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Association of Platelet Indices with neonatal sepsis - A Prospective observational study

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Introduction

Neonatal Sepsis, sepsis neonatorum and neonatal septicaemia is generalized systemic features of sepsis with pure growth of bacteria from one or more sites occurring in a baby aged less than 28 days.[1] As many as 2% of foetuses are infected in utero, & up to 10% of infants have infections in 1 month of life.[2] Infection may result in early spontaneous abortion, congenital malformation, intrauterine growth restriction, premature birth, stillbirth, acute or delayed disease in the neonatal period, or asymptomatic persistent infection with sequelae later in life. Infection is more common in the neonatal period than at any other time in life. This is partly attributable to exposure to a large number of organisms and also due to a relative failure of the neonatal host defenses to clear the microorganisms from blood and tissues.[3]

Systemic infection in the new-born is the commonest cause of neonatal mortality. Data from the National Neonatal Perinatal Database 2000 suggests that Klebsiella Pneumoniae and Staphylococcus aureus are the commonest causes of neonatal sepsis in India.[4] Clinical features of sepsis are nonspecific in neonates and a high index of suspicion is required for its identification. Although blood culture is the "Gold Standard" for the diagnosis of sepsis, reports are available after 48-72 hours and they may be affected by intrapartum antibiotic administration to the mother. Therefore, a complete blood cell count with differential count is widely used, either singly or in conjunction with other tests or clinical findings as a diagnostic tool for neonatal sepsis. Hematologic values at birth encompass broader ranges of normal than at any other time in life due to the dynamic nature of the developmental processes that precede and follow birth. The present study was undertaken to evaluate the relevance of thrombocytopenia, platelet indices, C-reactive protein

and blood culture as these parameters aid in the early diagnosis of neonatal sepsis.[5]

Aim

1) To determine the association between hyper-destructive and hypo-productive thrombocytopenia in neonatal sepsis.

Material & methods

This Prospective study was conducted in Koppal Institute of Medical Sciences, Koppal. from January 2021 to January 2022. Institute Ethics committee approval was taken prior to study initiation (IEC No: KIMS-Koppal/IEC/55/2020-21) Neonates with generalized systemic features of sepsis with pure growth of bacteria from one or more sites were included in this study.

Types of sepsis: Suspected sepsis: is a new born with clinical signs & symptoms of sepsis

Probable sepsis: when clinical & laboratory findings (like increased TLC, increased Absolute neutrophil count, CRP positivity, immature/ total mature neutrophil count > 0.2) are consistent with bacterial infection but blood culture is sterile.

Confirmed sepsis: is one clinical & laboratory findings are consistent with bacterial infection along with blood culture positive.

Early Onset Sepsis: Sepsis occurring within 72 hours of life.

Late Onset Sepsis: Sepsis occurring after 72 hours of life.

Inclusion Criteria

1) Neonatal age group (1-28 days) with sepsis & suspected/probable sepsis.

2) New-borns born to PIH mothers

Exclusion Criteria

1) Dysmorphic babies with genetic causes of thrombocytopenia

2) Drug induced causes of thrombocytopenia

All neonates with confirmed sepsis, suspected sepsis or probable sepsis admitted in NICU of Koppal Institute of Medical Sciences, Koppal were enrolled in the study. A detailed clinical history of each patient was recorded. After getting the informed assent from the parent or LAR, 2 ml of blood was collected by peripheral venepuncture using aseptic precautions. The blood samples were sent to the pathology laboratory in EDTA vacutainers. Samples were processed in the Sysmex KX21 automated analysers. The Platelet Distribution Width (PDW) indicates the platelet distribution width was measured at 20% relative height of the total height of the curve. An increased PDW is an indication for the anisocytosis of platelets. Standard PDW ranges from 9 to 14 fL. The P-LCR indicates the percentage of large platelets with a volume >12 fL. The standard range is 15–35 %. An increase of the parameter may be an indication for platelet aggregates, micro erythrocytes and giant platelets. Platelet count was crosschecked by slide method for any discrepancy; up to 1% discrepancy was accepted. In a peripheral smear finding of 3 to 10 platelets per oil immersion field is normal (multiply this by 15000 to 20000 to get rough platelet count) Pathologist crosschecked more than 1% discrepancy then final platelet count was confirmed. Then after obtaining values correlation of the PDW & P-LCR were studied.

Statistical analysis

Sample size was calculated based on the reported incidence of neonatal sepsis, as per pooled data hospital-based survey of NNPD survey incidence of neonatal

sepsis was 3% (30 per 1000), with absolute allowable error of 5% & 5% confidence level which was founded out to be 46.6% rounded off to 50. Above sample was calculated with formula 4pq/d2 where p is incidence, q is 1-p & d is precision. The quantitative parameters such as age and platelet indices, mean and median were computed and standard deviation was estimated as a measure of variation. Correlation was done between the baseline characteristics and the platelet indices. All statistical tests were done using GraphPad Prism.

Results

This study included a total of 100 neonates with sepsis and their platelet parameters were measured. Table 1 shows the baseline characteristics of the study participants. It was found that male neonates were high (59%) and female neonates were lower (41%). Most of them were pre-term deliveries (59%) and others were term deliveries (40%), Except one which was post-term (1%). Among them only 12% of the neonates were born to mothers who had PIH. PROM was associated only in 15% of the neonates. Thrombocytopenia a hallmark of sepsis was seen only in 8% of the neonates. Again, contrary to the conventional belief most neonates were born through LSCS and only 32% had normal vaginal delivery. Meconium-Stained Amniotic Fluid (MSAF) was seen only in 10% of the neonates. Culture positivity was seen in 42% of the neonates with Pseudomonas being the most common one in 21% among them. And CRP was also normal in 80% of the neonate's contrary to the convention. 4

Table 1: Baseline demographic characteristics of study participants

Parameter	Total n=100 (%)
Gender	
Male	59
Female	41
Gestational age	
Preterm	59
Term	40
Post-term	1
PIH	
Present	12
Absent	88
Mode of delivery	
LSCS	68
Normal vaginal	32
MSAF	
Yes	10
No	90
PROM	
Yes	15
No	85
Thrombocytopenia	
Present	8
Absent	92
Blood culture	
Candida	2
CONS	7
Klebsiella	12
Pseudomonas	21
Negative	58

Diagnosis CS PS SS	 42 27 31
CRP Elevated Normal	 20 80

CRP- C-Reactive Protein, LSCS- Lower Segment Caesarean Section, PIH- Pregnancy Induced Hypertension, MSAF- Meconium-stained Amniotic Fluid, CS- Confirmed sepsis, PS- probable sepsis, SS- Suspected sepsis

It is shown in table 2 that based on PDW, 19 had hyper destructive and only 2 had hypo productive thrombocytopenia with a total of 21. In case of P-LCR, 5 had hyper destructive and 5 had hypo productive out of the 10 who had deranged parameters. Only 4 neonates had both parameters deranged and were classified under hyper destructive & hypo productive throm Bo cytopenia.

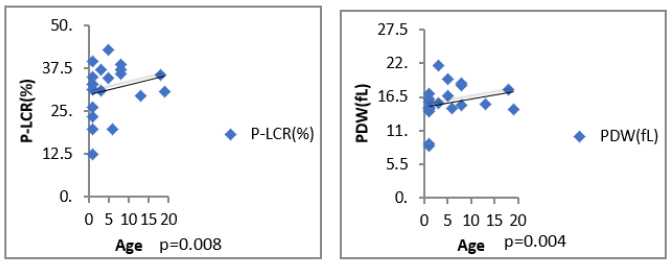
Table 2: Platelet indices in the study participants

Sn.	Platelet parameter	Hyper destructive (n)	Hypo productive (n)	Total (%)
1.	PDW	19	2	21
2.	P-LCR	5	5	10
3.	PDW+P-LCR	4	0	4

PDW- Platelet Distribution width, P-LCR- Platelet large cell ratio

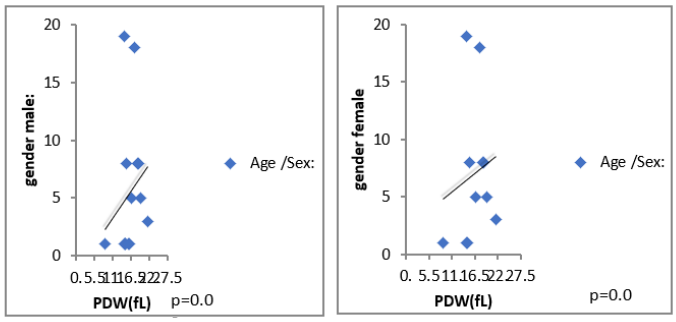
Figure 1: shows that there was a statistically significant correlation between age of the neonate and PDW and P-LCR.

Figure 1: Correlation between age and Platelet indices



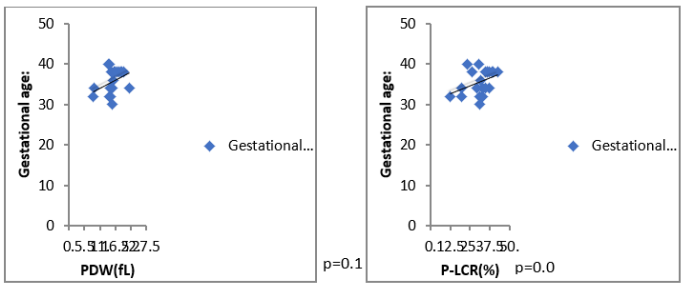
There was a significant correlation between male gender and PDW and female gender was found to have a statistically significant correlation with P-LCR as shown in figure 2.

Figure 2: Correlation between gender and platelet indices



Likewise, statistically significant correlation was seen with gestational age and P-LCR but not with PDW depicted in figure 3. Other Parameters were not statistically significant when analysed for correlation.

Figure 3: Correlation between gestational age and Platelet indices



Discussion

Sepsis is the commonest cause of neonatal mortality and is probably responsible for 30 to 50% of total neonatal deaths each year in developing countries.[4,6] One of the most difficult tasks faced by the neonatologist is to clinically differentiate between septicaemic and non-septicaemic cases. This is because several conditions like birth asphyxia, hypo glycaemia, hypothermia, prematurity and intracranial haemorrhage have clinical features similar to septicaemia. The gold standard for diagnosis of neonatal sepsis is a positive blood culture, which requires a minimum period of 48-72 hours and yields positive results in 25-70% of cases.[7-10] The current study was undertaken to evaluate thrombocytopenia and platelet indices variation and their correlation in neonatal septicaemia. Probable sepsis is blood parameters showing positivity (like increased TLC, increased Absolute neutrophil count, CRP positivity, immature/ total mature neutrophil count > 0.2) with blood culture negative. Neonatal survivors of sepsis can have severe neurologic Sequelae due to central nervous system (CNS) infection, as well as from secondary hypoxemia resulting from septic shock, persistent pulmonary hypertension, and severe parenchymal lung disease. So, it is of prime importance to detect sepsis early.[2]

Bacterial infection causes damage to vascular endothelium lining, thus accelerating adhesion, destruction, and removal of platelets. Sepsis also causes DIC; immune mediated destruction, and decreased production of platelets. About 10% to 60% of new-borns with proven bacterial invasion of the bloodstream or meninges have platelet counts of less than 100,000/mm3. Average duration of thrombocytopenia is about 1 week but can be as long as 2 to 3 weeks.[11,12]

Krishna B.V.et al. (2000)[13] and MA nucha V.et al. (2001)[14] performed immunoglobulin M estimation, CRP test and blood culture on neonates clinically suspected to have septicemia. They found CRP to have a higher sensitivity which was contrary to our study findings. A study done by Vamseedhar A.et al (2011)[15] showed that platelet indices and platelet counts had a significance in Pre-eclampsia and eclampsia. Platelet indices can have a significant impact on maternal and perinatal outcome. This finding is similar to our study.

Though our study showed significant correlation between platelet indices and the baseline parameters in development of sepsis, there were limitations in our study viz.: time taken from collection of the sample & processing can cause variation in the values slightly, the small sample size may not be sufficient to provide any conclusive result, positivity of CRP was very less in our study, may be because of time of sampling & cut off value of CRP is higher compared to other studied and serial values of platelet & platelet indices would yield a very good correlation.

Conclusion

In this study we conclude that there was thrombocytopenia in neonatal sepsis cases. Thrombocytopenia was more seen in new-borns born premature than term new-borns. Pseudomonas aeruginosa was the most common pathogen isolated in the blood culture followed by Klebsiella Pneumonia. Platelet indices had a significant correlation with the age, gender and gestational age of the neonate which in turn had an impact in the development of neonatal sepsis.

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