ORIGINAL ARTICLE

Study of Complete Blood Count (CBC) Indices and Immature Platelet Factors (IPF) in Predicting Severe Dengue and Their Role in Deciding Platelet Transfusion

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Abstract:

Background: The incidence of dengue in the world is rising rapidly. With a remarkable seroprevalence rate of 48.7 % in India, it is become an important infectious health concern now. Since supportive treatment and platelet transfusions are the crux of treatment, simple methods to detect trend of thrombocytopenia is needed which are easily available, accessible and reliable and can help in clinical decision making. Complete blood count with platelet indices and hematological parameters is a tool which can be explored in this regard. With this aim this study was undertaken. *Material and Method:* This is a retrospective observational cross-sectional study of 100 patients which were admitted in our institute in Solapur, Maharashtra. Complete blood count (CBC) along with routine parameters like liver function tests and renal function tests were collected. CBC markers were compiled and analysed by using statistical software SPSS v 20.0. Comparison between platelet counts was in three groups of mild, moderate and severe ($>1,00,000$, $21000-1,00,000$ and $< 20,000$) was done by ANOVA test and comparison with other indices was done using Pearson test. *Results:* Hemoglobin (15.18 ± 1.87 vs. 13.05 ± 1.61, $p < 0.001$) with slightly raised hematocrit, plateletcrit (0.02 ± 0.04 vs. 0.15 ± 0.04, $p < 0.0001$), MPV (9.91 ± 1.11 vs 9.82 ± 1.17, p value < 0.001) and IPF (15.60 ± 7.05 vs 6.94 ± 4.92 were significantly altered in dengue patients with platelets below 20,000 compared to platelets above 1,00,000.Conclusions: Platelet indices and IPF are useful parameters in dengue fever. Platelet counts, Pct, MPV, IPF, PDW and P-LCR are useful in monitoring trends in dengue fever and assessing severity so that clinical decisions for treatment and platelet transfusions can be taken.

Keywords: IPF-immature platelet factor, P-LCR- platelet large cell ratio, PCT-plateletcrit, MPV- mean platelet volume, HCT- hematocrit

Introduction:

Dengue fever is a mosquito borne viral infection found mostly in urban and semi-urban areas. The dengue virus is an RNA virus of Flaviviraedae family and is responsible for its causation. It has four serotypes. Infection can occur with any of the four serotypes and multiple sequential infections can occur. It is transmitted by female mosquito of species Aedes Aegypti and Aedes Albopictus. It is rapidly spreading all over the globe with more than 100 countries already being endemic for dengue1. The global incidence has dramatically increased in last two decades. There are an estimated 100-400 million infections each year. The word dengue is derived from the Swahili phrase ka-dinga pepo, meaning cramp like seizure. In India the first case of dengue was reported in Chennai in 1780 and the first virologically proven outbreak in Kolkata in 1963. Dengue has spread rapidly all across our country and has become endemic2. The overall seroprevalence of dengue infection in India was 48.7% (95% CI 43.5-54.0); increasing from 28.3% (21.5-36.2) among children aged 5-8 years to 41% (32.4-50.1) among children aged 9-17 years and to 56.2% (49.0-63.1) among individuals aged 18-45 years. The highest seroprevalence was in southern states (76.9%), followed by western (62.3%), and northern (60.3%) regions. Seroprevalence was greater in cities (70.9 %) than in rural regions (42.3 %)3,4. In a logistic regression model, the following factors were shown to be independently associated with confirmed dengue infection: retro-orbital ache, rash, platelet count < 2,40,000 cells/cumm, no sore throat, and no cough5. In India dengue surge is constantly increasing due to factors like inadequate financial and personnel resources, a lack of point-of-care diagnostics, and inadequate mosquito control methods. Also, India's urbanisation is proceeding at a dazzling speed and hence it becomes more important tocontrol spread of dengue under these circumstances. Although most dengue episodes are moderate, severe dengue carries a substantial risk of mortality if not treated properly. Hospital records in India show that dengue deaths have higher costs than severe hospitalised non-fatal dengue, and up to 90% expenditure may be out of

pocket, giving huge economic burden to the society. Dengue fever's direct and indirect expenses in India currently exceed three billion dollars6. Due to significant risk of bleeding and severe thrombocytopenia, Dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) are reversible vascular complications of dengue fever. To study the severity of the disease platelet counts are used. Thrombocytopenia can be caused by both bone marrow suppression and platelet breakdown. The most significant mechanism leading to thrombocytopenia in dengue infection is probably immune complex-mediated platelet degradation. There has been lot of research studies were increased on the role of platelet indices namely plateletcrit (PCT), mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR) due to the advent of automated hematology analyzers. The average size of platelets in the blood is represented by mean platelet volume (MPV). It ranges between 7.2 -11.7 fL. MPV of over normal size occurs in hyper destruction, when immature platelets become bigger in size and activity.MPV below normal value is a sign of platelet hypoproduction. Platelet counts are negatively linked to MPV. The various factors like age, smoking, alcohol can modify MPV. Platelet distribution width (PDW) is a prognostic factor for platelet anisocytosis. Reference levels range is between 8.3 – 56.6 %. It is the size distribution produced by megakaryocytes and increases upon platelet activation. It seems to be proportionally related MPV in healthy adults7. Plateletcrit (PCT) calculates total platelet mass as a percentage of blood volume occupied. The normal range is between 0.22 - 0.24 %. It is non-linearly correlated to the platelet count.Platelet larger cell ratio (P-LCR) is a percentage of all platelets with volume measuring over 12 fL circulating in the blood stream. It normally ranges between 15-35 %. It has a direct relation to MPV and PDW and inverse relation to platelet count. In comparison to MPV, P-LCR appears to be more responsive to changes in platelet size. Immature platelet fraction (IPF) indicates the percentage of immature platelets containing higher concentration of RNA released into the circulation. It ranges between 1.1-6.1 % in normal healthy individuals. Immature platelets are promptly discharged into the peripheral bloodstream, where they mature in about 24 hours. Their presence in the blood is an indirect sign of bone marrow function. It indicates platelet formation and precedes recovery by 3 days. Prophylactic platelet infusions are becoming more common in dengue-endemic nations, however due to the accompanying clinical hazards and financial expenses, controlled trials

must be conducted before this becomes standard of treatment. Improving early identification and risk prediction of severe disease is critical, particularly in places with a high case load, where appropriate allocation of limited resources is critical to the result. The present study was undertaken to assess the role of red cell indices and platelet indices alteration in dengue patients with thrombocytopenia and to identify these markers as predictive markers for thrombocytopenia or recovery.

Material and Methods:

A retrospective observational cross-section study of 100 admitted patients was conducted between June 2019 to October 2021 with clinical features of dengue fever who were serologically positive (NS1 antigen, ELISA /IgM antibody to dengue virus) in a tertiary care hospital in Solapur. Patients with a dengue positive serology to Non-Structural protein1 or IgM, Platelet count < 1.5 lakh/cumm were included in the study. The exclusion Criteria was IgG positive cases, Blood and coagulation Disorders affecting platelets like Idiopathic Thrombocytopenic purpura, Thrombotic Thrombocytopenic Purpura, DIC, Haematological malignancy which were excluded with appropriate positive investigations, Drug induced patients, Patients receiving platelet transfusion 5 days prior or during the study and Chronic diseases of liver, kidney. CBC samples were collected on day one, three and five for trends of platelets and also once or twice daily in cases of severe thrombocytopenia. Thrombocytopenia was divided as mild if platelets were > 100,000, moderate if between 20,000-100,000 and severe if less than 20,000.13,14,19 Blood samples were collected in EDTA-anticoagulated bulbs and samples were run within time to prevent artefactual change in platelet size on Sysmex XN 550, 5 part and Nihon Kohden celltac G, MEK-9100, 5 part. Low platelet counts were also confirmed on smears. Other baseline investigations like blood sugar, serum electrolytes, serum creatinine, liver function tests, electrocardiogram, HIV and HBsAg were done. Special tests like 2D-Echo and USG abdomen were done if further required. Descriptive statistics such as mean, standard deviation (SD) percentage/proportions was used to present the data. Pearson 'r' correlation coefficient was used to find correlation between platelet count with various other platelet indices and hematological parameters. Comparison of platelet count between groups was performed by ANOVA test. A p-value less than 0.05 were considered as significant. Data analysis was performed by using statistical software SPSS v 20.0.

Results:

In the present study, total of hundred patients were included. Of these 78 (78%) were males and 22 (22%) were females. Age related demographics showed 16 (16%) patients below the age of 20, 42 (42%) patients between age group 21-30, 23 (23%) patients between age group of 31-40, 12 (12%) patients between age group 41-50 and 7 (7%) patients between age group 51-60. The high male percentage could be due to their increased movement outdoors and more exposure whereas females are mostly indoors and well covered as well. Similarly total of 65 % of patients were between age group of 21-40 reflecting their active life style. Table 1 shows mean deviations of hematological and platelet parameters. The mean Hb was

Table 1 : Descriptive values

Parameters	Min.	Max.	Mean	Std. Deviation
HB (g/dl)	8.20	18.80	14.22	1.93
WBC (10^9/iL)	0.85	18.77	4.82	2.99
PLT (10^9/iL)	9.00	276.00	60.49	56.29
HCT (%)	23.10	53.70	42.05	5.72
PDW	6.80	23.80	14.23	3.42
MPV (fL)	8.00	13.60	10.40	1.27
PCT (ng/mL)	.01	.24	.0631	.05
PLCR (%)	10.20	80.60	35.89	10.71
IPF (%)	1.60	32.70	12.48	6.88

14.22 (SD=1.93), mean WBC count was 4.82 (SD =2.99), mean haematocrit was 42.05 (SD =5.72), average platelets were 60.49 (SD =56.29), mean PDW was 14.23(SD =3.42), mean MPV was 10.40 (SD =1.27), mean PCT was 0.06 (SD =0.05), mean P-LCR was 35.89 (SD =10.71) and mean IPF was 12.48 (SD = 6.88).

Table 2: Platelet count distribution

Platelet count	Number	Percentage
<20,000	21	21.0
20,000-100,000	58	58.0
>100,000	21	21.0
Total	100	100.0

Table 2 shows the platelet count distribution which is < 20,000 in 21 patients (21%), between 21,000 to 1,00,000 in 58 patients (58%) and > 1 lakh in 21 patients (21%). Table 3 showsplateletcrit (PCT) which was < 0.22 in 98 patients (98%) patients and between 0.22-0.24 in 2 patients (2%).

Table 3: Plateletcrit (PCT)

PCT	Number	Percentage
<0.22	98	98.0
0.22 - 0.24	2	2.0
Total	100	100.0

Table 4: P-LCR

PLCR	Number	Percentage
< 15	6	6.0
15 – 30	25	25.0
> 30	69	69.0
Total	100	100.0

Table 4 shows platelet large cell ratio (P-LCR) which was < 15 in 6 patients (6%), 15-30 in 25 patients (25%) and > 30 in 69 patients (69%). As shown in table 5 and 6, when various parameters were compared in different platelet group patients, a significant correlation was observedbetween Hb (p = 0.001), MPV (p = 0.001), PCT

Table 5 : Comparison of platelet parameters between groups

	PLT			p-value
	<20,000	20,000-100,000	>100,000	
HB	15.18 ± 1.87	14.30 ± 1.87	13.05 ± 1.61	0.001
WBC	5.03 ± 3.67	4.83 ± 2.74	4.60 ± 3.08	0.9
HCT	44.87 ± 5.95	42 ± 5.49	39.37 ± 5.03	0.01
PDW	13.36 ± 4.0	14.77 ± 3.22	13.63 ± 3.22	0.18
MPV	9.91 ± 1.11	10.79 ± 1.23	9.82 ± 1.17	0.001
PCT	0.02 ± 0.04	0.05 ± 0.02	0.15 ± 0.04	<0.0001
PLCR	32.63 ± 11.06	38.31 ± 10.25	32.51 ± 10.33	0.03
IPF	15.60 ± 7.05	13.37 ± 6.37	6.94 ± 4.92	<0.0001

(<0.0001) and IPF (< 0.0001). r values of Hb , MPV, and IPF show negative co-relation, r values (-0.36,-0.19, -0.54 respectively) whereas PCT shows a direct positive co-relation, r value (0.93). Significant negative hemoglobin co-relation may be due to severe hemoconcentration in severe dengue patients with platelets < 20,000. Values of WBC, HCT, PDW and P-LCR did not show any significant co-relation though WBCs showed a trend towards leucopenia and HCT, PDW and P-LCR showed higher side values in our study. WBC had a weak positive co-relation, r value (0.17), whereas HCT, PDW and P-LCR showed r values (-0.23, -0.11, -0.17 respectively) with non-significant p values.

Table 6 : Platelet count correlation with other parameters

Parameters	r-value	p-value
HB	-0.361	<0.0001
WBC	0.017	0.86
HCT	-0.293	0.003
PDW	-0.112	0.267
MPV	-0.194	0.05
PCT	0.931	<0.0001
PLCR	-0.172	0.087
IPF	-0.544	<0.0001

Disscussion:

There are three phases based on clinical viz. febrile period; critical phase, and recovery phase in dengue fever. It is during the critical phase where hemorrhagic manifestations are most common. Also moderate to severe thrombocytopenia is common during this phase. This lasts between days 4 and 7 of illness [8]. Many practitioners prefer transfusion of prophylactic platelets due to unpredictability of symptoms and also due to lack of clear guidelines to act in such circumstances, but there was lack of information to support this. On the contrary platelet count was shown to be associated with slower recovery with the group receiving transfusion. As a result, the length of stay increases. The transient increase in platelet count resulting from transfusion causes reduction in thrombopoietin levels which is stimulated by low platelet counts. Further few patients may have life threatening transfusion reactions[9].

Our study had high levels of hemoglobin with decreasing platelets showing negative r value (- 0.36) co-relation with p < 0.0001. This is probably due to concurrent capillary leakage in severe cases with hemoconcentration. Similar were findings with study by Dr Indumathi S et al [10].Mean platelet volume in our study had a mean value of 9.82 fL and weak negative co-relation, r value (-0.19) and p value

of 0.001 to 0.05 which is concurrent to findings in study by Nabila Afsar et al [11] and Anupama Brahma et al[12] and Rashmi Passi et al [13]. Plateletcrit showed significant positive and strong co-relation, r value (0.93) with p < 0.0001 which is similar to findings in study of P Mukker et al [14] and Vijay Kumar Meena et al [15].Immature platelet fraction (IPF) showed a strong negative co-relation, r value (- 0.54) and p < 0.0001 which is in accordance with a study by Looi et al[16] who found that IPF is higher in patients with severe dengue and could be an early recovery indicator.

A high PDW and P-LCR with a weak negative co-relation, r-value (- 0.11 and -0.17 respectively) with not significant p value was found in our study. The trends matched with those found with Wayez et al [17] and Navya et al [18].The strongest co-relation in our study group was found positively between low platelets and PCT and negatively between low platelets and IPF. It is understood from previous literature as well as from our findings that, there is a definite shift in parameters of CBC in dengue fever. Furthermore, these findings were also having chances to get altered due to lab methods. Also, at the time of admission patients are in various phases of dengue illness and prior treatment and drugs could have played a role which cannot be ascertained by history alone. Certain other factors e.g., platelet adhesion and complement activation and transient disruption in the function of the endothelial glycocalyx layer may play a role in platelet destruction and leaky capillaries. These platelet indices may just reflect the change in underlying pathogenesis. Further, these parameters may show different sensitivity and specificity to various types of dengue (D, DHF, DSS) as mentioned by Tau Hong Lee et al.[9]Nevertheless, these indices can play a vital role in knowing the severity of dengue and one can be prepared for its consequences and platelet transfusions in advance with their help.

Conclusion:

We conclude that low PCT, low MPV, low platelets, high PDW, high P-LCR are found in patients with moderate to severe dengue. High IPF with low platelets indicate active platelet production and possible rise in platelets over next 2-3 days.

Conflicts of interest: Nil
Sources of Support: Nil

References:

1. WHO Dengue and severe dengue. https://www.who.int/news-room/fact sheets/detail/dengue-and-severe-dengue Accessed on 05-04-2022.

2. Nivedita Gupta, Sakshi Srivastava, Amita Jain, Umesh C. Chaturvedi. Dengue in India. *Indian Journal of Medical Research* 2012 Sep; 136 (3): 373-390.

3. Annelies Wilder-Smith. Estimating the dengue burden in India. *The Lancet* 2019;7(8):E988-E989.

4. Murhekar MV et al. Burden of dengue infection in India - a cross sectional population based serosurvey. *The Lancet* 2019;7 (8):E1065-E1073.

5. Wilson ME, Chen LH. Dengue: update on epidemiology. *Current Infectious Disease Reports* 2015;17:1-8.

6. Shet A, Kang G. Dengue in India: towards a better understanding of priorities and progress. *International Journal of Infectious Diseases* 2019 ;84:S1-3.

7. Pogorzelska K, Krętowska A, Krawczuk-Rybak M, Sawicka-Żukowska M. Characteristics of platelet indices and their prognostic significance in selected medical condition–a systematic review. *Advances in Medical Sciences* 2020;65(2):310-315.

8. Cameron P. Simmons, Jeremy J. Farrar, Nguyen van Vinh Chau, Bridget Wills. Dengue. *New England Journal of Medicine* 2012;366:1423-1432.

9. Lee TH, Wong JG, Leo YS, Thein TL, Ng EL, Lee LK, Lye DC. Potential harm of prophylactic platelet transfusion in adult dengue patients. *PLoS Neglected Tropical Diseases* 2016;10(3):e0004576.

10. Indumathi S, Anoosha K, Lakshmi V. Assessment of Platelet Indices as Prognostic Markers in Seropositive Cases of Dengue. *Journal of Medical Science and Clinical Research* 2019;7(9):322-326.

11. Afsar N, Afroze IA, Humaira S, Abid Z. Use of mean platelet volume as an initial diagnostic marker in evaluation of dengue fever. *Annals Pathoogy and Laboratory Medicine* 2017;4(3):A310-313.

12. Brahma A, Ghosh PK, Goswami U, Majumdar S. Utility of mean platelet volume to predict significant thrombocytopenia and complications in dengue. *International Journal of Tropical Diseases* 2019;2(026):1-5.

13. Rashim Passi, B. P.Baviskar, R.R.Karle. Role of platelet parameters and hematological profile in dengue positive cases. *International Journal of Scientific Reports* 2022; 8(1):23-28.

14. Mukker P, Kiran S. Platelet indices evaluation in patients with dengue fever. *International Journal of Research in Medical Sciences* 2018;6(6):2054.

15. Meena VK, Bihari S, Meena SR. Diagnostic significance of platelet indices in dengue fever in endemic area. *International Journal of Research Review* 2020;7(2):315-319.

16. Looi KW, Matsui Y, Kono M, Samudi C, Kojima N, Ong JX, Tan CA, Ang CS, Tan PH, Shamnugam H, Sekaran SD. Evaluation of immature platelet fraction as a marker of dengue fever progression. *International Journal of Infectious Diseases* 2021 ;110:187- 194.

17. Ahmad W, Lubna Z, Mohsin A, Nishat A. Study of platelet indices in dengue fever with thrombocytopenia and correlation of immature platelet fraction (IPF) with platelet recovery. *Archieves of Hematological Case Reports Review* 2020;5(1):1-5.

18. Navya BN, Patil S, Kariappa TM. Role of platelet parameters in dengue positive cases-an observational study. *International Journal of Health Science Research* 2016;6(6):74-78.

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