ORIGINAL ARTICLE

RELATIONSHIP BETWEEN IMMATURE PLATELET FRACTION AND PLATELET COUNT AMONG PEDIATRIC PATIENTS WITH DENGUE FEVER: A PROSPECTIVE CROSS-SECTIONAL STUDY

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ABSTRACT

Background and Objectives: Immature platelet fraction (IPF) is a new hematologic parameter that reflects the rate of thrombopoiesis. It has been suggested to be a predictor of platelet recovery in patients with thrombocytopenia. This study aimed to determine the relationship between IPF and platelet count among pediatric patients with thrombocytopenia due to dengue fever.

Methods: This was a prospective cross-sectional study of 77 thrombocytopenic pediatric dengue fever patients. IPF was included in the daily complete blood count extraction. Baseline and daily IPF, platelet count, hematocrit, white blood cell count and presence of fever were recorded according to day of illness. The pattern of IPF in relation to the pattern of platelet count was analyzed. The proportion of patients showing platelet recovery at different time points was also determined. A receiver operating characteristic analysis was done to determine an IPF cut-off value predictive of platelet recovery within 24 hours.

Results: The IPF increased as the platelet count decreased. The highest increase in IPF coincided with the trough of platelet count. Eighty-seven percent of the patients showed platelet recovery after the increasing trend of IPF, 87% after the peak value and 95% after the decreasing trend. An IPF value of more than 6.6% was found to be predictive of platelet recovery within 24 hours, with a sensitivity of 45% and specificity of 70%.

Conclusion: There was an observed inverse relationship between IPF and platelet count but with a statistically weak correlation. The decreasing trend of IPF can be a possible good predictor of an increasing trend in platelet count. These findings suggest a possible role of IPF as an additional parameter to predict platelet recovery in pediatric dengue fever patients.

KEYWORDS: *Immature platelet fraction, thrombocytopenia, dengue fever*

INTRODUCTION

Dengue fever is a mosquito-borne illness that is a major public health concern internationally and locally due to its high morbidity and mortality.[1] In the Philippines, there were 200,415 suspected cases of dengue and 598 deaths in 2015, majority of whom were children.[2]

The clinical course of dengue virus infection ranges from an inapparent infection, mild febrile illness, to severe dengue hemorrhagic fever. Hematologically, the most common abnormalities are hemoconcentration, thrombocytopenia, prolonged bleeding time, and a moderately decreased prothrombin level.[3] Thrombocytopenia in dengue fever is multifactorial with bone marrow hypocellularity followed by immune-mediated peripheral destruction of platelets as the most common proposed mechanisms.[4,5,6,7]

Immature platelet fraction (IPF) is a new hematologic parameter that determines the percentage of new platelets released by the bone marrow by measuring reticulated platelets in peripheral blood. It reflects the rate of thrombopoiesis, as IPF level increases as the production of platelets increases.[8,9,10,11] IPF range is 4.1 $\pm$ 7 in neonates, 2.7 $\pm$ 1.3 in children and 1.1 to 6.1 in adults.[10] It is proven to differentiate between consumptive (peripheral destruction) versus productive (bone marrow failure) etiology of thrombocytopenia and has been suggested to be an early predictor of platelet recovery in various clinical conditions including dengue fever.[8,11,12,13,14,15]

There are no clear guidelines regarding blood transfusion for thrombocytopenia due to dengue fever. The World Health Organization (WHO) recommends that blood transfusion should be given as soon as severe bleeding is suspected or recognized in a hemodynamically unstable dengue patient.[16] However, there is no evidence that supports the practice of transfusing platelet concentrate and/or fresh frozen plasma for thrombocytopenia or severe bleeding in hemodynamically stable dengue patients.[16,17] It has been a common practice to consider blood transfusion when the platelet count is less than 50,000/µL in the presence of significant bleeding or when less than 10,000/µL with no bleeding.[7]

IPF as of today has no role in the management nor in the monitoring of dengue patients due to lack of available information and research. If IPF is proven to have significant association with platelet count recovery, it may have a role in the monitoring and management of dengue fever which can possibly lead to less unnecessary blood transfusion, blood test monitoring, financial cost and a shorter hospital stay.

This study aimed to determine the relationship between IPF and platelet count and whether IPF can be utilized as an indicator of platelet recovery among pediatric patients with dengue fever. Specifically, to describe the pattern of IPF in relation to the pattern of platelet count, hematocrit, white blood cell count (WBC) and fever throughout the course of dengue fever illness; to determine the proportion of patients showing platelet recovery within 24 hours after the increasing IPF trend, peak IPF and decreasing IPF trend; and to identify the IPF value that predicts platelet recovery within 24 hours.

METHODOLOGY

Subject Selection and Data Collection

This prospective cross-sectional study was conducted at Makati Medical Center, a tertiary private healthcare facility in the Philippines, from August 1 to October 31, 2016.

All private and service pediatric patients aged zero to 18 years old of any gender admitted with a diagnosis of dengue fever, positive dengue NS1 antigen and thrombocytopenia of less than 150,000/µL, were included in this study. Pediatric patients with a concomitant disease that may

cause thrombocytopenia such as primary immune thrombocytopenia, connective tissue disease, myelodysplastic syndrome, and those with intake of medications that may increase the risk of bleeding such as anticoagulants, antiplatelet agents, acetylsalicylic acid and ibuprofen were excluded. Those who had any blood product transfusion (fresh whole blood, packed red blood cell, platelet concentrate and fresh frozen plasma) prior to admission or during the course of illness were also excluded.

Upon the approval of the Institutional Review Board, all patients who satisfied the inclusion criteria were enrolled in the study. A written informed consent from the parents or guardian of legal age, and assent from patients seven years old and above were secured prior to enrollment. The cost of the IPF determination was shouldered by the study proponents. The study participants were allowed to drop out at any point during the study period.

General demographic and clinical data (age, sex, day of illness, presence of fever, platelet count, hematocrit, WBC count and presence of warning signs) upon enrollment to the study were taken. On subsequent days, the day of illness, presence of fever, IPF, platelet count, hematocrit and WBC count were recorded. IPF was requested to be included in the daily routine complete blood count (CBC) ordered by the attending physician. The blood samples were extracted daily at the patient's room by a certified medical technologist from the time of study enrollment until a platelet count of 150,000/µL or more was achieved, and/or until the attending physician ordered to discontinue CBC monitoring. Only one blood sample per day was required to determine both the CBC and IPF. When blood samples were drawn more frequently, IPF was included only on the first blood sample for the day. The study proponents decided to only include IPF on the first blood sample for the day for consistency and to minimize costs. Standard WHO dengue fever management was instituted, including the use of isotonic intravenous fluids.

IPF in this study was measured using the automated hematology analyzer, Sysmex XE-2100. It uses a carefully designed gating system in the optical (fluorescence) reticulocyte/platelet channel to quantify the IPF. This flow cytometric IPF determination uses a patented fluorescent dye containing polymethine and oxazine or oxadine. These dyes penetrate the cell membrane and stain the RNA in the red cell and platelet reticulocytes.[11,13,14]

Sample Size and Statistical Analysis

A minimum of 90 patients were needed for this study using the Cochran's equation ($n = z^2 * pq / error^2$) for determining sample size for proportions with precision of $\pm$ 5% at 95% level of confidence, where: n = sample size, z-value at 0.05 alpha level of significance is 1.96, p = 0.9375 (previous knowledge based on the study by Dadu et al.)[14], q = 1-p and error = 0.05.

Descriptive statistics were used to summarize the clinical characteristics of the patients. Frequency and proportion were used for nominal variables, median and range for ordinal variables, and mean and standard deviation for interval/ratio variables. Spearman's rank correlation was used to determine the correlation of the platelet count and IPF. The missing variables were neither replaced nor estimated. No imputation was performed so as not to underestimate nor overestimate percentage changes or trends. A receiver operating characteristic analysis was done to determine whether IPF was predictive of platelet recovery within 24 hours. The null hypothesis was rejected at 0.05 α-level of significance. STATA 12.0 was used for data analysis.

Operational Definition

1. Increasing IPF trend – an increase in IPF by more than 10% from its previous value.[14]
2. Decreasing IPF trend – a decrease in IPF by more than 10% from its previous value.[14]
3. Peak IPF value – the maximum IPF value reached while monitoring the IPF.[14]
4. Platelet recovery – the first day of increase in platelet by more than 14.1% without any blood transfusion.[11]

RESULTS

A total of 77 pediatric patients admitted for dengue fever were included in this study, which was 86% of the minimum sample size required. The mean age was 10.5 years and 52% were male. The subjects were enrolled on different days from the onset of illness, with 34 (44%) entering at the 4th day of illness. Sixty-seven percent of the patients were febrile upon enrollment. Their baseline platelet count ranged from 17,000 to 149,000. Table 1 provides the demographic and clinical characteristics of the enrolled patients.

TABLE 1. Demographic and clinical characteristics.

	Frequency (%); Mean ± SD; Median (Range)
Age	10.51 ± 4.86
1 to 5 years old	15 (19)
6 to 10 years old	19 (25)
11 to 18 years old	43 (56)
Sex	
Male	40 (52)
Female	37 (48)
Day of illness upon entry into the study	
Day 3 of illness	5 (7)
Day 4 of illness	34 (44)
Day 5 of illness	26 (34)
Day 6 of illness	12 (15)
Presence of fever upon entry into the study	67 (87)
Day 3 of illness	5 (7)
Day 4 of illness	34 (44)
Day 5 of illness	20 (26)
Day 6 of illness	8 (10)
Baseline platelet count (/µL)	106,000 (17,000 to 149,000)
Baseline hematocrit (%)	41.1 (33.2 to 52.8)
Baseline WBC count (x10^3/µL)	2.77 (0.93 to 9.4)
Presence of warning signs*	28 (36.4)
Abdominal pain/ tenderness	14 (50)
Persistent vomiting	10 (35.7)
Mucosal bleeding	5 (17.9)
Fluid accumulation (edema, ascites, pleural effusion)	1 (3.6)
Lethargy/restlessness	0
Liver enlargement > 2 cm	0
No urine output > 6 hours	0

** Multiple Response Variable*

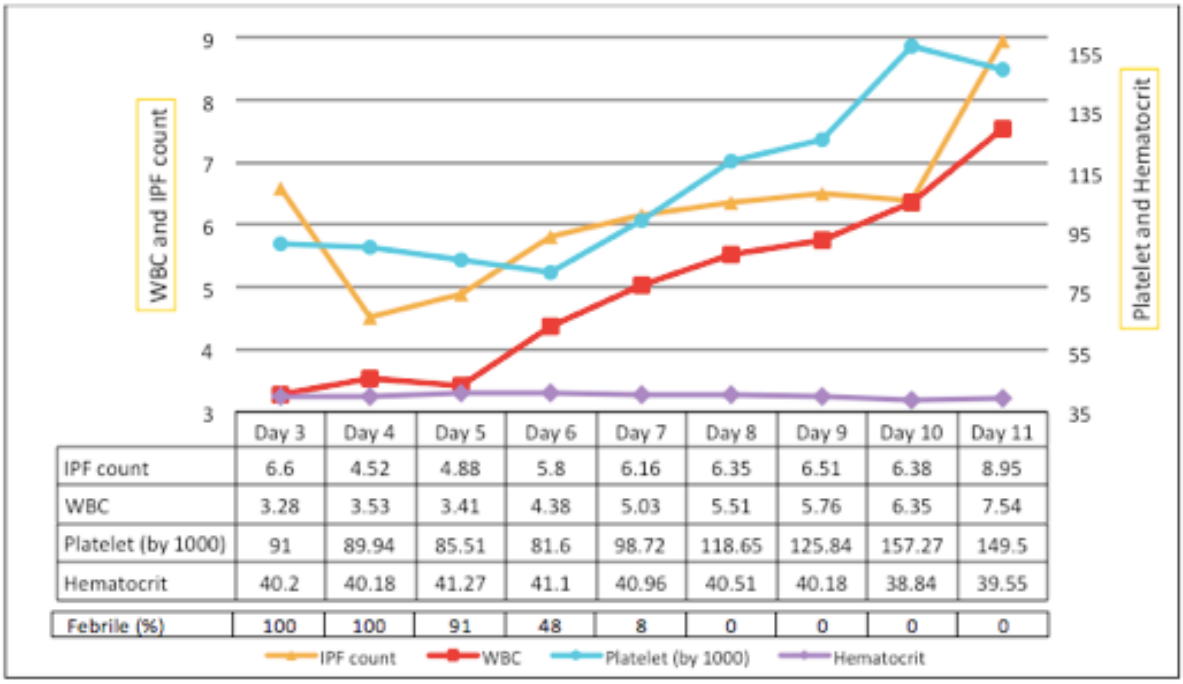
IPF, Platelet, Hematocrit, WBC and Fever Patterns

The average IPF increased steadily from day four to 11 of illness. The platelet count had reached its trough at day six of illness. The hematocrit count steadily decreased and the WBC count steadily increased. Resolution of fever started by day five of illness and by day eight, all patients were afebrile (Table 2).

TABLE 2. Average IPF, platelet count, hematocrit, WBC count and fever status according to day of illness.

Day of Illness	IPF (%)	Platelet (by 1000/µL)	Hematocrit (%)	WBC (x10^3/µL)	Febrile (%)
	Mean ± SD; Frequency (%)				
Day 3 (n=2)	6.6 ± 2.83	91 ± 33.94	40.2 ± 7.78	3.28 ± 1.22	2 (100)
Day 4 (n=17)	4.52 ± 2.32	89.94 ± 35.15	40.18 ± 3.84	3.53 ± 1.95	17 (100)
Day 5 (n=45)	4.88 ± 2.09	85.51 ± 33.08	41.27 ± 3.39	3.41 ± 1.74	41 (91)
Day 6 (n=67)	5.8 ± 2.11	81.6 ± 32.33	41.1 ± 3.55	4.38 ± 2.08	32 (48)
Day 7 (n=72)	6.16 ± 2.36	98.72 ± 44.9	40.96 ± 4.15	5.03 ± 1.99	6 (8)
Day 8 (n=55)	6.35 ± 2.23	118.65 ± 48.75	40.51 ± 4.2	5.51 ± 2.37	0
Day 9 (n=25)	6.51 ± 2.05	125.84 ± 57.96	40.18 ± 4.47	5.76 ± 1.89	0
Day 10 (n=11)	6.38 ± 2.76	157.27 ± 77.55	38.84 ± 5.45	6.35 ± 1.91	0
Day 11 (n=2)	8.95 ± 3.04	149.5 ± 7.78	39.55 ± 5.59	7.54 ± 2.48	0

Figure I illustrates the trend of IPF in relation to the trend of fever, platelet count, hematocrit and WBC count over days three to 11 of illness. Overall, there was a steady increase in IPF from day four to 11 of illness. The largest increase was observed on day six, where the median percentage change was at 32% from the previous day (Table 3). This coincides with the trough of platelet count (Table 2, Figure I).



	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
IPF count	6.6	4.52	4.88	5.8	6.16	6.35	6.51	6.38	8.95
WBC	3.28	3.53	3.41	4.38	5.03	5.51	5.76	6.35	7.54
Platelet (by 1000)	91	89.94	85.51	81.6	98.72	118.65	125.84	157.27	149.5
Hematocrit	40.2	40.18	41.27	41.1	40.96	40.51	40.18	38.84	39.55
Febrile (%)	100	100	91	48	8	0	0	0	0

FIGURE I. Pattern of IPF in relation to the platelet count, hematocrit, WBC count and fever according to day of illness.

TABLE 3. Percentage change in IPF from previous day according to day of illness.

Day of Illness	IPF (%)	% Change from previous day
	Mean ± SD; Median (Range)	
Day 3	6.6 ± 2.83	-
Day 4 (n=2)	4.52 ± 2.32	3 (2 to 5)
Day 5 (n=17)	4.88 ± 2.09	12 (-25 to 67)
Day 6 (n=44)	5.8 ± 2.11	32 (-73 to 274)
Day 7 (n=63)	6.16 ± 2.36	8 (-36 to 57.5)
Day 8 (n=55)	6.35 ± 2.23	2 (-57 to 96)
Day 9 (n=25)	6.51 ± 2.05	-10 (-34 to 55)
Day 10 (n=11)	6.38 ± 2.76	-16 (-63 to 27)
Day 11 (n=2)	8.95 ± 3.04	-19 (-29 to -9)

**Percentage change in IPF = [(current day IPF – previous day IPF)/previous day IPF]*100*

Since it is a common practice to consider blood transfusion when the platelet count is less than 50,000/µL in the presence of significant bleeding, or when less than 10,000/µL with no bleeding, a subgroup analysis for patients who had a platelet count of 50,000/µL and below was done to determine if there is a difference in the patterns of IPF, platelet count, hemocrit, WBC count and fever status among those patients. It was observed that the patterns of IPF, platelet count, hematocrit, WBC count and fever status of those with platelet count of 50,000/µL and below were the same when compared to those with platelet counts of more than 50,000/µL.

Of the 21 patients in the subgroup, nine presented with warning signs: four had abdominal pain, two had epistaxis, two had persistent vomiting and one had minimal pleural effusion. There were no cases of severe dengue, shock or death. And none of them were given fluid resuscitation or blood transfusion.

IPF and Platelet Recovery

The proportion of patients who showed platelet recovery at different time points is shown in Table 4. An increasing trend of IPF started to be evident by day seven of illness and was observed in 51% of the patients by day nine of illness. Twenty-four hours after the start of the increasing trend of IPF, 87% of the patients demonstrated platelet recovery.

For the decreasing trend of IPF, it started to be evident by day eight of illness and was observed in 39% of the patients. Twenty-four hours after the decreasing trend began, 95% of the patients demonstrated platelet recovery.

By day seven of illness, 62% of the patients had reached peak IPF value and 58% had recovering platelet counts. Twenty-four hours after the patients had reached peak IPF value, 87% of them demonstrated platelet recovery.

For all the time points mentioned, it was observed that the platelet and WBC counts showed an increasing trend while the hematocrit count showed a decreasing trend (Table 2, Figure I). Using the Spearman's correlation, there is a weak and indirect correlation between IPF and platelet count with a correlation coefficient of -0.23 (p-value of 0.000).

To determine the IPF value predictive of platelet recovery within 24 hours, a receiving operating characteristic analysis was done. From the 77 patients, a total of 296 readings of IPF were obtained. A receiver operating characteristic analysis was also done to determine the sensitivity and specificity of the different IPF cut-off points. The optimal criterion was an IPF of more than 6.6%, with a sensitivity of 45%, specificity of 70%, positive likelihood ratio of 1.49 and negative likelihood ratio of 0.79. This prescribed cut-off point has a Youden index J of 0.1548. The area under the curve is 0.592 (95% CI = 0.53 to 0.65, p value = 0.006).

TABLE 4. Cumulative frequency of patients that achieved increased or decreased IPF trend, peak IPF count, and proportion of patients that showed platelet recovery by day of illness.

Day of Illness	Increasing IPF trend	Reached peak IPF value	Decreasing IPF trend	With platelet recovery
	Cumulative frequency (%)			
Day 3	-	-	-	-
Day 4	-	1 (1)	-	-
Day 5	2 (3)	6 (8)	-	1 (1)
Day 6	11 (14)	25 (32)	-	14 (18)
Day 7	25 (32)	48 (62)	5 (6)	45 (58)
Day 8	35 (45)	69 (90)	10 (39)	67 (87)
Day 9	39 (51)	76 (99)	14 (18)	73 (95)
Day 10	-	77 (100)	19 (25)	74 (96)
Day 11	-	-	-	-

DISCUSSION

This prospective cross-sectional study of 77 hospitalized children with dengue fever who were dengue NS1 antigen test positive and thrombocytopenic (platelet count less than 150,000/µL) demonstrated an inverse relationship between IPF and platelet count, although this relationship showed a statistically weak correlation. Throughout the course of illness, measured by day, the IPF increased while the platelet count decreased. The trend of IPF at different time points (when it shows an increasing trend, peak value and decreasing trend) also suggested platelet recovery within 24 hours. An IPF value of more than 6.6% was found to be predictive of platelet recovery within 24 hours, although the sensitivity of this finding was at 45%.

There was an observed inverse relationship between IPF and platelet count. However, the correlation between IPF and platelet count was found to be statistically weak probably because the enrolled number of subjects in this study was below the minimum sample size required. Briggs et al. in 2004 assessed IPF in peripheral thrombocytopenia. Their study included 22 patients with immune thrombocytopenic purpura, 11 patients with thrombotic thrombocytopenic purpura, 12 pregnant women and 13 patients undergoing chemotherapy with decreasing platelet counts. There was a statistically significant inverse correlation of platelet count with IPF, as they saw that a decrease in platelet count was accompanied by an increase in IPF.[13] In 2014, a study by Dadu et al. showed that IPF has a strong correlation with recovery of platelet counts in patients with dengue fever. They evaluated the relationship of IPF with platelet recovery in 32 patients with dengue fever confirmed by dengue IgM and/or dengue NS1 antigen and with a complete blood count showing thrombocytopenia (defined as less than 150,000/µL) and concluded that IPF can be used to evaluate platelet recovery in patients with dengue fever.[14] In dengue fever, a rapid decrease in the platelet count is evident during the critical phase which can be between day three to seven of illness.[16,17] The thrombocytopenia in dengue fever is most commonly due to impaired thrombopoiesis and peripheral platelet destruction.[4,5,6,7] During the early phase of dengue fever (two to four days of dengue virus infection), the bone marrow shows hypocellularity and decrease in megakaryocyte maturation caused by direct infection of the

progenitor cells by the dengue virus and changes in bone marrow regulation.[4,6,7] During the defervescence phase, the progressive thrombocytopenia is due to the increase in peripheral platelet destruction caused by several factors which include autoimmune-induced platelet activation and destruction by anti-platelet autoantibodies, platelet consumption during an ongoing coagulopathy process and increased peripheral sequestration or hemophagocytosis.[5,6,7] As IPF measures young, reticulated platelets in the blood, it can be used as a marker for platelet production.[8,9,10,11] A high IPF indicates increased platelet production, while a low IPF indicates decreased platelet production as evident in this study wherein the largest increase in the IPF coincided with the lowest value of the platelet count, indicating an increase in thrombopoiesis.

In this study, it was also observed that as the IPF increased, the pattern of WBC count showed an increasing trend, while the hematocrit showed a decreasing trend throughout the days of illness. During the critical phase (day three to seven of illness) of dengue fever, aside from rapid decrease in platelet count, a decrease in WBC count and an increase in hematocrit count are also observed.[16,17] Damage on the structure of the endothelial cells by direct virus invasion or immune-mediated pathology results in increase in capillary permeability, plasma leakage, hemoconcentration, fluid effusion and consequently, the increase in hematocrit.[16,17,18] The decrease in WBC count is caused by bone marrow depression and hypocellularity during the period of viremia in dengue fever.[18]

The trend of IPF at different time points suggested platelet recovery within 24 hours. When the IPF reached an increasing trend, peak value and decreasing trend, 87%, 87%, and 95% of the patients showed platelet recovery after 24 hours respectively. The decreasing trend of IPF can be a possible good predictor of an increasing trend in platelet count since 95% of the patients showed platelet recovery 24 hours after this phase. This is similar to a study done by Dadu et al., wherein 94% of the 32 patients demonstrated platelet recovery within 24 to 48 hours during the increasing trend, 84% during the peak of IPF, 100% during the decreasing trend and 94% at a value of 10% or more.[14] These observed values can be explained by the presumed pathogenetic mechanisms of thrombocytopenia in dengue fever. The impaired thrombopoiesis and peripheral platelet destruction reduces the platelet count which leads to the production of more platelets by the bone marrow and subsequently causing an increase in the IPF.[4,5,6,7,14]

An IPF value of more than 6.6% was found in this study to be predictive of platelet recovery within 24 hours with a specificity of 70% and sensitivity of 45%. A study in 2014 involving 16 autologous stem cell transplant patients by Van der Linden et al. determined an IPF cut-off value of 5.3% (specificity of 98%, sensitivity of 47%, positive predictive value of 93%) to predict platelet recovery within two days.[11] On the other hand, an IPF cut-off value of 6.25% (specificity of 63%, sensitivity of 77%, positive predictive value 67%) was determined in a study by Suman et al. that same year to be statistically significant in predicting platelet recovery within 48 hours.[15] A known IPF cut-off value to predict platelet recovery would be useful in monitoring dengue fever patients and in helping physicians to decide if blood transfusion may be necessary since there are no clear guidelines for blood transfusion in pediatric dengue patients. According to the WHO, the prophylactic blood transfusion for severe thrombocytopenia in hemodynamically stable dengue patients are not effective and needed.[16] Observational studies have shown that preventive transfusions of platelet concentrate and fresh frozen plasma in dengue fever patients were not able to maintain the platelet counts or improve the coagulation

profile.[7,16,17] Moreover, the incidence of pulmonary edema and increased length of hospital stay was higher among patients who received transfusion.[7] Blood transfusion should be given in cases with suspected or severe bleeding in a hemodynamically unstable patient.[16] However, there is no defined recommendation for severely thrombocytopenic patients who are hemodynamically unstable but without severe bleeding. In these patients, IPF may be a potentially helpful parameter, though still needs further study, to aid in the decision-making process regarding its utility in deciding on blood transfusion.

CONCLUSION

There was an inverse relationship between IPF and platelet count, but this relationship showed a statistically weak correlation. The decreasing trend of IPF can be a possible good predictor of an increasing trend in platelet count based on the observed patterns. An IPF value of more than 6.6% was found to be predictive of platelet recovery within 24 hours but the sensitivity was only 45%. These findings provide support on the possible role of IPF as an additional parameter to predict platelet recovery in pediatric dengue fever patients.

LIMITATIONS AND RECOMMENDATIONS

Due to the relatively decreased number of dengue fever patients compared to previous years at Makati Medical Center, this study did not meet the required minimum sample size of 90. For future studies, a larger sample size and longer study period are recommended to increase the findings' reliability and accuracy. A clinical trial in a multicenter setting can be done to validate the IPF cut-off value identified in this study and possibly establish a role for IPF in pediatric dengue fever monitoring and management.

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