Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

PREDICTIVE VALUE OF IMMATURE PLATELET FRACTION IN PATIENTS WITH DENGUE FEVER AND IT'S OUTCOME

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Abstract

Background: Thrombocytopenia is a common complication in many disorders (such as aplastic anemia, ITP, dengue fever), the etiology being multifactorial. Immature platelet fraction (IPF) is a new parameter which is a measure of reticulated platelets that reflects the rate of thrombopoiesis. Here we tried to evaluate IPF as an indicator to predict the recovery of platelets in patients with dengue having thrombocytopenia. To estimate the levels of IPF in dengue. To correlate levels of IPF and recovery of thrombocytopenia. **Material and Methods:** A total of 110 patients suffering from dengue fever (as confirmed by NS1 antigen, IgM or IgG antibody positivity for dengue) were taken for the study. Detailed history and examination was done. The platelet count and IPF value of all these patients were evaluated on a daily basis and the relation between them was studied. **Results:** In our study about 89.1% of the patients showed improvement in platelet count within 24 hours of rise immature platelet fraction. Whereas rest 10.9 % of the patients showed platelet improvement between 24 hours to 48 hours after rise in immature platelet fraction.

About 77.3% of the patients showed improvement in platelet count within 24 hours after reaching the maximum immature platelet fraction. Whereas 13.6 % of the patients showed platelet improvement between 24 hours to 48 hours after reaching maximum immature platelet fraction. It was also observed that 9.1% of the patients showed platelet improvement between 48 hours to 72 hours after reaching maximum immature platelet fraction. **Conclusion:** In this study we would like to conclude that we can expect platelet recovery within 24 to 48 hours once the immature platelet fraction reaches maximum in most of the case while remaining show rising trend by the end of 72 hours, thus avoiding unnecessary platelet transfusions and transfusion related adverse effects.

Keywords: Immature platelet fraction, Dengue, platelet transfusion

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ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

Introduction

The various serotypes of the dengue virus are transmitted to humans through the bites of infected Aedes mosquitoes, principally Ae. Aegypti. Dengue virus infection is increasingly recognized as one of the world's emerging infectious diseases.[1,2,3,4] Currently dengue is regarded globally as the most important mosquito-borne viral disease and widely distributed throughout tropical and subtropical regions of the world. An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in dengue endemic countries.

Dengue in the future: Many experts hypothesize that dengue will increase in the future, including geographic expansion, incidence and reporting to WHO. It is therefore important to elaborate on some of the potential factors that drive dengue activity, as well as the global strategic direction to address this growth.

Dengue is a self-limited, systemic viral infection transmitted between humans by mosquitoes.[5] It is a systemic and dynamic disease. After the incubation period, the illness begins abruptly and is followed by the three phases - febrile, critical and recovery. A well-managed front-line response not only reduces the number of unnecessary hospital admissions but also saves the lives of dengue patients.

Pathogenic Mechanisms In Dengue Virus Infection,[6] – The hallmarks of dengue virus infections are haemorrhagic manifestations associated with thrombocytopenia and an increased vascular permeability.

Studies have suggested that dengue virus-induced bone marrow suppression decreases platelet synthesis, resulting in thrombocytopenia.[7] It is reported that coagulation abnormalities involve a combination of thrombocytopenia and increased fibrinolysis.

On the other hand, platelet kinetics in patients with dengue haemorrhagic fever increases platelet destruction as the major cause of thrombocytopenia. A recent investigation reported the presence of an anti-platelet autoantibody in sera from patients infected with dengue virus and thrombocytopenia. Another study demonstrated that both the dengue virus antigen and human immunoglobulin are present on the platelet surface in patients with a dengue haemorrhagic fever.[8]

An increased level of PAIgG is frequently observed in patients with chronic idiopathic thrombocytopenic purpura (ITP). It is recently demonstrated an inverse correlation between the levels of platelet-associated IgG (PAIgG) and the platelet count during the acute phase of secondary dengue infections.[9]

Immature Platelet Fraction: The immature platelet fraction (IPF) is the percentage of immature platelets compared to the total number of platelets.The immaturity is determined by two main characteristics: the large size of the platelets and their high fluorescence intensity.[10] Many fluorochromes had been tested but the more recent instruments use Oxadine.[11] The high fluorescence intensity of the immature platelets is due to the interaction between the RNA nucleic acids and the stain.[12] This interaction needs the perforation of the platelet by reagents, thus allowing the staining by Oxadine.[13] Data analysis is done by specially designed algorithm software (IPF-software). The suggested interval range goes from 0.3% as a minimum to 17.8% as a maximum value.[14]

Contribution of IPF in clinical practice: IPF and disseminated intravascular coagulation: In DIC, the IPF-% is predicted to increase.

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IPF and aplastic anemia: In aplastic anemia with an isolated thrombocytopenia presentation, the differential diagnosis with other etiology is difficult if invasive procedures are not used. IPF-% had been shown to increase in aplastic anemia.

IPF and cardiovascular disease: Several studies reported high IPF in patients with coronary artery disease; especially in patients with acute phase of coronary syndromes. IPF increase was more pronounced in smokers and diabetic patients compared to non-smokers and non-diabetic patients, respectively.

IPF and liver diseases: Thrombocytopenic patients with B or C hepatitis had elevated IPF values compared to non-thrombocytopenic infected patients.[15,16] IPF can also help to differentiate between the stages of hepatic fibrosis, as patients with cirrhosis have higher IPF values than patients with chronic hepatitis.

IPF and pregnancy complications: Physiologically, pregnancy is associated with an increase in IPF between 20 and 40 weeks of gestation but most values does not exceed 7.5%.

Adverse effects of platelet transfusion,[17] –

Frequency of moderate to severe PLT transfusion reactions – in one of the study 22% of patients (n = 132) had one or more moderate to severe transfusion reactions. Reactions occurred with 2.2 percent. The most common signs and symptoms were chills with rigors (143 episodes in 106 patients), urticarial eruptions (31 episodes in 24 patients), and an increase in temperature of at least 2∞C.For those patients who had a transfusion reaction, the mean number of PLT. Transfusions before the first reaction were 7 ± 6 transfusions (median, 5; interquartile range, 3- 10) and the mean number of days from the first PLT transfusion to the first transfusion reaction was 11 ± 10 days.

David C. Lye etal,[18] conducted a study which revealed that there were no significant benefits of prophylactic platelet transfusion among adult patients with dengue, which are similar to results of other studies involving different cohorts.

Muhammad etal,[19] conducted a study and revealed that platelet transfusion did not prevent development of severe bleeding or shorten time to cessation of bleeding and was associated with significant side effects. Therefore, platelet transfusion should not be routinely done in the management of dengue fever.

Objectives

1. To estimate the levels of immature platelet fraction in dengue patients.
2. To correlate levels of immature platelet fraction and recovery of thrombocytopenia.

Material and Methods

Study Design: This study is a prospective observational hospital based study, to study the immature platelet fraction in patients with dengue fever with thrombocytopenia and its outcome.

Study Area: This study was conducted in the department of General Medicine in S S Institute of Medical Sciences and Research Centre, a tertiary care teaching hospital located in Davangere, Karnataka.

Study population: Patients attending inpatient department of General medicine with dengue fever during the study period were included in the study.

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Study period: The study was conducted during the period of January 2019 to September 2020.

Inclusion criteria:

All fever patients who were positive for dengue serology with thrombocytopenia.

 Patients aged more than 18 years.

Exclusion criteria:

 Patients with Chronic liver disease.

 Patients with Haematological disorder.

 Patients with history of recent myocardial infarction.

 Patients who developed haemorrhagic manifestations.

 Patients with history of heavy alcohol consumption.

 Patients on chemotherapy drugs.

Sample Size $\frac{Z_{1-\alpha/2}^2 * p * (100-p)}{d^2}$

P	Recovery rate after attaining the peak IPF	-84.3%
1-α	Confidence level	-0.95
Z	Z value associated with confidence	-1.96
D	Absolute precision (Value less than P)	- 7%

Sample size 103.71 the rounded up to 110.

Ethical committee approval: Ethical committee approval was obtained for this study.

Data Collection: Patients attending the department of Emergency and General Medicine with fever were tested for dengue serology, and all the patients that came positive for dengue serology were included. Written informed consent was obtained from the study participants prior to the interview. Then CBC was sent and was looked for thrombocytopenia and only those with thrombocytopenia were included.

Data analysis: The statistical methods applied to analyse the data using chi square test and Fisher's t test. To validate the significance of admission AFI, the cases were grouped as those with under or more than 5 cm of AFI.A statistical package SPSS version 17.0 will be used and p value <0.05 is considered significant.

Results

In our study 30% i.e. 33 cases belonged to age group of 18 to 30 years of age. 19.1% i.e. 21 cases belonged to age group of 30 to 40 years of age. 17.3% i.e. 19 cases belonged to age group of 40 to 50 years of age. 14.5% i.e. 16 cases belonged to age group of 50 to 60 years of age. 10.9% i.e. 12 cases belonged to age group of 60 to 70 years of age.8.2% i.e. 9 cases belonged to age group of 18 to 30 years of age.

In our study, cases were predominantly male compared to females. There were 57.3% i.e. 63 cases out of 110 cases were males whereas 42.7% i.e. 47 cases were females.

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Table 1: Duration between rise in immature platelet fraction and rise in platelet count

Duration	Frequency	Percent
24	98	89.1
48	12	10.9
Total	110	100

In our study about 89.1% of the patients i.e. 98 cases showed improvement in platelet count within 24 hours of rise immature platelet fraction. Where increase in immature platelet fraction by 5% was consider as rise in immature platelet fraction. Whereas 10.9 % of the patients i.e. 12 cases showed platelet improvement between 24 hours to 48 hours after rise in immature platelet fraction. It was noted that all 100% patients i.e. 110 cases showed platelet count rise within 48 hours of rise in immature platelet fraction.

Table 2: Duration between highest immature platelet fraction and rise in platelet count

Duration	Frequency	Percent
24	85	77.3
48	15	13.6
72	10	9.1
Total 110		100

In our study about 77.3% of the patients i.e. 85 cases showed improvement in platelet count within 24 hours after reaching the maximum immature platelet fraction. Whereas 13.6 % of the patients i.e. 15 cases showed platelet improvement between 24 hours to 48 hours after reaching maximum immature platelet fraction. Thus by the end of 48 hours after reaching maximum immature platelet fraction 90.9% patients i.e. 100 cases showed rise in platelet count. It was also observed that 9.1% of the patients i.e. 10 cases showed platelet improvement between 48 hours to 72 hours after reaching maximum immature platelet fraction. Thus by the end of 72 hours after reaching maximum immature platelet fraction 100% patients i.e. 110 cases showed rise in platelet count.

Table 3: Duration between fall in immature platelet fraction and rise in platelet count

Duration	Frequency	Percent
24	110	100

In our study 100% of the cases i.e. 110 patients showed rise in platelet count with 24 hours of fall in immature platelet fraction, where fall in immature platelet fraction was defined by decline in immature platelet fraction by minimum of 5%.

Discussion

Thrombocytopenia is a common problem in dengue, which causes a lot of concern. The thrombocytopenia in dengue is primarily immune mediated and platelet transfusion is said to

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worsen the thrombocytopenia by an exalted immune response by a strong antigenic stimulus.[20]

Apart from that, the short life span of transfused platelets results only in a transient or non-sustained elevation of the platelet.[20,21] They can also induce hypersensitivity reactions and fluid-overload with complications such as pleural effusion, ascites and pulmonary oedema. 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.[22] The number of reticulated platelets reflects the rate of thrombopoiesis.[23] IPF levels rise as bone marrow production of platelets increases, and therefore, its measurement provides an assessment of bone marrow platelet production from a peripheral blood.

Immature platelet fraction has many applications in clinical practice mainly with chemotherapy however with respect to Dengue is minimal. Thrombocytopenia of moderate degree is a usual finding associated with dengue, the reasons for which are multifactorial, which include early transient marrow suppression with damage to megakaryocytes,[24] platelet aggregation to endothelial cells targeted by dengue fever viruses, hemophagocytosis , and finally, immune destruction of platelets, with dengue antibody complexes being found on their membrane. Our study shows that about 89.1% of the patients i.e. 98 cases showed improvement in platelet count within 24 hours of rise in immature platelet fraction. While the reference article showed rise in platelet count in 93.7%. (Increase in immature platelet fraction by 5% was consider as rise in immature platelet fraction). Whereas 10.9 % of the patients i.e. 12 cases showed platelet improvement between 24 hours to 48 hours after rise in immature platelet fraction. It was noted that all 100% patients i.e. 110 cases showed platelet count rise within 48 hours of rise in immature platelet fraction. In our study about 77.3% of the patients i.e. 85 cases showed improvement in platelet count within 24 hours after reaching the maximum immature platelet fraction. Whereas the reference article showed platelet rise in 84.3% of the cases within 24 hours.

13.6 % of the patients i.e. 15 cases showed platelet improvement between 24 hours to 48 hours after reaching maximum immature platelet fraction. Thus by the end of 48 hours after reaching maximum immature platelet fraction 90.9% patients i.e. 100 cases showed rise in platelet count. Whereas the reference article showed platelet rise in 100% cases by the end of 48 hours.

It was also observed that 9.1% of the patients i.e. 10 cases showed platelet improvement between 48 hours to 72 hours after reaching maximum immature platelet fraction. Thus by the end of 72 hours after reaching maximum immature platelet fraction 100% patients i.e. 110 cases showed rise in platelet count. Usually in patients with dengue, at a certain point, the IPF starts rising even though the platelets might be falling. This is due to a combination of pathogenic mechanisms; the peripheral destruction, which lowers the platelet count, stimulates the marrow to produce more platelets which causes an increase in the IPF. After a certain point when the IPF has peaked, then the platelets start coming up and IPF starts falling. If the patient is caught at this stage, where the first value was the peak value, it is obvious that the value on second day will be less than the peak (falling trend). This fall in the IPF is a strong predictor of an impending rise in platelet count. So if the platelets have not

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started recovering, then the time lag for recovery of platelets in such cases is < 24 h. It was observed that 100% patients show a recovery within 24 h of the fall.

Lucy etal,[25] did a study about preventive transfusion and found out that monitoring serial haematocrit, which reflects the degree of plasma leakage, rather than thrombocytopenia and coagulopathy resulted in reduced use of blood products, judicious intravenous therapy, a lower incidence of pulmonary edema. Thus instead of platelet count we replace that with immature platelet fraction to prevent those unnecessary transfusions.

David etal,[26] study showed adult patients with dengue and thrombocytopenia (≤20000 platelets per µL), prophylactic platelet transfusion was not superior to supportive care alone in the prevention of bleeding or improvement in platelet recovery, and might be associated with adverse events. So in such cases even with very low platelet count we can use immature platelet fraction to take a call on platelet transfusion.

Conclusion

In this study we would like to conclude that we can expect platelet recovery within 24 to 48 hours once the immature platelet fraction reaches maximum in most of the case while remaining show rising trend by the end of 72 hours, thus avoiding unnecessary platelet transfusions and transfusion related adverse effects. We can also conclude that platelet recovery is seen within 24 hours once immature platelet fraction starts declining.

Therefore IPF shows great promise of becoming a reliable future guide for decisions concerning platelet transfusions. There appears to be a direct co-relation between increases in base IPF levels and corresponding increases in platelet count. The time lag between increased IPF levels and corresponding increases in platelet count appears to be around 24–48 h in most of the patients with dengue. Therefore, measurement of IPF should be considered as routine practice to evaluate and monitor thrombocytopenia in patients with dengue.

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