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Original Research Paper

Significance of Platelet indices in dengue fever patients

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ABSTRACT:

Dengue is a tropical viral disease caused by dengue virus with varied clinical features ranging from dengue fever to dengue shock syndrome. During a dengue infection, there is thrombocytopenia with platelet activation and various theories are proposed for the same. The aim of our study was to record and analyze the variations in the platelet parameters such as MPV, PDW, Plateletocrit (PCT) and Platelet Count (PC) in dengue infected patients and to establish their significance in patient prognostication. Our study is the first of its kind reported from Kerala, India. Our study revealed a low MPV <9fl (8.5 fl) shows sensitivity for dengue fever. Moderate thrombocytopenia with increase in mean platelet volume indicated good marrow response and suggests a good prognosis. A sudden dip in MPV and increase in platelet count may be taken as an early indication of recovery and plan discharge accordingly. Mean Platelet Volume and Plateletcrit along with platelet count are reliable parameters to establish good patient prognosis in dengue infection.

KEYWORDS: Prognosis, Infection, Dengue, Platelet, MPV, PDW, Kerala , India

INTRODUCTION:

Dengue is a tropical viral disease caused by dengue virus (DEN-1, DEN2, DEN-3, DEN-4). They are arbovirus belonging to the genus flavivirus of the family flaviviridae. Mode of transmission is through the bite of Aedes aegypti mosquitoe [1,2]. Essentially the disease manifest in two forms :

a) Mild and self-limiting form known as Dengue Fever (DF) and

b) Fatal form known as Dengue Hemorrhagic Fever (DHF)/ Dengue Shock Syndrome (DSS).

The infection is detected by demonstrating the dengue antigens, dengue specific IgM / IgG antibodies or by isolating Non Structural Protein 1 (NS1). Thrombocytopenia (TCP) is another method to suspect a dengue infection which in severe cases invariably leads to spontaneous bleeding. Many theories were proposed on the mechanism of thrombocytopenia during dengue infection. Few that worth mention is [3-4] –

a) Direct bone marrow suppression by the virus,

b) Anti-dengue antibody-mediated platelet destruction,

c) Increased peripheral consumption of platelets and isolated viral replication in the platelet

Any form of platelet activation causes morphological changes in the platelet, resulting in pseudopodia formation and associated structural changes in the platelet[5]. This may be reflected in the platelet distribution width (PDW). In response to a

thrombocytopenia the marrow compensates the same by pushing out more immature/ giant platelets and is reflected as a spike in the Mean Platelet Volume (MPV), a measurement that describes the average size of the platelet in the blood and further help in assessing the bone marrow function, on megakaryopoesis and peripheral platelet destruction. Plateletcrit (PCT) measures the entire platelet volume (mature and immature) occupied by platelets in the blood [PCT(%) = (platelet count × MPV) / 10,000]. In homeostasis the amount of platelet population is maintained in equilibrium by regeneration and elimination. MPV is inversely propotional to platelet counts[6]. An accepted range of platelet distribution width is 9 – 13 fl, plateletcrit is 0.108 – 0.282 % and Mean Platelet Volume is 7.4 -10.4 fl. Although platelet count is the most basic measure of platelet health, in certain places, MPV enables a physician to detect a problem even before the recordable thrombocytopenia sets in. The aim of our study was to record and analyze the variations in the platelet parameters such as MPV, PDW, Plateletocrit (PCT) and Platelet Count (PC) in dengue infected patients and to establish their significance in patient prognostication. Our study is the first of its kind reported from Kerala, India.

Objective of the study

Record and analyze the interrelationship between platelet parameters – Platelet count (PC), Platelet Distribution Width (PDW), Mean Platelet volume (MPV) and Plateletcrit (PCT). Monitor the platelet parameters for three days from the day of admission. To determine whether any of these parameters could be used as an effective tool in patient prognostication.

MATERIAL AND METHODS:

The study is a retrospective observational study carried out from December 2018 to December 2020 in a tertiary care speciality hospital in Ernakulam , Kerala. The platelet parameters in dengue positive cases were analysed using five part analyzers (Beckman coulter DH 800 and Sysmex XN – 500) and data were noted for three days during hospital admission. The patients were monitored on these days for improvement. The first day of admission was taken as day 1 ; two days after admission was taken as day 2; day of discharge was taken as day 3.

Inclusion criteria: Patients with Dengue fever with serology positive (NS1 Ag and Ig G/Ig M) and those who had minimum of five days inpatient treatment.

Exclusion criteria: Children (<18yrs), Dengue Hemorrhagic Fever, Dengue Shock Syndrome and patients less than five days of hospital stay.

The literature adopts the following reference values as normal range. MPV: MPV between 9 and 12 fL for platelet counts 150 x 109 /L and MPV between 7.5 and 10 fL for platelet counts 400 x 109 /L the same is used for our study as well.

Statistical analysis: Data was entered in Microsoft Office Excel 2007. Categorical data were presented as numbers (percentage) and were compared among groups using Chi-square test. Groups compared for demographic data were presented as mean and standard deviation and were compared using student t-test, ANOVA and Post-Hoc Test, Tukey Test using SPSS, version 20 for Windows.

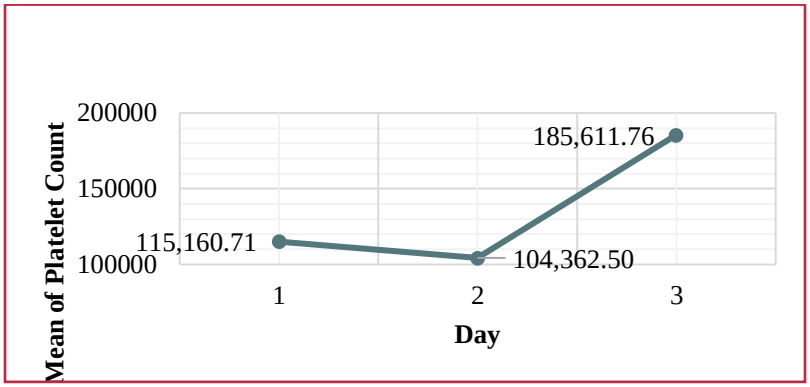
RESULTS:

There were a total of 381 Dengue positive cases over a period of 2 years out of which 112 cases fitting the criteria were selected for the study. There were 72 males and 40 females in the study. No significant gender-based variations were observed in any of the three days. Patients at the time of admission (Day 1 in our study) had a history of 3 days (mean value) of fever with no bleeding manifestations or any complications. Day 2 platelet values were taken two days after admission. Average hospital stay was 5 days. Patients improved over the period of hospitalization. Patients were discharged only after significant clinical improvement [negative serology for the infection and steady increase in platelet count (Day 3)]. The one way ANOVA operates with a null hypothesis which states the following:. $H_{0A,}$ $H_{0B,}$ $H_{0C,}$ $H_{0,}$: There is no significant difference in the values of Platelet count between the three days. However, the ANOVA results show that the value of F is significant for all four variables. Hence, all

four null hypotheses can be rejected, and we can come to a conclusion that there exist significant differences in the readings of the four variables across the three days of observation.

Platelet Count: Majority of the patients in this study got admitted during the third day of fever and at the time of admission the group showed mild thrombocytopenia (mean count of 115000). Mean value on day 2 was 1, 04,363 and on day 3 was 1, 85,611. From day 1 of study, there was a significant decrease in platelet count (day 2 of study) and gradually showed an improvement in platelet count nearing recovery (day 3). (Figure.1).

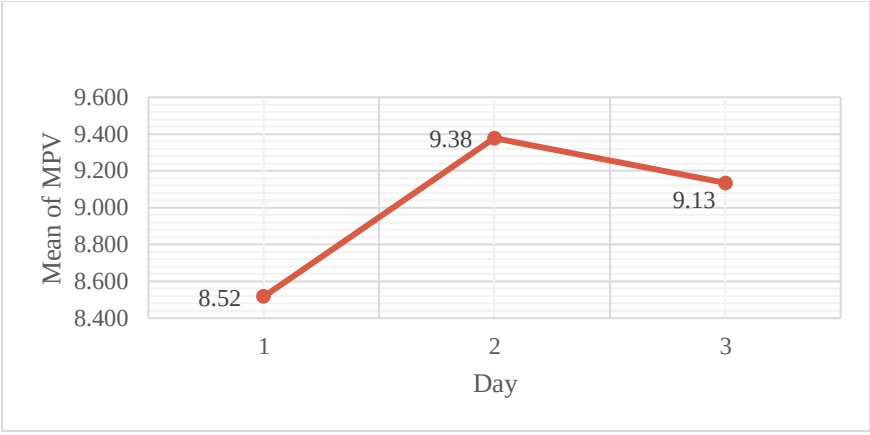
Figure 1: Means Plot (Platelet Count)



Legend . 1 : Graph shows variation in the platelet count with time . There is a decrease from day one to day two and increase from day 2 to day 3

Mean Platelet Volume: The overall mean value of MPV from our study was 9.81 ± 1.42 fl. The mean of MPV on day 1 was 8.5 fl, on day 2 it was 9.38 fl and on day 3 it was 9.13 fl. Mean platelet volume (MPV) in this study showed a significant spike from day 1 to day 2 and then a gradual dip from day 2 to day 3 (Figure.2). The value rises sharply between day 1 and day 2 by 0.86, before falling slightly by 0.244 between day 2 and day 3. MPV showed a statistically significant inverse correlation with platelet count. Initially there is increase in MPV, reaching a peak mean value of 9.38 fl followed by a gradual fall. When the platelet count decreased, MPV increased and vice versa.

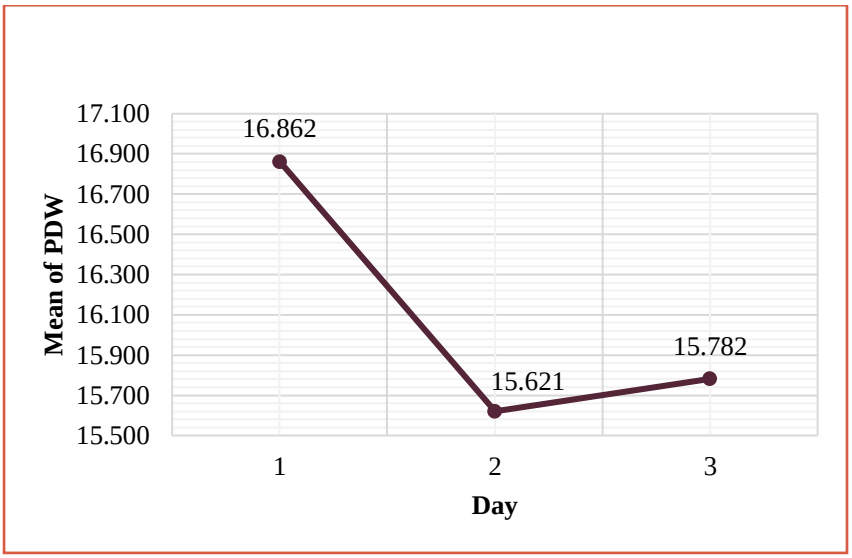
Figure 2: Means Plot (Mean Platelet Volume)



Legend .2 : Graph shows the trend of MPV with time. There is an increase in MPV initially followed by decrease or normalization

Platelet distribution width: Platelet Distribution Width (PDW) showed a sharp decreasing trend (by 1.24) since admission and gradual increase nearing recovery (by 0.16).(Figure. 3). PDW exhibited a negative correlation but was not statistically significant in our study (correlation coefficient -0.038). PDW showed a trend similar to platelet count and inverse relationship to MPV.

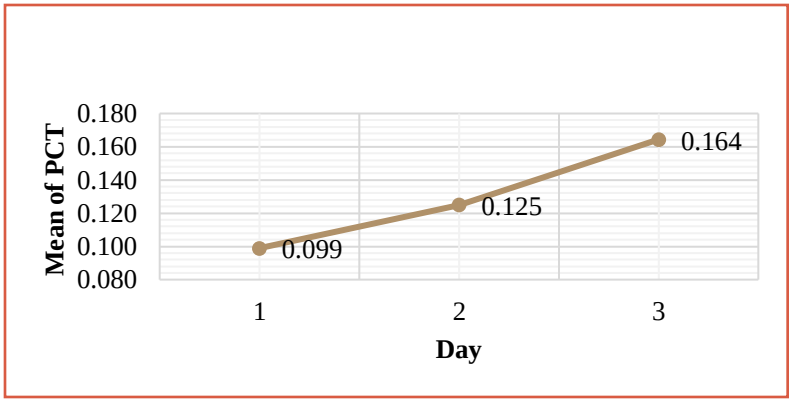
Figure 3: Means Plot (PDW)



Legend .3 : Graph showing trend in PDW with time. There is a gradual decrease followed by increase.

Plateletcrit: Mean of Plateletcrit (PCT) showed a significant gradual rising trend over the days (figure. 4).The increase is gradual over the first half but showed a sharp spike in the second half of hospital stay.

Figure 4: Means Plot (Platelet Distribution Width)



Legend .3 Graph showing trend of plateletcrit (PCT). PCT shows a gradual increase.

4.2 Bivariate Correlations (Overall):

Table 1: Correlations (Overall)

		Platelet Count	PDW	MPV	PCT
Platelet Count	Pearson Correlation	1	-.051	-.364**	.523**
	Sig. (2-tailed)		.376	.000	.000
	N	309	309	309	309
PDW	Pearson Correlation	-.051	1	-.331**	-.122*
	Sig. (2-tailed)	.376		.000	.033
	N	309	309	309	309
MPV	Pearson Correlation	-.364**	-.331**	1	-.141*
	Sig. (2-tailed)	.000	.000		.013
	N	309	309	309	309
PCT	Pearson Correlation	.523**	-.122*	-.141*	1
	Sig. (2-tailed)	.000	.033	.013	
	N	309	309	309	309
**. Correlation is significant at the 0.01 level (2-tailed).					
*. Correlation is significant at the 0.05 level (2-tailed).					

Legend . 5 : Table showing the correlation between Mean Platelet Volume, Plateletcrit Distribution Width, Plateletcrit and Platelet Count .

The overall correlation analysis between Platelet count (PC) and the other 3 variables shows that a statistically significant negative correlation exists between PC and MPV. The positive relationship between PC and PCT is statistically significant, with a Pearson correlation coefficient of 0.523 assuring a strong positive correlation. But the relationship between PC and PDW is not statistically significant overall (Table 1). In the case of PDW, the correlation coefficient has an inverse relationship with PC and is only getting stronger Mean platelet volume and Plateletocrit along with platelet count has emerged to be reliable parameters with good statistical significance in this study. In this study, patients present to the clinic on 3rd day (mean) of febrile illness. Critical phase with complications must appear within two days of admission. 95 % of our patients had an initial diagnosis of dengue fever and were admitted due to fever, thrombocytopenia, hypotension, or

vomiting. Patients were monitored daily with platelet counts and with supportive management. Average duration of hospital stay was 5 days and discharged only if the patient had been symptomatically better with adequate platelet count. Antibiotics were required in only 19.6 % of cases.

DISCUSSION:

Dengue fever is an acute disease with a broad spectrum of clinical manifestations, ranging from a clinically in apparent infection, an undifferentiated acute febrile illness, dengue fever (DF), to more severe forms, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Relying on biomarkers and clinical improvement has been the goal standard in patient management. The history of dengue virus in India dates back to 1946, the first major outbreak was reported in 1963 in Calcutta. The most common serotype prevalent in this geographical region is DENV-3. And since then many cases have been reported from all over the country [Ram et al., 1998; Dar et al., 1999; Joshi et al., 2000; Gore, 2005]. In a study conducted by Nandwani et al, TLC and low platelet count are established as predictors of fatal outcomes. Some hypotheses are postulated for immunopathogenesis of dengue which include antibody enhancement theory, T-cell activation of cross-reactive memory, and original antigenic sin. All hypotheses, to some extent, induce an overproduction of cytokine release. Function of platelets includes coagulation and hemostasis, angiogenesis, extracellular matrix synthesis, inflammation, and immune response.

Thrombocytopenia and dysfunction of platelets in dengue: Thrombocytopenia in dengue is attributed to reduced proliferative capacity of hematopoietic cells in bone marrow and/or increased destruction of platelets from peripheral blood. In a study by Wang et al, it was demonstrated that DENV-2 can bind to human platelets in the presence of virus-specific antibody and propose an immune-mediated clearance of platelets from blood. When platelet counts fall, circulating levels of TPO increase which is a useful indicator of megakaryopoiesis. Literature mentions DENV can induce thrombocytopenia through bone marrow suppression, lysis of megakaryocytes, and/or peripheral destruction of platelets. Proposed mechanisms suggest a direct infection of hematopoetic progenitor cells, stromal cells by DENV resulting in modification of bone marrow regulation. Some studies have also shown a hypocellularity in bone marrow, inhibition of megakaryocyte maturation, inhibition in the differentiation of multipotent stem cells eventually resulting in thrombocytopenia[7]. A study by Roa et al from Karnataka, concluded that co-infections with more than one serotype were not associated with disease severity in dengue infection and further highlighted the use of other biomarkers such as ferritin and serum AST and ALT to predict and to assess the disease severity. In their study the male to female preponderance was 2:1. In our study the male to female preponderance was 1:1. This may be attributed to a better literacy rate, outreach programs and disease awareness amongst women in Kerala. In a study conducted by Bashir et al which took in to consideration dengue fever, dengue hemorrhagic fever and dengue shock syndrome , low platelet count, MPV, and PDW was shown to be promising prognostic parameters in dengue in endemic areas. In their study, Low MPV of < 9 fl and high PDW of >13 fl and a high P- LCR value showed considerable sensitivity and specificity for dengue fever [8]. In another study by S R Dewi et al, the prevalence ratio of increased PDW were significantly 3.1 times in DHF than in DF [9]. Their study showed that PDW enhancement was a risk factor for worsening dengue infection. 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 hyper destructive thrombocytopenia when compared to hypoproductive thrombocytopenia[10]. Our study did not include hemorrhagic fever or shock syndrome. Mukker et al observed that MPV was decreased in dengue positive cases and was normal in control group. PDW was normal in control group but was increased in dengue infection[11] .Our study showed strong significant relationship between platelet count, MPV, and PCT in patient prognostication. PDW was not significant in our

study. Our study population included fully recovered individuals and was monitored three times. This may be because while during covalence, there may not be much of platelet degranulation and cytokine release which is one of the causes for complications in dengue. During the early phase of the disease, bone marrow displays hypocellularity and attenuation of megakaryocyte maturation[12] . Whenever there is an active bone marrow production, immature platelets are released into blood stream as a response to thrombocytopenia. It is an established fact that immature platelets have a larger size compared to mature platelets. Therefore, during active marrow response, mean platelet volume increases. In this study, there is a proportional inverse relationship between platelet count and mean platelet volume and is statistically significant. An increase in MPV was found few days after hospital admission associated with thrombocytopenia. All cases recovered subsequently with increase in platelet count with a decrease in MPV. During recovery phase, the platelet count is maintained or rises gradually. There is lesser peripheral destruction as evidenced by a steady rise in platelet count. The paper published by Kritika Sharma et al concluded that MPV has no relevance in the patient prognostication in dengue infection.[13] In our study, the lowest MPV was 8.5 fl with a corresponding mild thrombocytopenia. It gradually increased to a peak of 9. 4 fl and then decreased (normalize) to a value of 9.13fl. The thrombocytopenia continued over the days. From our study, we may arrive at a conclusion that, steady fall in MPV with rise in platelet count during the course of the illness indicates a good prognosis. A rise in MPV alone without increase in platelet count indicates good marrow response but continued immune platelet destruction from peripheral blood. No change in the value of MPV may suggest reduced or no marrow response. La Russa and Innis in 1995 demonstrated that DENV-induced bone marrow suppression depresses platelet synthesis. The events related to activation are not restricted to changes in morphology alone. During the exocytosis of constituents of platelet granules, expression of adhesion proteins, and secretion of cytokines and other immunological mediators, platelet assumes an activated state with configurational changes. However, in this study, there was a no significant correlation with PDW. PCT correlated with platelet count as expected with good statistical significance. 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 . A study by Ojha A et al describe a very clear association between activation status of platelets and their destruction/depletion from circulation in patients over the period of fever, beginning at day 4 of fever when they were admitted to the hospital with low platelet count (170000/μL)[14].Our study corroborates with their study.

CONCLUSION:

Platelets are cellular fragments derived from hematopoietic precursors megakaryocytes, primarily associated with coagulation and hemostasis and also with inflammation, immune response, angiogenesis, and extracellular matrix synthesis. Platelets are one of the major cell populations affected in dengue. Platelets could be considered cells that are active against the anti-DENV immune response. A Low MPV <9fl (8.5 fl) shows sensitivity for dengue fever. Moderate thrombocytopenia with increase in mean platelet volume indicated good marrow response and suggests a good prognosis. A sudden dip in MPV and increase in platelet count may be taken as an early indication of recovery and plan discharge accordingly. Mean platelet volume and Plateletocrit along with platelet count are reliable parameters to establish good patient prognosis in dengue infection.

Limitations of study:

This is a pilot study. The study cohort is assumed to be cases of primary dengue infection. A larger cohort study with follow up over a longer period, including different age groups and demographics is required to delineate the trend better.

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REFERENCES:

1. Dongre T, Karmarkar P; Hematological Parameters and Its Utility in Dengue-A Prospective Study, JDMS, 2015; 14(2)
2. Mehboo R, Munir M, Azeem A, Naeem S, Tariq AM, Ahmad JF; Low Platelet Count Associated with Dengue Hemorrhagic Fever, IJAC, 2013;1:29-34.
3. Aruna R, Prakash M; A comparative study on Dengue Virus infection: Serological markers Vs Platelet count, Int. J Curr. Rev. Aca. Rev. 2014; 2(12)
4. Prakash GM, Anikethana G V. Use of mean platelet volume and platelet distribution width in predicting trend in platelet count and bleeding risks in patients of dengue fever. Int J Adv Med 2016;3:611-3
5. Dewi YP. Mean Platelet Volume (MPV): Potential Predictor of Disease Severity in Dengue infection. In proceeding of: International Dengue Symposium. 2013 conference paper.
6. Azeredo, Monteiro, and de-Oliveira Pinto. Thrombocytopenia in Dengue: Interrelationship between Virus and the Imbalance between Coagulation and Fibrinolysis and Inflammatory Mediators: Mediators of Inflammation Volume 2015, Article ID 313842.
7. Leila J Noetzli, Shauna L French, and Kellie R Machlus. New Insights into the Differentiation of Megakaryocytes from Hematopoietic Progenitors: Arterioscler Thromb Vasc Biol. 2019 July ; 39(7): 1288–1300.
8. Bashir AB, Saeed OK, Mohammed BA, Ageep AK. Thrombocytopenia and bleeding manifestation among patients with dengue virus infection in port Sudan, Red sea state of Sudan. J Infect Dis Immun. 2015;7-13.
9. S R Dewi1, U Sukorini , and U S Intansari. Correlation of enhancement Platelet Distribution Width (PDW) with severity of dengue infection: IOP Conf. Series: Materials Science and Engineering 434 (2018) 012312
10. Borkataky S, Jain R, Gupta R, Singh S, Krishan G, Gupta K, et al. Role of platelet volume indices in the differential diagnosis of thrombocytopenia: a simple and inexpensive method. Hematology. 2009;14:182-6.
11. Mukker et al. Platelet indices evaluation in patients with dengue fever: Int J Res Med Sci. 2018 Jun;6(6)

12. Jayashree K, Manasa GC, Pallavi P, Manjunath GV. Evaluation of platelets as predictive parameters in dengue fever. Indian J Haematol Blood Transfus. 2011;27:127-30.
13. Kritika Sharma et al, Association of Platelet Counts and Mean Platelet Volume with Severity & Treatment Outcome in Dengue Fever. Journal of Clinical and Diagnostic Research. 2015 Nov, Vol-9(11): EC01-EC03
14. Ojha et al. Platelet activation determines the severity of thrombocytopenia in dengue infection: Scientific Reports, 7:41697