	6.3

Table 2: Serological distribution of the cases

Serology	Number	Percentage
NS1	115	33
IgM	141	40.46
IgG	23	6.65
NS1+IgM	28	8.03
NS1+IgG	5	1.43
IgM+IgG	36	10.43
Total	348	100

IgG: Immunoglobulin G, IgM: Immunoglobulin M

Table 3: Serological distribution along with platelet count

Serology	Total cases	Thrombocytopenia	Percentage
NS1 only	115	83	72.17
IgM only	141	78	55.3
IgG only	23	16	69.56
NS1+IgM	28	23	82.14
NS1+IgG	5	5	100
IgM+IgG	36	18	50
Total	348	223	64.23

IgG: Immunoglobulin G, IgM: Immunoglobulin M

cases had hemoglobin percentage above normal range for the particular age and sex followed by 145 (41.6%) cases had decreased hemoglobin percentage and 15 (4.4%) of the cases had hemoglobin percentage in the normal range.

Red blood cell (RBC) count ranged from 0.62 million/cumm to 7.79 million/cumm with the mean of 4.5 million/cumm. One hundred and seventy-seven (50.9%) cases had RBC count within the normal range, i.e., 4.5–6 million/cumm, 159 (45.63%) cases had decreased count, and 12 (3.47%) cases had increased count.

Total WBC count ranged from 240 to 30,280 cells/cumm with the mean of 7368 cells/cumm. Maximum number of cases, i.e., 186 (53.4%) had the count within normal range for the particular age followed by 97 (27.8%) cases had decreased count and 65 (18.8%) cases had increased count.

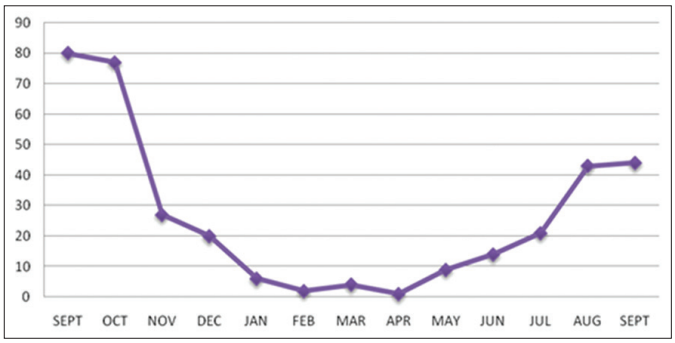

Figure 1: Month-wise distribution of cases

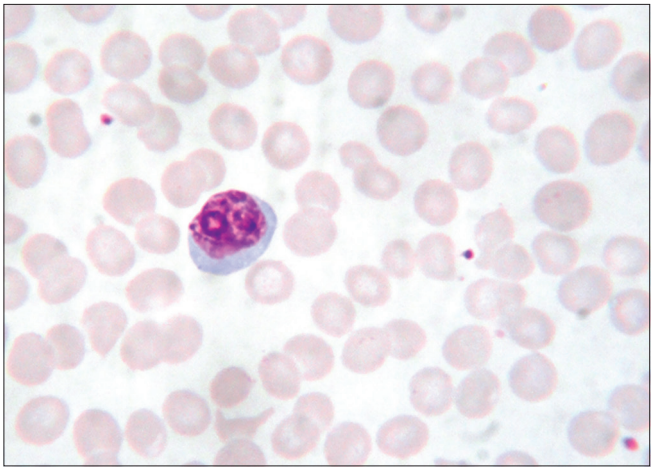

Figure 2: Atypical lymphocyte with prominent nucleolus (Leishman's stain, ×1000)

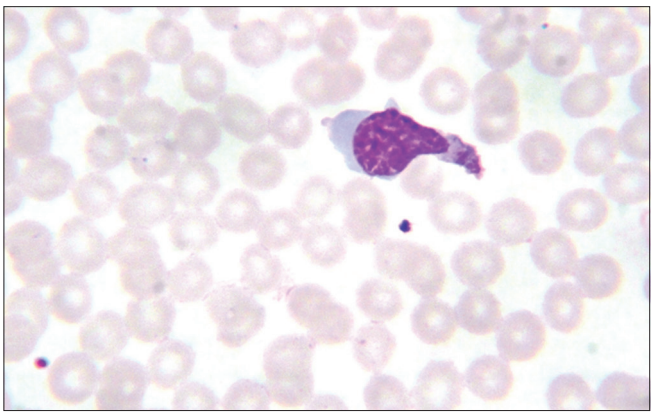

Figure 3: Atypical lymphocyte with cytoplasmic process (tailing) (Leishman's stain, ×1000)

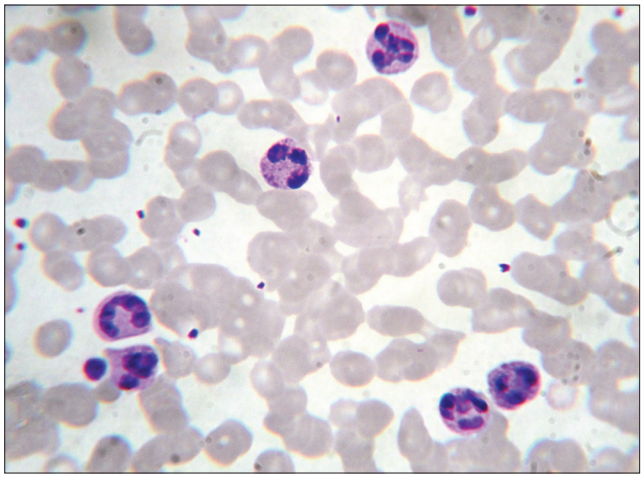

Figure 4: Neutrophilic leukocytosis (Leishman's stain, ×1000)

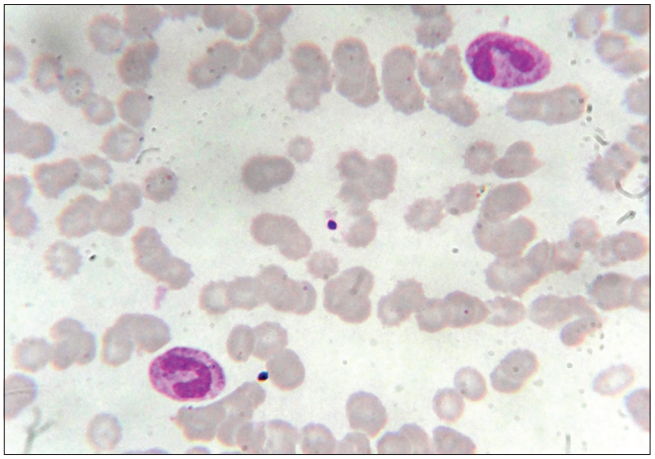

Figure 5: Band forms with toxic granules (Leishman's stain, ×1000)

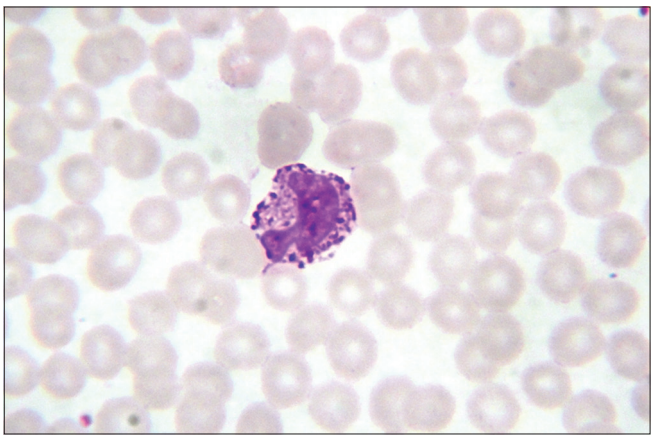

Figure 6: Metamyelocyte with toxic granules (Leishman's stain, ×1000)

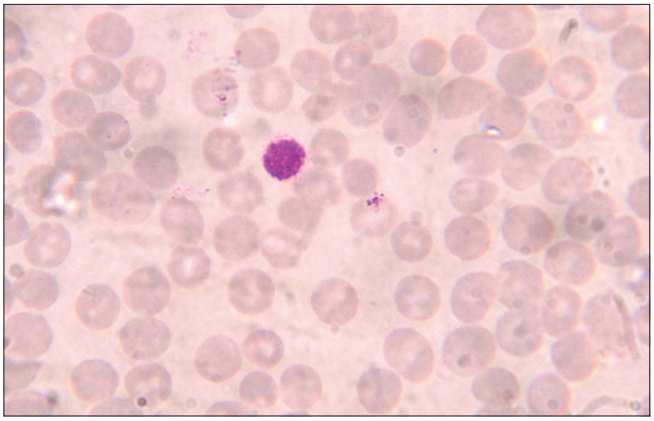

Figure 7: Giant platelet (Leishman's stain, ×1000)

Platelet count ranged from 2000 cells/cumm to 456,000 cells/cumm with the mean of 98,718 cells/cumm. Two hundred and twenty-three (64.13%) cases showed thrombocytopenia, i.e., platelet count below 100,000 cells/cumm and 125 (35.87%) cases showed platelet count above or equal to 100,000 cells/cumm. There were 38 (10.9%) cases with platelet count below 20,000 cells/cumm.

All the 17 cases of DSS had thrombocytopenia, followed by 30 of 37 (81.1%) cases of DHF, 25 of 39 (64.1%) cases of severe DF, and 151 of 255 (59.21%) DF cases had thrombocytopenia. The association of thrombocytopenia with DHF and DSS in our study was proved to be statistically highly significant with Chi- square test with $P < 0.001$.

(Chi square test – 14.63, Degree of freedom – 2, *P* value – 0.0001.)

Association of thrombocytopenia with NS1 was found to be higher by somatosensory evoked potential (SEP) test.

Further, analysis of NS1 alone (72.17%) versus NS1 plus IgM (82.14%) showed that thrombocytopenia was associated excellently with both NS1 and IgM both positive compared to NS1 alone.

Peripheral smear study was done in 333 cases of 348 in the present study. The most common finding seen on the peripheral smear examination was the presence of atypical lymphocytes in 252 (75.67%) cases. These showed various morphological features such as prominent nucleolus, clumped chromatin, dense basophilic cytoplasm, irregular cytoplasmic process (tail), and indentation of cytoplasmic border by RBCs (Turkcell) [Figure 2, 3]. The other findings were toxic granules within the neutrophils in 117 (35.13%) cases, shift to left of granulocytes in 17 (4.95%) cases, and >5% of band forms in 48 (14.4%) of the cases [Figure 4, 5, 6]. Occasional large platelets were noted in 84 (25.22%) of cases and normoblasts were noted in 22 (6.6%) cases [Figure 7].

Outcome

Of 348 cases, 8 (2.29%) cases got discharged against medical advice, for which outcome was not available; however, the condition was unchanged. Three hundred and twenty-eight (94.26%) cases recovered and 12 (3.45%) cases expired. Two hundred and eighty-eight of 294 cases (99.6%) of DF and severe DF recovered, and six cases lost to follow-up. All the cases (100%) of DHF recovered and three cases of 17 (17.64%) of DSS cases recovered and majority, i.e., 12 of 17 (70.6%) cases of DSS expired.

Eight of 348 cases were lost to follow-up. Among the remaining, 12 (100%) fatal cases had thrombocytopenia followed by 206 recovered cases, of 328 (62.80%) had thrombocytopenia.

DISCUSSION

Dengue is caused by a virus belonging to the Flaviviridae family (single-stranded, positive, and non-segmented RNA virus). Infection with one serotype confers immunity to only that serotype, and hence, a person may be infected up to 4 times. Humans are the main reservoir of dengue virus.[2]

The most common age group affected in our study was 21–30 years and males outnumbered females which is comparable to that of studies done by Neeraja *et al*.[7] and Kumar *et al*.,[8] and the reason for male preponderance is said to be due to their clothing habits or outdoor activities. Environment being more favorable for breeding of the vector during monsoon and post-monsoon season, it explains the increase in number of cases during rainy season. Clinical features were same as that of Neeraja *et al*.,[7] Kumar *et al*.,[8] and Banerjee *et al*.[2] Serologically more than one marker was detected in 19.89% of the cases [Table 2]. Studies done by Dumortier *et al*.[9] showed only 11.72% of cases of anemia which was much lower and Banerjee *et al*.[2] observed 51.85% of cases of anemia in their study which was higher than that of our study. These two studies have not mentioned the number of cases with normal and increased hemoglobin percentage. With this, it can be stated that hemoglobin percentage is not associated with dengue spectrum. Wide range of RBC distribution was noted as there will be initial halt in erythropoiesis for a few days in a typical case of dengue, which may not be reflected in the peripheral blood level of the red cells because of its long half-life. Later on, there will be accelerated erythropoiesis.[10] However, the duration of illness was not taken into account in our study. We found thrombocytopenia in 100% of DSS cases followed by 81.1% of cases of DHF and 64.1% of cases of DF cases. Nelson *et al*.[10] studied thrombocytopenia in 49% of non-shock cases and in 90% in the shock group. The association of thrombocytopenia with DHF and DSS in our study has been proved to be statistically significant ($P < 0.001$).

In the present study, 82.14% of cases which were positive for both NS1 and IgM had thrombocytopenia followed by 72.17% of cases which were positive for NS1 alone showed thrombocytopenia [Table 3]. The study done by Kulkarni *et al*.[11] observed that 94.12% of cases which were positive for both NS1 and IgM and 79.2% of cases which were positive for NS1 alone had thrombocytopenia. Similarly, in the present study, association of thrombocytopenia with NS1 was found to be higher by SEP test. Further, analysis of NS1 alone (72.17%) versus NS1 plus IgM (82.14%) showed that thrombocytopenia was associated excellently with cases where both NS1 and IgM were positive compared to NS1 alone. In the present study, leukocytosis was observed in 18.8% of the cases, normal count was observed in 53.4% of cases, and leukopenia in 27.8% of cases. However, no study has made a note on leukocytosis and normal count. Leukopenia is seen in 15.38% of the cases in a study done by Malavige *et al*.[12] Oliveira *et al*.,[13] Mahmood *et al*.,[14] and Wilder-Smith *et al*.[6] observed leukopenia in much higher number of cases. This can be due to the fact that infection in our study may be caused by less virulent serotype as explained by Banerjee *et al*.[2] The peripheral smear was studied for the morphology and most commonly found that morphological change was atypical lymphocytes, seen in 75.64% of the cases. About 66.9% of atypical lymphocytes were seen in the study done by Oliveira *et al*.[13] and 49% in the study done by Liu *et al*.[15] Atypical lymphocytes were observed also by Kalayanarooj *et al*.,[16] but the absolute number of atypical lymphocytes in their study was not significant. The other morphological changes observed were toxic granules, shift to left which included metamyelocytes, myelocytes, and band forms (more than 5% of band forms were seen in 14.4% of cases) and giant platelets. However, no study has been found regarding the other changes mentioned. In the present study, cases which got discharged against medical advice were considered as lost to follow-up. About 94.26% of cases recovered and 3.45% of cases had fatal outcome. The data obtained in our study were comparable with the study done by Kumar *et al*.,[8] in which 97.6% of the cases of 455 recovered and 2.45% of cases had a fatal outcome.

A logistic regression analysis was conducted to predict the outcome in 340 patients of DF (excluding the cases which were lost to follow-up) using the hematological parameters as predictors. The parameters were age, sex, platelet count, hemoglobin, hematocrit, total leukocyte count, total neutrophil count, and total lymphocyte count. A test of the full model against a constant only model was statistically significant, indicating that the predictors as a set reliably distinguished between death and survival as outcomes (Chi-square = 33.746, $P < 0.004$ with df = 15). Nagelkerke's R^2 of 0.341 indicated a modest relationship between prediction and grouping. The Hosmer and Lemeshow test of goodness of fit did not demonstrate a significance which strengthens the model ($P = 0.096$ at df = 8). Prediction success overall was 96.8% (15.4% for death and 100% for survival). The Wald criterion demonstrated that only platelet count made a significant contribution to prediction ($P = 0.015$). Increased lymphocyte count was the next predictor approaching significance at $P = 0.090$. Exponential value indicates that when the platelet count falls by one unit (one thousand), the odds ratio is 1.019 times as large for death and the same is 5.465 times for one unit (one thousand) increase in lymphocyte count.

About 99.6% of DF and severe DF recovered and 2.04% of cases lost to follow-up. All the cases (100%) of DHF recovered and majority, i.e., 70.6% of cases of DSS expired. The study done by Neeraja *et al*.[7] showed 100% (85 of 85) of recovery in DF cases. About 60% (3 of 5) in the DHF cases and 80% (8 of 10) of the DSS cases had fatal outcome which was comparable in case of outcome associated with DF and DSS of our study.

Strength

- Serological panel in our study consisted NS1 antigen, IgM and IgG antibodies. Considering very high sensitivity of NS1 antigen in dengue diagnosis it can be stated that we would have missed the diagnosis in 33% of cases, had we not included NS1 antigen in the test panel.
- Association of thrombocytopenia with NS1 alone and NS1 and IgM-positive cases was statistically significant which has a major impact on timely intervention which affects the morbidity and mortality associated with dengue.

Limitation

Hemoconcentration of greater than 20% of the baseline signifies one of the diagnosing criteria for DHF and DSS. However, in our study, categorization of hematocrit toward increased levels was not possible due to the non-availability of pre-illness hematocrit, i.e., baseline hematocrit value of the particular patient. There will be modification of the hematocrit in such instances due to pre-diagnostic treatment such as blood transfusion or colloid transfusion and non-availability of serial hematocrits.

CONCLUSION

Dengue is a disease affecting younger age group with male preponderance. It has seasonal variability with maximum number of cases occurring in monsoon and post-monsoon season. Dengue affects most of the organ systems such as gastrointestinal and hepatobiliary system, respiratory system, cardiovascular system, and the nervous system. Major hematological complications in dengue are bleeding tendency, thrombocytopenia, and plasma leakage. Thrombocytopenia and hematocrit concentration have been shown to be major prognostic markers. DSS is the independent risk factor for

death in dengue. Other causes may be multiorgan failure and concurrent bacteremia.

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