Original Article

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Patterns of Neonatal Thrombocytopenia with A Note on Platelet Indices and Optical Technology – A Cross-Sectional Study

Uma Rani Pallivilla*, Vahini Gudeli, Maadhurika Ledalla and Rajendra Jalagam

Alluri Sitaram Raju academy of Medical Sciences, Eluru.

Abstract

Introduction: Neonatal thrombocytopenia is defined as a platelet count <150 × 109/L regardless of gestational age. It results from hypo-proliferation in marrow or peripheral destruction of platelets. Platelet count can be rapidly measured using automated hematology analyzers, but peripheral smear remains the best method. The causes of neonatal thrombocytopenia are defined by time of presentation into foetal (mainly TORCH infections), early (< 3 days), and late. The present study highlights the pattern and severity of neonatal thrombocytopenia in the study hospital, adding the importance of platelet indices and optical technology.

Aim: To study the patterns and severity of neonatal thrombocytopenia, platelet indices and to measure the accuracy of platelet count by optical technology methods against peripheral smear.

Materials and methods: A cross-sectional study done for a period of 8 months from December 2018 to July 2019, at ASRAM medical college, Eluru. During this period the blood samples of 113 critical cases of newborns, admitted with thrombocytopenia, were collected in Ethylenediamine tetra acetic acid (EDTA) vials. The platelet count was analyzed by two automated analyzers, Sysmex XN1000 and Horiba ABX Pentra XL 80. The Leishman-stained films were examined under a light microscope. ANOVA test was used to find the mean difference between platelet counts, sensitivity, and specificity, and accuracy was calculated by MEDCALC CALCULATOR.

Results: Out of the total of 113 critical cases of new-borns admitted to Neonatal intensive care unit (NICU) at the institute, 85 presented with thrombocytopenia. The variation of platelet indices was noted in 40 cases, blood cultures were collected in 77 cases. Thrombocytopenia showing platelet count less than 20,000/ mm3 is considered very severe, with 30.5% (26 cases) of the total number. Elevated platelet indices were noted at 47.5%. The common clinical diagnosis was neonatal sepsis (42.3%), followed by neonatal jaundice (18.8%). The light scatterer principle of platelet evaluation proved to have better accuracy than electrical impedance, in comparison to peripheral smear findings.

Conclusion: The study concludes that neonatal septicemia is the major cause of neonatal thrombocytopenia as proved by correlating platelet count with platelet indices. And also, the usage of automated hemogram report with platelet indices, as a source of information to suspect the etiology of thrombocytopenia there by preventing adverse outcomes. The principle of the Optical Light scatterer technique in an automated analyzer gives better results than the electrical impedance technique for detecting platelet count value, though peripheral smear examination is mandatory for confirmation.

Keywords: New-born, Platelet count, Light scatterer, Electrical impedance.

Introduction

Thrombocytopenia is defined as a platelet count below 150 ×109/L. In neonates, the most frequent causes of thrombocytopenia are infection, birth asphyxia, preterm, meconium aspiration syndrome, intrauterine growth retardation, immune-related diseases, and respiratory distress syndrome [1]. It increases mortality, especially in septicemic cases. Multiple disease processes cause thrombocytopenia in neonates and these are classified as early-onset (<72 hours) and late-onset (>72 hours) neonatal thrombocytopenia [2].

Thrombopoiesis is a complex process, that results in the production of thrombopoietin as a stimulus for the generation and proliferation of megakaryocyte progenitors. Platelet transfusions (PT) in neonatal thrombocytopenia (NT) are commonly administered to reduce the risk of bleeding. However, there are few evidence-based guidelines to inform clinicians' decision-making processes [3]. Sepsis

is not a homogenous entity, as it derelicts the pathogenic and clinical differences between the various causative micro-organisms, clinical syndromes, and presentations of neonatal sepsis [4].

Detection of thrombocytopenia is a useful initial assessment for every sick neonate as it is considered one of the complications of the disease process, however in a few cases, it is noted accidentally. Thrombocytopenia is so prevalent that it is often ignored and assumed that it will resolve spontaneously. However, if not detected early it can result in devastating complications [5,6].

Platelet indices such as MPV, PDW, and plateletcrit may be a vital marker for the identification of haemostatic disorders in neonates [7]. The frequency of thrombocytopenia and its association with platelet indices in culture-proven neonatal septicemic patients were noted in a previous study [8]. Obtaining accurate, precise, and reliable platelet count with calibrated haematology analyzers is observed generally, but different haematology analyzers give varying results makes comparison difficult [9]. Traditional microscope methods give the most reliable results. Thereby, in many disorders, platelet count estimation appears to be an important element of the diagnostic and treatment process.

There is no existing concrete definition for mild, moderate, and severe thrombocytopenia. Usually, it is considered that platelet counts higher than 50x109/L do not cause any clinical problems unless platelet dysfunction coexists. Medical help is usually required when platelet counts are less than 30x109/L or patient suffering from spontaneous bruising. However, clinically significant bleeding does not usually occur until the platelet count is less than 10x109/L [8,10]. The clinical findings usually include petechiae, purpura, ecchymosis, hematuria, intestinal and respiratory haemorrhage, and intracranial haemorrhage [11].

The evaluation and management of thrombocytopenia, a marker of the underlying disease is a frequent challenge for neonatologists [12]. Though it is frequent among sick neonates, very little is known about the underlying pathogenetic mechanism of thrombocytopenia [13,14].

Increased platelet consumption or sequestration is considered the major mechanisms in about 25–35% of cases of neonatal thrombocytopenia. Overall, 15–20% of neonatal thrombocytopenias present at birth mostly result from the transplacental passage of maternal platelet alloantibodies and autoantibodies [15].

In the present study, platelet count less than 20x109/L was considered severe thrombocytopenia [4], and a comparison of the platelet count in haematology analyzers working with different working principles was observed.

Automated counting appears controversial, especially in the thrombocytopenic samples or other cases with morphological abnormalities of platelet aggregates or any other small particles mimicking platelet particles like debris and red cell fragments could generate electrical or optical signals [16].In patients with abnormal thrombocytes where platelet transfusion is required, the reliability of platelet count is necessary for appropriate treatment along with elevated platelet indices which indicates a propensity for platelet activation [17].

The current study was done to estimate the patterns, prevalence, severity, and etiological factors of neonatal thrombocytopenia, making a note of the importance of platelet indices along with evaluation of the accurate method for platelet count estimation among two automated haematology analyzers.

Material and methods

This cross-sectional study was conducted from December 2018 to July 2019 at ASRAM medical college, Eluru. Ethics committee approval was taken bearing number IEC/ASR/APPROVAL NO/35/2019.

Inclusion criteria: All the newborns, less than 4 weeks, presenting with thrombocytopenia were included in the study.

Exclusion criteria: Babies who died and babies with normal platelet count were excluded from the study. The 85 samples reported in this study were free of platelet clumps, hence errors due to falsely low platelet count were eliminated.

Sample size calculation: n=4pq/l2 where prevalence was taken as 60% similar to a previous study reported by Nandyal SS et al [3].

P=60%, q=100-p, 100-60=40, q=40 and error were taken as 18%. 18% of p= 10.8 so, L2= 116. N=4x60x40/116= 82. Finally, rounded off to 85 subjects who were enrolled to compensate for probable dropouts.

113 critically ill neonates of which 85 presented with thrombocytopenia were included in the study. Cases with less than 20,000/L platelet count were considered very severe thrombocytopenia cases, similar to a previous study by Ree IM et al [4].

The bacterial culture reports by the agar plate method were

also collected in 77 cases (90.5%).

Venous blood was collected from each neonate into an ethylenediaminetetraacetic acid (EDTA) anticoagulant and kept on a roller mixer for constant mixing. The platelet count of the sample was studied in two automated analyzers i.e Sysmex XN1000 and Horiba ABX pentra XL 80. Leishman-stained blood films were examined under the light microscope, to rule out platelet clumps and ensure that the platelets were spread evenly before the actual count was reported. Changes occurring in Mean platelet volume (MPV), Platelet distribution width (PDW), and plateletcrit values were obtained in both the automated analyzers.

The platelet count value for every case is observed with two analyzers having different techniques and finalizing the platelet count value with peripheral smear. It is considered positive if the platelet count is less than 20,000/cumm.

Date of admission, diagnosis, platelet count, MPV (Mean Platelet value), PDW (Platelet Distribution width), and blood culture reports were recorded on a data form. The provisional clinical diagnosis was provided for every case by the pediatric department.

Statistical analysis: The data was extended into excel 2000 and analysis was done by Medcalc 20.006 (trial version), sensitivity, specificity, and ANOVA were calculated. The sensitivity of Sysmex XN 1500 (light scatterer technology) obtained was 100.00% and specificity was 53.85%, whereas the sensitivity of Horiba Pentre (Electrical impedance) was 93.85% and specificity was 7.69%. Thereby the accuracy of the Platelet count values observed by the Sysmex analyzer is 81.54% compared to Horiba's 59.38%.

A p-value of less than 0.05 was considered statistically significant. For measuring thrombocytopenia peripheral smear is taken as a standard method.

Result

Out of 113 consecutive neonatal admissions in NICU at a tertiary health care hospital, 85 neonates were found to have thrombocytopenia due to various etiological factors as per the blood samples analyzed.

The overall prevalence of neonatal thrombocytopenia was 75%.

Among neonates with thrombocytopenia 19 cases (22.3%) had mild thrombocytopenia (platelets count >1-1.5 lakh/µL), 30 cases (35.2%) had moderate thrombocytopenia (platelets count 51,000 - ≤1lakh/µL), 10 (11.7%) had severe thrombocytopenia (platelets count21,000 - 50,000/µL) and 26 neonates (30.5%) had very severe thrombocytopenia (platelets count ≤20,000/µL) [table1].

Early-onset thrombocytopenia (< 3days of age) was seen in 40 (47.1%) cases and late-onset thrombocytopenia (3-28 days) in 45 (52.9%) cases.

Male preponderance was noted, with 44 (51.7%) male babies admitted to NICU while female babies were about 41 (48.2%) [table2]. The most common causes of neonatal thrombocytopenia were neonatal septicaemia (42.3%) followed by neonatal jaundice (18.8%) and respiratory distress syndrome (17.6%) [table2].

The platelet indices were elevated in 43cases (50.5%) cases (Table 3). Of the 43cases, elevation of MPV (9-13fl) was observed in 24 cases (28.2%), elevated PDW (9-17fl) was observed in 39cases (45.8%) and plateletcrit (0.22-0.24%) was noted in 7cases (8.2%).

The above variation was most observed in thrombocytopenic sick newborns associated with neonatal sepsis.

Table 1: The severity of Neonatal Thrombocytopenia

	Mild Thrombocytopenia (>1.01-1.49 lakh/µl)	Moderate Thrombocytopenia (51,000 -1 lakh/µl)	Severe Thrombocytopenia (21,000 - 50,000/µl)	Very Severe Thrombocytopenia (≤20,000/µl)	Total	Mean ±Sd (Platelet Count In Lakhs)
D1-7	16 (18.8%)	20 (23.5%)	9 (10.5%)	19 (22.3%)	64 (75.2%)	0.7582±0.7852
D 8-14	2 (2.3%)	7 (8.2%)	1 (1.1%)	7 (8.2%)	17 (20%)	0.5876±0.4378
D 15 – 21	-	1 (1.1%)	-	-	1 (1.1%)	
D22 -28	1 (1.1%)	2 (2.3%)	-	-	3 (3.4%)	1.1±0.1732
Total	19 (22.3%)	30 (35.2%)	10 (11.7%)	26 (30.5%)	85	-
Mean ±SD	1.2578±0.08377	1.0466 ±0.9107	0.39±0.8755	0.1392± 0.5183	-	-

The other common conditions showing elevated platelet indices were empyema, meconium aspiration syndrome, immune thrombocytopenic purpura, disseminated intravascular coagulation, and hypoxic-ischemic encephalopathy (table 3).

It was observed that the mean and SD between Horiba (54692.31+6.76E+09), Sysmex (80483.52+1.77E+10) and peripheral smear (140186.8+6.61E+10) greatly varied[table/fig4]. Thereby a comparison between the platelet count values obtained from two different haematology analyzers, taking peripheral smear as a standard. (Table 5). The sensitivity and specificity for evaluating the best technology for platelet count measuring were done. The study showed statistical significance with a P-value of 0.003 (table 4).

65 out of 85 cases showed thrombocytopenia and were considered positive, using Light scatterer technology when compared with peripheral smear. Whereas 61 of 85 patients showed thrombocytopenia using electrical impedance technology when compared with peripheral smear taking it as standard assessment [table 5]. Thereby sensitivity of Sysmex XN 1500 (light scatterer technology) obtained was 100.00% and specificity is 53.85%, whereas the sensitivity of Horiba Pentra (Electrical impedance) is 93.85% and specificity is 7.69%. Thereby the accuracy of the Platelet count values obtained by Sysmex is 81.54%, compared to Horiba is 59.38%.

Table 2: Gender characteristics and provisional clinical diagnosis of the neonates

Characteristics	Number of cases (%)
Gender	
Male	44 (51.7%)
Female	41 (48.2 %)
Clinical diagnosis	
Neonatal sepsis	36 (42.3%)
Respiratory distress syndrome	15 (17.6%)
Neonatal jaundice	16 (18.8%)
Perinatal asphyxia	9 (10.6%)
Meconium aspiration syndrome	5 (5.8 %)
Others*	4 (4.7%)
*** Two cases of immune thrombocytopenic purpura, one case of severe anaemia, one case of Dengue**	

Table 3: Distribution of cases with variation in platelet indices:

Clinical conditions with Elevated platelet indices	No. of cases (%)	Clinical conditions with Normal platelet indices	No. of Cases (%)
Empyema	2 (2.4%)	Status epilepticus	2 (2.4%)
Neonatal sepsis	33 (38.8%)	Respiratory distress syndrome	16 (18.8%)
Meconium aspiration syndrome	2 (2.4%)	Perinatal asphyxia	4 (4.7%)
ITP	2 (2.4%)	Others	18 (21%)
DIC	2 (2.4%)	NEC	2 (2.4%)
HIE	2 (2.4%)		

Table 4: Variation in platelet counts among three methods.

Groups	*Count*	*Sum*	*Average*	*Variance*		
Platelet count by Horiba	91	4977000	54692.31	6.76E+09		
Platelet count by Sysmex	91	7324000	80483.52	1.77E+10		
Platelet count by Peripheral Smear Examination	91	12757000	140186.8	6.61E+10		
ANOVA						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.50E+11	2	1.75E+11	5.794488	0.00344	3.02922
Within Groups	8.15E+12	270	3.02E+10			
Total	8.50E+12	272				

Culture reports were observed in 77 cases (90.5%) of which 32 (41.6%) were gram-positive, 8 (10.4%) were gram-negative and 37 (48%) were sterile.

Table 5: Comparison of Optical technology methods with Peripheral smear test.

Light scatter method	Peripheral smear-positive	Peripheral smear-negative	Total
Thrombocytopenia present	65	12	77
Thrombocytopenia absent	0	14	14
Total	65	26	91
Electrical impedance method			
Thrombocytopenia present	61	24	85
Thrombocytopenia absent	4	2	6
Total	65	26	91

Discussion

Thrombocytopenia is one of the most common haematological abnormalities noted in NICU, as it can be caused due multiple pathological mechanisms. Clinically presentation varies from mild bleeding manifestations to DIC. There is a high prevalence (60%) of neonatal thrombocytopenia in the present study, which is in concordance with the study done by Sonam S et al [6] (63.8%).

Table 6: Comparison of Patterns of neonatal thrombocytopenia

Studies	Early (< 3 days)	Late (3-28days)
Meena SL et al [2]	51 (51%)	49 (49%)
Elisabeth et al [3]	128 (73%)	47 (27%)
Sonam S et al [6]	43 (43.4%)	56 (56.5%)
Khalessi et al [11]	42 (67.7%)	20 (32.3%)
Jeremiah et al [13]	112 (84.84%)	20 (15.16%)
Present study	40 (47.1%)	45 (52.9%)

Neonates presenting with thrombocytopenia were more in the late neonatal age group comprising 45 (52.9%), similar to Sonam et al study 56 (56.5%) [6], whereas other studies like Khalessi et al [11] documented 42 (67.7%) and Jeremiah et al [13] documented 112 (84.84%) thrombocytopenia cases in early neonatal age group (table 6).

It is observed that M: F ratio was 1:1 in the present study, whereas some other studies showed it to be 1.8:1 and 1.4:1 with slight male preponderance [1,7] [table7].

Etiological profile in the present study showed 36 (42.3%) cases presenting with neonatal septicemia as the most

Table 7: Comparison of present study findings with other related studies.

Characteristics	Present study	Jeremiah et al study [13]	Elisabeth et al study [3]	M Sandeep et al study [7]	Meena sl et al. [2]	Kripa Nath Mishra et al [1]	Sonam s et al [6]
Gender							
Male	44 (51.7%)	56 (42.4%)	-	35 (58.3%)	-	65 (65%)	-
Female	41 (48.2%)	76 (57.6%)	-	25 (41.67%)	-	35 (35%)	-
Clinical diagnosis							
Neonatal sepsis	36 (42.3%)	22 (16.7%)	175 (47%)	-	53 (53%)	64 (64%)	22 (22.2%)
Respiratory distress syndrome	15 (17.6%)	-	-	-	15 (15%)	18 (16.7%)	14 (14.1%)
Neonatal jaundice	16 (18.8%)	26 (19.7%)	-	-	6 (6%)	18 (16.6%)	5 (5%)
Perinatal asphyxia	9 (10.6%)	44 (33.3%)	95 (25%)	-	11 (11%)	-	8 (8%)
Meconium aspiration syndrome	5 (5.8 %)	-		-	10 (10%)	-	7 (7 %)
Necrotizing enterocolitis	-	-	16 (4.1%)	-	5 (5%)	-	-
Congenital anomalies	-	-	15 (3.9%)	-	-	-	-
Prematurity							38 (38.3%)
Others	4 (4.7%)	14 (10.6%)	34 (8.9%)	-	-	-	43

common cause of neonatal thrombocytopenia followed by neonatal jaundice (18.8%) similar to Elisabeth et al [3], Meena SL et al [2] and Kripa Nath Mishra et al [1]. According to Jeremiah et al [13], birth asphyxia (33.3%) was most common which is followed by jaundice (19.7%).

As a part of supplementary analysis elevation of platelet

indices like PDW, MPV and PLT were noted in 43 cases. The platelet indices especially MPV are a measure of the average size of platelet particles in the blood. PDW is an indicator of variation in platelet size [4,5]. Present study showed elevated PDW (45.8%), MPV (28.2%) and PLT (8.2%). Thereby PDW appears more sensitive to estimating changes in platelet size like Sandeep et al study [5].

In the present study, 26 cases (30.5%) of very severe thrombocytopenia were observed in comparison with Ree IMC et al [4] who noted 34 cases (7%). Monitoring critically ill neonates presenting with thrombocytopenia is very much essential as any of them can clinically deteriorate. Peripheral smear is known as the best method for platelet count monitoring, however, of the two automated analysers, SysmexXN1500 (optical light scatterer technology) has given optimal platelet count value and appears more sensitive.

Automated analyser I (Sysmex XN1500) works on the principle of the fluorescence-based light scatterer and other Automated analysers II (Horiba Pentra ABX XL80) work on the principle of electrical impedance [1s7,18,19]. In cases where clinicians would take the risk of waiting for platelet transfusion when the platelet count is less than 10,000/L, the light scatterer technology will be much helpful.

Raimundo et al [15] observed that the use of multiple light scatterer technology rather than impedance technology had improved the ability to differentiate platelet particles, which is similar to our study findings.

Limitations:

Out of the total samples sent for the culture, we received culture reports for 77 cases only and it was observed that there is contamination of some samples. Thereby culture reports were excluded from the study.

Conclusion

Neonatal thrombocytopenia is the most common clinical condition in NICU and it could be used as a prognostic marker for various underlying pathogenetic mechanisms. The prevalence of neonatal thrombocytopenia is 60% and the most common etiological factor was neonatal septicemia. Late-onset neonatal thrombocytopenia (52.9%) cases were slightly more common than early-onset neonatal thrombocytopenia in the present study. The increasing number of severe and very severe thrombocytopenia cases alerts us to monitor every case admitted to NICU, due to varied etiopathogenetic mechanisms resulting in thrombocytopenia. PDW helps in the process. Sysmex XN 1500 (Light scatterer technique) is more sensitive in platelet count measurement and monitoring thrombocytopenia cases.

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Corresponding Author:

Dr P. Uma Rani, Professor of Pathology,
Alluri Sitaram Raju academy of Medical Sciences,
Eluru.

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