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ROLE OF PLATELET PARAMETERS IN DENGUE POSITIVE CASES – A SINGLE CENTRE EXPERIENCE

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ABSTRACT
Aim: To study the role of platelet parameters like platelet count (PLT), Mean platelet volume (MPV) and Platelet distribution width (PDW) in dengue positive infection and also to note their relationship with the severity of the disease.

Objectives: 1. Take Dengue positive patients
2. Get CBC, CXR, USG of Abdomen.
3. See Relationship of Platelet parameters with severity of disease in the form of pleural effusion , ascites, bleeding manifestation, DHF, DSS.

Material and Methodology: The study was done on 78 dengue positive cases during the outbreak of dengue infection over a period of 2 months between JULY 2018 to AUGUST 2018. The platelet parameter like Platelet count, Mean platelet volume, Platelet distribution width were noted using BC 3000 plus Mindray Automated Hematology Analyzer) along with routine blood parameters hemoglobin(Hb), hematocrit, WBC, serum bilirubin, liver enzymes (AST, ALT) and was compared with severity of disease in the form of in the form of pleural effusion , ascites, bleeding manifestation, DHF, DSS. These 100 patients were also grouped into three according to the platelet count (<20,000, 20,000-1,00,000, >1,00,000), and PDW and MPV were compared in these groups.

Inclusion Criteria: All patients with clinical features and serologically positive dengue infection above age of >18 years were included.

Exclusion Criteria: Other causes of thrombocytopenia like due to infections other than dengue, megaloblastic anaemia, cirrhosis etc.

Conclusion: The study focuses the importance of platelet parameters in dengue infection. Significant difference was observed between severity of the thrombocytopenia and severity of the disease (P value -0.013). Platelet count is thus a predictive parameter of DF/DHF/DSS. Low MPV (13fl) shows sensitivity for dengue fever thus reflecting a predictive marker for diagnosing dengue fever in endemic area.

KEYWORDS :

INTRODUCTION:

Dengue is the most important merging tropical viral disease in the world today. Dengue is caused by one of the four serotypes of the dengue virus (DEN-1,DEN2,DEN-3,DEN-4) also referred to as an arbovirus (arthropod-borne viruses) that belongs to the genus flavivirus of the family flaviviridae. It is transmitted by mosquitoes of the genus Aedes aegypti.(1,2)

Over the past few years, dengue has emerged as a serious public health concern especially in India. It is estimated that around 2.5 billion people, in urban areas of tropical countries, are at a risk of developing dengue infection.(3) The emergence of dengue in India has gone into epidemic proportions and dengue outbreaks are frequently engulfing different parts of the country in both urban and rural populations.

The WHO 2009 classification divides dengue fever into two groups: uncomplicated and severe; 2 though the 1997 WHO classification is still widely used, classifying dengue in to 3 groups: dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).(4,5) One of the most common laboratory findings in dengue is thrombocytopenia.

Platelet indices like MPV, PDW and P-LCR have been investigated as prospective platelet activation indicators.4 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. The normal range for MPV in Indian population is from 8.6-15.5 fL(6)

PDW is a marker of volume variability in platelets size and is elevated in the presence of platelet anisocytosis.7 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(.8-12)

The study of platelets would have a substantial impact on reducing the mortality and morbidity associated with dengue. Hence the present study is planned to analyze the platelet parameters in dengue illness and to correlate the same with clinical course.

MATERIAL AND METHODS:

This prospective observational study was carried out in a tertiary care ccentre in Kohlapur, D Y Patil Hospital and Research Centre from August 2018 to October 2018. All patients above 12 years with confirmed dengue, who were either hospitalized or managed as outdoor patients with NS1 (nonstructural protein) antigen and/ or IgM dengue antibody positivity were included in the study. The patients with concomitant malaria, typhoid, leptospirosis etc were excluded from the study. Detailed history and careful clinical examination was performed on each patient. Laboratory investigations done were hemoglobin, total and differential leucocyte counts, platelet count, hematocrit, liver function tests, blood urea and serum creatinine,ECG, chest radiograph and ultrasound scan of abdomen. Blood counts were monitored periodically as and when required till resolution. Other differential diagnosis were excluded by appropriate tests. . The data is collected in a specially designed proforma for the study. It is transformed to a master chart which will then be subjected to analysis The study was approved by hospital ethics committee and informed consent was obtained from each patient.

The patients were divided in three groups-DF,DHF,DSS. These 100 patients were also grouped into three according to the platelet count (<20,000, 20,000-1,00,000, >1,00,000), and PDW and MPV were compared in these groups.

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 & leucopenia.

Dengue haemorrhagic fever- all must be present:-
1. Fever, or recent history of acute fever.
2. Haemorrhagic manifestations
3. Thrombocytopenia (100,000/mm^3 or less).
4. Evidence of plasma leakage due to increased capillary

permeability manifested by one or more of the following:
•   >20% rise in haemotocrit for age and sex.
•   >20% drop in haemotocrit following treatment with fluids as compared to baseline.
•   Signs of plasma leakage (pleural effusion, ascites or hypoproteinaemia).

Dengue shock syndrome- Four criteria for Dengue Haemorrhagic Fever with signs of circulatory failure.

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.

INCLUSION CRITERIA :
All patients with clinical features and serologically positive dengue infection above age of >18 years were included.

EXCLUSION CRITERIA Other causes of thrombocytopenia like due to infections other than dengue, megaloblastic anaemia, cirrhosis etc.

Statistical analysis
All data are described as means with standard deviations or numbers with percentages. Statistical analysis was performed by Chi Square test done by using the Statistical Package for Social Sciences (SPSS 21) with $p < 0.05$ taken as statistically significant. Wicoxon's signed rank test and ANOVA were also applied wherever applicable.

RESULTS
Out of total 78 dengue cases, commonest presentation was dengue fever (DF) (68%) followed by dengue haemorrhagic fever (DHF) (23%) and least were dengue shock syndrome (DSS) (9%). Males were in higher proportion as compared to females (with males 70.5% and 29.48% females) and M:F = 2.4:1. No significant difference was observed according to sex with severity of the cases. Cases of dengue were highest in 21 to 40 age group (younger age group). Proportion of cases decreased as age increased.

Table 1- Distribution of study subjects according to age and gender

Age-group	Males	Females	Total
0-20	11	5	16
21-40	30	11	41
41-60	10	4	14
61-80	4	3	7
Total	55	23	78

Of all the 78 patients of dengue, 62 (79.48%) patients had NS1Ag positive. Among them IgM antibody was raised in 13

(16.66%) patients. Out of 78 patients of dengue, 4 (5.12%) patients had raised IgG antibody level.

Both NS1 and IgM were positive in 5(6.41%) patients, NS1 and IgG in 3(3.84%) patients, IgG and IgM in 3 (3.84%) patients. All three antigens were positive in 2 (2.56%) patients.

Table 2-Distribution of study subjects according to diagnostic parameters

Diagnostic parameter	Positive patients	Percentage
NS1	62	79.48
IgM	13	16.66
IgG	4	5.12
NS1&IgM	5	6.41
NS1 &IgG	3	3.84
IgG & IgM	3	3.84
All positive	2	2.56

Platelet counts results were grouped under 4 groups <20,000, 21-50,000, 50,000-1,00,000, and >1 lakhs. Of the patients with thrombocytopenia (i.e. platelet count < 1 lakh) 5 patients had platelet count <20,000 (sever thrombocytopenia) 3 patients had platelet count between 21,000- 50,000 (moderate thrombocytopenia), 20 patients had platelet count between 51,000-1 lakh (mild thrombocytopenia) while remaining cases in our study had platelet count > 1 lakhs (47 cases). There was no significant difference in severe thrombocytopenia among different age group. Chi square was done and p value was found to be 0.991 which signifies that, it is not statistically significant.

Table 3 Distribution of study subjects according to platelet count and age

Age-group	Platelet count				Total
	0-20000	21000-50000	51000-100000	>100000	
0-20	0	1	4	13	18
21-40	1	0	14	23	41
41-60	2	2	2	8	14
61-80	2	0	0	3	5
Total	5	3	20	47	78

The seropositive patients were followed clinically for the symptoms of DHF/DSS and they were correlated with the respective platelet counts. Dengue fever (DF) cases were noted in 72 cases and dengue hemorrhagic fever (DHF) cases were noted in 5 cases and dengue shock syndrome(DSS) were seen in 1 case. Among the 72 cases of DF about 19 cases had mild thrombocytopenia followed by severe thrombocytopenia in 2 cases and moderate thrombocytopenia in 1 case while remaining 47 cases had platelet count > 1 lakh. Among 5 cases of DHF, 1 case had mild thrombocytopenia, 2 cases had moderate thrombocytopenia and remaining 2 cases had severe thrombocytopenia. In our study, one DSS case was seen with severe thrombocytopenia. Significant difference was observed between severity of the thrombocytopenia and severity of the disease, (P value -0.013)

Table 4 Distribution of study subjects according to platelet count severity of disease

platelet count	Severity of disease			Total
	DF	DHF	DSS	
0-20000	2	2	1	5
21000-50000	1	2	0	3
51000-100000	19	1	0	20
>100000	47	0	0	47
Total	72	5	1	78

Among 78 dengue positive cases, low MPV (<9fl) which indicates bone marrow suppression was seen in 15 (19.23%) patients of all the cases. Of which 66.6% were diagnosed as DF while 26.6% were of DHF and remaining were diagnosed as DSS. The rest of the 63(87.17%) patients showed high MPV (>9fl). (Table 5)

Table 5 Distribution of study subjects according to severity of disease and mean platelet volume

Mean platelet volume	Severity of disease			Total
	DF	DHF	DSS	
Low ≤9	10	4	1	15
High >9	62	1	0	63
Total	72	5	1	78

A high PDW (>13fl) which indicates as useful marker for platelet activation was seen in 87% 0f cases, of which 62 cases were DF while 5 cases were of DHF and one case of DSS. The remaining 13% of cases showed low PDW (<13fl), of which all were having DF

Table 6 Distribution of study subjects according to severity of disease and mean platelet distribution width

platelet distribution width	Severity of disease			Total
	DF	DHF	DSS	
Low ≤13	12	0	0	12
High >13	62	5	1	68
Total	72	5	1	78

DISCUSSION:

DF is a self limited febrile illness; DHF is characterized by prominenthaemorrhagic manifestations associated with thrombocytopenia and an increased vascular permeability. The clinical diagnosis of DHF especially in the early phase of illness is not easy. Laboratory findings such as thrombocytopenia and a rising hematocrit in DHF cases are usually observed by day 3 or 4 of the illness. [13] 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 [14]

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 [15-17] 11 of 61 patients studied by Mitrakul et al, had destruction as a main cause for thrombocytopenia, as revealed by platelet kinetic study.

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 megakaryocyte 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 [18] The present effort for finding simple and widely used platelet activation indices focused on the fact that platelet activation causes morphologic changes of platelets, including both the spherical shape and pseudopodia formation. Platelets with increased number and size of pseudopodia differ in size, possibly affecting platelet distribution width (PDW). [19,20]

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 [14]

According to the study conducted by Jayashree K et al there was a significant association between platelet counts and severity of the disease which is similar to our study, thus concluding that platelet count can be used as predictive parameters for diagnosing DF/DHF/DSS [13]

CONCLUSION

The study focuses the importance of platelet parameters in dengue infection. Significant difference was observed between severity of the thrombocytopenia and severity of the disease (P value -0.013). Platelet count is thus a predictive parameter of DF/DHF/DSS. Low MPV (13fl) shows sensitivity for dengue fever thus reflecting a predictive marker for diagnosing dengue fever in endemic area.Low MPV <9 fl and high PDW >13fl shows considerable sensitivity for dengue fever.

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