

Thrombocytopenia in Dengue Illness: Destruction, Suppression and Composite Platelet Index: A Retrospective Study

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ABSTRACT

Background: Dengue is a common viral illness which can be fatal. Thrombocytopenia is the common hematological abnormality which can result in catastrophic bleeding. The present study attempts to look at the magnitude of different mechanisms causing thrombocytopenia with the help of platelet indices.

Methods: This was a retrospective study undertaken in a tertiary care center in south India. Of all the adult patients admitted for the treatment of Dengue illness between June 2013 to May 2014, Platelet indices such as Mean platelet volume (MPV) and Platelet large cell ratio (P – LCR) of 98 patients were collected and analyzed. Proportion of patients with increased MPV (> 11.5) and P – LCR (>37) was then computed.

Result: Among the dengue patients, 56.1% had raised MPV (>11.5) and 59.2% had raised Platelet large cell ratio (>37). This suggested that more than half of the patients had platelet destruction and the rest had bone marrow suppression. There was significant increase in Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and platelet Large Cell Ratio (LCR) in Dengue patients compared to the controls.

We devised a composite platelet index (CPLI) by multiplying platelet count with MPV which we believe could be a better indicator of bleeding than either alone. In our study we found the median composite platelet index in dengue patients was 5.9 compared to 27. 2 in the controls.

Conclusion: Peripheral destruction and bone marrow suppression is seen in equal proportion of patients with dengue illness.

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Introduction

Dengue is a common epidemic illness seen in both pediatric and adult population. The severity of the disease can vary from mild fever to death. The most common hematological abnormalities observed are hemoconcentration and thrombocytopenia. Thrombocytopenia in dengue is due to multiple mechanisms such as peripheral destruction and bone marrow suppression to a variable degree. Gupta et.al have extensively reviewed dengue infection in Indian scenario ^[1]

Platelet counts below normal value define thrombocytopenia in dengue patients but do not indicate the underlying pathomechanism. Automated analyzers measure various platelet indices such as Mean platelet volume (MPV), Platelet distribution width (PDW) and Platelet large cell ratio (P-LCR), and their clinical usefulness has recently been recognized. ^[2,3,4]

With these indices, it is possible to assess the functional state of the bone marrow. Increased MPV and platelet large cell ratio indicate that the bone marrow is active and releasing the immature larger platelets at a higher rate than usual. Conversely, in thrombocytopenia due to hypo functional bone marrow, these indices remain unchanged. ^[5] With the help of these indices, we can infer the predominant mechanism of thrombocytopenia operating in dengue patients without bone marrow examination. So far studies have suggested different mechanisms of thrombocytopenia in dengue illness but we do not have the information about the proportion of patients developing thrombocytopenia due to various mechanisms. This study was undertaken to estimate the magnitude of different mechanisms of thrombocytopenia based on the different platelet parameters.

Primary objective: To estimate the magnitude of peripheral platelet destruction and bone marrow suppression suggested by depression of two or more cell lines in peripheral blood smear, causing thrombocytopenia in dengue patients.

Secondary objectives:

1. To compare platelet indices between dengue patients and controls
2. To compare the gender differences in platelet indices in dengue patients
3. To assess the correlation between MPV and platelet count
4. To derive a composite platelet index for prediction of bleeding in dengue patients

Materials and Methods

Study design: This is a retrospective analytical study undertaken in a tertiary care referral hospital in south India.

Inclusion criteria: Patients who were detected positive for dengue NS1 antigen, IgM, or both IgG and IgM antibodies by Immunochromatography (Standard Diagnostic inc. Biotec, Dengue duo, Gyeibgggu-do, Republic of Korea) and who were evaluated for platelet indices were included in the study.

Subjects: We screened 368 patients of seropositive dengue cases over a period of one year from June 2013 to May 2014. Out of these, 98 patients satisfying the inclusion criteria were selected. One hundred and sixteen age matched controls were selected from normal healthy persons enrolled in master health checkup.

Sample Processing: The blood sample was collected in EDTA vacutainer and transported to the laboratory via chute system. As soon as the sample was received in the laboratory, initial identification formalities were completed and subjected to analysis immediately. The average time taken from collection of sample to start processing is 40 mins in our hospital. The platelet count, PDW, MPV and P-LCR were analyzed by automated analyzer Sysmex XN-1000 (Sysmex America, Inc. in Lincolnshire, Illinois) and the results were compared.

Data Collection: All the results after approval is stored in hospital database. This was accessed using the inpatient number obtained from medical record section.

Composite Platelet index (CPLI). This is a conceptual index which we derived by multiplying platelet count (as decimal) with the Mean platelet volume (e.g. Platelet count of $1.5 \times 10^5 \text{ mm}^3$ and MPV of 10 fl, $\text{CPLI} = 1.5 \times 10 = 15$). The rationale behind this index is, fresh and large platelets would be more functional and hence counter the disadvantage of lower platelet count to certain extent. This means for a given platelet count, higher CPLI indicates higher MPV and hence better function and protection from bleeding.

Sample Size: In one of the study, the prevalence of severe neutropenia is reported to be 11.8% among dengue patients. ^[6] Assuming this to be due to suppression of bone marrow, confidence level of 90%, margin of error of 5%, we needed 113 patients. We had a total of 116 patients who fulfilled the inclusion criteria. However, 18 of them were below 12 years of age and constituted pediatric population. Since we did not have any control in that age group, we excluded them from the study. Thus 98 cases remained in the study. One hundred and sixteen patients from master health checkup who were healthy without any medical or surgical illness and not on any medication that could affect platelet counts. We had a total of 214 subjects.

Statistical Analysis: Continuous variables were analyzed with Student t test for comparison of means. In case of skewed distributions, the data was summarized as Median and interquartile range. Mann Whitney U test was used for comparison. Categorical variables were reported as proportions. Comparison of categorical variables was done using either Chi square test or Fischer exact test as appropriate. Analysis was done using Microsoft Excel 2013 and SPSS 20. A p value of less than 0.05 was considered statistically significant. Simple linear correlation between platelet count and mean platelet volume was calculated as Pearson product moment correlation.

Ethics: This study was approved by institutional ethical committee.

Result

Median age of cases was 27 years and that of controls was 31 years. Males were higher in number among cases with 63.8% (Male: Female = 1: 0.57) compared to control group which was 57.8% (Male: Female = 1: 0.73). Table 1 depicts the baseline characteristics of the study population. Mean platelet count in Dengue patients was 0.53×10^5 mm³ which was significantly lower compared to control group (mean 2.84×10^5 mm³). Other platelet indices such as platelet distribution width, mean platelet volume and platelet large cell ratio (P – LCR) were significantly lower in the control group. Table 2 depicts the comparison of platelet indices between cases and controls.

Table 1: Baseline characteristics.

	Cases (N = 98)	Control (N = 116)	P value
Age Median(IQR)	29.5 (21 – 40.8)	31 (26 - 38)	0.234
Males, n (%)	64 (65.3)	67 (57.8)	0.258
Females, n (%)	34 (34.7)	49 (42.2)	

Table 2: Comparison of Platelet indices between Dengue cases and controls.

Platelet indices Mean (SD)	Cases	Control	Mean diff	95% CI	P value *
Platelet count ($\times 10^5$ mm ³)	0.53 (0.28)	2.84 (0.75)	-2.31	- 2.45 to -2.16	0.0001
Platelet distribution width	15.1 (2.9)	10.8 (1.6)	4.3	3.7 to 4.9	0.0001
Mean platelet volume (Femtolitre)	11.7 (0.97)	9.9 (0.81)	1.8	1.57 to 2.02	0.0001
Platelet large cell ratio	38.3 (7.5)	23.7 (6.3)	14.6	12.8 to 16.3	0.0001

* Student 't' Test. SD - Standard deviation, CI – Confidence interval

Table 3: Comparison of proportion of patients with high MPV and P – LCR.

	Cases (%)	Control (%)	P Value *
MPV > 11.5	54 (55)	4 (3.4)	0.0001
MPV < 11.5	44 (45)	112 (96.6)	
P - LCR > 37	56(57)	4 (3.4)	0.0001
P - LCR < 37	42 (43)	112 (96.6)	

* Fischer exact test, MPV – Mean platelet volume, P –LCR – Platelet large cell ratio

Our control population had a mean MPV of 9.9 fl, with a standard deviation of 0.81. Mean plus 2 standard deviation yielded a value of 11.5 fl which was considered as upper limit in our study. Similarly, the value for platelet large cell ratio was obtained, which was 37. There were 53.4% of Patients with MPV more than 11.5fl and 56% of patients with P – LCR greater than 37 (Table 3). This was significantly higher than in control population (P = 0.0001). The proportion of patients with thrombocytopenia due to bone marrow suppression was just less than half.

The new Composite platelet index (CPLI) was computed by multiplying the platelet count and MPV. The median CPLI in dengue patients was 5.9 and in controls was 27.2 (Fig 1). Comparison of the medians using Mann Whitney U test yielded a Z value of -13.1, U of 0 and P of 0.0001 suggesting statistically significant difference.

Our study did not find any significant difference in platelet indices between males and females. The mean difference in platelet count between the genders was 0.04×10^5 , being low in males with a 95% Confidence interval of -0.14 to 0.06. Similarly, the mean difference in platelet distribution width was -0.9 fl (95% CI -0.97 to 0.17), mean platelet volume was zero (95% CI -0.35 to +0.35) and platelet large cell ratio was -0.9 (95% CI -3.62 to 1.82).

We did not find any significant linear correlation between platelet count and mean platelet volume in both the cases and the control groups. The correlation 'r' was 0.12 in cases and -0.1 in control group.

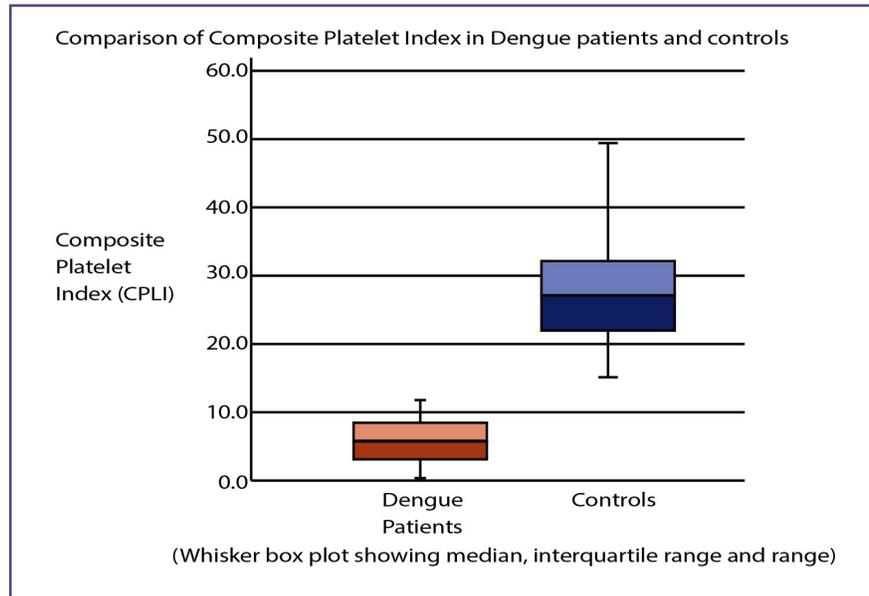


Fig. 1: Comparison of Composite Platelet Index in Dengue patients and controls.

Discussion

Dengue fever is commonly associated with thrombocytopenia.^[7, 8] Our study also reaffirmed such a finding. Out of the 350 patients screened, 98 (28%) had thrombocytopenia with platelet count of less than 1×10^5 hence necessitating request for platelet indices.

Anti-platelet antibodies are shown to increase during the acute phase of dengue infection resulting in thrombocytopenia.^[9] A study by Bashir AB et al on platelet indices in dengue patients had low platelet count, high PDW and high P-LCR.^[10] Our study showed similar results. It is well known that the platelets are destroyed stimulating the bone marrow activity. This results in varied sized platelets and hence higher PDW.

Hitherto, many authors have suggested multiple mechanisms of thrombocytopenia in Dengue patients.^[11, 12] However, there is no sufficient information on the magnitude of different mechanisms of thrombocytopenia in these patients. In our study we have addressed this issue. We found that both peripheral platelet destruction and bone marrow suppression to be equally predominant mechanisms in dengue patients. This could help clinicians in adopting strategies to counter immune mechanism with drugs such as Steroids or using blood products as temporary measure.^[13, 14]

Several authors have studied the mechanism of thrombocytopenia in experimental and in vitro studies.^[15,16,17,18] Viral infection of the non-erythrocytic cells and

release of cytokines which suppress the bone marrow is documented in these studies. In our study we studied this phenomenon with the help of platelet indices.

The composite platelet index proposed by us is based on very simple rationale that active bone marrow as noticed by increased MPV could be protective against bleeding in dengue patients. However, this has to be corrected to the platelet count for standardization. In this study we have not correlated this index with bleeding. In future, studies have to be designed to look into this potential predictive indicator, which will throw more light on the utility of this index in regular clinical scenarios.

MPV raises in thrombocytopenia due to peripheral platelet destruction. Hence we expected a negative linear correlation between platelet count and MPV. However, in our study we did not find any significant linear correlation between platelet count and MPV. This could be explained by the fact that there could be variable amount of bone marrow suppression even in patients with peripheral platelet destruction.

It is interesting to note that corticosteroid use in dengue infection has produced inconsistent results in clinical studies. Extensive review by Rajapakse and team have detailed 12 studies with five of them favoring the use of steroids and rest seven not showing any benefit.^[19] This raises the possibility of steroids being effective in a subset of patients who possible have immune mediated thrombocytopenia. Our new index CPLI could be of use to

categorize these patients with thrombocytopenia as due to peripheral destruction or otherwise. This could set a stage for further evaluation of benefits of steroids in dengue patients. Recently it has been shown that Dengue virus bind to platelet and become more infectious.^[20] The role of such a mechanism on platelet indices, and therapeutic value of immunosuppressant is yet to be studied.

In a large retrospective study in New Delhi, it has been shown that there is poor correlation between thrombocytopenia alone and clinical bleeding.^[21] Other authors have shown that it is possible to predict bleeding with the help of platelet indices instead of platelet count alone, which are now easily available from most of the analyzer.^[4] Our effort is to further fine tune these indices to identify the subset of patients with dengue illness and thrombocytopenia who could develop clinical bleeding.

Limitations:

Sample did not include the patients where platelet indices were not requested and hence not available.

Conclusion

Platelet indices suggest that both peripheral destruction of platelets and possible bone marrow suppression play role in causing thrombocytopenia in Dengue patients. In nearly half of the Dengue Patients Mean platelet volume is raised suggesting platelet destruction. In rest of the patients, hematological parameters suggest decreased production which could account for thrombocytopenia.

Dengue patients have decreased platelet count, increased MPV, PDW and P-LCR compared to controls. No statistical significance was found between the platelet indices in men and women with dengue infection

No linear correlation was found between MPV and platelet count in dengue patients, which suggests variable suppression of the marrow.

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None

Competing Interests

None Declared

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