

Peripheral cell line suppression in dengue infection in relation to serological status



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ABSTRACT

Background: Dengue infection occurs in epidemic and can be fatal. Bleeding due to thrombocytopenia and hypovolemic shock due to capillary leak is the predominant clinical feature. Bone marrow suppression as a cause of thrombocytopenia and neutropenia is well documented. This is generally recognised as decrease in the count of formed elements in the peripheral blood. However, relation of such a phenomenon with the serological stage of the disease is less studied. **Aims and Objective:** We aimed to quantify the magnitude of peripheral cell line suppression in relation to serological status of dengue illness. **Materials and Methods:** This was a retrospective study. Dengue infection was diagnosed by clinical and serological parameters. The proportion of patients with peripheral cell line suppression and their association with the different serological phases was described. **Results:** Records of 219 Dengue positive patients were analysed retrospectively. Most common serological status was positive IgG which was noted in 68.9%, this was followed by NS1 antigen positivity in 39.7% of the patients. Around 40% of the patients had peripheral cell line suppression of two or more formed elements at the time of diagnosis. Sixty-four percent of them had low platelet and WBC counts. All the three formed elements were decreased in 14% of our patients. Of these, 23% were in the initial phase of the illness and 33.3% during post primary phase. **Conclusion:** Decrease in the formed elements in the peripheral blood suggesting bone marrow suppression is seen in nearly 40% of the patients with Dengue infection at the time of diagnosis. This was highest during post primary phase followed by initial phase of the illness.

Key words: Thrombocytopenia, Neutropenia, Dengue infection, Bone marrow suppression

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INTRODUCTION

Dengue is a regularly occurring epidemic in India. Many of the patients have non-fatal illness and recover with minimal medical intervention. The exact hospitalisation rates are not known. However, WHO states that, nearly 50000 hospitalisations occur worldwide for severe Dengue infection. Mortality in well-treated Dengue infection is reported to be less than 1%.¹ The two predominant pathogenic mechanisms that can cause death in Dengue infection is bleeding due to thrombocytopenia and hypovolemic shock due to capillary leak. Animal experiments have demonstrated that the thrombocytopenia in Dengue is predominantly due to decreased production of megakaryocytes.²

However, current evidences suggest that complex immune mechanisms play a significant role in causing thrombocytopenia.³⁻⁵

Many of the patients with thrombocytopenia also demonstrate reduction in the counts of other cell lines indicating hypo functioning bone marrow.⁶ Such a phenomenon is now increasingly being recognised. The extent and pattern of bone marrow suppression as inferred by decrease in different cell lines in the peripheral blood in Dengue infected patients and its relation to different serological phase of the illness is yet to be characterised. This information could be of significant value as it enables clinicians in management of cases. This study intended to provide information on the magnitude and pattern of cell

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line suppression, and relation to the different phases of Dengue infection.

Primary objective

To describe the magnitude of formed element suppression in the peripheral blood in relation to serological stage of Dengue infection.

Secondary objective

To describe the pattern of different cell lines affected in patients diagnosed with Dengue infection.

MATERIALS AND METHODS

Study design

This is a retrospective descriptive study conducted in a tertiary care centre. Study period was from January 2014 to December 2014.

Inclusion criteria

Patients aged more than one year admitted with fever and had serological diagnosis of Dengue infection.

Exclusion criteria

Patients who were diagnosed as Dengue elsewhere and admitted to the hospital for further management.

Subjects and investigations

All the patients who full filled the inclusion criteria were included in the analysis. Patients who were admitted with fever and had at least one serological evidence of Dengue infection such as positive NS1 antigen or Dengue IgM or IgG antibody were considered to be suffering from Dengue infection.

Serological investigations

Serological investigations were done in the department of microbiology on clotted blood samples. NS1 antigen, IgM and IgG antibodies were detected by Immunochromatography technique (Standard Diagnostic Inc. Bioline, Dengue duo, Gyeibgggu-do, Republic of Korea). The results were reported as positive or negative.

Haematological investigations

All the haematological investigations were done on EDTA samples. The analysis was done by the automated cell counter SysmexXN- 1000(Sysmex America, Inc. in Lincolnshire, Illinois). Haemoglobin was expressed as Gram/dl, RBC, WBC and platelet counts were expressed as cells/mm³. Bone marrow suppression was presumed as the cause of decrease in two or more formed elements in the peripheral blood. The RBC count of 4 million/mm³, WBC count of 4000/mm³ and platelet count of 150000/mm³

were considered to be the lowest values below which they were considered to be reduced.

Data collection

The medical record numbers of all the patients diagnosed as Dengue between Jan 2014 and Dec 2014 (both inclusive), were obtained from medical records department. These numbers were individually searched in the repository of laboratory information system of our hospital. The results of serological investigations were noted. Later the haematological values of the sample sent at the time of diagnosis were noted. Subsequent haematological values were not obtained for this study. These values were entered in Microsoft excel and later analysed.

Ethical consideration

This study was approved by the institutional ethical committee with exemption for patient consent.

Sample size

Our initial observation revealed that bone marrow suppression in Dengue infection ranged from 25 to 30%. Assuming arate of 25% with the margin of error 5% and confidence limits of 90%, we needed 201 cases for this study.

Statistical analysis

The continuous variables with normal distribution were reported as mean and standard deviation. Continuous variables with skewed distribution were reported as median and inter quartile range. Categorical variables were reported as proportions. All the analysis was done using Microsoft Excel 2013.

RESULTS

We had a total of 368 patients diagnosed and treated for Dengue infection in our hospital during the study period. Out of them, 144 patients had the diagnosis done elsewhere and were admitted to our hospital for management alone. We could not get complete data needed in five patients. We had 219 subjects for final analysis.

The median age of patients was 23 years and 56% were males. The base line characteristics of our study subjects are depicted in Table 1. We had 41 children aged less than 12 years which constituted 18.7%. Our youngest patient was 1 year and oldest was 79 years. Lowest haemoglobin noted in our study was 6.2 gm/dl and highest was 21.5 gm/dl. Lowest WBC count noted was 500/mm³ and highest was 18500/mm³. Lowest platelet count was 5000/mm³ and highest was 394000/mm³.

68.9% of our patients had IgG antibody positive at diagnosis followed by NS1 antigen in 39.7%. Detailed

serological profile of our study subjects is depicted in Table 2.

Haematological profile: Twenty (9.1%) of our study subjects had Haemoglobin of less than 10 gm/dl, whereas 72 (32.9%) had haemoglobin of greater than 14 gm/dl. Fifty-nine (26.9%) of the patients had low haematocrit of less than 35% and 32 (14.6%) had a haematocrit greater than 45%. Seventy-two (32.9%) had WBC count below 4000/mm³ and 18 (8.2%) had the counts above 11000/mm³. One hundred and seventy-six (80.4%) had thrombocytopenia with platelet count of less than 150000/mm³ and 69 (31.5%) patients had a platelet count of less than 50000/mm³.

Bone marrow suppression as inferred by decrease in two or more cell lines in the peripheral blood was seen in 87 (39.7%) of our study subjects. Most of them had decreased platelet and WBC counts. All the three cell lines were decreased in 12 (5%) of our subjects. Table 3 depicts the peripheral cell line suppression profile in our study subjects. Twenty-nine (33.3%) of the patients with two or more cell line suppression had only IgG positive. This was followed by 20 (23%) who had only NS1 antigen positive. Figure 1 depicts the proportion of patients with peripheral cell line suppression associated different serological phase of Dengue infection.

Table 1: Baseline characteristics	
Total (N)	219
Age (yrs) - median (IQR)	23 (14-35.5)
Gender Male - n (%)	123 (56)
Gender Female - n (%)	96 (44)
Hb (gm/dl) Mean (SD)	13.1 (2.5)
WBC/cumm - Median (IQR)	5075 (3315-7953)
Platelet count - Median (IQR)	80000 (36000-130000)

Table 2: Serological profile in dengue illness	
Positive serological parameter at diagnosis	Number (%)
NS1 Antigen	87 (39.7)
IgM antibody	54 (24.7)
IgG antibody	151 (68.9)
NS1 Antigen & IgM antibody	18 (8.2)
NS1 Antigen & IgG antibody	34 (15.5)
IgM antibody & IgG antibody	28 (12.8)

Table 3: Peripheral cell line suppression profile in dengue illness	
Decreased cell lines in the peripheral blood	Number (% of total cases)
Platelet & WBC	56 (26)
Platelet & RBC	19 (9)
RBC & WBC	0 (0)
Platelet, WBC & RBC	12 (5)
Total	87 (39.7)

DISCUSSION

Bone marrow involvement in Dengue has been a recognised phenomenon and several animal and human studies have demonstrated the mechanism of bone marrow suppression.^{7,8} This will be reflected in the peripheral blood as reduced counts of two or more than two cell lines.

Studies have also demonstrated that the Dengue virus have a tropism for hematopoietic cells and can proliferate in these cell lines without causing cytopathic effect. This enables them to sustain and propagate in this cell lines.⁸ Our study showed that less than half of the study subjects had indirect evidence of bone marrow suppression at the time of diagnosis.

The pattern of changes that occur in bone marrow is studied in experimental set up. This study has shown that the cell lines in the bone marrow get suppressed in the initial stage in viral infection and the nadir is seen on day 4 or 5 of clinical manifestation of fever.⁹ However, this suppressive effect can be seen up to 10 days after the cell lines get infected.¹⁰ These experimental findings have been reflected in the real life scenario as seen in our study. Maximum number of patients had the multiple cell line suppression during the IgG phase of the illness, which is typically seen about 10 days after infections. Second higher peak in the number of patients was seen in the early phase of clinical illness where only NS1 antigen was positive. This again supports the earlier finding that the bone marrow suppression starts about 4 to 5 days after infection which coincides with the onset of fever following incubation period.

Dengue virus does affect all the cell lines in the bone marrow with an increased tropism for erythrocytic line.⁸ Given this fact, it is reasonable to expect predominant decrease in the peripheral blood red cell counts. However, antibody response to the viral infection and an immunological cascade results in capillary leak^{5,11,12} and hence result

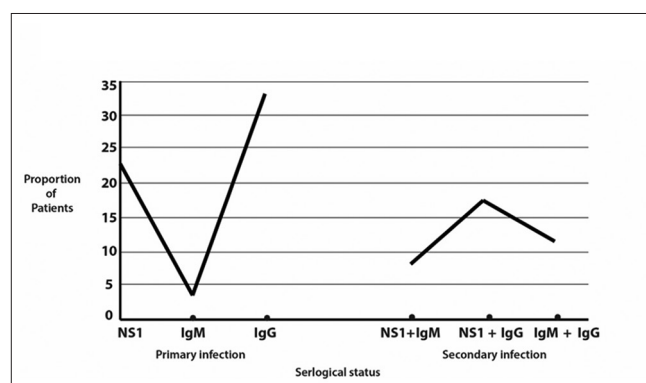


Figure 1: Proportion of patients with decreased peripheral cell line (2 or >2) in relation to various serological phases of dengue infection

in hemoconcentration. This can spuriously elevate the peripheral blood red cell count. In our study we had 26.9% of our patients having haematocrit below 35%. This could be explained by the fact that either patients were significantly anaemic prior to contracting this infection or there was bone marrow suppression as a predominant pathogenic mechanism and capillary leak was insignificant.

These patients could form a subset whose thrombocytopenia may theoretically respond to corticosteroids as demonstrated in few clinical studies.^{13,14} Conversely majority of the clinical studies have failed to demonstrate this benefit.¹⁵ Future studies segregating the patients with thrombocytopenia with different mechanisms and targeting the treatment may provide the answers to the dogma of steroid usage in dengue infection.

CONCLUSION

Nearly 40% of the patients will have decrease in multiple cell lines in the peripheral blood suggesting bone marrow suppression at the time of diagnosis of Dengue infection. Highest proportion (33%) is seen during post primary phase where only IgG antibody will be positive followed by patients in the initial stage of the infection where NS1 antigen alone will be positive (23%).

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Authors Contribution:

VK - Concept, review of literature, manuscript preparation and critical review of final manuscript; **RR** - Review of literature, collection of data and manuscript preparation; **SMD** - Concept, study design, statistical analysis, manuscript preparation and its critical review.

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