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Original Research Article

Immature platelet fraction in children infected with dengue fever

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ABSTRACT

Background: Millions are infected with dengue every year. Early diagnosis of dengue infection is important for proper treatment of DHF and DSS to avoid fatal outcome. Thrombocytopenia is a common hematological abnormality in dengue, which demands platelet transfusion in most of the severe dengue cases. Platelet transfusion though life-saving has its own hazards. Hence, we can use some new parameter like immature platelet fraction (IPF) which is a measure of reticulated platelets that reflects the rate of thrombopoiesis. The risk of platelet transfusion may be decreased by rapid identification of immature platelet fraction. This study was performed to establish reference of IPF values for the assessment of thrombopoiesis.
Methods: Blood samples from 150 children were obtained on day of illness 3, 5 and 7. The IPF is identified by sysmex XE2100 hematology analyser in the reticulocyte channel using a fluorescent dye and a carefully designed gating system and counted by a special software termed IPF master7. IPF values against platelet count were assessed separately on day 3, 5 and 7.
Results: The reference intervals of IPF > 8 % and IPF < 8 % were assessed against platelet count. Increase in IPF favored increase in platelet count on day 5 which was statistically significant with the p value <0.001.
Conclusions: A rapid and inexpensive automated measurement of IPF can be integrated as a standard parameter to evaluate the thrombopoietic state of the bone marrow. From the study it can be concluded that IPF is an important predictor of increase in platelet count. Increase in IPF>8 % suggests that platelet count will be increased in next 24 to 48hrs indicating that further blood transfusion will not be required.

Keywords: Blood transfusion, Dengue hemorrhagic fever (DHF), Dengue shock syndrome (DSS), Immature platelet fraction (IPF), Platelet count

INTRODUCTION

Dengue virus (DENV) infection is one of the mosquito-borne viral diseases with a major impact on public health, globally.[1] World Health Organization (WHO) data suggest that at least 100 countries are endemic of dengue viral transmission. DENV produce a spectrum of clinical illnesses ranging from a classical dengue fever (DF) to cruel and potentially fatal complications known as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[2] The average case fatal outcome rate is around 5%and mainly among children and young adults.[3] Early diagnosis of DENV infection is important for proper treatment of DHF and DSS to avoid these fatal outcomes. Several dengue vaccines are in an advanced stage of development such as Sanofi Pasteur's ChimeriVax-DENV vaccine previously entered phase 3 clinical testing.[4-6]

Dengue is transmitted by the infective bite of Aedes aegypti mosquito. A persondevelops disease after 5-6 days of being bitten by an infective mosquito. It occurs in two forms: Dengue Fever and (DHF).[7] The classical clinical presentation is characterized by the unexpected onset of headache, myalgia and high fever, retro-orbital pain and hemorrhagic manifestations. Unlike the classical

presentation dengue hemorrhagic fever is characterized by fluid leakage into the interstitium.[8]

Dengue virus belongs to the genus Flavivirus (group B arbovirus, RNA virus) and comprises structural and nonstructural proteins.[9] DENV infection can be diagnosed and confirmed by NS1 antigen positive, IgM antibody positive, viral nucleic acid by PCR and isolation of dengue virus in serum, plasma or leukocyte.[10]

Recent report by the ministry of health, in India there were 45,490 dengue cases in 2016 out of which 4,436 were in Karnataka alone, its clear evidence that the incidence rate and death rate has been increasing every year.[7] The lack of scientific basis for the proper supervision of cases with hemorrhagic symptom, especially regarding blood transfusion procedures are contributing to the high death rate.

Thrombocytopenia is a common hematological abnormality in dengue, which demands platelet transfusion in most of the severe dengue cases. Platelet transfusion though life-saving has its own hazards.[11] Hence, we can use some new parameter like Immature platelet fraction (IPF) which is a measure of reticulated platelets that reflects the rate of thrombopoiesis.[12] Reticulated platelets contain RNA and are newly released platelets that are larger, more physiologically active and are the analogue of the red cell reticulocyte.[13]

The IPF% could predict the timing of platelet recovery. In general, the platelet recovery time is 1-2 days of IPF increase.[14,15] The cut off value above which platelet recovery is expected is yet to be scientifically validated.

The IPF can be identified by Sysmex XE2100 hematology analyzer in the reticulocyte channel using a fluorescent dye and a carefully designed gating system and counted by a special software termed IPF master.[7]

Immature platelets fraction (IPF%) is raised in diseases where there is increased platelet destruction and conditions such as Idiopathic Thrombocytopenic Purpura (ITP) Aplastic anemia (AA) and is case of marrow failure.[13]

Thrombocytopenia in critically ill dengue patients, warrants for platelet transfusion.

However, unnecessary transfusions are best avoided due to heightened risk from alloimmunization, immunosuppression, transmission of infectious diseases and graft versus host disease.

METHODS

Patient inclusion criteria

Hospital-based prospective study was conducted for a period of 18 months (January 2017 to August 2018). All children aged up to 18 years admitted under paediatrics at Rajarajeswari medical college and hospital who were infected with dengue with platelet count less than or equal to 50,000 were assessed. Detailed history was elicited to rule out patient with chronic infection or mixed infection, patients diagnosed with hematological disorder such as idiopathic thrombocytopenic purpura and aplastic anemia. The study was carried out with the informed consent signed by parents.

Patient blood sampling

Peripheral blood samples were collected from the patients in Department of Paediatrics, Rajarajeswari medical college and hospital. The veins in the antecubital fossa or dorsum of the hand were identified and a tourniquet applied to make the veins visible. The area was then cleansed with an alcohol swab and allowed to air dry, 3-5mL of blood was drawn from each febrile patient using a sterile needle and stored in EDTA plasma aliquoted tube and kept at -70 ºC.

All samples were analysed within 4 hours ofcollection. IPF measurement was carried out using Sysmex XE-2100 (Sysmex, Kobe, Japan).

Statistical analysis

Data was analyzed using SPSS 22 version software(IBM SPSS Statistics, Somers NY, USA).

Categorical data was represented in the form of Frequencies and proportions. Chi-square test or Fischer's exact test (for 2x2 tables only) was used as test of significance for qualitative data. Continuous data was represented as mean and standard deviation.

Pearson correlation was done to find the correlation between two quantitative variables and qualitative variables respectively.[16-19] p value of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

RESULTS

A total number of 150 patients were included in the study and we further classified our data based on dengue serotype, by day of illness admitted and relationship of IPF with Platelet count on Day 3, 5 and 7.

Population distribution based on dengue serotypes

Three dengue serotypes including IgM positive, NS1 positive and both IgM and NS1 positive cases were considered for the present study as dengue affected populations.

The results of dengue serotypes are shown in Table 1. In the study 40 cases with 26.7% were IgM positive, 50 cases with 33.3% were NS1 antigen positive and 60 cases

with 40% were NS1 antigen and IgM positive. This shows the predominant cases were found to be detected by both tests positive.

Table 1: Dengue serology among subjects.

Dengue serology	Number of patients	Patient in %
IgM +ve	40	26.7
NS1AG & IgM +ve	60	40.0
NS1AG +ve	50	33.3
Total	150	100.0

Population distribution by the day of illness

Day of illness characteristics of the study population are depicted in Table 2. 29 (19.3 %) children were admitted on 3rd day of illness. 73 (48.7 %) children were admitted on 5th day of illness and 48 (32 %) children were admitted on 7th day of illness.

Table 2: Day of illness among subjects.

Days of illness	Number of patients	Patient in %
3rd	29	19.3
5th	73	48.7
7th	48	32.0
Total	150	

Relationship of IPF with Platelet count Day 3

Pearson correlation was performed based on the IPF% with platelet count on day 3 and depicted in Table 3.

Table 3: Correlation between IPF on day 3 with platelet count on day 3.

		IPF day 3	Platelet count on day 3
IPF Day 3	Pearson correlation	1	0.333
	P value		0.152
	N	20	20

In the study there was a positive correlation between IPF with platelet count on Day 3. Briefly, as the increases in IPF there was an increase in platelet count and with decrease in IPF there was a decrease in platelet count.However the correlation was not that statistically significant (Figure 1).

Relationship of IPF with platelet count Day 5

Pearson correlation was performed based on the IPF% with platelet count on day 5 and depicted in Table 4. In the study there was a positive correlation between IPF with platelet count on Day 5. Briefly, as the increases in IPF there was also an increase in platelet count and with decrease in IPF there was a decrease in platelet count. The correlation was statistically highly significant with the p value <0.001 (Figure 2).

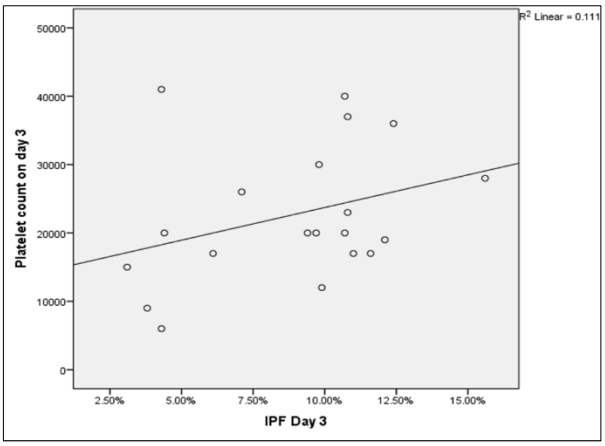

Figure 1: Scatter plot showing correlation between IPF with platelet count on day 3.

Table 4: Correlation between IPF on day 5 with platelet count on day 5.

		IPF day 5	Platelet count on day 5
IPF day 5	Pearson Correlation	1	0.408**
	P value		<0.001*
	N	84	83

**Correlation is significant at the 0.01 level (2-tailed).

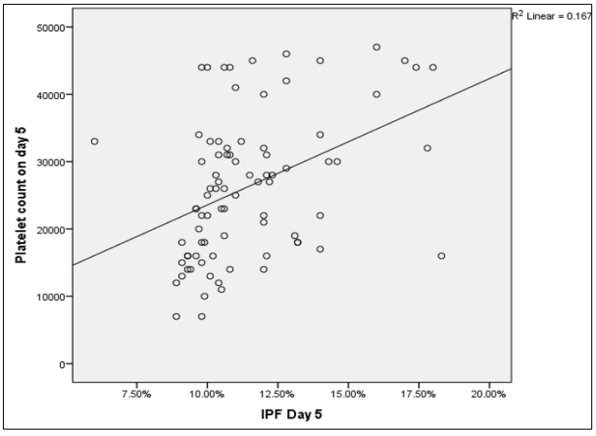

Figure 2: Scatter plot showing correlation between IPF with platelet count on day 5.

Relationship of IPF with Platelet count Day 7

Pearson correlation was performed based on the IPF% with platelet count on day 7 and depicted in Table 5. In the study there was a positive correlation between IPF with platelet count on Day 7. Briefly, as the increases in IPF there was also an increase in platelet count and with

decrease in IPF there was a decrease in platelet count. The correlation was not that statistically significant with p value of 0.07. (Figure 3).

Table 5: Correlation between IPF on day 7 with platelet count on day 7.

		IPF Day 5	Platelet count on day 7
IPF Day 7	Pearson Correlation	1	0.365**
	P value		0.07*
	N	54	54

**. Correlation is significant at the 0.01 level (2-tailed)

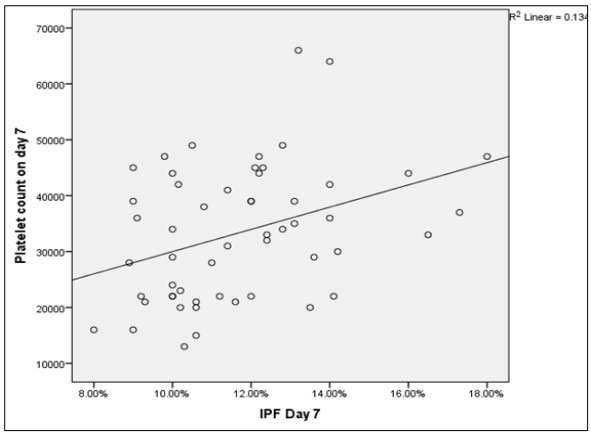

Figure 3: Scatter plot showing correlation between IPF with platelet count on day 7.

DISCUSSION

The study demonstrated that the IPF% value predicts the timing of platelet recovery following dengue infection. Treatment of dengue remains compassionate in the absence of targeted antiviral treatment or approved vaccines.

A major component of dengue is a result of immune-pathology. In the process of attempting to eradicate dengue virus, changes in physiology occur, result in a good outcome after a period of acute disease, but in cases where the host physiology is not robust enough to withstand the change (e.g., in children or older adults), and may results in permanent damage.[20]

Prophylactic platelet transfusions are given in dengue fever with thrombocytopenia to prevent hemorrhagic complications. There is lack of evidence-based guidelines for transfusion support in patients with dengue fever. This contributes to inappropriate use of blood components and blood centers constantly face the challenge of inventory management during dengue outbreaks. Platelet transfusion though life-saving it has its own hazards. Hence, there is a necessity to use some new marker like Immature Platelet Fraction (IPF) which is a measure of reticulated platelets that reflects the rate of thrombopoiesis. The risk of platelet transfusion may be decreased by rapid identification of Immature Platelet Fraction. In present study on day 5, there was a positive correlation between IPF with platelet count, as the increases in IPF there was also an increase in platelet count. The correlation is statistically significant with the p value <0.001. Day 7 also is favoring the positive correlation between IPF with platelet count. Our results were very similar to that of the previous studies, where they had also found a significant rise in the mean IPF after 12 hours of illness.[21]

Many studies have been carried out to find the value of IPF to find platelet recovery on patients receiving chemotherapy.[22] However, to the best of our knowledge, there is very limited studies carried out in dengue affected children. Hence IPF shows great promise of becoming a reliable future guide for decisions concerning platelet transfusions. Finding in our present study shows that there is a strong positive correlation between IPF and platelet count. As a result, IPF could be measured as regular practice to assess the thrombopoiesis in children with dengue.

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