

ROLE OF PLATELET INDICES IN DENGUE FEVER CASES IN CORRELATION WITH SEROLOGICAL MARKERS – A STUDY OF 100 CASES

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ABSTRACT

Objective: This study aims to evaluate the utility of various platelet indices, i.e., platelet count, mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT) in dengue fever and to study different serological markers (NS1 Ag, IgM, and IgG) and compare them with platelet counts.

Methods: This was a retrospective, observational, and cross-sectional study done over 6 months. A total of 100 cases with symptoms of dengue and seropositive for any of the rapid tests (NS1 Ag, Ig M, and Ig G antibodies) were included. Platelet count, MPV, (PDW), and (PCT) were determined using six-part Hematology analyzer. Based on the platelet count, all dengue seropositive cases were divided into 5 groups, and platelet indices were analyzed and tested for statistical significance using Chi-square test and Anova test.

Results: Thrombocytopenia was more common in the NS1 Ag-only reactive cases. Dengue hemorrhagic fever (DHF) cases (12) had significantly low MPV, high PDW, and low PCT. Statistical analysis between mean MPV, mean PDW, mean PCT, and platelet count among various groups was significant. Low MPV, high PDW, and low PCT were more evident when platelet count was < 1lakh.

Conclusion: Our study showed that thrombocytopenia was more common in the NS1 Ag-only reactive cases than other serological markers. A Low MPV with a spike in PDW, and a plummeted PCT in cases with thrombocytopenia indicate severity of dengue infection. These platelet indices act as surrogate markers in predicting the risk of bleeding.

Keywords: Dengue fever, Platelet count, Mean platelet volume, Platelet distribution width, Plateletcrit, NS1 antigen

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INTRODUCTION

Dengue fever is a mosquito-borne viral disease transmitted by the vector *Aedes aegypti* mosquito [1]. Most of the dengue cases are reported from Southeast Asia and the western Pacific regions. India is regularly reporting incidence outbreaks of dengue infection [2]. Dengue is endemic in India with increasing incidence. It is an arboviral disease and belongs to genus flavivirus of the flaviviridae family. There are four serotypes of dengue virus which are DENV1, DENV2, DENV3, and DENV4. Incubation period is 4–6 days [3]. The spectrum of dengue infection ranges from being asymptomatic to symptomatic which includes dengue fever (DF), dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Clinical features include fever, retro-orbital pain, and myalgia. Severe cases present with rash, abdominal pain, vomiting, respiratory discomfort, and bleeding manifestations. Thrombocytopenia is commonly associated with dengue infection [3,4]. Rapid and accurate diagnosis of dengue plays a cardinal role in the management, including reducing the risk of complications and associated case fatality. Various laboratory investigations available for diagnosis of dengue infection are isolation of virus, viral RNA detection by RT-PCR, serological tests, viral antigen detection by ELISA, rapid diagnostic tests, and analysis of hematological parameters [4].

Commercially available rapid diagnostic tests contain test kits for the detection of NS1 antigen, anti-dengue IgM, and IgG antibodies. Non-structural protein1 (NS1) is a viral antigen that can be detected in patients with both primary and secondary infection up to 6 days after the onset of illness. It is an early diagnostic marker of dengue infection. Other laboratory investigations include assessment of various hematological parameters such as platelet count (PC) and platelet indices such as mean platelet volume (MPV), platelet distribution width (PDW), Plateletcrit (PCT) [4].

Aims and objectives

1. To study the age and sex-wise distribution of the seropositive dengue fever cases.
2. To study different serological markers (NS1 Ag, IgM, and IgG) and compare them with platelet counts.
3. To compare the mean of different platelet indices (MPV, PDW, and PCT) with various groups of platelet count.

METHODS

This was a retrospective, observational, and cross-sectional study done for 6 months from September 2019 to February 2020 in the Department of Pathology in a tertiary care hospital. A total of one hundred patients (both outpatient and inpatient) having both symptoms of dengue fever and seropositive for any of the rapid diagnostic tests (i.e., any of the NS1 antigen, Ig M antibody, or IgG antibody) were included in this study. Seronegative patients were excluded from this study. All the relevant clinical information was obtained from the hospital database. Rapid diagnostic tests were done in all cases with symptoms of dengue infection for detection of NS1 antigen, IgM, and IgG antibodies using the immunochromatography test kit. The platelet parameters were measured using the Horiba Yumizen H500 automated hematology analyzer on venous samples collected in K3EDTA. The samples were analyzed within 2 h from venipuncture after collecting in vacutainers, to avoid EDTA-induced hematological changes. The parameters analyzed were platelet count (PC), mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT). Data were analyzed using statistical tools available in Microsoft Office Excel2010 for Windows 10 with the Chi-square test and ANOVA test. A p-value of less than 0.05 was considered statistically significant. This study was approved by Internal Ethics Committee, and the patient consent had been waived off as the required data, without any identifiable patient information, was obtained from the hospital database.

RESULTS

A total of 100 dengue seropositive cases were included in this study. The age of the patients in this study ranged from the 7th day of life to 68 years. The maximum number of cases was in the age group of 21–30 years (26%), followed by 0–20 years (22%) and 31–40 years (19%). There were 54 cases (54%) of males and 46 cases (46%) of females, with male-to-female ratio of 1.17: 1 (Table 1).

Based on the platelet count, cases were divided into five groups, i.e., <0.25, >0.25 to 0.50, >0.50 to 1, >1–1.5 and >1.5 (lakhs/mm³). Normal platelet count (>1.5 lakh) was seen in 26 cases (26%), and thrombocytopenia (platelet count <1.5 lakh) in 74 cases (74%). Out of 100 cases, 56 cases (56%) were positive for NS1 Ag only, with thrombocytopenia evident in 44 cases (78.6%). 26 (26%) cases tested positive for IgM only with thrombocytopenia apparent in 14 cases (53.8%). 2 (2%) cases emerged positive for IgG only, of which Thrombocytopenia was noted in one case (50%) (Table 2).

Cases with low MPV (<9 fl) were 59 (59%), normal MPV (9 to 13 fl) were 41 (41%), and no cases with high MPV (>13 fl). Low PDW (<9 fl) was seen in 4 cases (4%), normal PDW (9–14 fl) in 44 cases (44%), and high PDW (>14 fl) in 52 cases (52%). Also, cases with low Plateletcrit (PCT) (<0.15%) were 76 (76%), normal PCT (0.15 to 0.4%) were 21 (21%), and high PCT (>0.4%) were 3 cases (3%). Bleeding manifestations were seen in 12 cases (16.2%) among patients with thrombocytopenia only, of which hematuria was the most common feature. In DHF, 9 cases had low MPV, 12 cases had high PDW, and 12 cases had low PCT. Platelet indices in DF and DHF were incorporated in Table 3. Bleeding manifestations were seen in 10 cases where platelet count is <0.5 lakhs/cumm (Fig. 1).

It was observed that with a fall in platelet count, MPV also showed a similar decrease coupled with a decline in PCT and an increment in PDW in patients with bleeding manifestations. Statistical analysis between mean MPV and platelet count amongst various groups is found to be significant ($p < 0.0001$). Similar statistical significance was also observed when mean PDW and mean PCT were compared with various groups of platelet count, respectively (Table 4).

DISCUSSION

Dengue fever is one of the major endemic diseases in India, causing public health problems. Thrombocytopenia in dengue depends on many factors varying from a reactive immune or non-immune response against platelets to a decreased platelet production secondary to bone marrow suppression. It has been proposed in several studies that not only direct infection of megakaryocytes by the dengue virus but also increased peripheral destruction of platelets could lead to thrombocytopenia. Along with platelet count, platelet indices such as MPV, PDW, and PCT were considered as platelet activation markers [5–8]. MPV refers to the average size of platelets. It is an indicator of regenerative platelets and bone marrow activation/suppression. It also acts as an independent predictor of bleeding. PDW is a measure of variation in platelet size and it increases with platelet activation. It also indicates heterogeneity of platelet morphology. PCT is a measure of platelet biomass that is derived from MPV and platelet count, thus, it is directly proportional to MPV [2,9].

A total of 100 dengue seropositive cases were studied. Predominantly 45% of the cases were seen in 21–40 years age group which is similar to the studies by Navya *et al.* [10] (40%) and Mukker *et al.* [11] (36.3%). There were 54 (54%) males and 46 (46%) females with a slight male preponderance (M: F=1.17: 1) analogous to that of Hardeva *et al.* [6] (1.94:1) and Shailja *et al.* [1] (2.63:1). Thrombocytopenia was discerned in 74 cases (74%) and normal platelet count in 26 cases (26%). Majority of the cases (33%) with thrombocytopenia were in the group with a platelet count between >0.50 and 1 lakh akin to that of Navya *et al.* [10] (42%), S.B. Chiranth *et al.* [9] (36.8%) and Shailja *et al.* [1] (36%). Out of 100 cases, dengue fever (DF) was seen in 88 cases

Table 1: Age and sex distribution of dengue cases

Age (years)	Male	Female	Total	Percentage
0–20	14	8	22	22
21–30	17	9	26	26
31–40	9	10	19	19
41–50	7	6	13	13
>50	7	13	20	20
Total	54	46	100	100

Table 2: Disposition of cases with Thrombocytopenia in Dengue seropositive patients

Positive serological markers	Number of cases	No. of cases with platelet count below 1.5 lakh per mm ³
NS1 Ag only	56	46 (82%)
Ig M only	26	14 (54%)
Ig G only	02	01 (50%)
NS1 Ag and Ig M	12	11 (92%)
Ig M and Ig G	01	-
NS1 Ag and Ig G	01	-
NS1 Ag, Ig M and Ig G	02	02 (100%)
Total	100	74 (100%)

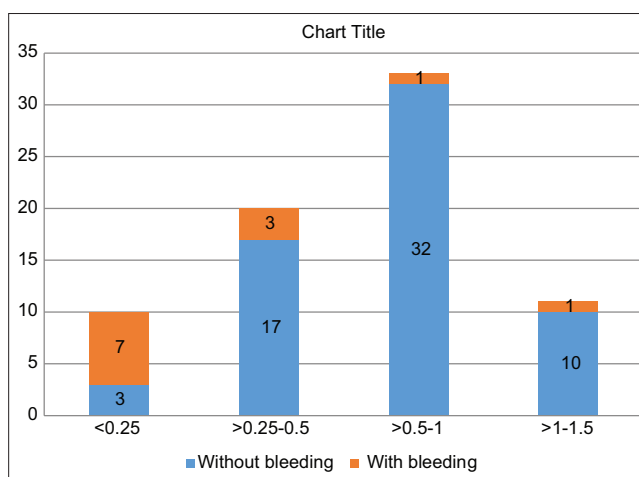


Fig. 1: Distribution of cases with or without bleeding across various groups of thrombocytopenia (platelet count in lakhs/cumm)

(88%) and dengue hemorrhagic fever (DHF) was seen in 12 cases (12%).

Our study showed that thrombocytopenia was more common in the NS1 Ag only reactive cases than other serological markers. Chi-square test proved that the association of thrombocytopenia with NS1 antigen only was high enough with a statistical p value of < 0.0001 , which is in accordance with the studies by Kulkarni *et al.* [12].

In the present study, maximum number of dengue serology positive cases were associated with low MPV (<9 fl) in 59% of cases, high PDW (>14 fl) in 52% of cases, and low PCT (<0.15%) in 76% of cases. In a study done by Hardeva *et al.* [6], low MPV, high PDW, and low PCT were seen in 70%, 78%, and 81% of cases, respectively. Similar findings were also seen in the study done by Navya *et al.* [10], showing low MPV (72%) and high PDW (92%). Studies done by Shailja *et al.* [1] showed normal MPV (74.5%), high PDW (72%), low PCT (100%), and Khatri *et al.* [13] reported as Normal MPV (92.4%), Normal PDW (83%), and High PCT (98.1%) (Table 5).

Our study showed a low MPV, high PDW, and low PCT in cases with platelet count less than one lakh, which reflects statistical significance

Table 3: Platelet indices in dengue seropositive cases

	MPV			PDW			PCT		
	Low	Normal	High	Low	Normal	High	Low	Normal	High
DF	50	38	0	4	44	40	64	21	3
DHF	9	3	0	0	0	12	12	0	0
Total	59	41	0	0	44	52	76	21	3

Table 4: Comparison between mean of various platelet indices versus five groups of platelet count

Platelet indices	Platelet count (lakhs per mm ³)					p-value
	<0.25	>0.25-0.50	>0.50-1	>1-1.50	>1.50	
Mean MPV (fL)	8.1	8.4	8.6	9.1	10.0	<0.0001
Mean PDW (fL)	15.1	15.8	15.3	13.2	12.7	<0.0001
Mean PCT (%)	0.03	0.03	0.06	0.12	0.24	<0.0001

Table 5: Comparison of platelet indices with other studies

Other studies	MPV			PDW			PCT		
	Low	Normal	High	Low	Normal	High	Low	Normal	High
Hardeva et al.[6] (n=200)	140	60	0	0	44	156	162	38	0
Shailja et al.[1] (n=200)	0	149	51	0	56	144	200	0	0
Khatri et al.[13] (n=106)	3	98	5	0	88	18	104	2	0
Navya et al.[10] (n=100)	72	28	0	0	8	92	-	-	-
Present study (n=100)	59	41	0	4	44	52	76	21	3

with a $p < 0.0001$, in comparison to higher platelet count groups. Similar results were seen in studies done by Mukkar *et al.* [11], Hardeva *et al.* [6], and Chiranth *et al.* [9] showing decreasing MPV, increasing PDW, and decreasing PCT levels noteworthy in patients with thrombocytopenia of platelet count less than one lakh. High MPV with thrombocytopenia is due to peripheral platelet destruction and bone marrow activation. Normal or low MPV with thrombocytopenia is due to decreased platelet production and bone marrow suppression [14,15]. Many studies have observed that high MPV with severe thrombocytopenia is associated with less risk of bleeding and low MPV with a high chance of bleeding [16,17].

Common causes of bleeding in dengue infection include abnormal platelet function, thrombocytopenia, hyperfibrinolysis, and reduced synthesis of coagulation factors. In our study, 12 (16.2%) cases had bleeding manifestations among patients with thrombocytopenia. These include hematuria (60%) as the most common feature, followed by petechiae (45%), gum bleeding (25%), epistaxis (25%), and melena (10%). This study observed that the risk of mucocutaneous bleeding increased with a sharp fall in platelet count with a corresponding decline in MPV, PCT, and increased PDW. In a study done by Bashir *et al.* [18], bleeding manifestations were more with low MPV and low platelet count. In severe thrombocytopenia, bleeding severity may also increase. PDW is of some value in differentiating essential thrombocythaemia from secondary or reactive thrombocytosis. Increase in PDW reflects the activation of megakaryocytes with increased thrombopoiesis and the release of platelets of varying sizes from the bone marrow. In this study, high PDW was observed with a platelet count of less than one lakh/mm³ in accordance with Navya *et al.* [10] and Hardeva *et al.* [6]. All cases of DHF showed high PDW akin to PCT was found to be declining with a drop in the platelet count in this study, with all cases of DHF showing a significant reduction in PCT. There is also some evidence supporting that PCT predicts the risk of bleeding in patients with thrombocytopenia [8,19].

Thrombocytopenia is not directly proportional to the risk of bleeding and platelet transfusions may be unwarranted in every case of thrombocytopenia. The present study demonstrates that decreasing MPV, decreasing PCT, and increasing PDW levels are more objective

than simple platelet counts in assessing the degree of severity of dengue infection and risk of bleeding when associated with thrombocytopenia. Thus, these platelet parameters would be helpful in the early diagnosis of dengue hemorrhagic fever.

CONCLUSION

This study eventually showed that the maximum number of dengue serology positive cases belong to 2nd and 3rd decades with slight male preponderance. NS1 Ag only reactive cases exhibited more degree of thrombocytopenia, with majority of them in the group with a platelet count between >0.50 to 1 lakh/mm³. Along with platelet count, MPV, PDW and PCT are useful in monitoring dengue fever. A low MPV coupled with a spike in PDW, and a plummeted PCT in cases with thrombocytopenia indicate suppression of thrombopoiesis in the bone marrow and may trigger bleeding. Thus, these platelet parameters play a significant role in predicting the severity of the disease.

AUTHOR'S CONTRIBUTION

All three authors contributed equally to conceptualizing the research proposal, literature review, data collection and analysis, and manuscript writing.

CONFLICTS OF INTEREST

Nil.

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Nil.

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