Research Article

Study of platelet indices in dengue fever with thrombocytopenia and correlation of immature platelet fraction (IPF) with platelet recovery

Ahmad Wayez[1], Lubna Zafar[2] , Mohsin Aijaz[3]* and Nishat Afroz[4]

[1]Junior resident, Department of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh (202002), U.P, India

[2]Assistant Professor, Department of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh (202002), U.P, India

[3]Senior resident, Department of Pathology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh (202002), U.P, India

[4]Professor, Department of Pathology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh (202002), U.P, India

Introduction

Dengue fever is the most rapidly spreading mosquito-borne viral disease in the world. An estimated 50 million infections per year occur across approximately 100 countries. The incidence has increased 30-fold with increasing geographic expansion with potential for further spread [1].

The primary dengue vector Aedes aegypti mosquito has become widely distributed across tropical and subtropical latitudes. Based on the antigenic differences, dengue virus can be divided into four different serotypes, DEN 1–4 [2]. 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 [3]. One of the most common laboratory findings in dengue is thrombocytopenia [2]. The complex mechanism of thrombocytopenia remains unclear. Possible mechanisms of thrombocytopenia could be, direct bone marrow suppression by the virus; antidengue antibody-mediated platelet destruction, peripheral consumption of platelets and isolated viral replication in the platelet.

Recently, novel platelet indices such as Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), and Platelet to Large Cell Ratio (PLCR) have been investigated as prospective platelet activation markers [4]. Mean Platelet Volume is a surrogate marker of bone marrow activity; a high MPV indicates increased megakaryocyte activity. A low MPV indicates marrow suppression. Platelets with increased number and size of pseudopodia differ in size, possibly affecting platelet distribution width (PDW) which increases during platelet activation [4].

Platelet to Large Cell Ratio (PLCR) is significantly decreased in patients with thrombocytosis than in normal while it is increased in thrombocytopenia. PLCR was inversely related to platelet count and directly related to PDW and MPV [5]. The immature platelet fraction (IPF) is a new parameter which is an automated measure of reticulated platelets in peripheral

blood. Reticulated platelets contain RNA and are newly released platelets that are larger, more physiologically active and are the analogue of the red cell reticulocyte [6]. The number of reticulated platelets reflects the rate of thrombopoiesis [7].

Thrombocytopenia in patients with dengue may cause a steep fall in platelet count, warranting platelet transfusion. The transfusion may be avoided when the platelet count is set to rise. This brings us to the issue of how we can reliably predict the rise in the platelet count. IPF count holds great promise of being this predictor. Our study aims to evaluate the platelet parameters in patients of dengue fever having thrombocytopenia and also to correlate them with platelet recovery. This may be of great assistance in the management of thrombocytopenic dengue fever patients in future.

Materials and methods

Study design

This study was a hospital based observational study. The patients who presented with dengue fever with thrombocytopenia in OPD and IPD in the Department of Medicine from December 2016 to October 2018 were taken in study. The informed consent was obtained from each patient prior to commencement of this study. The detailed history and examination was carried out and each patient was investigated as per designed proforma.

Inclusion Criteria

1. Patients with a positive dengue serology (IgM or Non-Structural protein 1 antigen-NS1).

2. Platelet count <1 lakh/cumm.

Exclusion Criteria

1. IgG positive cases. 2. Inherited Disorders of thrombocytopenia. 3. Idiopathic Thrombocytopenic Purpura. 4. Thrombotic Thrombocytopenic Purpura 5. Aplastic Anemia. 6. Malignancy (Systemic/haematological) 7. Drug Induced 8. Disseminated Intravascular Coagulation. 9. Patients who received platelets transfusion during the study. 10. Patients on antiplatelet medications. 11. Chronic diseases (Chronic Liver Disease, Chronic Kidney Disease and chronic alcoholics).

Investigations

A venous blood sample of 3 ml was collected from each patient and transported to the laboratory immediately. A commercially available strip test (SD Bio line Dengue Duo) was used according to manufacturer instructions to detect NS1 antigen and anti-dengue immunoglobulin IgM and IgG. A total of 106 cases were studied, based on positive dengue test. We studied the complete blood count, various platelet parameters, Liver Function Test, Renal Function Test at presentation in all dengue positive patients. CBC and various Platelet parameters like platelet count, MPV, PCT and PDW of these patients were assessed by using Automated Hematology Analyzer (Nihon Kohden MEK-6420p Coulter machine). The Sysmex XE-2100 (Sysmex, Kobe, Japan) was used to measure the IPF. This fully

automated hematology analyzer uses a carefully designed gating system in the optical (fluorescence) reticulocyte/platelet channel to reliably quantitate the IPF. All the patients were categorized into four groups based on platelet count and for every group mean of the platelet indices was taken. Platelet counts and various platelet parameters were also studied after 24 hrs and 48 hrs of admission and were compared to note their relationship with the severity of the disease.

Statistical analysis

All the data were collected, tabulated using the statistical package for the social sciences (SPSS version 20). The results were presented as mean SD or percentage. The test used in the study was Chi square test, Fisher exact test, t test and Pearson product moment correlation analysis. A p-value of less than 0.05 was considered as statistically significant.

Ethical consideration

Ethical clearance of this study was approved from the Institutional Ethical Review Committee. Data regarding the age and sex was recorded in predesigned forms.

Observation and results

The present prospective observational study was conducted from December 2016 to October 2018. A total number of 106 patients of dengue fever, who presented in J.N Medical College and Hospital, Aligarh Muslim University and met the inclusion criteria, as mentioned in materials and methods were enrolled in the study after obtaining informed consent.

All of our 106 patients were NS1 antigen positive. Seventy-one patients were NS1 antigen positive only and 35 patients were positive for both NS1 and IgM antibody. IgG only cases were excluded from our study.

Fever was the major complaint, seen in almost all patients at presentation (100% cases). It was accompanied by vomiting (10.38%), gum bleed (9.43%), generalized bodyache (8.49%), rash (6.6%), epistaxis (3.77%), jaundice and pruritus (2.83%). Some patients had abdominal pain with hepatomegaly or splenomegaly (Table 1).

In our study, 90 cases (84.9% of total study population) were anaemic. Leucopenia (<4000) was observed in 27% of cases. When haematocrit was studied, 45 patients had haematocrit below normal range and 61 patients had hematocrit within normal range. Abnormal liver enzymes were observed in 24.52% (26 cases) of patients. A total of 29 patients (27%) had deranged serum creatinine.

As shown in the Table 2 various platelet parameters were calculated with respect to different groups of platelet count on the day of admission. For platelet count <25000/cu.mm, the mean IPF was 13.28±6.11%, for 26000-50000/cu.mm, it was 14.43±5.71%. The mean IPF for the platelet range of 51000-75000/cu.mm was 17.70±8.06% (Table 2).

The mean MPV for platelet count <25000/cu.mm was 8.61±1.32 fl. The mean MPV for platelet count in the range

26000-50000/cu.mm was 8.77±1.47 fl. The mean MPV for platelet count in the range 51000-75000/cu.mm was 9.95±2.05fl (Table 2).

Table 1: Presenting clinical features of patients in the study group.

S.No	Presenting Clinical Features	Number	Percentage %
1	Fever	106	100
2	Vomiting	16	15.09
3	Gum Bleed	11	10.38
4	Generalised Bodyache	10	9.43
5	Rash	9	8.49
6	Epistaxis	7	6.6
7	Jaundice	4	3.77
8	Abdominal Pain, hepatosplenomegaly	3	2.83
9	Headache, retro-orbital pain	3	2.83
10	Pedal edema	0	0

Table 2: Mean Platelet parameters in relation to platelet count at presentation at day 1.

Platelet Count/ (cumm) on day 1	< 25000	25000-50000	51000-75000	76000-100000	correlation coefficient r	p-value
IPF %	13.28±6.11%	14.43±5.71%	17.70±8.06%	0		
MPV(fl)	8.61±1.32fl	8.77±1.47fl	9.95±2.05fl	0	0.124	<0.5
PDW%	17.67±1.61%	17.22±1.77%	16.40±1.69%	0	-0.19	<0.5
PCT%	0.04±0.02%	0.05±0.02%	0.09±0.02%	0	0.238	<0.5

The mean PDW for the platelet count <25000/cu.mm was 17.67±1.61%. The mean PDW for the platelet count in the range of 26000-50000/cu.mm was 17.22±1.77%. The mean PDW for the platelet count in the range of 51000-76000/cu.mm was 16.40±1.69%.

The mean value for the PCT for platelet count <25000/cu.mm was 0.04±0.02%. The mean value for the PCT for the platelet count in the range of 26000-50000/cu.mm was 0.05±0.02. The mean value for the platelet count range 51000-75000/cu.mm was 0.09±0.02%.

When mean MPV was correlated with platelet count at presentation, the correlation coefficient *r* was 0.124 and *p* value was <0.05, thus showing positive correlation. Similarly when the plateletcrit was correlated with platelet count at presentation, the correlation coefficient was 0.238 and the *p* value was again <0.05 indicating that it is also positively correlated. On the other hand, when the PDW was correlated with platelet count at presentation, its correlation coefficient was -0.19 with the *p* value <0.05 thus, it was negatively correlated.

The normal range of IPF is 0.3-8%. In our study it was found that majority of our patients (90 cases, 84.90%) had IPF more than 8%, that is above the normal range and only 16 cases (15.10 %) had IPF value less than 8%. When IPF value was correlated with platelet count after 24 hrs of presentation (day 2), the correlation coefficient r was 0.133 and *p* value was

<0.05 (significant), thus positive significant correlation was seen. Similarly after 48 hrs (day 3), correlation coefficient r was 0.303 and *p* value was <0.01 (Table 3). It was seen that IPF had positive correlation with platelet count, hence when IPF was above the normal limit i.e. >8%, platelet count showed increasing trend.

Table 3: Correlation between Immature Platelet Fraction and Mean Platelet count after 24 hrs and 48 hrs.

		PLTD2 (cumm)	PLTD3 (cumm)
IPF	Platelet Count	54157.3±18317.21	71078.65±23390.56
	Correlation Coefficient	0.133	0.303
	P-Value	*P*<0.05	*P*<0.01

PLTD2: Mean Platelet Count at day 2
PLTD3: Mean Platelet Count at day 3

Discussion

Dengue fever is one of the major public health problems. One of the major concerns of dengue fever is thrombocytopenia. Both doctor and patients anxiously wait for an increase in the platelet count. It is often seen that many a times unnecessary platelet transfusion takes place exposing the patients to many adverse reactions related to transfusion. So there have been several studies going on and many have come up with the novel markers to predict the recovery of platelets.

The platelets activity and regeneration has been assessed in terms of their Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Plateletcrit (PCT) and Immature Platelet Fraction (IPF). Out of all the parameters IPF has shown promising results in diseases like ITP and after immunosuppressive therapy. The measurement of IPF is simple and non-invasive and provides rapid information about the bone marrow megakaryocyte activity and platelet life span. The present work has been extrapolated to study the platelet indices specially the role of IPF in dengue fever patients. There are various studies carried in South East Asian region and Mediterranean region on this parameter of immature platelet fraction and its correlation with platelet recovery. Also several studies are there regarding platelet indices such as Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Plateletcrit (PCT) [8].

In our study, a total of 106 patients of various age groups were included. The maximum number of patients were in the age group of 15-30 years (Table 4), which was similar to the studies by Ukey, et al., and Cecilia, et al., in which maximum cases were noted in 15-30 years & 21-30 years respectively [9,10].

In the present study, 71 patients (66.9% of the total population), were found to be NS1 positive alone. Rest of the 35 patients (33.1%), showed both NS1 and IgM positivity (Table 5). In the study conducted by Navya, et al., 2016, 90 patients out of 100, were NS1 positive [11]. This is in accordance with our study.

Haematological parameters like haemoglobin, Total Leucocyte Counts (TLC) and platelets at presentation and the

haematocrit were also studied (Table 6). We found that 84.9% patients were anaemic. In the study by Singh and Kothari, 2016; it was found that 71 patients (46%) were anemic and 79 patients (51%) had normal haemoglobin [12]. When total leukocyte count was considered, leucopenia was seen in 27.35% patients. Only 8.49% cases showed TLC within normal range (Table 6). In the study by Singh and Kothari., 2016, Leukopenia was present in 35.06% patients, which was comparable to our study, while leucocytosis was present in only 4.55% patients [12]. In our study, 64.15% patients showed leucocytosis. The reason for leucocytosis may be superimposed infection. Considering the platelets count, majority of our patients (104 cases; 98.11%) had their platelet counts less than 50000/cu.mm and only 2 cases (1.88%) presented with platelet counts above 50000/cu.mm (Table 6). The complex mechanism of thrombocytopenia remains controversial. Various mechanisms have been proposed like direct bone marrow suppression by the virus; anti-dengue antibody-mediated platelet destruction, peripheral consumption of platelets and isolated replication of virus in the platelet [13]. The release of high levels of platelet-activating factor may induce platelet consumption and enhance adhesiveness of endothelial cells resulting in thrombocytopenia [14]. Thrombocytopenia can also be due to increased peripheral destruction, inadequate production or abnormal pooling [15].

Out of total 106 patients, 45(42.45%) patients had their

Table 4: Age and sex distribution of patients.

S.No	Age (Years)	Male (n)	Female (n)
1	15-30	41	29
2	31-45	13	9
3	46-60	3	8
4	>60	2	1

Table 5: Dengue serology of patients.

S.No	Dengue Serology	No of Patients	Percentage %
1	Only NS1+ve	71	66.9
2	NS1+IgM+ve	35	33.1
3	IgM+ve and IgG+ve	0	0
4	NS1,IgM+ve and IgG+ve	0	0

Table 6: Haematological parameters.

Haematological Parameters	Groups	No. of Patients (n)	Percentage %
Haemoglobin (Hb)	Normal (male > 13g/dl), (female >12g/dl)	16	15.09
	Anemia (male<13gm/dl, female <12 gm/dl)	90	84.9
Total Leucocyte Count (TLC)	Normal (4000 11000/ cumm)	09	8.49
	Leucopenia (<4000/cumm)	29	27.35
	Leucocytosis (>11000/cumm)	68	64.15
Platelets	<50000/cumm	104	98.11
	>50000/cumm	02	1.88
Haematocrit (Hct)	Normal (36-56)%	61	57.54
	Low <36%	45	42.45
	Raised >56%	00	00

haematocrit below 36 and 61(57.54%) patients had their haematocrit within normal range and none of the patients in the study population had raised haematocrit (Table 6). The haematocrit is very important parameters in the management of dengue fever.

Immature Platelet Fraction (IPF) is an automated measure of reticulated platelets in peripheral blood. In our study, we observed that 90 (84.91%) patients had IPF more than 8% and only 16 (15.09%) patients had IPF below 8% (Table 2). When the IPF was correlated with the platelet counts on day 2 and day 3, a significant positive correlation was found (Table 3), similar to the study shown by Dadu, et al., 2014 [8]. The recovery criteria taken by us was in accordance with WHO, where a platelet count cut off value of 50000/cumm was taken for platelets recovery. Those patients whose platelet count reached more than 50000/cumm either on the second or third day of presentation were said to have recovered. These patients are haemodynamically stable and can be discharged from hospital [16].

In the present study (Table 2), we divided the platelets counts into 4 classes. Mean MPV, PDW and PCT values were taken for the different classes of platelet count. We can see from table 2, the value of mean MPV increases as the platelets count increases. This is in accordance with the study conducted by Bashir, et al., 2015, Anuradha, et al., 2014, Navya, et al., 2016 and Mukker, et al., 2018 [17,18,11,19] They also observed low value of mean platelet volume (MPV) with low platelet count. Navya, et al.,2016 in their study group of 100 patients, saw a mean MPV of <9 fl in 72% of patients. A mean MPV of <9 fl indicates bone marrow suppression. Thus postulating transient bone marrow suppression by dengue virus as one of the mechanisms of thrombocytopenia in patients of dengue fever [11]. Khandal, and Raghuraman., 2017 in their study also found that low MPV <9fl has strong correlation with low platelet count, similar to our study [20]. We observed the correlation coefficient (r) of 0.124 and the p value <0.05 (Table 2). This suggests a direct relationship of platelet count with mean MPV. Thus we can conclude that low MPV is associated with low platelet count and may be used as a probable indicator for dengue in endemic areas as also suggested by Bashir, et al., in 2015 [17]. A high MPV indicates increased megakaryocytic activity, whereas low MPV indicates marrow suppression and increased risk of bleeding. Thus MPV is surrogate marker of bone marrow activity. Correlation of platelet count and MPV with bleeding and severity of the disease can potentially help in predicting outcome.

In the present study, when PDW was correlated with platelet count at presentation (Table 2), a negative correlation between the mean PDW and the platelet count was found. The correlation coefficient was r-0.019, and the p value is <0.05. Thus the present study shows that low platelet count was associated with high PDW. This was in accordance with study by Navya, et al., 2016 and Mukker, et al., 2018 [11,19]. Similar results were also observed in a case control study by Bashir, et al., 2015 who found normal value of PDW in control group while it was increased in dengue infection. [17]. Navya, et al., 2016, in their study observed high PDW >13fl in 92% of cases [11]. PDW is a useful marker for platelet activation (Table 2).

When PCT was correlated with platelet count at presentation (Table 2). correlation coefficient value (r) 0.238 and the p value <0.05 were found. Thus it showed a positive correlation between the PCT and the low platelet count. In another study conducted by Hardeva, et al., in 2016, it was found that plateletcrit was on the lower side of the normal range in dengue fever patients [22]. We also observed that low platelet count was associated with low plateletcrit (Table 2). Low value of plateletcrit may be used as probable indicator for dengue in endemic area [21].

Conclusion

The following conclusion were drawn from the present study:

- The Mean Platelet Volume and Plateletcrit has positive correlation with low platelet count. Low MPV (<9fl) with low platelet count implies marrow suppression as a mechanism of thrombocytopenia. Whereas Platelet Distribution Width has a negative correlation with low platelet count. High value of Platelet Distribution Width (>13%) is seen in patients with low platelet count.

- The Immature Platelet Fraction (IPF) can be used as predictive marker for platelet recovery. The platelet count tends to recover within 24 to 48 hours in dengue patient if the value of Immature Platelet Fraction (IPF) is more than 8%.

Limitations

We did not evaluate the parameters sequentially and also patients with major bleeding manifestations were excluded. Therefore, there is need for further studies which include more subjects and varied manifestations of dengue fever.

References

1. Mackenzie JS, Gubler DJ, Petersen LR (2004) Emerging flaviviruses: the spread and resurgence of Japanese encephalitis. West Nile and dengue viruses. Nat Med 10: S98-S109. **Link:** http://bit.ly/36iCjZV

2. Chuang YC, Lin YS, Liu CC, Liu HS, Liao SH, et al. (2013) Factors contributing to the disturbance of coagulation and fibrinolysis in dengue virus infection. J Formos Med Assoc 112: 12-17. **Link:** http://bit.ly/38wLWWn

3. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, et al. (2010) Dengue: a continuing global thread. Nat Rev Microbiol 8: S7-S16. **Link:** http://bit.ly/2GiJEhD

4. Vagdatli E, Gounari E, Lazaridou E, Katsibourlia E, Tsikopoulou F, et al. (2010) Platelet distribution width: a simple, practical and specific marker of activation of coagulation. Hippokratia 14: 28-32. **Link:** http://bit.ly/3aBGJ1q

5. Babu E, Basu D (2004) Platelet large cell ratio in the differential diagnosis of abnormal platelet counts. Indian J Pathol Microbiol 47: 202-205. **Link:** http://bit.ly/2RhSl22

6. Briggs C, Hart D, Kunka S, Oguni S, Machin S (2006) Immature platelet fraction measurement: a future guide to platelet transfusion requirement after haematopoietic stem cell transplantation. Transfus Med 16: 101-109. **Link:** http://bit.ly/38BRQ8R

7. Briggs C (2010) Quality counts: new parameters in blood cell counting. Int J Lab Hematol 31: 277-297. **Link:** http://bit.ly/38vazCS

8. Dadu T, Sehgal K, Joshi M, Khodaiji S (2014) Evaluation of the immature platelet fraction as an indicator of platelet recovery in dengue patients. Int J Lab Hem 36: 499-504. **Link:** http://bit.ly/37wC9PS

9. Ukey PM, Bondade SA, Paunipagar PV, Powar RM, Akulwar SL (2010) Study of Seroprevalence of Dengue Fever in Central India. Indian J Community Med 35: 517-519. **Link:** http://bit.ly/2RhNGNw

10. Cecilia D (2014) Current status of dengue and chikungunya in India. WHO South-East Asia. Public Health 3: 22-26. **Link:** http://bit.ly/2RHfZnF

11. Navya BN, Patil S, Kariappa TM (2016) Role of platelet parameters in dengue positive cases - an observational study. Int J Health Sci Res 6: 74-78. **Link:** http://bit.ly/38yMf36

12. Singh O, Kothari DC (2016) Haematological Profile of Dengue Viral Infection-Study of Recent Epidemic-2013 in North West Rajasthan. IOSR Journal of Dental and Medical Sciences 15: 20-23. **Link:** http://bit.ly/2Rhx3BB

13. Gupta E, Dar L, Kapoor G, Broor S (2006) The changing epidemiology of dengue in Delhi, India. Virol J 3: 92. **Link:** http://bit.ly/2viEK21

14. Dongre T, Karmarkar P (2015) Hematological Parameters and Its Utility in Dengue-A Prospective Study. JDMS 14: 31-34. **Link:** http://bit.ly/37mpkYn

15. Reddy SR, Khan IM, Phansalkar DM (2015) Platelet Distribution Width (PDW) in Thrombocytopenia. Indian Medical Gazette 169-174. **Link:** http://bit.ly/2NPU6kE

16. (2009) Dengue Guidelines for diagnosis, treatment, prevention and control. 2nd Edition, World Health Organization. Geneva 1-144. **Link:** http://bit.ly/2Rhy8cD

17. Bashir AB, Saeed OK, Mohammed BA, Ageep AK (2015) Role of Platelet Indices in Patients with Dengue Infection in Red Sea State, Sudan. IJSR 4: l573-1536. **Link:** http://bit.ly/2RJm4Qd

18. Anuradha M, Dandekar RH (2014) Screening and manifestations of seropositive dengue fever patients in perambalur: a hospital based study. International J Med Sci Pub Health 3: 745-748. **Link:** http://bit.ly/37mvLuB

19. Mukker P, Kiran S (2018) Platelet indices evaluation in patients with dengue fever. Int J Res Med Sci 6: 2054-2059. **Link:** http://bit.ly/2RkTTsb

20. Khandal A, Raghuraman D (2017) Rising Mean Platelet Volume (MPV) Heralding Platelets Recovery in Dengue?. American Journal of Clinical Medicine Research 5: 59-63. **Link:** https://go.aws/2GfzhuP

21. Hardeva RN, Shyam LM, Sahil P (2016) Evaluation of platelet indices in patients with dengue infection. Int J Sci Res 5: 78-81.