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Original Research Article

PLATELET INDICES ASSAY AS AN INDICATOR OF RECOVERY IN DENGUE-AFFECTED CHILDREN

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Abstract

Background: Dengue thrombocytopenia is complex, varies in severity, and is extremely transitory. Contrary to patients with aplastic anaemia and chemotherapy, dengue patients seldom require platelet transfusions. Predicting platelet count recovery in dengue patients will assist save needless transfusions. We evaluated the numerous platelet parameters to forecast dengue's recovery of platelet count. **Materials and Methods:** A total of 80 patients hospitalized at the Institute of Child Health in Chennai with dengue fever (as determined by NS1 antigen or IgM antibody positive for dengue) were used in the research. The platelet count, IPF value, platelet crit, and mean platelet volume were assessed and compared on three consecutive days. **Result:** The mean platelet counts on the three consecutive days were 43536.2, 75405.8, and 88187. There was a significant rise in platelets, as expected. Mean platelet volume and platelet crit mostly remained within normal range, showed a flat trend line during these three days, and were insignificant. The immature platelet fraction had mean values of 14.34, 11.08, and 9.31 on these days and showed a falling trend line, significant correlation with the rise in platelet counts. Our study's reference normal value for IPF was less than 5%. **Conclusion:** The study concludes that IPF can be used to evaluate the recovery of platelet counts in patients with dengue, whereas the other platelet indicators are not significant. IPF can be used early to predict the following recovery in platelets, thus guiding decisions concerning platelet transfusions.

INTRODUCTION

The Flaviviridae family includes the dengue virus and other mosquito-borne illnesses. Dengue viruses are tiny, spherical viruses with a single strand of RNA and a lipid coat. Dengue was a rare illness that seldom caused outbreaks during the 19th century.[1] Its frequency has surged 30-fold over 50 years, and five out of the six WHO areas have had large outbreaks. Dengue is an ongoing problem in 112 nations worldwide.[2] According to estimates, there are 100 million cases of dengue fever and 500,000 cases of dengue hemorrhagic fever (DHF) each year, with a case fatality rate of 0.5% to 3.5% in Asian countries.[3] Aedes mosquitoes mostly spread dengue, specifically Aedes aegypti, Aedes albopictus, and Aedes polynesiensis. Aegypti is the main and most significant vector. However, depending on the region, A albopictus and A polynesiensis may also function as vectors.[2]

The Swahili term "Kadinga pepo," which means "cramp-like seizures produced by the evil spirit," is where the word "dengue" first appeared. The Spanish word "dengue," which refers to the gait of someone who has dengue fever and is experiencing bone discomfort, may have been the source of the Swahili word "dinga." In 1779, the illness was given a name.[4] In 1780, the first recorded dengue epidemics struck simultaneously in North America, Asia, and Africa. Undifferentiated fever, dengue fever, DHF, or dengue shock syndrome can result from dengue infections, which can also be asymptomatic. In most cases of dengue fever, platelet counts and blood biochemistry are normal. Leucopenia, thrombocytopenia, and elevated liver enzymes can yet occur.[1]

A common dengue-related finding is a moderate thrombocytopenia, which has several causes, including early transient marrow suppression with megakaryocyte damage, platelet aggregation to endothelial cells, hemophagocytosis, and ultimately immune destruction of platelets with dengue antibody complexes found on their membrane.[5] With complex etiology, thrombocytopenia is a frequent complication of several diseases (including aplastic anaemia, ITP, and dengue fever). A novel measurement called the Immature Platelet Fraction (IPF) measures reticulated platelets and represents

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the pace of thrombopoiesis.[1] IPF is a measure of the quantity of reticulated (young) platelets. Comparable to reticulocytes. With the aid of flow cytometry and a fluorescent dye like oxazine, the RNA of these platelets may be precisely quantitated. In several institutions worldwide, IPF is already being used to track instances of ITP, TTP, and bone marrow transplants to predict recovery from thrombocytopenia precisely.[6]

In cases with dengue fever without bleeding symptoms, a preventative platelet transfusion may be administered at levels below 10,000.[7] There are numerous uses for immature platelet fraction in clinical settings. Numerous research has been conducted to determine the value of IPF in predicting platelet recovery. The majority of the studies focus on chemotherapy patients.[1] Ingram & Coopersmith (1969) initially identified the immature platelet fraction in the peripheral blood of dogs that had experienced acute blood loss, referring to platelets having elevated RNA content.[3] Moderate thrombocytopenia is a frequent finding in patients with dengue. The causes of this condition are multifactorial and include early transient megakaryocyte damage and marrow suppression, platelet aggregation to endothelial cells targeted by dengue fever viruses, hemophagocytosis, and ultimately immune destruction of platelets with the discovery of dengue antibody complexes on their membrane. IPF typically uses a reference range of 1% to 5%.[1]

Most of the literature has been reported on chemotherapeutic patients undergoing IPF in the West and Japan.[5] This is one of the first studies to research the utility of Immature Platelet Fraction and to predict the value in platelet recovery in dengue patients.

The current study aims to fill the gap and correlate the association between the IPF, other platelet indices, and platelet recovery of dengue-affected children, preventing unnecessary platelet transfusion.

Aim

The objective was to assess the platelet indices, such as platelet count, mean platelet volume, platelet crit, and IPF, in dengue-affected children under the age of 12 on days 1, 2, and 3 of the critical periods and to determine whether there is a statistically significant correlation between the different platelet indices.

The secondary end-point was to use the immature platelet fraction to gauge healing in children with dengue.

MATERIALS AND METHODS

A prospective study was conducted at the Institute of Child Health and Hospital for Children, Egmore's pediatric medical ward, for a year from May 2018 to April 2019 based on children who met the inclusion criteria and were hospitalized as inpatients. A total of 80 patients were included in the study after their consent and permission were taken.

Inclusion criteria: Children under 12 years of age, IgM Dengue-positive children, post-day 5 of illness, and Ns1antigen-positive children between days 1 to 4 of illness were included.

Exclusion criteria: Children with thrombocytopenia due to other causes, such as Immune thrombocytopenia, Leukemia, Platelet disorders, and Aplastic anaemia, were excluded.

According to the WHO's definition of dengue fever, children hospitalized as inpatients with complaints of fever, joint pain, and warning indications like abdominal pain, vomiting, decreased urine output, bleeding presentations, and rashes were recruited. A clinical examination was performed after obtaining informed consent, and detailed history of the patient was noted. IgM ELISA or NS1 antigen was used to confirm the diagnosis of dengue by serology. Following the confirmation, aseptically collected peripheral venous blood samples were taken on days 1, 2, and 3 of the critical period and sent for platelet indices, peripheral smear, and complete blood count analysis. In K2 EDTA tubes, peripheral blood samples were taken (Beckton Dickinson, Franklin Lakes, NJ, USA). All samples were examined using The Sysmex XE-2100 analyzer (Sysmex, Kobe, Japan) within 4 hours of collection. A predesigned excel sheet proforma was used to collect all the results, which were then analyzed.

An Excel sheet will be used to enter the data. The statistical program SPSS was used to analyze the data. Outcome variables were proportionately stated with a 95% confidence level.

RESULTS

Of the 80 children chosen for this study, 42 (52.5%) were male, and 38 (47.5%) were female. This investigation revealed a male predominance. The 80 kids tested positive for either the NS1 antigen (33.7%) or dengue IgM (66.3%).

When we compared the mean platelet volume and platelet count in 80 children, we discovered that even when the platelet count was abnormal or the platelet count trend was abnormal, the mean platelet volume was found to be in the normal range (9.3-12.4 fl) (Figure 1) and there were no significant changes in MPV with the change in platelet count ($p>0.05$). [Table 1].

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Table 1: Comparison of Mean platelet volume (FL) with Platelet count ON days 1,2,3 of the critical phase

Mean platelet volume FL day 1	Platelet count day 1		Total	Platelet count day 2		Total	Platelet count day 3		Total
	Abnormal	Normal		Abnormal	Normal		Abnormal	Normal	
Low (<9.3)	9	0	9	6	2	8	4	3	7
Percentage	11.4%	0%	11.3%	11.1%	13.3%	11.1%	14.3%	5.8%	8.7%
Normal (9.3 to 12.4)	69	1	70	48	24	72	23	48	71
Percentage	87.3%	100%	87.5%	88.9%	86.7%	88.9%	82.1%	92.3%	88.8%
High (>12.4)	1	0	1	0	0	0	1	1	2
Percentage	1.3%	0%	1.3%	0	0	0	3.6%	1.9%	2.5%
Total	79	1	80	54	26	80	28	52	80
Chi-square	0.145			0.254			1.107		
P value	0.930			0.614			0.575		

Table 2: Comparison of platelet crit with Platelet count on day 1,2,3 of the critical phase

Platelet crit	Platelet count day 1		Total	Platelet count day 2		Total	Platelet count day 3		total
	Abnormal	Normal		Abnormal	normal		Abnormal	normal	
Low (<0.15)	17	0	17	10	6	16	4	18	22
Percentage	21.6%	0%	21.2%	18.5%	23.1%	20%	14.3%	34.6%	27.5%
Normal (0.15 to 0.64)	60	1	61	42	20	62	24	34	58
Percentage	75.9%	100%	76.3%	77.8%	76.9%	77.5%	85.7%	65.4%	72.5%
High (>0.64)	2	0	2	2	0	2	0	0	0
Percentage	2.5%	0%	2.5%	3.7%	0%	2.5%	0	0	0
Total	79	1	80	54	26	80	28	52	80
Chi-square	0.315			0.577			2.834		
P value	0.854			0.750			0.092		

Table 3: Comparison of immature platelet fraction (IPF) with Platelet count on day 1,2,3 of a critical phase

IPF	Platelet count day 1		Total	Platelet count day 2		Total	Platelet count day 3		total
	Abnormal	Normal		Abnormal	Normal		Abnormal	Normal	
Normal (1% to 5%)	19	0	19	0	10	10	0	36	36
Percentage	24.1%	0%	23.7%	0%	38.5%	12.5%	0%	69.23%	45%
Abnormal (>5%)	60	1	61	54	16	70	28	16	44
Percentage	75.9%	100%	76.3%	100%	61.5%	87.5%	100%	30.67%	55%
Total	79	1	80	54	26	80	28	52	80
Chi-square	39.494			23.657			14.922		
P value	<0.001			<0.001			<0.001		

Table 4: Comparison of the mean platelet indices on three days of the critical illness period

	Mean platelet count		Mean platelet volume		Mean platelet CRIT		Mean immature platelet fraction	
Platelet count	Mean ± SD	Paired t-test P value	Mean ± SD	Paired t-test P value	Mean ± SD	Paired t-test P value	Mean ± SD	Paired t-test P value
Day 1	43536.23 ± 22348.56	(baseline)	10.46 ± 0.97	(baseline)	0.29 ± 0.16	(baseline)	14.34 ± 4.45	(baseline)
Day 2	75405.80 ± 41518.36	<0.001	10.39 ± 0.87	0.689	0.30 ± 0.16	0.582	11.08 ± 5.21	<0.001
Day 3	88187.50 ± 35589.06	<0.001	10.69 ± 1.07	0.161	0.29 ± 0.16	0.673	9.31 ± 4.43	<0.001

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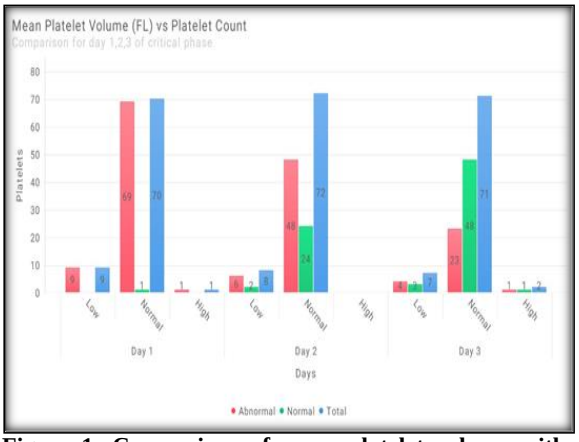

Figure 1: Comparison of mean platelet volume with platelet count

When we contrasted the platelet crit and platelet count in the 80 children (Figure 2). We discovered that there was no discernible relationship between the changes in the platelet crit and the platelet count because the majority of them remained within normal ranges (0.15 to 0.64), even when the platelet count changed ($p > 0.05$). [Table 2].

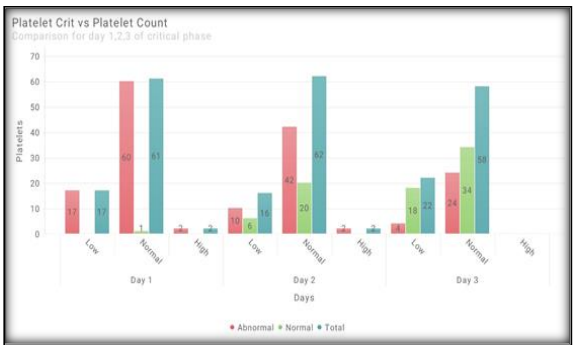

Figure 1: Comparison of platelet crit with platelet count

On the first day of the follow-up period, we noted that the change in the IPF correlates with the platelet count, which is a substantial relationship. When the platelet count is low, as it is in 80 children on day one, the immature platelet percentage is higher than the usual range (5%). (Figure 3). And this difference is statistically significant with the change in platelet count (P-value 0.001). We discovered that the change in IPF corresponded significantly with the change in platelet count when we repeated the IPF for the follow-up period for two additional days [Table 3].

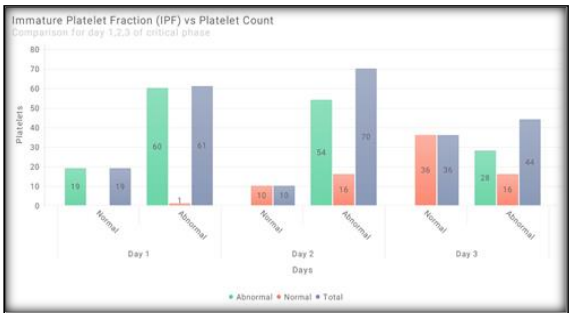

Figure 3: Comparison of IPF with platelet count

Following up on 80 kids for three days, we discovered that there had been a substantial shift in the platelet count (P-value 0.0001), and it had been trending upward as the days went on. The immature platelet percentage among the 80 kids in the three-day follow-up also changed significantly (P-value (0.0001)), and as the days went by, it trended downward (Figure 4). The other two markers, platelet volume and concentration, which remained at their normal levels during the follow-up period, were determined to be non-significant [Table 4].

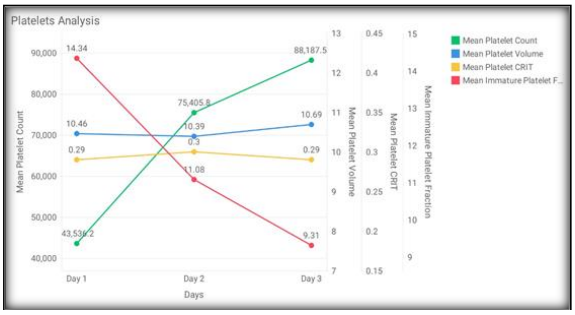

Figure 4: Trend curve comparing platelet indices with the platelet counts on three days of a critical phase

DISCUSSION

Dengue fever is a growing public health concern in most tropical countries. In India, epidemics are becoming more frequent. One of the genus flavivirus's four serotypes (DEN1, DEN2, DEN3, and DEN4) is closely related. But antigenically diverse causes dengue fever (DF) and dengue hemorrhagic fever (DHF). People who live in dengue-endemic areas have a lifetime risk of developing four different dengue infections because cross-immunity is not provided by infection with one of these serotypes.[9]

Our study showed male predominance. 38 (47.5%) were women, and 42 (52.5%) were men. According to research, DF was more prevalent in men (58%) and was most prevalent in children between 6 and 10 years old.[10] This is consistent with research conducted in Belgium.[11] In DHF instances, day three or day four of the disease is often when laboratory abnormalities like thrombocytopenia and an increasing hematocrit are noticed. One of the diagnostic criteria for DHF is thrombocytopenia, which may be infrequently seen in DF but is a recurring symptom.[12] Mean platelet volume and platelet crit, two of the platelet markers we used in our research, show no discernible relationship to platelet counts and, consequently, to patients' recovery. This is in line with the studies done by Sharma et al.[13]

A statistically significant correlation between platelet count changes and the immature platelet fraction was discovered by our investigation. During the 3-day follow-up in our research, the platelet count had a rising trend, whereas the IPF had a

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declining trend. The findings of our study are thus comparable with the results of Dadu T et al.[6] It has been established that there is a substantial (p 0.001) correlation between thrombocytopenia and dengue infection. 98% of the patients had thrombocytopenia overall.[13] The marrow stimulation to create more platelets usually causes the IPF to rise at a particular point in dengue patients, even if the platelets may be dropping. After a specific time, when the IPF has peaked, the platelets begin to rise, and the IPF starts to decline. This decline in the IPF (falling trend) is a reliable indicator of an imminent increase in platelet count if the patient is identified at this time.

Automated measurement of reticulated platelets in peripheral blood is the immature platelet fraction (% IPF), a novel parameter.[6] The reference range for IPF utilized in our study was 1-5%, which is close to the reference ranges used by Dadu et al.[6] The reference range in the study was determined to be 0.7-4.3%, which is comparable to Briggs' reference range of 1.1-6.1% of the total platelet count.[14] All 80 children displayed one or more warning signals, although stomach discomfort and vomiting were more common than other symptoms. After determining that 80 kids met the requirements for participation, blood samples were collected and submitted for analysis of the platelet markers.[1]

The absence of precise reference values for platelet indices in children is one of the study's weaknesses. Therefore, we were forced to adopt values taken from earlier adult investigations. According to research, a normal sample with IPF within the usual range had a CV of 10.09 percent. The CV was 7.89% to 10.02% for samples with low platelet count and high IPF. As would be predicted, samples with a high IPF% had lower CV.[6] The findings of a study were very similar to Briggs', where the coefficients of variation (CV) for IPF in three samples from people whose platelet counts fell within the healthy reference range ranged from 6.82% to 11.39%. For three samples with low platelet counts and high IPF, the CV ranged from 6.92% to 14.27%.[14] In the absence of generally acknowledged recommendations or reference values for the immature platelet fraction in children, we were forced to depend on the results of earlier related investigations.[15] This difference could be attributed to differences in the statistical tools used to derive the reference ranges.

The Chi-square test was used for the comparison, and the p-value was higher than 0.05. It was discovered that the immature platelet fraction among the 80 kids in the three-day follow-up showed a significant change in the IPF P-value (0.0001) and a declining trend as the day went on. There were no appreciable changes in MPV with the shift in platelet count, and the mean platelet volume was within the normal range. It has been established that there is a substantial (p0.001) correlation between thrombocytopenia and dengue infection. 98% of patients had thrombocytopenia, similar to the findings of Sharma et al.[13] A study revealed that 89% of the patients had thrombocytopenia.[16] A study showed in individuals with dengue, and there seems to be a 24-48 h lag between rising IPF levels and matching rising platelet counts. Therefore, measuring IPF should be regarded as a standard procedure to assess and track thrombocytopenia in dengue patients.[6]

The study identifies a significant relationship between IPF with an increased platelet count, which can be used to identify the prognosis of dengue. An early prompt diagnosis can help prevent the need for platelet transfusion in children.

CONCLUSION

As a result of our study, we can say that the immature platelet percentage significantly correlates with the increase in platelet count, one of the key indicators of dengue recovery. Inversely associated patterns include declining IPF and increasing platelet count. The immature platelet fraction can be employed early to forecast platelet recovery and prevent unnecessary transfusions. As a result, IPF should be measured often to assess and track thrombocytopenia and its recovery in dengue patients.

Limitations

There is a lack of information and availability of data to be used as a reference for platelet indices among children; hence, we have used the derived values from the previous study conducted among adult patients.

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