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Variation in Platelet Count and Platelet Indices with Their Clinical Correlation in Neonatal Sepsis

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Abstract: **Introduction:** Neonatal sepsis is a major cause of neonatal morbidity and mortality. Early diagnosis remains challenging because clinical manifestations are often nonspecific and conventional diagnostic methods such as blood culture are time-consuming. Platelet count and platelet indices have emerged as potential adjunctive biomarkers for diagnosis and prognosis in neonatal sepsis. **Aim:** To evaluate variations in platelet count, mean platelet volume (MPV), and platelet distribution width (PDW) and determine their clinical correlation in neonatal sepsis. **Methodology:** A hospital-based observational cross-sectional study was conducted among 100 neonates (0–28 days) with suspected sepsis admitted to the neonatal unit of Dr. B.C. Roy PGIPS, Kolkata, from February 2021 to October 2022. Complete blood count, platelet indices, sepsis screen, and blood culture were performed. Platelet count <1.5 lakh/cumm, MPV >10.8 fL, and PDW >13% were considered abnormal. Diagnostic performance and prognostic significance were analyzed using appropriate statistical tests and ROC curve analysis. **Results:** Among 100 neonates, 55% were culture-positive and 56% had late-onset sepsis. Thrombocytopenia was present in 97% of cases, while elevated MPV and PDW were observed in 57.35% and 58.82% respectively. Severe thrombocytopenia was significantly associated with mortality (77.78%, $p<0.0001$). Expired neonates had significantly lower platelet counts and higher MPV and PDW values than survivors ($p<0.001$). For predicting mortality, MPV >12.1 fL demonstrated 84.21% sensitivity and 91.84% specificity (AUC=0.913), while PDW >15.3% showed 84.21% sensitivity and 87.76% specificity (AUC=0.908). MPV emerged as the best predictor of mortality. **Conclusion:** Thrombocytopenia, elevated MPV, and increased PDW are significantly associated with neonatal sepsis and adverse outcomes. These inexpensive and readily available platelet parameters may serve as useful adjunctive diagnostic and prognostic markers in neonatal sepsis, particularly in resource-limited settings.

Keywords: Neonatal sepsis, Thrombocytopenia, Platelet count, Mean platelet volume, Platelet distribution width, Mortality, Biomarkers, Neonate.

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INTRODUCTION

Neonatal sepsis is a disease process, which represents the systemic response to bacteria entering the blood stream during the first 28 days of life which is the leading cause of mortality and morbidity in the immediate postnatal period which is partly attributable to exposure to large number of organisms and also due to relative failure of the neonatal host defences to clear microorganisms from blood and tissues [1]. The earliest signs of neonatal sepsis are often subtle and non-specific and thus the diagnosis of neonatal sepsis is highly dependent on biochemical and microbiological parameters, blood culture being the gold standard investigation. Supreetha *et al* reported that definite diagnosis of septicemia by a positive blood culture takes a minimum period of 48-72 hours and yielded a positive result in 30-70 % of cases [2]. To overcome these limitations, we usually rely on sepsis screen.

Sepsis screen and blood culture have variable sensitivity and specificity. Moreover, CRP increases in other inflammatory conditions as well. Hisamudddin *et al* reported 70% diagnostic accuracy of CRP levels for diagnosis of neonatal sepsis and suggested that CRP levels alone is not specific enough to be relied upon as the only indicator of neonatal sepsis [3].

Hematological imbalance is a frequent association of neonatal sepsis, Thrombocytopenia (platelet count < 1.5lakh/cumm) being the one of the commonest early laboratories finding. Changes in platelet indices like mean platelet volume (MPV) and platelet distribution width (PDW), plateletcrit are also noticed in sepsis. Lower platelet counts and high MPV, PDW have been reported in patients with sepsis than controls [4]. Interestingly, these indices have been found to be useful in the prognosis of adverse clinical outcomes including mortality [5,6] but none of the available studies compare platelet indices with the existing sepsis screen in prediction of neonatal septicaemia.

METHODOLOGY

This was a single center observational cross-sectional study conducted over a period of 21 months (February 2021-October 2022) after obtaining approval from institutional ethics committee. The study was done in neonatal ward, special newborn care unit (SNCU) and neonatal intensive care unit (NICU) of DR B.C Roy PGIPS, Kolkata. 100 babies of 0-28 days having clinical suspicion of sepsis with positive sepsis screen [7] as per systemic inflammatory response syndrome (SIRS) criteria[8] were included in study. Secondary causes of neonatal thrombocytopenia other than sepsis such as neonatal alloimmune thrombocytopenia, auto immune thrombocytopenia such as neonatal lupus, immune thrombocytopenic purpura, perinatal asphyxia, perinatal infections, chromosomal abnormalities, inborn errors of metabolism, neonatal enterocolitis, thrombocytopenia absent radii syndrome (TAR), viral infections, central line thrombosis, drug induced thrombocytopenia and babies with platelet disorders and major congenital anomalies were excluded from the study.

A complete blood count with platelet indices, sepsis screen was sent. Blood for culture and sensitivity was also sent. The blood samples were sent to the pathology department in EDTA vacutainers and was collected by peripheral venipuncture using aseptic precautions. The routine haematological investigations were performed in automated cell count machine-SYSMEX, XN350, 5 PARTS with standard calibrations. 1-3 ml of venous sample was taken in blood culture bottle under all aseptic conditions. The blood culture and sensitivity report were done by BACTEC method in BacT/ALERT 3D machine with 60 incubation wells. Neonates were divided into early onset sepsis (EOS) and late onset sepsis (LOS) group based on the onset of symptoms. Again, based on the reports of blood culture and sepsis, they were classified as culture positive sepsis and screen positive sepsis. Platelet parameters with MPV >10.8fL, PDW >13% and TPC <1.5 lakh/cumm were considered positive. Grading of thrombocytopenia was as per standardization, mild thrombocytopenia being 1-1.49 lakhs/cumm whereas moderate being 0.5-1 lakh/cumm and severe stated as < 0.5 lakh/cumm [9]. Once the result came, we compared variation in platelet count and platelet indices in EOS versus LOS as well as gram positive sepsis and gram-negative sepsis.

STATISTICAL ANALYSIS

The presentation of the Categorical variables was done in the form of number and percentage (%) and they were analysed through Chi-square test and Fisher's exact test. On the other hand, the quantitative data were presented as the means ± SD and as median with 25th and 75th percentiles (interquartile range) and their analysis were done through independent t test for two groups and ANOVA test for more than two groups. The association of the variables which were qSensitivity, specificity, positive predictive value and negative predictive value was calculated of decreased platelet count, high MPV, high PDW and (decreased platelet count + high MPV + high PDW) for predicting screen positive sepsis and mortality. Receiver operating characteristic curve was used to find cut off point, sensitivity, specificity, PPV and NPV of MPV (fL) and PDW (%) for predicting mortality. The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, ver 25.0. For statistical significance, p value of less than 0.05 was considered statistically significant.

RESULTS

The study was conducted at Dr. B.C. Roy PGIPS, Kolkata, involving 100 neonates suspected of neonatal sepsis, fulfilling SIRS criteria and having a positive sepsis screen whose mean age was 5.45±6 days with a male predominance of 2:1. Most of the cases were diagnosed as LONS, and culture positivity was found in 55% of cases of which gram-negative organisms contributed the most. A detailed baseline clinico-demographical profile is outlined in Table 1.

Table 1: Demographic and Laboratory parameters of neonates with sepsis

Parameter	Category	Frequency (%)	Mean ± SD	Median (25th-75th percentile)	Range
Age (days)	1-10 days	84	5.45 ± 6	3 (1-7)	01-27
	11-20 days	11			

	21-30 days	5			
Gender	Male	67			
	Female	33			
Gestational Age (weeks)	Pre term	34	35.98 ± 3.01	37 (34-38)	27-42
	Late pre term	9			
	Term	55			
	Post term	2			
Onset of Sepsis	Early onset	44			
	Late onset	56			
Sepsis Type	Culture positive	55			
	Screen positive	45			
Blood Culture Result	No growth	45			
	Klebsiella pneumoniae	13			
	Staphylococcus aureus	10			
	Serratia marcescens	8			
	Enterobacter cloacae complex	6			
	Acinetobacter baumannii	5			
	Pseudomonas aeruginosa	5			
	Staphylococcus hemolyticus	5			
	Burkholderia cepacia	2			
	Candida sp	1			
Gram Type	Gram negative	39			
	Gram positive	15			
Platelet Count (in lakhs) (cells/mm³)	Normal	3	0.74 ± 0.4	0.8 (0.39-1.02)	0.1-2.1
	Mild	28			
	Moderate	34			
	Severe	35			
MPV (fL)	Normal (≤10.8 fL)	29	11.29 ± 2.22	11.1 (9.65-12.25)	8-17.8
	Increased (>10.8 fL)	39			
PDW (%)	Normal (≤13%)	28	14.55 ± 3.36	13.35 (11.8-17.25)	9.1-21.4
	Increased (>13%)	40			
Outcome	Discharged	64			
	Expired	36			
Platelet Count (in lakhs cells/mm³) [For Gram negative sepsis(n=39) & for Gram positive sepsis(n=15)]	Normal	Gram-negative: 1(2.56%), Gram-positive: 0(0%)	0.62 ± 0.38	0.55 (0.285-0.938)	0.1-1.8
	Mild	Gram-negative: 8(20.51%), Gram-positive: 3(20%)			
	Moderate	Gram-negative: 12(30.77%), Gram-positive: 6(40%)			
	Severe	Gram-negative: 18(46.15%), Gram-positive: 6(40%)			

Platelet count and platelet indices when compared between the term and preterm neonates with suspected sepsis did not yield any statistically significant result. The mean platelet count, MPV, PDW among term neonates were 0.74 ± 0.45 lakhs cells/cumm, 11.41 ± 2.61 fL, 14.55 ± 3.36 % and that of preterm neonates were 0.75 ± 0.42 lakhs cells/cumm, 11.12 ± 1.53 fL, 14.32 ± 3.37 % consecutively. The aforementioned data when compared intergroup did not show any statistical significance with a p-value of 0.918, 0.597, 0.644 consecutively. Table 2 summarizes the variation of platelet count among three subgroups, gram positive and gram-negative sepsis, early and late onset neonatal sepsis and culture and screen positive sepsis.

Table 2: Intergroup comparisons of different platelet indices and their statistical significances

Parameters	Culture Positive Sepsis (n=55)	Screen Positive Sepsis (n=45)	P-value	EONS (n=44)	LONS (n=56)	P-value	Gram Negative Sepsis (n=39)	Gram Positive Sepsis (n=15)	P-value
Total Platelet Count (lakhs/cumm)	**0.61 ± 0.39**	**0.91 ± 0.44**		**0.86 ± 0.48**	**0.66 ± 0.38**		**0.62 ± 0.41**	**0.62 ± 0.32**	
Normal decrease	1 (1.82%)	2 (4.44%)	**0.0004**	3 (6.82%)	0 (0%)	**0.054**	1 (2.56%)	0 (0%)	**0.975**
Mild decrease	11 (20.00%)	17 (37.78%)		16 (36.36%)	12 (21.43%)		8 (20.51%)	3 (20.00%)	
Moderate decrease	18 (32.73%)	16 (35.56%)		13 (29.55%)	21 (37.50%)		12 (30.77%)	6 (40.00%)	
Severe decrease	25 (45.45%)	10 (22.22%)	**0.046**	12 (27.27%)	23 (41.07%)	**0.024**	18 (46.15%)	6 (40.00%)	**0.943**
Mean Platelet Volume (fL)	**11.15 ± 2.39**	**11.44 ± 2.06**	**0.586**	**11.22 ± 2.50**	**11.36 ± 1.94**	**0.8**	**11.27 ± 2.61**	**10.64 ± 1.11**	**0.341**
Normal (≤10.8 fL)	16 (45.71%)	13 (39.39%)		15 (44.12%)	14 (41.18%)		13 (46.43%)	3 (42.86%)	
Increased (>10.8 fL)	19 (54.29%)	20 (60.61%)	**0.598**	19 (55.88%)	20 (58.82%)	**0.806**	15 (53.57%)	4 (57.14%)	**1**
Platelet Distribution Width (%)	**14.60 ± 3.80**	**14.49 ± 2.90**	**0.891**	**14.16 ± 3.09**	**14.93 ± 3.63**	**0.351**	**15.10 ± 4.04**	**12.60 ± 1.50**	**0.014**
Normal (≤13%)	15 (42.86%)	13 (39.39%)		15 (44.12%)	13 (38.24%)		12 (42.86%)	3 (42.86%)	
Increased (>13%)	20 (57.14%)	20 (60.61%)	**0.772**	19 (55.88%)	21 (61.76%)	**0.622**	16 (57.14%)	4 (57.14%)	**1**

When we compared culture positive sepsis with screen positive sepsis, no significant differences in platelet count, MPV and PDW were noted (P > 0.05 for all parameters).Similar results were observed with platelet count, MPV and PDW with respect to gram negative sepsis versus gram positive sepsis and EONS versus LONS (P value> 0.05 for all parameters).Moreover, for screen positive sepsis, sensitivity of thrombocytopenia, MPV, and PDW were 95.56%, 60.61%, and 60.61% respectively. Diagnostic accuracy of thrombocytopenia, high MPV and PDW also were 44%, 52.94%, and 51.47% respectively. When used in combination, they had a sensitivity of 52.94% and diagnostic accuracy of 52.17% (Table 3, Figure 1)

Table 3: Sensitivity, specificity, positive predictive value and negative predictive value of platelet count and indices alone and in combination in screen positive sepsis

Diagnostic Parameter	Decreased Platelet Count	High MPV (>10.8 fL)	High PDW (>13%)	Decreased Platelet Count + High MPV + High PDW
Sensitivity (95% CI)	95.56% (84.85–99.46)	60.61% (42.14–77.09)	60.61% (42.14–77.09)	52.94% (35.13–70.22)
Specificity (95% CI)	1.82% (0.05–9.72)	45.71% (28.83–63.35)	42.86% (26.32–60.65)	51.43% (33.99–68.62)
AUC (95% CI)	0.49 (0.39–0.59)	0.53 (0.41–0.65)	0.52 (0.39–0.64)	0.52 (0.40–0.64)
Positive Predictive Value (PPV) (95% CI)	44.33% (34.24–54.77)	51.28% (34.78–67.58)	50.00% (33.80–66.20)	51.43% (33.99–68.62)
Negative Predictive Value (NPV) (95% CI)	33.33% (0.84–90.57)	55.17% (35.69–73.55)	53.57% (33.87–72.49)	52.94% (35.13–70.22)
Diagnostic Accuracy	44.00%	52.94%	51.47%	52.17%

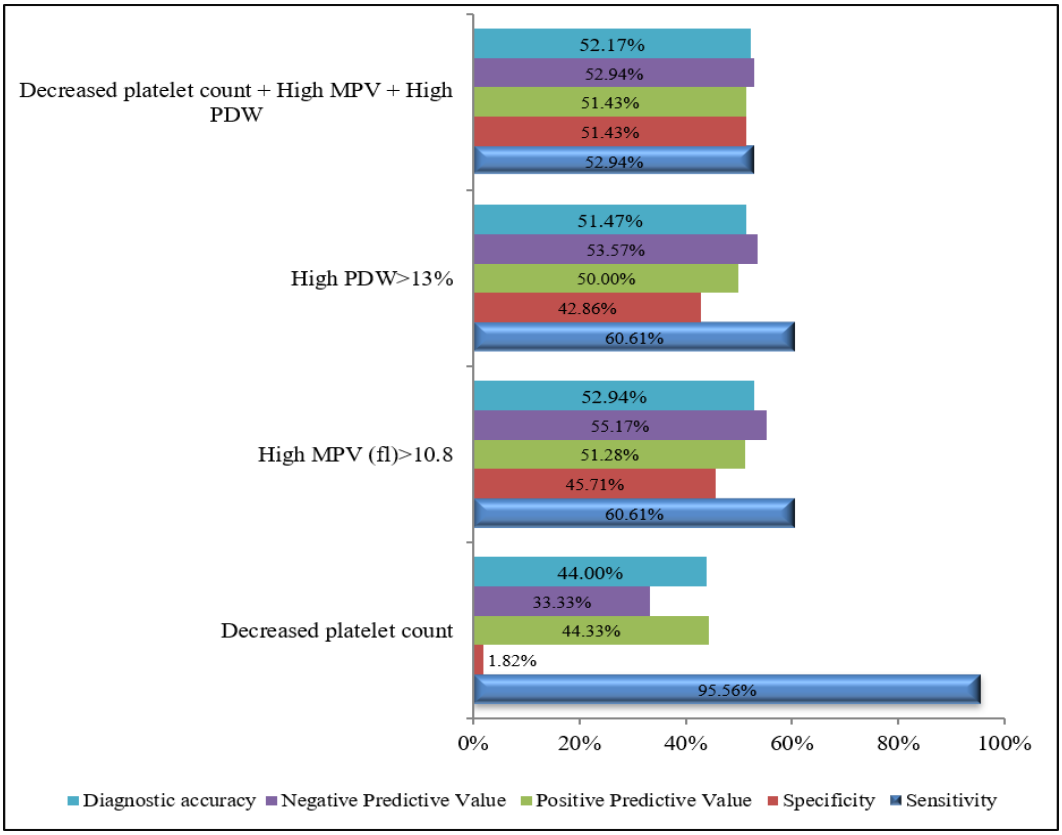

Figure 1: Comparative bar diagram depicting sensitivity, specificity, positive predictive value and negative predictive value of platelet count and indices alone and in combination in screen positive sepsis

In our study, we found significant correlation of thrombocytopenia, elevated MPV and PDW with mortality (p value <0.0001, p value 0.0009 and p value 0.0001 respectively). Also to predict mortality, thrombocytopenia, MPV and PDW showed a sensitivity of 100%, 89.47%, and 94.74% respectively. Besides, when used in combination, they had a sensitivity of 89.47%. Regarding specificity, PPV, NPV as a mortality indicator MPV shows 91.84%, 80%, 93.7% diagnostic validity whereas for PDW they are 87.76%, 72.7%, 93.5% respectively. Receiver operating characteristic (ROC) curve analysis demonstrated an area under curve (AUC) of 0.913 for MPV and 0.908 for PDW (p value <0.0001), showing their significant discriminative power to anticipate mortality.

Table 4: Overview of platelet count and platelet indices as mortality predictor in neonatal sepsis

Parameters	**Discharged**	**Expired**	**P-value**
Total Platelet Count (lakhs/cumm)	**n = 64**	**n = 36**	
Mean ± SD	0.94 ± 0.36	0.40 ± 0.34	**<0.0001**
Normal decrease	3 (4.69%)	0 (0%)	**<0.0001**
Mild decrease	25 (39.06%)	3 (8.33%)	
Moderate decrease	29 (45.31%)	5 (13.89%)	
Severe decrease	7 (10.94%)	28 (77.78%)	
Mean Platelet Volume (fL)	**n = 49**	**n = 19**	
Mean ± SD	10.37 ± 1.28	13.66 ± 2.40	**<0.0001**
Normal (≤10.8 fL)	27 (55.10%)	2 (10.53%)	**0.0009**
Increased (>10.8 fL)	22 (44.90%)	17 (89.47%)	
Platelet Distribution Width (%)	**n = 49**	**n = 19**	
Mean ± SD	13.11 ± 2.12	18.26 ± 3.16	**<0.0001**
Normal (≤13%)	27 (55.10%)	1 (5.26%)	**0.0001**
Increased (>13%)	22 (44.90%)	18 (94.74%)	

Table 4 describes the in-depth review of different platelet indices as mortality predictors in neonatal sepsis. A Cut off value of MPV >12.1 fl was identified as optimal, providing a sensitivity of 84.21% and specificity of 91.84% (Figure 2a). Likewise, PDW at cut-off value of >15.31% had a sensitivity of 84.21% and specificity of 87.76% (Figure 2b). There is always a tradeoff between sensitivity and specificity (any increase in sensitivity will be accompanied by decrease in specificity). So, we chose that variable as best in which combination of sensitivity and specificity gives the maximum PPV i.e., max AUC. So, overall MPV was the best predictor of mortality.

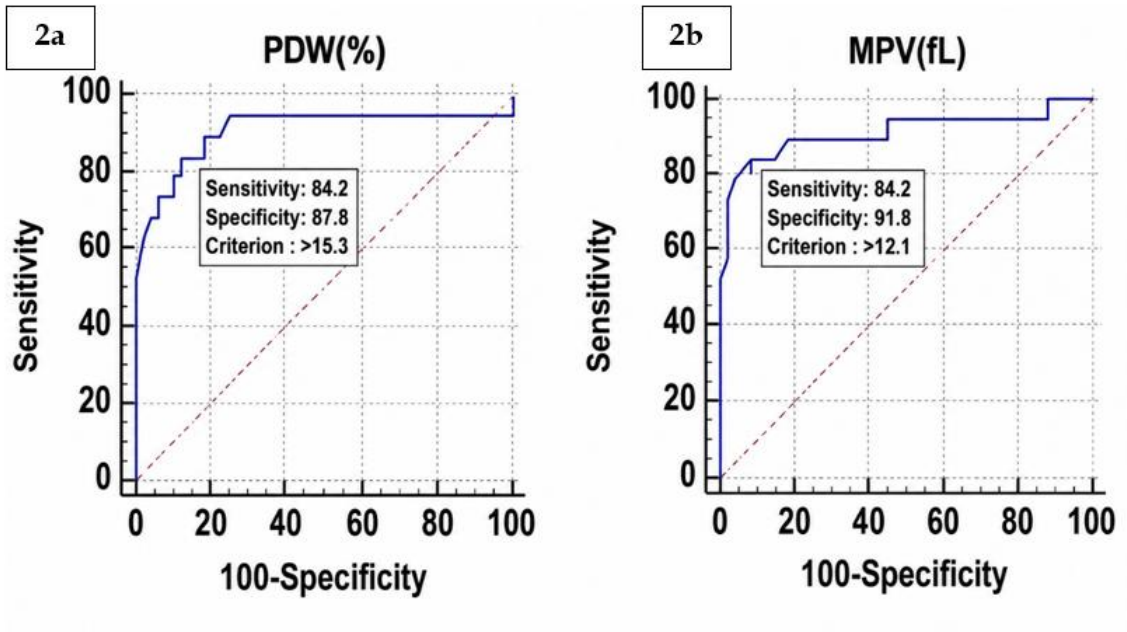

Figure 2: Receiver operating characteristic curve of MPV (fL) and PDW (%) for predicting mortality

DISCUSSION

Neonatal sepsis remains one of the leading causes of morbidity and mortality. Diagnosing it remains one of the challenges that pediatrician is facing, because clinical findings are non-specific and vague [10]. Blood culture has always been the gold standard for the diagnosis of neonatal sepsis, but it takes a minimum period of 48-72 hours with a moderate yield. To overcome these limitations, we usually rely on sepsis screen. Sepsis screen and blood culture have variable sensitivity and specificity. Moreover, CRP increases in other inflammatory conditions as well. So, it is not specific enough to be relied upon as the only indicator of neonatal sepsis [3]. This necessitates alternative diagnostic tests for neonatal sepsis with good sensitivity and specificity. Platelet count and platelet indices is evolving as a sensitive marker in recent studies. But it is not utilized in day-to-day practice.

In this study conducted in the neonatal unit, 100 neonates with clinical features of sepsis fulfilling the SIRS criteria were evaluated to assess the diagnostic potential of platelet count and platelet indices. This study is based on the variation of platelet count and platelet indices in neonatal sepsis. The probable pathophysiology is sepsis induced endothelial damage and formation of microthrombi, which lead to the consumption of platelets. The imbalance between the consumption and production from the bone marrow leads to low platelet count in neonatal sepsis. The relationship between platelet indices and various diseases has been the study of interest because of their rapid and widespread availability. The exact mechanism of elevated MPV and PDW in neonatal sepsis are not entirely clear. It has been reported during activation process, platelets change shape from discoid form to spherical form increasing MPV. During this process, they acquire pseudopodia which can increase PDW value.

The demographic profile of our study depicted 84 (84%) of newborns belonged to the group 1-10 days, 67(67%) were male and 33(33%) were female. Out of 100 enrolled neonates, [56(56%)] had LOS and [44(44%)] had EOS. Eman M. Rabie Shehab El Din *et al* [11] had done a study on epidemiology of neonatal sepsis which showed LOS (55.8%) was more common than EOS (44.2%). Among 55 culture positive sepsis, [39 (72.22%)] were gram negative sepsis, [15(27.78%)] were gram positive sepsis and [1(1%)] was fungal sepsis highlighting the fact that gram negative sepsis is more prevalent than gram positive sepsis in present study population. The commonest organism isolated was Klebsiella pneumoniae [13(13%)] followed by Staphylococcus aureus [10(10.00%)]. A similar bacteriological profile of neonatal sepsis was also reported by various studies [12-16].

Thrombocytopenia was seen in majority of the septic newborns out of which 35%, 34% and 28% had severe, moderate and mild thrombocytopenia respectively, consistent with findings of Singh S *et al* [15]. Our study showed neonates with mild to moderate thrombocytopenia had the highest rate of survivability [25(39.06%)], [29(45.31%)] respectively, while the neonates with severe thrombocytopenia showed the highest rate of mortality [28(77.78%)]. Association of outcome versus thrombocytopenia was statistically significant (p value <0.0001). Mean ±SD of platelet count in discharged was 0.94±0.36, which was significantly higher as compared to expired 0.4±3.4 (p value= < 0.0001). Charki S *et al* [13] reported increased mortality in neonates having severe thrombocytopenia. Additionally, Choudhary D.K *et al* [16] found a higher mortality rate in severe thrombocytopenic neonates.

[39(57.35%)] and [40(58.82%)] of neonates under study had high MPV and PDW respectively, indicating their increased level in neonatal sepsis. Studies by Sharma N *et al* [17], Tabassum H [12], Patil A *et al* [14] were in consonance with the above finding. Likewise, statistically significant elevated MPV and PDW was noted in expired babies in comparison to survivors (p value =0.0009, p value =0.0001). Mean ±SD of MPV (fl) in expired neonates was 13.66±2.4, which was significantly higher as compared to discharged neonates 10.37±1.28 (p value < 0.001). Similarly, mean ±SD of PDW (%) in expired babies was 18.26±3.16, which was significantly higher than discharged babies 13.11±2.12 (p value < 0.001). To predict mortality, our study had a sensitivity of 100%, 89.47% and 94.74% for thrombocytopenia, high MPV and high PDW respectively. When used in combination they had a sensitivity of 89.47%. There is sparse literature on platelet parameters as a marker to determine outcome. More studies are needed to corroborate this finding. In a study done by Catal F, MPV value of 10.75fL was determined as the cut off value for patients with high risk of mortality at diagnosis of sepsis with a sensitivity of 95.2% and a specificity of 84.9% [19]. On ROC curve analysis, present study showed a sensitivity of 84.21% and specificity of 91.84% at a cut off value of > 12.1fL for MPV (p value<0.0001). PDW at a cut off value of >15.3% had a sensitivity of 84.21%, specificity of 87.76% (p value <0.0001). This portrays the fact that both parameters had significant discriminatory power to predict mortality. As AUC was highest for MPV (0.913), it was considered as the best predictor of mortality.

CONCLUSION

The future prospects of using platelet count and platelet indices in neonatal sepsis appear promising for early diagnosis, prognosis and monitoring of disease progression. Main advantages of these parameters lie in their noninvasive, inexpensive nature. They can be readily available from routine complete blood count and useful in resource limited settings where other markers (e.g. CRP, procalcitonin, IL-6) may not be accessible. Owing to the high sensitivity of thrombocytopenia, elevated MPV and PDW in our study, platelet parameters alone or in combination can be used as a sensitive screening marker along with existing sepsis screen to rule out sepsis. Moreover, our study results also showed their high sensitivity pattern to predict mortality in newborns having sepsis. Thus, they can also be used as a prognostic marker in neonatal sepsis when used alone or in combination. However, more research should be done in establishing standardized reference ranges for platelet indices in neonates and integrating them with clinical scoring systems and biomarker panels to improve diagnostic accuracy.

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