

## Original Research Article

# Platelet indices evaluation in patients with dengue fever

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### ABSTRACT

**Background:** Platelet indices (PIs)-Platecrit, mean platelet volume (MPV) and platelet distribution width (PDW)-are a group of platelet parameters obtained as a part of complete blood count using automated hematology analyzers. Evidence suggests that PIs may have diagnostic and prognostic value in febrile thrombocytopenia. This study aims to understand the profile of PIs in dengue fever. Aims and objectives was to study the platelet indices in patients with dengue fever.

**Methods:** The present study is a retrospective observational study conducted in a tertiary hospital in Kerala. Platelet count, mean platelet volume (MPV), platecrit and Platelet distribution width (PDW) along with routine blood parameters hemoglobin(Hb), hematocrit, WBC, Platelet count, serum bilirubin, liver enzymes (AST, ALT) of 123 patients were collected. These 123 patients were grouped into three according to the platelet count (<20000, 20000-100000, >100000). All the test results are available in hospital database. This was accessed using inpatient numbers obtained from medical records department of our institution. All analysis was done using free to use software R and values were rounded off to the nearest decimal point. Non normally distributed parameters were expressed as median (IQR). Parameters which did not follow normal distribution were analyzed with Kruskal Wallis test and the values are expressed as mean (SD) and a p value <0.05 was considered statistically significant.

**Results:** Platelet indices PDW ( $57\pm 13.8$  vs.  $55.4\pm 6.9$ , p value 0.001) and MPV ( $9.2\pm 0.09$  vs.  $13.8\pm 1.3$ fL, p value <0.001) values were significantly altered in dengue fever with platelet counts below 20,000 compared to platelet count more than one lakh group. Similarly, the Platelet index (MPV $\times$ PDW\PLC $\times$ PCT), MPV\PLC, MPV\Platecrit, PDW\PLC and PDW\Platecrit ratio showed statistically significant difference between the different platelet groups.

**Conclusions:** Platelet indices are useful parameters in dengue infection. Other than platelet count, PDW, MPV, platecrit are useful to monitor dengue fever.

**Keywords:** Dengue, Platelet, Mastoid process, PLT, PCT, PDW, MPV

### INTRODUCTION

Dengue fever (DF) is an acute febrile disease characterized by sudden onset of fever of 3-5 days, intense headache, myalgia, joint pain, retro-orbital pain, anorexia, gastrointestinal disturbances and rash.<sup>1</sup> Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are life threatening reversible vascular

complications of DF and are associated with severe thrombocytopenia, bleeding and increased vascular permeability.<sup>2</sup> The decreasing platelet counts have found to predict the severity of the disease.<sup>3</sup> So, there is a need to study platelets profile and understand its importance so that adverse outcomes of this rapidly spreading disease can be controlled to a great extent.

**Platelet indices**

Platelet indices like MPV, PDW and P-LCR have been investigated as prospective platelet activation indicators.<sup>4</sup> Platelet volume, a marker of platelet function and activity is obtained as mean platelet volume (MPV) by laboratory haematology analyzers. When platelet production is reduced, newer platelets become larger and more active, and MPV levels rise.

During platelet activation their shape changes from biconcave discs to more spherical, and also pseudopod formation commonly occurs that leads to MPV increase during platelet activation. MPV is a useful independent predictor of bleeding. It is surrogate indicator of bone marrow activity; a high MPV is suggestive of increased megakaryocyte activity. A low MPV indicates bone marrow suppression and suggests a risk of bleeding. Correlation of parameters like platelet count and MPV with bleeding and severity of the disease can be a predictor of disease outcome.<sup>5</sup> The normal range for MPV in Indian population is from 8.6-15.5 fL.<sup>6</sup>

PDW is a marker of volume variability in platelets size and is elevated in the presence of platelet anisocytosis.<sup>7</sup> The PDW reported varies with reference intervals ranging from 8.3 to 56.6%. PDW directly measures variability in platelet size, changes occurring with platelet activation, and suggests the heterogeneity in platelet morphology.<sup>8-12</sup>

Platecrit is a measurement of total platelet mass. Platecrit is useful for detecting platelet quantitative abnormalities. PCT is the volume occupied by platelets in the blood as a percentage and calculated by the formula  $PCT = \text{platelet count} \times MPV / 10,000$ . The normal range for PCT is 0.22-0.24%.<sup>13-16</sup>

**METHODS**

A retrospective study of 123 patients admitted between June 2017 to March 2018 with clinical features of dengue fever (WHO definition) and serological positivity (NS1 antigen, ELISA/IgM antibody to the dengue virus) in a tertiary hospital in Kerala.

Subjects were divided into three groups according to their platelet count (<20,000, 20000 to 100000, >100000). Platelet indices (MPV, PDW, PCT) were studied in each group. The CBCs were performed using a Siemens RXL MAX fully automatic hematology analyzer (US). Ratio of MPV/PLC, MPV/Platecrit and similarly PDW/PLC, PDW/platecrit and platelet Index (MPV x PDW/platelet count x PCT) were calculated as these ratios and platelet index could be candidate markers of severity of illness. The utility of these ratios were also looked into in our study.

**Inclusion criteria**

All patients above 18 years with clinical features of dengue with serologically positive infection were included.

**Exclusion criteria**

- Evidence for cirrhosis,
- Sepsis or other active infections,
- On antiplatelet drugs or NSAIDS.

**WHO criteria**

*Clinical criteria for diagnosis of dengue fever*

Dengue fever-classical dengue fever is an acute febrile viral disease frequently presenting with two or more of the following-headache, bone or joint pain, muscular pain, retro-orbital pain, rash and leucopenia.

**RESULTS**

Baseline characteristics of the patients are shown in Table 1. In table 2, of the total 123 dengue cases, age wise distribution shows maximum cases in the age group 41-50 years (33.1%), least in the age group 21-30 years (21%).

**Table 1: Baseline characteristics of the patients.**

Parameter	Median (IQR)
Age	44 (36-55)
Hb	13.4 (12.1-14.6)
WBC	4925 (3400-7425)
Bilirubin	1.8 (1-2.4)
AST	92 (53.3-125.5)
ALT	76.5 (48-113.8)
Haematocrit	39.1 (35.2-41.8)
Platelet count	29500 (15000-85750)
Platelet distribution width (%)	54.1 (48.3-59.4)
Platecrit (%)	0.04 (0.02-0.1)
Mean platelet volume (fL)	11.1 (8.7-12.4)

**Table 2: Age distribution among the study participants.**

Age groups (years)	N (%)
≤20	1 (0.8)
21 - 30	21 (16.9)
31 - 40	24 (19.4)
41 - 50	41 (33.1)
51 - 60	29(23.4)
61 - 70	7 (5.6)
71 - 80	1 (0.8)

Table 3 shows 52 of them had platelet counts below 20,000, 69 patients had counts between 20,000 to 100000 and 3 patients had counts above 100000.

Table 4 shows comparison of platelet indices and ratios among the three groups based on platelet count. Comparing the platelet indices it was found that in

patients with platelet count below 20,000 the mean PDW was 57%, whereas those with platelet count between 20,000 to 100000, the mean PDW was 51.4% and 55.4% in those with platelet count above 100000 which showed statistically significant difference between the groups (p value 0.001).

**Table 3: Comparisons of the baseline parameters between the three groups.**

Parameter	Platelet count	n	Mean (SD)	p value
Hemoglobin (gms)	< 20000	52	13.4 (2.5)	0.3
	20000-100000	69	12.9 (2)	
	> 100000	3	13 (0.4)	
Haematocrit	< 20000	52	39.4 (7.6)	0.005 *
	20000-100000	68	37.5 (4.3)	
	> 100000	3	36.5 (0.6)	
WBC count	< 20000	51	5676.9 (2061.5)	0.09
	20000-100000	68	5519.5 (3629.4)	
	> 100000	3	6950 (391.3)	
Serum Bilirubin	< 20000	52	1.9 (0.6)	0.02 *
	20000-100000	69	1.8 (1.2)	
	> 100000	3	0.4 (0.2)	
AST	< 20000	52	87.1 (47.4)	0.2
	20000-100000	69	193.9 (550.9)	
	> 100000	3	119.3 (87)	
ALT	< 20000	52	72.4 (33.5)	0.2
	20000-100000	69	153.9 (404.9)	
	> 100000	3	87.3 (32.6)	

**Table 4: Comparison of the platelet indices between the three groups.**

Platelet parameter	Platelet count	n	Mean (SD)	p value
Platelet distribution width (%)	< 20000	52	57 (13.8)	0.001*
	20000-100000	69	51.4 (8.6)	
	> 100000	3	55.4 (6.9)	
Platecrit (%)	< 20000	52	0.02 (0.01)	< 0.001*
	20000-100000	69	0.1 (0.07)	
	> 100000	3	0.1 (0.1)	
Mean platelet volume (fL)	< 20000	52	9.2 (1.9)	< 0.001*
	20000-100000	69	12 (1.9)	
	> 100000	3	13.8 (1.3)	
MPV/PLC	< 20000	52	0.0008 (0.0004)	< 0.001*
	20000-100000	69	0.0002 (0.0001)	
	> 100000	3	0.0001 (0.00003)	
MPV/Platecrit	< 20000	52	583.4 (250.3)	< 0.001*
	20000-100000	69	196 (212.9)	
	> 100000	3	3198.4 (5395.5)	
Platelet distribution width/ platelet count	< 20000	52	0.005 (0.003)	< 0.001*
	20000-100000	69	0.001 (0.0007)	
	> 100000	3	0.0005 (0.0001)	
Platelet distribution width/ Platecrit	< 20000	52	3586.1 (1815.8)	< 0.001*
	20000-100000	69	881.1 (975.9)	
	> 100000	3	14330.6 (24271.8)	
Platelet Index	< 20000	52	3 (3)	< 0.001*
	20000-100000	69	0.2 (0.3)	
	> 100000	3	1.7 (2.9)	

In the table 4 as expected the hematocrit values were higher in patients with platelet count below 20,000 (p value 0.005) and serum bilirubin values were elevated in those with platelet count below 20,000 (p value 0.02) both of which were statistically significant.

The mean MPV values were 9.2fl in patients with platelet count below 20,000 as compared to 12fl in the platelet count group of 20,000 to 100000 and 13.8fl in patients with platelet count above 100000. There was statistically significant difference between the groups (p value 0.001).

Comparison of the platelet index (MPV x PDW\PLC x PCT) shows a higher value in patients whose platelet count was below 20,000 as compared to patients with a count between 20,000 to 100000 and above 100000, which was statistically significant (p value<0.001).

Similarly, the MPV\PLC ratio and MPV\Platecrit ratio shows statistically significant difference between the three platelet groups. The MPV\PLC value was higher (0.0008) in the platelet count <20,000 group as compared to the other two groups.

The MPV\Platecrit value was significantly high in the platelet count>100000 group as compared to those with platelet count <20,000 and 20,000 and 100000 (p value <0.001).

On comparing the ratio between the platelet distribution width and platelet count amongst the three platelet groups it was found to have a higher value (0.0005) in the 100000 platelet group as compared to that in the group 20,000 to 100000(0.001) and <20,000 group (0.005), which was statistically significant (p value<0.001).

Similarly, the platelet distribution width to platecrit ratio was significantly different between the three groups with high value in the 100000 platelet count group as compared to the other two groups (p value<0.001) (Table 4).

## DISCUSSION

Dengue fever is a growing public health concern in tropical countries like India.<sup>17</sup> It is assessed that there are as of now 50-100 million instances of dengue consistently around the world, including more than 500,000 announced instances of dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS).<sup>18</sup>

In spite of the fact that in India dengue was first reported in 1940s, the first case of confirmed dengue infection from Kerala was recorded in 1997 only. Since 2001 occurrence of dengue increased in Kerala and outbreaks were reported repeatedly from most of the central and southern districts. In the year 2017, approximately 18,700 cases of dengue have been confirmed from India which was quiet alarming. Kerala was at the top of the list

which was having the highest number reported from India. The total number of dengue patients in Kerala was 9104 followed by Tamil Nadu 4174, Karnataka 1945 cases, Gujarat 616, Andhra Pradesh 606, West Bengal 469, and Delhi 100 till 2<sup>nd</sup> July 2017. Thrombocytopenia has dependably been one of the criteria utilized by WHO rules as a potential pointer of clinical severity.<sup>19</sup> Development of thrombocytopenia in dengue patients mainly rests on two events: decreased production of platelets in the bone marrow and/or increased destruction and clearance of platelets from peripheral blood.<sup>20</sup> Studies report that the platelet activation (with elevated surface P-selectin) and apoptosis (with increased caspases and phosphatidylserine (PS) expression) are associated in the early days of dengue infection.<sup>21,22</sup> Reports also show that the activation of complement factor C3 followed by binding of C5b-9 complex to platelet surface is significantly linked with platelet destruction and thrombocytopenia in these patients.<sup>23-25</sup> 11 of 61 patients studied by Mitrakul et al, had destruction as a main cause for thrombocytopenia, as revealed by platelet kinetic study. A low MPV implies marrow suppression as a cause of thrombocytopenia and a rising MPV heralds the improvement in platelet count. A low MPV in Indian subset of patients has been reported in more than two third of the Navya et al, study group of DF patients.

Other studies didn't find consistent correlation with MPV and Dengue severity, that might be partly due to different pathogenesis or lack of a similar design to observe the MPV and platelets serially till recovery. Serially observing the MPV and platelets may guide a clinician in an important subset of patients in DF and severe dengue where the mechanism of thrombocytopenia is largely marrow suppression-initial MPV significantly low and the thrombocytopenia recovery following the MPV. Increased MPV indicates increased platelet diameter, which can be used as a marker of production rate and platelet activation. During activation, platelets' shapes change from biconcave discs to spherical, and a pronounced pseudopod formation occurs that leads to MPV increase during platelet activation.<sup>26</sup> Platelets with increased number and size of pseudopodia differ in size, possibly affecting platelet distribution width (PDW) which increases during platelet activation.

Low platelet count, low PCT and high PDW may be used as predictor of severity of Dengue infection. Shah et al and Borkataky et al, found a higher PDW in hyperdestructive thrombocytopenia when compared to hypoproductive thrombocytopenia.<sup>27,28</sup>

In our study, the mean platelet count was 29,500. Study by Anuradha M et al, showed thrombocytopenia in 89% of total dengue patients.<sup>29</sup> Our study revealed a low MPV and high PDW in patients with platelet counts below 20,000 compared to higher platelet count groups. Similar result was seen in a case control study by Bashir AB et al.

They found MPV was decreased in dengue positive cases and was normal in control group. PDW was normal in control group while it was increased in dengue infection.<sup>30</sup> This study is in accordance with our study.

Navya et al, studied the relationship between platelet parameters like platelet count, MPV and PDW and severity of the disease (DF/DHF/DSS). Significant difference was observed between severity of the thrombocytopenia and severity of the disease (P value = 0.013). Dengue positive cases were associated with low MPV and high PDW values in 72% cases and 92% cases respectively. They found Low MPV (13fl) shows sensitivity for dengue fever thus reflecting a predictive marker for diagnosing dengue fever in endemic area.<sup>20</sup>

Sharma K et al, studied 200 Dengue fever cases. Out of which, 68% cases were of DF, 23% DHF and 9% DSS i.e. classical dengue fever was most common presentation. 98% cases of dengue had thrombocytopenia. MPV showed no significant correlation with severity, serology and treatment outcome, thus excluding its role in dengue cases. No significant difference was observed in Mean between MPV at the time of minimal platelet counts and at discharge in dengue cases except in dengue fever cases.<sup>31</sup>

In the study, done by Dewi et al, there was no significant difference in MPV between DF, DHF and DSS (9.18±1.5 fL vs. 8.94±1.94 fL vs. 8.57±1.03 fL, p =0.761).<sup>32</sup> Wiwanitkit et al, found that MPV for patients with DHF is not decreased and appears to be similar to that for the general healthy population.<sup>5</sup>

In our study we have studied the ratios of the platelet indices in the three platelet groups (<20,000, 20,000 to 100000 and >100000) The Platelet index (MPVxPDW\PLCxPCT) was 3 in the 20,000 group as compared to a value of 1.7 in the 100000 group (p value <0.001). The changes in platelet ratios also reflect the bone marrow involvement of dengue virus. Dengue is also called "break bone fever" because it is either directly and/or indirectly involved in dengue manifestations. Interestingly, early bone marrow suppression has long been recognized as a common clinical feature in dengue infected patients.

An early investigation of dengue cases in Southeast Asia suggested that the bone marrow mass is at its nadir prior to the onset of fever and at its peak 2-3 days later (the time when symptoms become severe and patients seek professional help in the hospital). Cumulative data supporting the involvement of bone marrow in dengue virus infection include factors like isolation of virus from bone marrow of patients with dengue fever. Occurrence of bone marrow aplasia following dengue fever. *Ex vivo* experimental studies showing dengue fever survival in hemopoietic cells and virus replication in leukocytes derived from the bone marrow. Dengue virus infection transmission via contaminated bone marrow has also

been documented.<sup>33</sup> Similarly, the ratios of the platelet indices and platelet index have been compared in a case control study in a pediatric population as a predictor of mortality.<sup>34</sup> None of the other studies in dengue infection have taken into account these ratios. Further prospective studies are needed to assess the utility of these indices in dengue fever.

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