

Significance of Platelets and its Indices in Patients with Dengue Fever

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ABSTRACT

Background: Dengue is a viral infection affecting different parts of the world, particularly the under developed countries including Pakistan. Changes in hematological parameters are frequently observed in dengue viral infection out of which the changes in the structure of platelets and their levels are a predominant factor in dengue viral infection. In view of the foresaid, this study was conducted to find the platelet levels and indices in confirmed cases suffering from dengue fever.

Methods: This descriptive cross-sectional study was conducted in Peshawar in a duration of five months from September to December 2022. A total of five hundred patients were screened for dengue viral infection. Blood samples were collected from all suspected dengue infected patients and processed for complete blood count (CBC). Percentage and mean standard deviation were calculated and data were expressed in tables and graphs.

Results: Among total suspected patients, one hundred fifty patients were found positive in which highest number of patients were male (65.3%) than female patients (34.7%). The minimum hemoglobin, white blood cells, and platelets were 6.9 g/dl, 2400 g/dl, and 43000 g/dl respectively. Low level of hemoglobin was found in 9.4% patients, 25.3% patients had below normal white blood cell count, and 30.4% patients were observed with less than normal platelet count. Few patients were noted with lower mean platelets volume and plateletcrit than normal.

Conclusion: The study showed a significant impact of dengue viral infection on complete blood count. The hemoglobin, WBC, platelets, and platelet indices were potentially affected with dengue viral infection. It is necessary to design a strategy for early diagnosis of dengue infected patients for timely treatment, which can together save patients from severe impact of dengue viral infection such as dengue shock syndrome which could be fatal.

Keywords: Dengue viral infection, Hematological parameters, Platelets, Mean Platelets Volume, Plateletcrit, Platelet distributive width.

INTRODUCTION

The first documented signs of Dengue fever were cited in the Chinese dynasty (265 to 420AD). This disease was called "water poison" as it was connected to airborne arthropods¹. The word "Dengue" stands for "cramp like seizure" and derived from the Swahili word "Ka-dinga pepo". During 1780's dengue outbreaks were officially documented after outbreaks reported in Asia, Africa and North America. Gupta et al., reported the first clinical case in Philadelphia during an epidemic and also invented the word "break bone fever" due to the most prominent symptoms of myalgia and arthralgia². Dengue is a life threatening infection affecting the entire globe and involving more than 100 countries³. Worldwide dengue affects 100 million people annually out of which 250000 cases of dengue hemorrhagic fever and 24000 deaths⁴. The most common and important symptoms of dengue viral infection include vomiting, diarrhea, rash, joint and muscle pains, nose and mouth bleeding and severe headache⁵.

Dengue virus belongs to the Flaviviridae family, an RNA virus⁶, with 11000 nucleotide bases in its genetic material⁷, and spherical in shape surrounded by a nucleocapsid. It has three structural protein genes for coding the nucleocapsid, membrane associated protein, envelope protein and seven nonstructural protein genes⁷. Viral neutralization and hemagglutination are the basic functions of glycoprotein envelope⁸. Four antigenically interrelated serotypes have been recognized for Dengue virus⁹.

For severe dengue viral infection, thrombocytopenia is one of the major indicators which also contributes to bleeding one of the most important symptoms. Platelet count may or may not be directly related to hemorrhagic manifestation¹⁰. From 3rd to 7th day of Dengue fever the number of platelets starts to decrease while from 8th to 10th day it starts to normalize. Abnormal pooling of platelets causes thrombocytopenia¹¹, the possible mechanism for which could be antidengue antibody mediated platelets, peripheral consumption of platelets, direct bone marrow suppression and isolated viral replication in the platelets¹².

Mean platelets volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR) are the platelets indices

which are the indicators for platelet activation¹³. MPV is surrogate indicator of bone marrow and useful independent predictor of bleeding. Due to increase megakaryocyte activities level of MPV become high, and risk of bleeding due to low MPV as it indicates bone marrow suppression¹⁴. Due to PDW we can measure the variability in the size of platelets, the changes that occur in platelets activation and heterogeneity in platelets morphology¹⁵. For total mass of platelets we use plateletcrit also useful for detecting abnormalities in platelets¹⁶. The total volume of platelets in blood which is called PCT are calculated by formula "platelet count x MPV / 10,000"¹⁷. The normal range of PCT is 0.022024%¹⁸ and that for MPV is 8.9 -11.8fl and that of PDW range 10.0% - 17.9%^{19,20}.

Platelet deficiency or function defect may result in clinical bleeding for which both clinical and research laboratories assessment is essential for platelets count¹, 50,000 to 4,50,000/mm³ to be in the normal range of platelets in a healthy person²¹. Clinical manifestations mild when platelets count is more 20,000/mm³ but when this number decreases below 10,000/mm³ the risk of life threatening bleeding increases like gastrointestinal bleeding or intracranial hemorrhage²². There are different methods for platelet estimation such as "using counting chamber for manual counting" "evaluation in peripheral smear" recommended by the international committee for Standardization in Hematology for assessing platelet count²³. Peripheral blood smears is the traditional and accurate method for estimating platelet count and provides adequate quality of results²⁴. The earliest prognosticator of severe dengue is decline in white cell count with sudden platelet drop which precedes plasma leakage²⁵. In thrombosis, hemostasis and in the process of inflammation the platelets, a nucleated type of blood cell play an important role. From clinical studies the role of platelets in the pathogenesis of clumping parasitized red blood cells and bleeding in severe dengue infection has been reported²⁶. DHF and DSS the severe form of Dengue infection are due to platelet dysfunction²⁷. Improving platelet count is the prognostic indicator for recovery from severe dengue²⁸. Platelets differ in size having increased number and size of pseudopodia affect platelet distribution width which increases during platelet activation. Due to

platelet activation the morphology of the cells change which can be evaluated on the basis of MPV and PDW²⁹. For diagnosis and prognosis parameters such as total white cell count, hematocrit, atypical lymphocytes which are the parts of complete blood count aid better management of the patient³⁰. This study aimed at finding the role of platelets indices in severe dengue.

MATERIALS AND METHODS

Study Design and Setting: This descriptive study was conducted in district Peshawar located in the province Khyber Pakhtunkhwa in Pakistan. Total area of district Peshawar is 1,257 square kilometers. During 2022 dengue severity was recorded in the main health center of Lady Reading Hospital Peshawar.

Ethical Approval: With the permission of the Medical Superintendent of Lady Reading Hospital Peshawar we collected the data of the patients including in our study.

Sample Size: 500 were screened for dengue among the total.

Data Collection Procedure: Extra details were also taken from each patients on separate sheets. Month, age, category and type of diagnostic test performed all were recorded in investigation proforma.

Blood Collection Procedure: 2.5 ml blood for serotyping was collected in gel tube and 3ml was collected in EDTA tube for hematological parameters like platelets count, total leukocyte count, hemoglobin level, mean platelet volume, mean corpuscular volume and hematocrit were performed in automated hematology analyzer. Minimum level of Platelets count was also recorded during the illness. For dengue confirmation serological tests done by ELISA technique for NS 1, IgM, IgG antibodies. By electrical impedance method platelet count and size was calculated using an automated counter.

Following standard protocol we made blood smears by using Leishman's stain and blood collected in EDTA vacutainer tubes. For rough calculation of platelet count the average of ten successive oil immersion fields was calculated which was then multiplied by 20,000. The yield of platelets count estimated in lacs/mm and this method is reliable method for estimation of platelets. Platelet count less than 150 x 10⁹/L was defined as thrombocytopenia and defined as thrombocytosis when count more than 450 x 10⁹/L, meaning that the normal range of platelet count was 150 x10⁹/L to 450 x 10⁹/L. We further subdivided the thrombocytopenia into three categories based upon the number of platelets like mild whose range from 100 to 150 moderate range from 50 to 100 and severe in which platelets were below 50 x 10⁹/L. With the help of autoanalyzer mindray BC-500 we measured the mean platelets volume (MPV) and Platelets Distribution Width (PDW) and set ranges for their parameters. The average size of platelets was calculated by machine called as MPV while platelet anisocytosis was termed as PDW. Both in MPV and PDW patients the paired student t test for the comparison between manual and automated platelets was applied while for the comparison between thrombocytopenic and nonthrombocytopenic unpaired student t test was used. To study the correlation between automated and manual platelets count Pearson's correlation coefficient was used for which the P value ≤ 0.05 was considered as significant.

Statistical Analysis: The frequency (percentage) and mean standard deviation were expressed on categorical and quantitative variables. To compare quantitative data between groups independent t – test was performed. To compare categorical parameters with selected variables Chi square test was used. Statistical software package SPSS, version 20.0 IBM Crop. IBM SPSS statistics was used for statistical analysis.

RESULTS

Males dominated the gender spectrum, with 98% of valid responses being males. Meanwhile, women made up a smaller percentage of the total, with only 34.7% (Table 1).

Table 1: Gender-wise distribution of dengue infected patients

Gender	Frequency	Percent
Valid		
Male	98	65.3
Female	52	34.7
Total	150	100.0

The results in the below table show the characteristics of platelets and blood cells. The first row shows the Minimum and Maximum values for each category, and the second row provides the standard deviation for each value. The third row lists the categories in order from most to least common, and the fourth row shows the values in Percentiles. The last column shows the results in N, which is the number of values in each category (Table 2).

Table 2: Descriptive data of blood parameters

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
Hb	150	6.9	18.5	14.733	2.0745
WBC	150	2400.0	17400.0	5837.333	2320.4821
Platelets	150	43.0	347.0	182.027	63.0894
Valid N (listwise)	150				

The hemoglobin frequency percent indicates the percentage of individuals whose hemoglobin is within the acceptable range. In this case, 4 out of 2.7% of the participants had hemoglobin levels below the acceptable range of 9.1-12.0 grams per deciliter. Meanwhile, 10 out of 6.7% of the participants had hemoglobin levels within the acceptable range of 9.1-12.0 grams per deciliter. As for the total hemoglobin amount, 136 out of 150 grams were within the acceptable range (Table 3).

Table 3: Patients categorized based on hemoglobin level

Hemoglobin	Frequency	Percent
Valid		
<9	4	2.7
9.1-12.0	10	6.7
>12.0	136	90.7
Total	150	100.0

The results for patient characterization on the basis of WBC levels, showed that out of 150 people, 38 (25.3%) had WBC counts of less than 4,000, 110 (73.3%) had WBC counts between 4,000 and 11,000, while only 2 (1.3%) had WBC counts higher than 11,000 (Table 4).

Table 4: Patients categorized based on WBC level

WBC	Frequency	Percent
Valid		
<4000	38	25.3
4000-11000	110	73.3
>11000	2	1.3
Total	150	100.0

The frequency of platelet levels was measured in 102 individuals. Platelets had a level of less than 150000 in 31 instances (30.4%) while they had a level of 150000-450000 in 71 instances (69.6%). Overall, platelet levels were measured in all 102 individuals (Table 5).

Table 5: Patients categorized based on Platelets level

Platelets	Frequency	Percent
Valid		
<150000	31	30.4
150000-450000	71	69.6
Total	102	100.0

The results of the study showed that the mean platelet volume (MPV) of the 150 participants had an average of 8.492, with a standard deviation of .7255. The minimum MPV for participants was 6.9 and the maximum was 10.5. For plateletcrit (PCT), the average value for the 150 participants was .1519, with a standard deviation of .04773. The lowest PCT value recorded was .04 and the highest PCT value was .25. Finally, the platelet distributive width (PDW) had an average of 16.876 and a standard

deviation of .8072. The lowest PDW value for participants was 14.1 and the highest was 19.1 (Table 6).

Table 6: Descriptive data of platelet indices

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
MPV	150	6.9	10.5	8.492	.7255
PCT	150	.04	.25	.1519	.04773
PDW	150	14.1	19.1	16.876	.8072
Valid N (listwise)	150				

On the basis of data we divide MPV as lower, normal and high class in which we found that 116 patients were in the normal range and 32 patients were in high class while only two patients were in the lower (Table 7).

Table 7: MPV level among different gender groups

Gender * MPV Cross- tabulation					
Gender		MPV			Total
		<7	7-9	>9	
	Male	2	72	24	98
	Female	0	44	8	52
Total		2	116	32	150

The majority of the patients in our study had low PCT baseline value. PCT of the patients who had transfusion were found to be low with a mean value of 0.04%, and this had a statistically significant association ($P < 0.001$) (Table 8).

Table 8: PCT level among different gender groups

Gender * PCT Cross tabulation					
Gender		PCT			Total
		<0.22	0.22-0.24	>0.24	
	Male	90	6	2	98
	Female	48	4	0	52
Total		138	10	2	150

The normal value of PDW is between 8.3 and 56.6 fL. The majority (99.6%) of the patients had normal PDW value as the baseline. There was no statistically significant association for the same (Table 9).

Table 9: PDW level among different gender groups

Gender * PDW Cross tabulation			
Gender		PDW	Total
		2.0	
	Male	98	98
	Female	52	52
Total		150	150

DISCUSSION

One of the major public health problems is dengue fever. Multifactorial mechanisms like thrombopathy, coagulopathy and vasculopathy may be involved in Dengue fever or dengue hemorrhage fever (DF/DHF). Many factors can contribute to the onset of thrombocytopenia in DF, varying from a reactive immune response against platelets to decreased platelet production. In tropical and subtropical regions of the world dengue fever is one of the growing public health concerns. Around the world 50 – 100 million of dengue cases are assessed in recent times in which dengue hemorrhage fever and dengue shock syndrome are announced in more than 5,00,000 cases³¹. The results of the present study showed that MPV and platelet count were lower in Dengue patients whereas the level of PDW was significantly higher in Dengue patients. The serial observation may guide a clinician in diagnosing patients with dengue and severe thrombocytopenia. In these patients, the mechanism of thrombocytopenia is largely due to marrow suppression. The contradictory results show that MPV with thrombocytopenia has been evaluated as a diagnostic tool in different clinical scenarios related to dengue. Many studies show that MPV has sufficient specificity and sensitivity to aplastic

anemia, thrombocytopenia and bone marrow disease³². The increased level of MPV can be used as marker as it indicates increase in platelet diameter. During platelet activation the level of MPV increases because the change in shape of the platelets from biconcave disc to almost spherical. This also affects the platelet distribution width because the number and size of pseudopodia differ during platelet activation³³. In endemic areas low MPV and high PDW are used as predictive markers for the diagnosis of dengue fever as concluded by Navya BN et al which is the same as the results obtained in our study³⁴. Study conducted by Jayashree K et al showed similar results as shown in our study that there was significant association seen in platelet count and severity of the dengue disease from which we concluded that for diagnosing of DF/DHF/DSS platelet count can be used as predictive parameters. Similar results was reported in the study conducted by Bashir AB et al in which he found that MPV was decreased in dengue positive patient, his results also showed that in dengue patients the level of PDW was increased which is similar to the results obtained in our study³⁵. Due to decrease in platelet count many cases presented with bleeding manifestations did not bleed subsequently which indicated weak correlation between thrombocytopenia and bleeding tendencies, Sharma et al. also reported in their study that aggregation of the abnormal platelets rather than reduction in their numbers was the cause of bleeding diathesis along with the cytokine mediated endothelial injury. The most important therapy for the management of DF/ DHF is the immediate replacement of plasma loss by rapid volume expanders. As in our study low MPV, high PDW and low PCT was similar to the study conducted by Mukker et al which showed similar variation in platelets indices in Dengue patients. For diagnosing dengue fever the Platelets indices were used as predictive markers because they show sensitivity to dengue fever. Due to decreased production of platelets in the bone marrow or increased destruction and clearance of platelets from peripheral blood were considered as the main cause of low platelet count in Dengue patients³⁶.

CONCLUSION

This study examined the significance of platelets and their indices in dengue viral infections. Lower platelet count and PCT are observed with dengue viral infection, whereas PDW does not fluctuate. MPV and PDW are also useful predictors of dengue infection. Platelets and their indices may be able to predict the outcome and need for platelet transfusions in patients infected with dengue viral infections. Platelet indices can also be useful to monitor in assessing the severity of dengue fever.

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