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Are Platelet Indices Useful Tools for Diagnosis of Dengue Infection? Mystery Unveiled: A Prospective Case Control Study

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Abstract

Introduction: Dengue is a major public health problem in tropical countries like India. The role of novel platelet indices is not well established for routine clinical use in dengue infection. Our study was undertaken to compare the platelet indices PLT count, MPV, PDW and PCT in serologically positive Dengue cases with that of control group and to assess the differences in platelet indices, if any between serologically antigen and antibody positive Dengue cases. *Methods*: Serologically NS1 antigen, IgM, IgG antibody positive 521 dengue cases detected by rapid ICT card test and 158 serologically dengue negative controls with normal platelet counts were studied. The PLT count, MPV, PCT and PDW in both case and control groups were measured by Beckman LH780 hematology analyzer and compared by statistical analysis. *Results*: PLT count and PCT were significantly lower (P< 0.005), MPV and PDW were significantly higher (P<0.005, 0.384 respectively) in dengue cases when compared to controls. Low PLT count, high MPV and low PCT were significantly associated with NS1 antigen, IgM, IgG antibody positivity. PLT count <150x10^9/L was sensitive, MPV >8.5FL and PCT <0.14% were specific for dengue infection. PDW was not an accurate marker.*Conclusion*: Low PLT count, high MPV and low PCT were significantly associated with NS1 antigen, IgM and IgG antibody positivity. Platelet count <150x10^9/L showed sensitivity, MPV > 8.5FL and PCT <0.14% showed specificity for dengue infection.

Keywords : Dengue; Mean Platelet Volume; Platelet Count; Plateletcrit; Platelet Distribution Width.

Introduction

Dengue fever is an acute, potentially fatal arboviral infection that can culminate into Dengue hemorrhagic fever and Dengue shock syndrome [1]. It is the most rapidly spreading mosquito borne viral disease in the world, more common in tropical countries like India [2]. An estimated 50 million infections occur per year approximately across 100 countries [3]. The hallmark of Dengue infection is low platelet count or thrombocytopenia[4]. The causes of thrombocytopenia are anti-Dengue antibody mediated destruction of platelets, peripheral consumption of platelets and lastly direct bone marrow suppression by the virus [5]. In addition to platelet count (PLT count), other new platelet indices like Mean platelet volume (MPV), Platelet distribution width (PDW), Platelet large cell ratio (P-LCR), Plateletcrit (PCT) and Immature platelet fraction (IPF) are being evaluated by many researchers. All these novel platelet indices are indicators of platelet kinetics and are readily available in the new generation automated hematology analyzers [6]. The utility of these new platelet indices to differentiate between hypo productive and hyper destructive or immune thrombocytopenia has been well established by various studies [7,8]. However, apart from platelet count, the other platelet indices are not used or evaluated routinely to aid the clinical diagnosis of Dengue.

There are very few studies available in literature to establish the utility of these platelet indices in Dengue infection and in day to day clinical practice [9,10]. Further, on extensively searching the literature, we found that no study has compared the differences in platelet indices between NS1 antigen and IgM, IgG

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antibody positive cases. Hence, our study has been designed with an aim to compare the platelet indices like PLTcount, MPV, PDW and PCT in serologically positive Dengue cases with that of control group. Further, we aim to assess the differences in platelet indices, if any between serologically antigen and antibody positive Dengue cases.

Materials and Methods

This is a prospective study which was conducted at our institution, a teaching hospital and research center, for a period of four months from June 2015 to September 2015. The above mentioned period was considered ideal for the study as there was outbreak of Dengue due to monsoon. The platelet indices namely PLT count, MPV, PDW and PCT were measured by Beckman LH780, a fully automated Hematology analyzer. The venous EDTA blood samples from 521 cases and 158 controls were analyzed for the platelet indices.To prevent deterioration and degeneration of the samples, both case and control samples were analyzed within one hour of collection.

The inclusion criteria for cases were patients who were either NS1 antigen or IgM, IgG antibody positive serologically. The patients who were symptomatic clinically and presented with Dengue like features but serologically negative for Dengue were excluded. The controls were chosen from out patient database and were serologically Dengue negative with normal platelet counts ranging from 150- 400x 10^9/L and normal CBC parameters. The controls chosen were individuals belonging to both genders and spread across all age groups. The Dengue test on cases was performed using the SD BiolineDengue Duo rapid immunochromatographic (ICT) card test.

The relevant data regarding age, gender, Dengue parameters was collected in a predetermined electronic format. The data was analyzed using SPSS software version 20. The four platelet indices PLTcount, MPV, PDW and PCT in case and control groups were compared using mean and Chi-square tests. A P value of <0.05 was considered statistically significant. The ROC (receiver operator characteristic) curve analysis was done to determine the sensitivity and specificity of the platelet indices in Dengue infection.

Results

This study is a case control study conducted at Vydehi institute of medical sciences and research center, Bengaluru, India. The total number of serologically positive Dengue patients in the study was 521 with 397 (76.2%) males and 124 (23.8%) females. The age of the patients ranged from 6 months to 92 years with a mean of 27 years. The number of controls were 158 with 90 (57%) males and 68 (43%) females.

The ageof the control group ranged from 1 year to 85 years with a mean of 36 years. The detailed age and gender wise distribution of Dengue positive cases and controls is shown in table 1. The case and control samples were analyzed in Beckman LH780 analyzer for platelet count (PLT count), mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT). The differences in the test and control group means and their corresponding P values for all the four platelet indices is depicted in table 2. The mean platelet count in case and control groups was 120 X 10^9/L and 254 X 10^9/L. The mean MPV was 9.23fL and 9.92 fL, mean PCT was 0.12% and 0.23% , mean PDW was 16.9fL and 16.82 fLrespectively.

The mean PLT count and mean PCT in case group was lower than the control group, whereas mean MPV and mean PDW was higher when compared to control group. The P values were significant for platelet count, MPV and PCT indices, whereas P value for PDW was not statistically significant (table 2).

In addition the number of Dengue positive cases were further categorized Dengue parameter wise and gender wise as shown in table 3.

The number of NS1 antigen positive cases (248/47.6%) was highest in the study, followed by IgM + IgG positive cases (78/15%). The least number of caseswere of NS1 antigen + IgG positive category (9/1.7%). The highest number of both male and female cases was NS1 antigen positive (201/38.6% and 47/9.02%).

The statistical analysis of all the four platelet indices was done in NS1 antigen positive, IgM positive and IgG positive cases as summarized intable 4, using Pearson Chi-square test and P values. The P values derived for PLT count, MPV and PCT were considered statistically significant in NS1 antigen, IgM and IgG antibody positive cases and insignificant for PDW.

The ROC (receiver operator characteristic) curve is shown in Figure 1. MPV>8.5fL showed sensitivity for Dengue infection, whereas platelet count<150x10^9/L and PCT< 0.14% showed specificity for Dengue infection. PDW was not an accurate platelet index in our study.

Table 1: Age and gender wise distribution of Dengue positive cases and controls

Gender	Age group (yrs)	Number of cases	Number of Controls	Total
Females	0-10	20	5	25
	11-20	16	14	30
	21-30	45	17	62
	31-40	24	12	36
	41-50	10	8	18
	51-60	6	8	14
	61-70	3	3	6
	71-80	1	1	2
	81-100	1	0	1
	Total	**126 (64.9%)**	**68 (35.1%)**	**194 (100%)**
Males	0-10	26	9	35
	11-20	65	9	74
	21-30	197	12	209
	31-40	67	19	86
	41-50	30	13	43
	51-60	6	18	24
	61-70	3	6	9
	71-80	1	3	4
	81-100	0	1	1
	Total	**395 (81.4%)**	**90 (18.6%)**	**485 (100%)**

Legends: yrs: years

Table 2: Case and control group means and P values of platelet indices

Platelet Index	Case group mean	Control group mean	P value
PLT count (X 10^9/L)	120.47	253.61	0.000
MPV (fL)	9.92	9.23	0.000
PCT (%)	0.12	0.23	0.000
PDW (fL)	16.9	16.82	0.384

Legends: PLT count: Platelet count; MPV: Mean platelet volume; PCT: Plateletcrit; PDW: platelet distribution width; fL: Femtoliter

Table 3: Number of Dengue positive cases- Parameter and gender wise.

Positive Dengue parameter	Number of Females	Number of males	Total
NS1 Antigen	47	201	248 (47.6%)
NS1 antigen +IgG	2	7	9 (1.7%)
NS1 antigen +IgM	18	39	57 (10.9%)
NS1 antigen +IgG+ IgM	15	40	55 (10.6%)
IgG	21	41	62 (11.9%)
IgM	3	9	12 (2.3%)
IgM + IgG	18	60	78 (15%)
Total	124	397	521 (100%)

Legends: NS1: Non structural protein 1; IgG: Immunoglobulin G; IgM: Immunoglobulin M

Table 4: Statistical analysis of platelet indices in NS1 antigen, IgM and IgG positive cases.

Platelet index	Pearson Chi square test value	P value
	NS1 antigen positive cases	
PLT count	236.298	0.000
MPV	41.376	0.000
PCT	169.128	0.000
	IgM positive cases	
PLT count	241.332	0.000
MPV	40.288	0.000
PCT	174.077	0.000
	IgG positive cases	
PLT count	241.592	0.000
MPV	41.489	0.000
PCT	177.707	0.000

Legends: PLT count: Platelet count; MPV: Mean platelet volume; PCT: Plateletcrit NS1: Non structural protein1; IgG: Immunoglobulin G; IgM: Immunoglobulin M

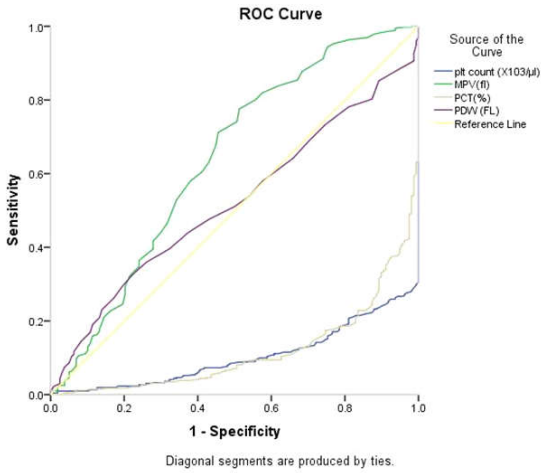

Fig. 1: ROC Curve for platelet indices in Dengue infection

ROC: Receptor Operator Characteristic; PLT count: Platelet count; MPV: Mean platelet volume; PCT: Plateletcrit; PDW: platelet distribution width

Discussion

Dengue is a fatal infection and a major challenge to public health in tropical countries like India where over 2.5 billion people are at a risk of infection [2]. Outbreaks of Dengue infection occur in several parts of India mostly during the monsoons. The morbidity and mortality in Dengue is associated with frequent laboratory finding of low platelet count or thrombocytopenia which occurs due to combined mechanisms of hyper destruction of platelets and hypo production in the bone marrow [4,5]. In addition to low platelet counts in Dengue, there are significant changes in other platelet indices as studied by few researchers [9,10]. These platelet indices are mean platelet volume (MPV), plateletcrit(PCT), Platelet distribution width (PDW), Platelet large cell ratio (P-LCR), Immature platelet fraction (IPF) etc. These novel platelet indices are readily available in all the new generation automated hematology analyzers. All these indices have been investigated and proved to be platelet activation markers. These indices are not in routine clinical use to evaluate cases of Dengue. Of these indices, PLT count, MPV, PCT and PDW were available in Beckman LH780 analyzer in our set up and hence were considered for the current study on Dengue.

The role of platelet count in Dengue has been evaluated by many researchers time and again. Low platelet count or thrombocytopenia showed considerable sensitivity for Dengue virus infection in a study by Bashir AB and Saeed OK et al [9]. Many factors contribute to thrombocytopenia in Dengue like reactive immune response against platelets to decreased platelet production, bone marrow depression in acute stage, direct infection of megakaryocytes by Dengue virus leading to an increased destruction of platelets. In a study by Bashir AB and Mohammed BA et al, low platelet counts were statistically significant in Dengue [11]. In a study by Jayashree K et al, platelet count was considered predictive as well as a recovery parameter of Dengue fever, DHF and DSS [12]. Further, an association of thrombocytopenia with NS1 antigen was found to be higher in a study by Kulkarni RD et al [1]. No study in literature has elaborated the association of other platelet indices like MPV, PCT and PDW in NS1 antigen, IgM and IgG antibody positive cases of Dengue which we have catered to in our study.

MPV is considered as a marker of bone marrow activity and platelet activation. It is also a common surrogate marker of bleeding. MPV increases with increased megakaryocytic activity in the bone marrow. Low MPV is associated with marrow suppression and increased risk of bleeding [13]. There are contradictory outcomes of studies on MPV in Dengue. Studies by WiwanitkitVet al, DewiYP et al, and Sharma KS et al show no significant correlation of MPV with severity and serology of Dengue [13,14,15]. The study by Bashir AB and Saeed OK et al showed low MPV as a probable indicator for Dengue in an endemic area and a cut off MPV of <9 fL showed considerable sensitivity for Dengue fever in their study [9].

PCT is a platelet index which has been least studied. PCT parameter is available only in Beckman LH series automated analyzers by and large. PCT is a measure of total platelet mass. Values of PCT vary depending on MPV and PLT count. In a study conducted by Chandrasekhar V et al, a cut off value of 0.2- 0.36% of PCT showed sensitivity and specificity of 97% and 80% respectively in thrombocytopenia cases. The plateletcrit hence can be used along with platelet counts to determine if patient needs platelet transfusions and also as a screening tool for detection of platelet quantitative disorders [16]. Its role in diagnosis of Dengue is not yet established by any study in literature.

PDW is a marker of platelet activation. The activated platelets show many morphological changes like assuming spherical shape with presence of pseudopodia and hence seem larger in size. Thus, platelets with increased number and size of pseudopodia show high or altered PDW values [6]. In a study by Bashir AB and Saeed OK et al, PDW levels were higher in Dengue patients when compared to controls. High PDW values of >13 fL showed considerable sensitivity for Dengue fever in their study [9]. PDW also gives valuable information regarding

mechanism of platelet destruction as per study by Reddy RS et al [17].

In our study, a total of 521 cases which were Dengue positive serologically by rapid ICT card test were studied. The platelet indices in these cases were compared with 158 controls,who were Dengue negative by serology and had normal platelet counts of 150-400x10^9/L. The age and gender details in our study correlated with study by Bashir AB and Saeed OK et al [9]. The maximum number of cases in our study were in the age group of 21-30 years in both males and females. The mean PLT count for case group in our study was 120x10^9/L, which was significantly lower (P<0.005) than control group mean of 253x10^9/L. Similar findings were noted in the study by Bashir AB and Saeed OK et al, Tathe SS et al and Jyothi P et al [9,18,19]. The case group mean for PLT count in our study was marginally higher due to the fact that majority of our cases (47.6%) were NS1 antigen positive and platelet count does not significantly drop in early stage of the disease when NS1 antigen is positive. This has been proved by Kulkarni RS et al in their study that platelet count drops below 100x10^9/L when simultaneously NS1 antigen and IgM antibody are positive [1]. The mean MPV in our study was 9.92 fL and 9.23 fL for case and control group respectively. The mean MPV was significantly higher in case group (P<0.005). This finding of our study correlated with findings by Khaleel KJ et al, Reddy RS et al and Katti TV et al [8,17,20]. However, MPV was lower in case group in the study by Bashir AB and Saeed OK et al.[9] The differences in MPV in our study and study by Bashir AB and Saeed OK et al are due to differences in marrow activity in the Dengue cases in these studies. High MPV in our study in the case group indicates increased megakaryocytic activity due to peripheral destruction of platelets. Since different studies have shown contradictory results of MPV in Dengue, we believe that mechanism and pathogenesis of low platelet counts in Dengue affect MPV values. In our study, high MPV is suggestive of a destructive etiology for low platelet count than marrow suppression or combined causes. The mean PCT values in the present study were 0.12% and 0.23% for case and control groups respectively. The mean PCT forthe case group was significantly lower (P<0.005). This finding correlated well with the study by Nehara HR et al and ChandrasekharV et al [10,16]. PCT is a measure of platelet mass because it is derived from MPV and absolute platelet count. Since MPV was high in our study, PCT values were marginally high when compared to mean PCT values of the case group in the study by Nehara HR et al [10]. The mean PDW in our study was 16.9 fL and 16.82 fL for case group and control groups respectively. The mean PDW was higher in case group but not statistically significant (P=0.384) because of high mean PDW values in controls. In a study by Kim KY et al, platelet size was heterogeneous even in controls due to ageing platelets or due to heterogeneous demarcation of megakaryocytes in the marrow [21]. These findings explain the presence of high PDW in controls in our study. The ROC curve analysis showed that MPV > 8.5 fL is highly sensitive for Dengue infection whereas platelet count < 150x10^9/L and PCT<0.14% are highly specific for Dengue. PDW was not an accurate platelet index for diagnosis of Dengue infection in our study. Low platelet count, low PCT and high MPV were good predictors of Dengue infection in our study.

In addition to these findings of PLT count, MPV, PCT and PDW in Dengue cases, we studied the significance of platelet indices in NS1 antigen, IgM and IgG antibody positive cases. The Dengue positive cases were categorized parameter wise(Table 3), of which majority of the cases were NS1 antigen positive (47.6%). These findings correlated with findings of study Tathe SS et al [18]. The least common category was combined NS1 antigen and IgG antibody positive cases(1.7%). This combination indicates occurrence of secondary infection in an old case of Dengue possibly by a different serotype and hence these cases were the least in number in the study. The PLT count, MPV, PCT and PDW were statistically analyzed in NS1 antigen, IgM and IgG antibody positive cases. Low PLT count, high MPV and low PCT were found to be statistically significant (P<0.005) in antigen and antibody positive cases whereas PDW was not significant. These findings have not been evaluated in any study till date and need to be further studied as they may prove to be of clinical relevance in predicting severity and outcome of disease in Dengue patients.

Conclusion

Platelet count, MPV and PCT can be used as good indicators for dengue infection. Low PLT count, high MPV and low PCT are significantly associated with NS1 antigen, IgM and IgG antibody positivity. MPV > 8.5 fL showed sensitivity whereas and PLT count < 150x 10^9/L and PCT < 0.14% showed specificity for dengue infection.

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