Original Article

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Paediatrics Section

Quantitative Analysis of Thrombocytes in Neonates in a Tertiary Care Centre of Southern India- A Retrospective Study

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ABSTRACT

Introduction: Thrombocytopenia is defined as a platelet count of less than 150×10^9/L, which is also applicable to newborn infants. It may be a result of increased platelet destruction (immune and non immune), decreased platelet production and a combination of both or unknown. A platelet count of more than 500×10^9/L is considered thrombocytosis, classified as primary or essential and secondary or reactive. Abnormal platelet count being quite common, is most often neglected and it can lead to devastating complications if untreated.

Aim: To study the incidence of abnormal platelet counts and to determine the risk factors associated with abnormal platelet counts in neonates admitted to the Neonatal Intensive Care Unit (NICU).

Materials and Methods: A retrospective analysis of platelet count of all neonates admitted to a tertiary NICU of RL Jalappa Hospital, Tamaka, Karnataka, India, between July 2020 to June 2021 was done to determine the incidence and risk factors associated with abnormal platelet count (thrombocytopenia and thrombocytosis). Data of all the neonates were collected retrospectively from July to August 2021 and analysed. A total of 562 neonates were included in the study. Categorical data was taken and expressed in the form of frequencies and percentages. Data were entered as frequencies using Microsoft excel version 2203 and analysed using Statistical Package for Social Sciences (SPSS) software version 22.0. Chi-square and Fischer's exact test were used to determining the significance of the data. The p-value <0.05 was considered statistically significant.

Results: Out of 629 neonates admitted, 562 neonates were included in the study. Total 209 neonates had thrombocytopenia, with early thrombocytopenia being in 94 (45%) and late being in 115 (55%). Thrombocytosis was seen in only 15 neonates where 7 (46.6%) had early, and 8 (53.3%) had late thrombocytosis. The most common risk factor associated with both cases was sepsis. Placental insufficiency had a statistically significant association with thrombocytopenia (p<0.001**). Term babies had a higher incidence of thrombocytopenia and thrombocytosis and the association with thrombocytopenia was statistically significant (p=0.043*).

Conclusion: Based on the present study, it can be concluded that abnormal platelet count is quite a common finding in sick neonates. Thrombocytopenia is more common than thrombocytosis. The most common risk factor is sepsis, it should not be neglected, and appropriate treatment should be initiated once an abnormal platelet count is detected in neonates.

Keywords: Platelet count, Sepsis, Thrombocytopenia, Thrombocytosis

INTRODUCTION

Platelet count abnormality, especially thrombocytopenia, is a common finding in neonates admitted to the Neonatal Intensive Care Unit (NICU), but thrombocytosis is unusual in the neonatal period. Thrombocytopenia is most common in sick newborns and preterm babies. The incidence of thrombocytopenia is about 1-5% at birth, but in sick neonates, the incidence is about 20-50%. Thrombocytopenia is defined as a platelet count of less than 150×10^9/L, which is also applicable to newborn infants [1]. Severe thrombocytopenia is a platelet count of less than 50×10^9/L seen in only 0.1-0.5% of newborns [1,2].

Causes of thrombocytopenia could be due to increased destruction or decreased production. Increased destruction might be because of immune causes, which include autoantibodies (primary immune thrombocytopenia or secondary to conditions like Systemic Lupus Erythematosus (SLE) or alloantibodies (neonatal alloimmune thrombocytopenia). The most common non immune causes are intrauterine infections, severe hyperbilirubinaemia severe haemolytic disease of newborns, polycythemia with hyperviscosity syndrome, hypersplenism, disseminated intravascular coagulation, necrotising enterocolitis, intrauterine growth restriction, among others. Decreased production of platelets is attributable to bone marrow failure conditions [2,3].

A platelet count of more than 500×10^9/L is considered thrombocytosis, classified as primary or essential and secondary or reactive. Thrombocytosis is more common in very preterm neonates who are primarily reactive and has an incidence of about 3-13% [4-6]. The exact mechanism and aetiology of thrombocytosis are not truly clear. It is postulated that low Thrombopoietin (TPO) clearance in neonates leads to increased levels of TPO, resulting in subsequent thrombocytopenia [6].

Abnormal platelet count being quite common, is most often neglected and it can lead to devastating complications, if untreated. Thrombocytosis, being less common, has limited literature regarding its prevalence and risk factors [5,6]. Understanding the associated factors is much needed to initiate imminent treatment. Hence, this study was undertaken to determine the incidence and the risk factors of abnormal platelet counts in the tertiary NICU of RL Jalappa Hospital, Tamaka, Karnataka, India.

MATERIALS AND METHODS

The present retrospective observational study was done in the tertiary NICU of RL Jalappa Hospital, Tamaka, Karnataka, India. The study was proceeded after taking the institutional ethical clearance, with the certificate number being DMC/KLR/IEC/393/2021-22.

Inclusion criteria: All the inborn and outborn neonates were admitted to NICU for over one year from July 2020 to June 2021 were included in the study.

Exclusion criteria: Those neonates in whom the investigations could not be done or Complete Blood Count (CBC) was done less than twice were excluded. The reasons might be early discharge, parental unwillingness continue the treatment or death or referral to a higher centre.

Data Collection

Investigations of the study patients, like CBC, C-Reactive Protein (CRP), blood culture and Arterial Blood Gas (ABG) analysis were collected. Toxoplasmosis, Rubella Cytomegalovirus, Herpessimplex, and HIV (TORCH) screening and screening for immune thrombocytopenia were done in selected cases. The CBC was done at least twice to determine the onset of abnormal platelet count, whether early or late. The data were collected retrospectively between the period July to August 2021, using predetermined, internally validated proforma by review of case sheets and the reports were accessed through a web-based, comprehensive hospital information management system, using the Unique Hospital Identification (UHID) a number of each patient. The incidence of early and late onset abnormal platelet count (thrombocytopenia and thrombocytosis) over one year was calculated. The association with common risk factors like placental insufficiency, sepsis including TORCH infections, perinatal asphyxia, gestational diabetes mellitus, and immune and non immune causes of thrombocytopenia was calculated. These were again stratified based on the age of onset and gender.

Thrombocytopenia: Thrombocytopenia was defined as a platelet count of less than 150×10^9/L, which is also applicable to newborn infants [1].

Early onset thrombocytopenia: onset within 72 hours

Late onset thrombocytopenia: onset after 72 hours

Thrombocytosis: A platelet count of more than 500×10^9/L was considered as thrombocytosis [6].

Early Onset thrombocytosis: onset within 72 hours

Late Onset thrombocytosis: onset after 72 hours

Probable sepsis: Those with C-Reactive Protein (CRP) positive or White Blood Cell (WBC) count >20,000/mm^3 or WBC count <5,000/mm^3.

Culture proven sepsis: Those with blood culture growing any organism within five days of incubation.

TORCH (Toxoplasmosis, Other agents, Rubella, Cytomegalovirus, Herpes Simplex Virus) infections: Those with clinical features of or laboratory evidence of TORCH infections.

Placental insufficiency: Those who were born Small for Gestational Age (SGA) or Intra Uterine Growth Retardation (IUGR) or with doppler changes in antenatal doppler scan suggestive of placental insufficiency.

Perinatal asphyxia: The guidelines of the American Academy of Paediatrics (AAP) and the American College of Obstetrics and Gynaecology (ACOG) consider all of the following criteria in diagnosing asphyxia:

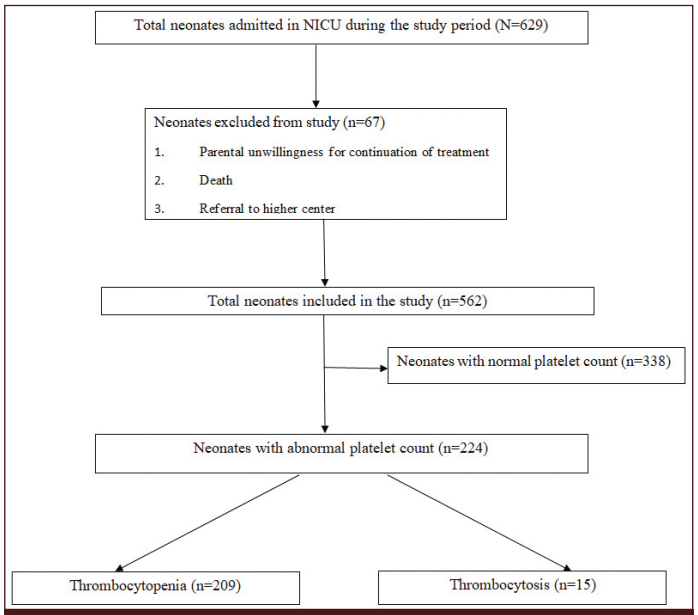
- Profound metabolic or mixed acidemia (pH <7.00) in an umbilical artery blood sample, if obtained.
- Persistence of an Apgar score of 0-3 for longer than five minutes.
- Neonatal neurologic sequelae (e.g., seizures, coma, hypotonia).
- Multiple organs involvement [7,8].

STATISTICAL ANALYSIS

Data were entered as frequencies and analysed using Microsoft excel version 2203. Incidence was computed in the form of percentages. Categorical data was represented in the form of tables. Data were analysed using Statistical Package for Social Sciences (SPSS) software version 22.0. Categorical data was represented in the form of frequencies and percentages. The Chi-square test or Fischer's exact test (for 2×2 tables only) was used as a test of significance for qualitative data. The p-value <0.05 (Probability that the result is true) was considered statistically significant after assuming all the rules of statistical tests.

RESULTS

The total number of neonates admitted to NICU during the study period was 629, out of which 562 were included in the study. The [Table/Fig-1] shows the flow diagram depicting the number of neonates included in the study, along with the proportion of neonates, with thrombocytopenia and thrombocytosis.



[Table/Fig-1]: Flow diagram depicting the number of neonates included in the present study, along with the proportion of neonates, with thrombocytopenia and thrombocytosis.

Abnormal platelet count was seen in 224 neonates and 338 neonates had normal platelet counts. In the present study, 209 neonates had thrombocytopenia (early and late), accounting for 37% of neonates included in the study. Among the neonates with thrombocytopenia, early and late thrombocytopenia was noted in 94 (45%) and 115 (55%) neonates, respectively. Thrombocytosis was seen in only fifteen neonates accounting for 2.6% of neonates in the study; early and late thrombocytosis was seen in 7 (46.6%) and 8 (53.3%) neonates, respectively. Males had a slightly higher incidence of platelet abnormality, thrombocytopenia and thrombocytosis. However, in the present study, the incidence of thrombocytopenia was higher than thrombocytosis [Table/Fig-2].

Sepsis was the major risk factor for thrombocytopenia, followed by placental insufficiency, perinatal asphyxia and gestational diabetes mellitus. Placental insufficiency had a statistically significant association with thrombocytopenia (p<0.001**). There were no cases of TORCH infections or congenital anomalies or autoimmune conditions [Table/Fig-3].

Demographic details of neonates	Number (%)
Neonates included in the study (n=562)	
Males	317 (56.4%)
Females	245 (43.6%)
Thrombocytopenia (n=209)	
a. Early onset thrombocytopenia (n=94)	
Males	64 (68%)
Females	30 (32%)
b. Late onset thrombocytopenia (n=115)	
Males	66 (57.3%)
Females	49 (42.6%)
Thrombocytosis (n=15)	
a. Early onset thrombocytosis (n=7)	
Males	6 (85.7%)
Females	1 (14.2%)
b. Late onset thrombocytosis (n=8)	
Males	4 (50%)
Female	4 (50%)

[Table/Fig-2]: Neonatal demographic characteristics.

Risk factors	Thrombocytopenia (n=209)		p-value
	Early onset n=94 (45%)	Late onset n=115 (55%)	
Sepsis	72 (76.6%)	97 (84.3%)	0.156
a. Probable sepsis	71 (75.5%)	92 (80%)	
b. Culture proven sepsis	1 (1%)	5 (4.3%)	
c. TORCH infections	-	-	
Placental insufficiency	15 (16%)	4 (3.4%)	<0.001**
Perinatal asphyxia	6 (6.3%)	12 (10.4%)	0.298
Infant of diabetic mother	1 (1%)	2 (1.7%)	0.683
Congenital anomalies	-	-	-
Autoimmune conditions	-	-	-
Risk factors	**Thrombocytosis (n=15)**		**p-value**
	Early onset n=7 (46.7%)	**Late onset n=8 (53.3%)**	
Sepsis	6 (85.7%)	6 (75%)	0.604
a. Probable sepsis	6 (85.7%)	5 (62.5%)	
b. Culture proven sepsis	-	1 (12.5%)	
c. TORCH infections	-	-	
Placental insufficiency	1 (14.2%)	1 (12.5%)	0.919
Perinatal asphyxia	-	1 (12.5%)	-
Infant of diabetic mother	-	-	-
Congenital anomalies	-	-	-
Autoimmune conditions	-	-	-

[Table/Fig-3]: Risk factors for abnormal platelet counts.

Thrombocytosis was mainly associated with sepsis [Table/Fig-3]. The abnormal platelet count could be observed in different gestational period. In which the term babies had higher incidence of thrombocytopenia and thrombocytosis with the association of thrombocytopenia p=0.043, which is statistically significant [Table/Fig-4].

Gestation	Thrombocytopenia (n=209)		p-value
	Early onset n=94 (45%)	Late onset n=115 (55%)	
Preterm (<34 weeks)	23 (24.4%)	47 (40.8%)	0.043*
Late preterm (34-36 weeks 6 days)	7 (7.4%)	7 (6%)	
Term (>37 weeks)	64 (68%)	61 (53%)	
Gestation	**Thrombocytosis (n=15)**		**p-value**
	Early onset n=7 (46.7%)	**Late onset n=8 (53.3%)**	
Preterm (<34 weeks)	1 (14.2%)	3 (37.5%)	0.683
Late preterm (34-36 weeks 6 days)	1 (14.2%)	0	
Term (>37 weeks)	5 (71.4%)	5 (62.5%)	-

[Table/Fig-4]: Abnormal platelet count in relation to gestational age. As per gestational age, term babies had higher incidence of thrombocytopenia as well as thrombocytosis and the association with thrombocytopenia was statistically significant (p=0.043*)

DISCUSSION

Platelet count is an essential determinant of neonatal outcome in neonates admitted to NICU; thrombocytopenia is one of the frequent haematological abnormalities in the NICU [1]. The present study had 562 neonates, among which 224 neonates had abnormal platelet counts and the remaining 338 neonates had normal platelet counts. Among 224 neonates, 209 neonates had thrombocytopenia and 15 had thrombocytosis. As per present study analysis, the incidence of thrombocytopenia in neonates admitted to NICU during the study period was 37%. Baer VL et al., had studied the incidence of thrombocytopenia and found it to be 22%, whereas a similar study done by Gupta A et al., had an incidence of thrombocytopenia of 70% in neonates admitted to NICU [9,10]. A similar study by Eslami Z et al., found an incidence of thrombocytopenia of 28.5%, while Gupta AK et al., found the incidence of neonatal thrombocytopenia to be 16.7% [11,12]. Ayadi ID et al., had an incidence of 12.4% of neonatal thrombocytopenia on observing 808 neonates admitted to the NICU [13]. Rathi P, has found in his study an incidence of 27.2% of neonatal thrombocytopenia [14]. This wide variation in the incidence of thrombocytopenia from 12-70% in different studies was probably due to confounding factors associated with thrombocytopenia. Nevertheless, thrombocytopenia has been a common finding in neonates admitted to NICU.

The incidence of thrombocytosis in the present study was found to be 3% which was comparable with other studies having an incidence of 3-13% [6]. Matsubara K et al., evaluated causes for thrombocytosis in children less than five years and found an incidence of 12.5% in the neonatal period, which peaked at one month of age [15]. The wide range of thrombocytosis may be due to the scarcity of literature regarding the condition [5,6,15]. The mechanism of increased platelet count is also not clear. Overall, it is less commonly seen compared to thrombocytopenia.

The current study showed that thrombocytopenia was more common in male neonates than in female neonates, both in the early and late thrombocytopenia group (68% vs 32% and 57% vs 43% respectively). A study done by Rathi P, found an incidence of neonatal thrombocytopenia was 59% among male babies as compared to 41% among female neonates [14]. There was no significant gender difference in any of the above studies [11-13]. As in the present study, the proportion of male babies were more at the beginning of the study, which may have led to this finding. Based on the findings, there was not much variation in the gender and the incidence of low platelet count, and the difference seen was not found to be statistically significant.

In the present study, the incidence of late onset thrombocytopenia 41% was more compared to early onset thrombocytopenia 24% among preterm babies. It could be because most of the preterm neonates had sepsis as the confounding factor for thrombocytopenia. Rathi P, had an incidence of 45% among preterm neonates while 55% of term neonates [14]. Resch E et al., in a similar study found that the early onset thrombocytopenia was 87.1% and late onset thrombocytopenia in 76% of preterm neonates [16]. Among term neonates with thrombocytopenia, the incidence of early onset thrombocytopenia was more compared to late onset thrombocytopenia as most of our term neonates had either placental insufficiency or hypoxic event during the delivery as the cause for thrombocytopenia. In other studies, placental insufficiency and perinatal asphyxia are the most important cause of early onset thrombocytopenia. In another study done by Gebreselassie HA et al., they found the incidence of neonatal thrombocytopenia to be 55.8%, with the majority being late onset thrombocytopenia secondary to sepsis. They also concluded prematurity, maternal hypertension and low birth weight as other risk factors for thrombocytopenia [17].

In a study done by Pulmamidi RK and Yendamuri RM, they found that the early onset neonatal thrombocytopenia was 47% and late onset thrombocytopenia was 53%, with sepsis, birth asphyxia and prematurity being the important causes [18]. One of the studies, found sepsis, placental insufficiency and asphyxia as the major causes of early onset thrombocytopenia, while sepsis was the major cause of late onset thrombocytopenia [19]. Rathi P, in his study found early and late onset thrombocytopenia to be 56% and 44% respectively; prematurity, sepsis, respiratory distress and placental insufficiency was the crucial cause [14]. A study was done by Tirupathi K et al., found early onset thrombocytopenia of 44% and late onset thrombocytopenia of 56%, during sepsis and birth asphyxia accounted for 48% and 20%, respectively [19]. Madavi D et al., found sepsis, prematurity and placental insufficiency as the major causes of neonatal thrombocytopenia [20]. In the present study, sepsis accounted for most early and late onset thrombocytopenia group cases. This was followed by placental insufficiency and perinatal asphyxia. Most of the studies had a similar incidences of early and late onset thrombocytopenia; the risk factors were also like other studies. The few dissimilarities that are seen in the different studies could be because of many confounding factors shows the review of literature on thrombocytopenia in neonates [Table/Fig-5].

Authors	Study period	Incidence of thrombocytopenia	Associated factors
Resch E et al., [16]	1990-2012	84.1%-Early onset 15.9%-Late onset	Prematurity, sepsis, asphyxia
Matsubara K et al., [4]	1997-2007	58%	High TPO levels
Gupta AK et al., [12]	2001-2004	16.7%	Low birth weight, pregnancy induced hypertension, IUGR, sepsis
Wiedmeier SE et al., [5]	2004-2013	31%	SGA
Eslami Z et al., [11]	2010-2011	28.5%	Sepsis, IUGR, perinatal asphyxia, prematurity
Ayadi ID et al., [13]	2010-2013	74.1%	Sepsis, IUGR
Madavi D et al., [20]	2018-2019	45%	Prematurity, sepsis, IUGR, NEC
Gebreselassie HA et al., [17]	2019	55.8%	Sepsis, atresia
Rathi P [14]	2019	27.2%	Prematurity, sepsis, respiratory distress

[Table/Fig-5]: The table shows the review of literature on thrombocytopenia in neonates [4,5,11-14,16,17,20].
IUGR: Intra uterine growth retardation; TPO: Thrombopoietin; SGA: Small for gestational age; PROM: Premature rupture of membranes; NEC: Necrotising enterocolitis

In the present study, neonatal thrombocytosis was seen in 15 neonates, early onset was seen in seven neonates and late onset was seen in eight neonates. Sepsis and placental insufficiency were the most important causes in both early and late onset thrombocytosis. This was comparable with the study by Özyürek E et al., who found that anaemia, high risk pregnancy, prematurity and infections were major causes of neonatal thrombocytosis [21]. The [Table/Fig-6] shows the literature review on thrombocytes in neonates.

Authors	Study period	Incidence of thrombocytosis	Associated factors
Matsubara K et al., [4]	1998-2000	38%	Thrombopoietin (TPO)
Matsubara K et al., [15]	2000-2012	12.5%	Infection, preterm
Ayadi ID et al., [13]	2010-2013	12.4%	Intra Uterine Growth Restriction (IUGR), Early onset neonatal sepsis and maternal Pregnancy Induced Hypertension (PIH)
Del Ray Hurtado de Mendoza B et al., [6]	2011-2014	32.6%	Prematurity, low birth weight

[Table/Fig-6]: The table shows the review of literature on thrombocytosis in neonates [4,6,13,15].

Overall, there are very few studies conducted to evaluate thrombocytosis, most probably due to the low incidence of the condition. Nevertheless, it is still an important finding and its association with conditions like sepsis and prematurity make it essential to identify any thrombocytosis and the treatment should be initiated.

Limitation(s)

Other criteria for probable sepsis like absolute neutrophil count, micro Erythrocyte Sediment Ratio (ESR), immature to total neutrophil ratio as well as newer modalities like Interleukin-6 (IL-6) and procalcitonin were not measured.

CONCLUSION(S)

In conclusion, the abnormal platelet count is a frequent finding in neonates admitted to NICU, with thrombocytopenia being more prevalent than thrombocytosis. Sepsis, placental insufficiency and perinatal asphyxia are the most common associated factors for thrombocytopenia. Thrombocytosis was associated with sepsis, probably as reactive thrombocytosis.

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