Volume : 5 | Issue : 7 | July 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

Evaluation of Platelet Indices in Patients With Dengue Infections

Medical Science

KEYWORDS : Mean platelet volume, Dengue, thrombocytopenia, Platelet distribution width, Bikaner

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ABSTRACT

Recently, novel platelet indices such as MPV, PDW, and P-LCR have been investigated as prospective platelet activation markers. The aim of our study was to investigate the platelet indices in dengue patients and to assess the role of it in the severity dengue infection. The platelet parameters of 200 patients were compared with 200 apparent healthy normal individuals. In our study, there was a significantly lower Platelet count and PCT in DHF group as compared to DF group (P<0.0016 and <0.0166 respectively) and significantly higher PDW in DHF group as compared DF group (P<0.0008). High P-LCR, High PDW and low PCT were more sensitive for DHF. In conclusion, low platelet count, low PCT and high PDW may be used as predictor of severity of Dengue infection. Low MPV, low PCT, high P-LCR and high PDW showing considerable sensitivity and specificity for dengue fever.

Introduction

Dengue fever is an acute infectious disease caused by four serotypes of dengue virus, and is the most prevalent mosquito-borne viral disease in humans, occurring in tropical and subtropical countries of the world where over 2.5 billion people are at risk of infection.1 The World Health Organization has estimated 50 million cases of dengue fever and several hundred thousand cases of dengue hemorrhagic fever occur each year, depending on the epidemic activity.2 Some 1.8 billion of the population at risk for dengue worldwide live in member states of the WHO South-East Asia Region and Western Pacific Region, which bear nearly 75% of the current global disease burden due to dengue.3

Dengue has a wide spectrum of clinical presentations, often with unpredictable clinical evolution and outcome. While most patients recover following a self-limiting non severe clinical course, a small proportion progress to severe disease, mostly characterized by plasma leakage with or without hemorrhage. Early recognition of dengue is challenging because the initial symptoms are often non-specific, viremia may be below detectable levels and serological tests confirm dengue late in the course of illness.4 The World Health Organization (WHO) classified the clinical presentations of DHF into four severity grades based on laboratory data: Grade I: fever with positive tourniquet test; Grade II: plus mild spontaneous bleeding; Grade III: presence of weak and rapid pulse; and Grade IV: profound shock with undetectable pulse. The last two are considered DSS.5 Prompt diagnosis during the febrile stage is essential for adjusting appropriate management.6

Severity of the illness is determined by various risk factors such as age, pre-existing illness, infecting serotype, and secondary infection. A second infection with a different serotype leads to more severe form of the disease than the primary infection.7 One of the most common laboratory findings in dengue is thrombocytopenia. The complex mechanism of thrombocytopenia remains unclear. Possible mechanisms of thrombocytopenia could be, direct bone marrow suppression by the virus; anti-dengue antibody-mediated platelet destruction, peripheral consumption of platelets and isolated viral replication in the platelet. Thrombocytopenia leads to bleeding although the platelet count may not directly correlate with the bleeding manifestation.8

Recently, novel platelet indices such as MPV, PDW, and P-LCR have been investigated as prospective platelet activation markers.9 Platelet volume, a marker of platelet function and activity is measured as mean platelet volume (MPV) by hematology analyzers. MPV can be used as independent predictors of bleeding. It is surrogate marker of bone marrow activity; a high MPV indicates increased megakaryocytic activity. A low MPV indicates marrow suppression and increased risk of bleeding. Correlation of platelet count and MPV with bleeding and severity of the disease can potentially predict outcome.10 Platelets with increased number and size of pseudopodia differ in size, possibly affecting platelet distribution width (PDW) which increases during platelet activation.9 Furthermore, platelet activation alters the morphology of these cells, which can be evaluated on the basis of mean platelet volume (MPV) and platelet distribution width (PDW), Another platelet parameter is plateletcrit (PCT), which is a reliable measurement of platelet biomass because it combines the MPV with the absolute platelet count.11 Platelet large cell ratio (P-LCR) was significantly decreased in patients with thrombocytosis than in normal while it was increased in thrombocytopenia. P-LCR was inversely related to platelet count and directly related to PDW and MPV.12 The aim of our study was to investigate the platelet indices in patients compared to the control and to assess the role of it in the severity of dengue infection and its diagnostic significance.

Material and Method

This study was conducted prospectively for a period from July 2015 to November 2015 during the recent outbreak of dengue in Sardar Patel Medical college, Bikaner, Rajasthan, India. The platelet parameters were measured by the Sysmex KX-21N (Sysmex Corporation, B 7151, MF 9/2008, Japan) semi automated hematology analyzer on venous samples collected in EDTA from 200 patients as a clinical

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sample and 200 apparent healthy normal individuals as control sample. In the inclusion criteria all patients with clinical features and serologically positive dengue infection were included. The exclusion criteria include patient's serologically negative dengue and if routine laboratory testing suggested a bacterial, parasite or any viral infection other than dengue infection or any other disease. The specimens were analysed within 1 hour from venesection. The parameters analysed included PLT, MPV, PDW, P-LCR and PCT. Observations were considered valid only if the specimens were analysed within 1 hour from venesection, to avoid the problems occurring when EDTA collected samples are analysed.

Patients were classified as dengue fever, dengue hemorrhagic fever or dengue shock syndrome according to WHO guidelines and laboratory diagnosis of dengue was established by demonstration of IgM and IgG immune chromatographic Rapid strip test (Pan Bio, Australia) sensitivity 95.6 and specificity 96.

Statistical analysis
Data were analyzed using a statistical software GraphPad Prism version 6.05 for window. Measurements of laboratory data and platelet parameters of patients with DF, DHF were statistically tested by comparing mean and Chi-square test which ever was appropriate. A P value less than 0.05 was considered statistically significant. The sensitivity and specificity of relevant platelet indices parameters as an indicator of dengue infection were also assessed by performing receptor operating curve (ROC) analysis.

Ethical Consideration
Ethical clearance of this study was approved from the regional Ethical Review Committee (ERC). Data regarding the age and sex was recorded in predesigned forms.

Result
This is a case control analytical study conducted in Sardar Patel Medical college, Bikaner, Rajasthan, India. The total number of the confirmed diagnosed dengue patients were 200. The age of the patients in this study was between 16 – 65 years (mean age 29.69 ± 12.87 years). 200 individuals, age and sex matched, were selected as control group. The control individual aged between 16 – 65 years (mean age 40.43 ± 16.22 years). Of the 200 clinical patients, (132) 66% were males and (68) 34% were female. In control group, (120) 60% were males and (80) 40% were females. Demographics data were obtained from patients with dengue virus infection include residence and occupation. 74% patients belongs from Urban area and 26% belong from Rural area. The student was the most common segment of occupation affected (39%), followed by house wife (18), the laborer (17%) and traders (8%).

All of the Patients presented were analysed for PLT, PCT, MPV, PDW, and P-LCR. Median MPV was 8.25 (range 5.7 – 10.4 fl), median PDW was 16.8 (range 14.2 – 18.9 fl), median P-LCR was 46.18 (range 16.1 – 49.1%), median PLT was 36x103/µl (range 12x103/µl – 196x103/µl) and median PCT was 0.038% (0.012-0.167). Low MPV which indicates bone marrow suppression was noted in 71.95% of patients with DF and 61.11% in patients with DHF. A high PDW which indicates as useful marker for platelet activation was seen in 73.17% of patients with DF and 100% in DHF patients. High P-LCR that correlated with thrombocytopenia was observed in 84.15% of patients with DF and 77.78% in DHF patients (Table 1).

The differences between the patient group and the control group in MPV, PCT and PLT were found to be significantly lower in the patient group (P< 0.0001), while the differences between patient group and control in PDW and P-LCR were found to be significant higher in patient group (P< 0.0001) (Table 2). There was a significantly lower Platelet count and PCT in DHF group as compared to DF group (P<0.0016 and <0.0166 respectively) and significantly higher PDW in DHF group as compared DF group (P<0.0008)) (Table 3). There was a significant negative correlation with mean platelet count and PDW and P-LCR (P<0.0017; r= -0.3097 and P<0.0001; r= -0.8005) and significant positive correlation with mean platelet count and PCT (P< 0.0001; r=0.6829) (Table 4). Table 5 shows that high P-LCR and low PCT more sensitive for DHF than DF and equally specific for both DF and DHF. High PDW is more sensitive for DHF and more specific for DF and low MPV is more sensitive for DF and equally specific for DF and DHF.

Discussion
Dengue fever is one of the major public health problems. Defect in DF/DHF are multifactorial mechanisms that include thrombopathy, coagulopathy and vasculopathy. Thrombopathy means the thrombocytopenia and platelet dysfunction. Many factors can contribute to the onset of thrombocytopenia in DF, varying from a reactive immune response against platelets to decreased platelet production. The depression in the bone marrow observed in dengue fever in the acute stage may account for thrombocytopenia. In addition, direct infection of megakaryocytes by dengue virus could lead to an increased destruction of platelets.13 Platelets are involved in hemostasis, tissue repairing, and infection. To our knowledge, there are only few studies investigating changes in platelet indices during dengue infection. MPV has been evaluated as a diagnostic tool in different conditions with thrombocytopenia with contradictory results. It has been demonstrated that MPV has sufficient sensitivity and specificity to discriminate aplastic anemia, bone marrow disease, hypoproductive thrombocytopenia, and bone marrow metastasis of solid tumor.14–17 However, it has been reported that although MPV may be used as an initial suggestion of bone marrow disease in thrombocytopenic patient, it has limited sensitivity and specificity.18 As the platelets are natural sources of growth factors like platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1) or transforming growth factor β (TGF-β), they have important role in inflammation, angiogenesis, repair and regeneration of the tissues.19

In our study we found that MPV, Plateletcrit and Platelet count were lower in dengue fever patients as compared to controls and PDW and P-LCR levels were higher in dengue fever patients compared to controls. However, the alteration in MPV level in dengue infection was studied before by Wiwanikit V and found that MPV in patients with dengue hemorrhagic fever was not decreased and appear to be similar to general healthy population.14 Bashir AB et al also found that MPV and Platelet count were lower in dengue fever patients when compared to controls and PDW levels were higher in dengue fever patients compared to controls.20 We noticed that MPV level was significantly lower and PDW level was significantly higher in the dengue fever, which reflect that decreasing MPV and increasing PDW levels may predict dengue fever. Other researchers such as Niethammer et al have upheld the diagnostic value of platelet histogram maximum rather than MPV to differentiate between idiopathic thrombocytopenic purpura (ITP) and hypo productive thrombocytopenia.21,22 Ntiaos et al have concluded that MPV and PDW can be safely relied on for a positive diagnosis of ITP. MPV and

PDW were superior to P-LCR.23 A different approach was adopted by Kurata et al who assessed reticulated platelet which are newly released from the marrow by using RNA-binding dyes and flow cytometric analysis, these immature platelets were successful as a discriminating guide to determine the etiology of thrombocytopenia.24

We also found that high P-LCR (>42%) and low PCT(<0.15%) more sensitive for DHF (89% and 100%) than DF(98% and 84%) and equally specific for both DF and DHF(95%). High PDW (>15 fl) is more sensitive for DHF (100% v 95%) and more specific for DF (90% v 85%) and low MPV (<9 fl) is more sensitive for DF (71% v 67%) and equally specific for DF and DHF (95%). Bashir AB et al also found that low MPV <9 fl and high PDW >13fl shows considerable sensitivity for dengue fever.20 Trupti V. Katti et al found that platelet indices showed linear relationship i.e., decrease (MPV < 6 fl, PDW < 10) with decreasing platelet count in most hypo production group and inverse relationship i.e., Increase (MPV > 10 fl, PDW > 16) with decreasing platelet count was observed in most cases of hyper destruction group.25 Yanxia Gao et al in their study concluded that the rise in MPV, and to a lesser extent an increase in PLCR and PDW, is indicative of a worse prognosis in patients with septic shock.26 Mohamed AR et al found that thrombocytopenia occurred in (67.5) % of malarial cases and platelet indices in this study showed (90.2) % had low MPV and (81.3) % had low PCT.27 We also compared platelet indices between DF and DHF group and found that there was a significantly lower Platelet count and PCT in DHF group as compared to DF group and significantly higher PDW in DHF group as compared DF group. We also found a significant negative correlation with mean platelet count and PDW and P-LCR and significant positive correlation with mean platelet count and PCT.

Conclusion

In conclusion, significant differences were observed in the MPV, PDW, P-LCR, PCT and PLT in patients with dengue infection. Low platelet count, low MPV, low PCT, high PDW and high P-LCR may be used as probable indicators for dengue in endemic area. Low platelet count, low PCT and high PDW may be used as predicter of severity of Dengue infection Low MPV <9 fl, low PCT <0.15%, high P-LCR >42% and high PDW >15 fl shows considerable sensitivity and specificity for dengue fever.

Table 1: Platelet indices in the case group of the study

Diagnosis	Low MPV	Normal MPV	High MPV	Total
DF	118 (71.95%)	46 (28.05%)	0	164 (82%)
DHF	22 (61.11)	14 (38.89)	0	36 (18%)
Total	140 (70%)	60 (30%)	0	200 (100%)
	Low PDW	Normal PDW	High PDW	Total
DF	0	44 (26.83%)	120 (73.17%)	164 (82%)
DHF	0	0	36 (100%)	36 (18%)
Total	0	44 (22%)	156 (78%)	200 (100%)
	Low P-LCR	Normal P-LCR	High P-LCR	Total
DF	6 (3.66%)	20 (12.19%)	138 (84.15%)	164 (82%)
DHF	2 (5.55%)	6 (16.67%)	28 (77.78%)	36 (18%)
Total	8 (4%)	26 (13%)	166 (83%)	200 (100%)

MPV, mean platelet volume; PDW, platelet distribution width, P-LCR: platelet large cell ratio DF, dengue fever; DHF, dengue haemorrhaic fever

Table 2: The difference of platelet indices between case and control groups in the study

Parameter	Test group (mean)	Control group (mean)	P-value	Significance
MPV	8.28 ± 1.012	9.92 ± 0.576	< 0.0001	Extremely significant
PDW	16.69 ± 0.858	14.02 ± 1.060	< 0.0001	Extremely significant
P-LCR	43.96 ± 6.566	32.63 ± 7.363	< 0.0001	Extremely significant
Platelet count	43.87 ± 27.47	291.06 ± 80.41	< 0.0001	Extremely significant
PCT	0.0454 ± 0.0249	0.272 ± 0.0725	< 0.0001	Extremely significant

Table 3: Comparision of platelet indices between DF and DHF groups

Parameter	DF (mean)	DHF (mean)	P-value	Significance
MPV (fl)	8.313 ± 1.004	8.133 ± 1.064	< 0.4971	Not significant
PDW (fl)	16.56 ± 0.779	17.29 ± 0.964	< 0.0008	Very significant
P-LCR (%)	43.79 ± 7.131	44.72 ± 2.829	< 0.5913	Not significant
Platelet count (10^3 /mm^3)	47.86 ± 28.43	25.67 ± 10.68	< 0.0016	Very significant
PCT (%)	0.0482 ± 0.0259	0.0328 ± 0.0148	< 0.0166	Significant

Table 4: Correlation between mean platelet count and platelet indices in study group

Correlation with mean Platelet count	'r' Value	P Value	Significance
PDW	-0.3097	0.0017	Very significant
MPV	0.1668	0.0972	Not significant
P-LCR	-0.8005	<0.0001	Extremely significant
PCT	0.6829	<0.0001	Extremely significant

Table 5: Statistical analysis of Platelet indices for diagnosis of Dengue fever (DF) and Dengue hemorrhagic fever (DHF)

Parameters	Sensitivity (%)	Specificity(%)	LR
MPV < 9 fl			
DF	71	95	14.15
DHF	67	95	13.33
PDW > 15 fl			
DF	95	90	9.512
DHF	100	85	6.667
P-LCR > 42 %			
DF	84	95	16.83
DHF	89	95	17.78
PCT < 0.15%			
DF	98	95	19.76
DHF	100	95	20.00

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