Study of Platelet Parameters with Different Isolated Organism in Neonatal Bacterial Sepsis

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RKP and AUS designed the study, analysed the data and drafted the manuscript. Authors CC, RI, NM collected the data, analysed the data. All the authors managed literature search, helped in final writing of manuscript and approved the final draft. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Thrombocytopenia is a common hematological abnormality observed in neonatal sepsis and considered as early, nonspecific marker of sepsis. The studies related to organism specific platelet response are few.
Objective: To assess the prevalence of thrombocytopenia and to study changes in various platelet parameters in relation to different isolated organism.
Methods: A prospective observational study involving neonates with 1st episode of culture positive sepsis was done over a period of 18 months. The platelet parameters studied were incidence, degree, duration of thrombocytopenia; mean platelet volume and platelet nadir among neonate with specific organism isolated.
Results: Out of 114 culture positive sepsis 31% Klebsiella, 30% Pseudomonas, 13% Citrobacter, 18% CONS, 4% Staphylococcus Aureus (S Aureus) and 3% had Acinetobacter sepsis. Overall prevalence of thrombocytopenia was 88%, of which klebsiella and S. Aureus sepsis observed 100% prevalence of thrombocytopenia followed by 88% pseudomonas, 80% coagulase negative staphylococci (CONS), 75% Acinetobacter and 66% Citrobacter sepsis. The proportion of severe

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degree of thrombocytopenia (18% Vs 4%), higher MPV (61% Vs 54%) and longer duration of thrombocytopenia (3.63 ± 0.49 Vs 2.95 ± 0.52) was observed more with Gram negative sepsis than with Gram positive sepsis and statistically significant difference in platelet nadir was observed with Gram negative sepsis. Severe degree of thrombocytopenia was seen in 50% neonates with Acinetobacter and 23% with Klebsiella sepsis. Acinetobacter, Klebsiella and Pseudomonas sepsis had higher mean MPV value, longer duration of thrombocytopenia and lowest platelet nadir. The platelet parameters were less affected with Gram positive organism.

Conclusion: Thrombocytopenia is a frequent occurrence in neonates with sepsis especially with Gram negative organism. Sepsis with Acinetobacter, Klebsiella & Pseudomonas organism was associated with prolonged duration, higher MPV and lower platelet count as compared to other isolated organisms.

1. INTRODUCTION

Neonatal sepsis is an important cause of thrombocytopenia [1,2]. Bacterial, fungal and viral infections all have been associated with neonatal thrombocytopenia [3,4]. Thrombocytopenia is usually of late onset type with bacterial sepsis and it is more severe and prolonged [5]. In the period in which sepsis is diagnosed, 25% neonates show thrombocytopenia and by 36 – 48 hours of onset of sepsis majority of neonates develop thrombocytopenia [6,7]. Thus thrombocytopenia is considered as an early but nonspecific marker of sepsis in neonate [8]. Fungal sepsis is associated with greater degree of thrombocytopenia than bacterial sepsis and among bacterial infection Gram negative sepsis has greater degree of thrombocytopenia than Gram positive sepsis [9,10]. The early diagnosis of sepsis in neonate is important to interrupt the cascade of events leading to shock and multi-organ failure. Also identification of causative organism for sepsis helps in proper selection of antibiotic treatment and improving the outcome. However isolation of organism by blood culture takes time. Keeping in view of these facts, this study was planned to find the proportion & severity of thrombocytopenia as well as pattern of changes in platelet parameter in relation to different organism isolated.

2. MATERIALS AND METHODS

2.1 Study Place, Setting and Duration

A cross sectional prospective observational study was conducted at neonatal tertiary care center over a period of 18 months.

2.2 Study Subjects

Neonates were included in the study based on fulfillment of criteria of neonatal sepsis. Neonatal sepsis was considered if: presence of more than 2 clinical features suggestive of sepsis with positive C - reactive protein (CRP) and positive blood culture. The clinical features suggestive of sepsis considered were: hypothermia/fever; poor cry/poor feeding/refusal to suck; lethargy; fast breathing/respiratory distress/apnoea; abdominal distension/diarrhoea/vomiting; sclerema; prolonged capillary refill time; bulging anterior fontanel/convulsion.

Neonates with major congenital malformation, chromosomal anomalies were excluded. Neonates with maternal thrombocytopenia were excluded. Maternal history of pregnancy induced hypertension (PIH), any infection, drug intake affecting platelet count were also excluded. Neonates who took discharge against medical advice were also excluded from the study.

To avoid any confounding effect on platelet count from previous episode of sepsis, only first episode of sepsis was considered in analysis.

2.3 Data Collection

Baseline neonatal characteristics like gestational age, birth weight, gender, mode of delivery, APGAR score was noted for all enrolled babies. Neonates were examined clinically for primary illness according to standard format. As per our neonatal unit policy baseline investigation of complete blood count (CBC), CRP and Blood culture were sent at the time of admission and subsequently on clinical suspicion of sepsis. Platelet count was repeated twice a week or at time of sampling for other investigations in thrombocytopenic neonates until platelet count was normalized. Blood for investigation was collected by vene-puncture under strict asepsis. CBC was done by automated cell counter. Blood culture was done by BACTEC method.

Platelet parameters studied were platelet count at baseline & at onset of sepsis; degree of thrombocytopenia; duration of thrombocytopenia; mean platelet volume (MPV) and platelet nadir. The platelet count at the onset of sepsis was considered as platelet count coinciding with blood sample showing positive culture report. Thrombocytopenia was defined as platelet count less than 150,000 / cmm and graded as mild if platelet count between 50,000 to 150,000 / cmm, moderate if counts were between 20,000 to 50,000 / cmm and severe if count was less than 20,000/ cmm. The normal range for MPV was 7.4 to 11.4 fentolitre (fl) (as per lab reference value). Duration of thrombocytopenia was the number of continuous days that the platelet count remained below 150,000/cmm. Platelet nadir means lowest platelet count noted during the period of thrombocytopenia.

Identification of isolated organism was done based on colony characteristic after overnight incubation on sheep blood agar.

2.4 Statistical analysis

Descriptive statistical analysis was done to analyze data. Qualitative data were represented by percentage and quantitative data were represented by mean with standard deviation. Chi-square test for qualitative data and Independent 't' test was applied for quantitative data. Statistical analysis was done using Open-Epi software.

3. RESULTS AND DISCUSSION

Tables 1,2. Total 114 babies with culture positive sepsis were analyzed. Mean gestational age of study group was 35.39 ± 3.48 weeks; mean birth weight was 1.953 ± 0.65 kg; male: female ratio was 1.32: 1. Mode of delivery was normal vaginal delivery (NVD) in 67% neonates. Mean APGAR score of study group was 7.68 ± 1.24. Our study observed early onset sepsis in 69% babies and Gram negative sepsis in 77% babies.

Table 3. Overall thrombocytopenia was observed in 88% babies with 52% having moderate to severe degree of thrombocytopenia. The proportion of severe degree of thrombocytopenia (18% Vs 4%), higher MPV (61% Vs 54%) and longer duration of thrombocytopenia (3.63 ± 0.49 Vs 2.95 ± 0.52) was observed more with Gram negative sepsis than with Gram positive sepsis, but statistical significance was not found. We observed statistically significant platelet nadir with Gram negative sepsis.

Table 4. Out of 114 neonates with culture positive sepsis, Gm negative sepsis was observed in (88) 77% and Gm positive sepsis was observed in (26)23%. The proportion of isolated organisms was: 31% klebsiella, 30% pseudomonas, 19% Coagulase- negative Staphylococci (CONS), 13% Citrobacter , 4% S. Aureus & 3% Acinetobacter.

Table 1. Baseline characteristics: Demographic profile

Neonatal characteristics	n (%) N = 114
Gestational age	
< 28 week	4 (4)
28– 32 week	27 (26)
33– 37 week	27 (26)
> 37 week	56 (44)
Mean gestational age = 35.39 ± 3.48	
Birth weight	
< 1 Kg	8 (7)
1 – 2 Kg	55 (48)
2 – 3 Kg	44 (39)
➢ 3 Kg	7(6)
Mean Birth weight = 1.953 ± 0.65	
Gender	
Male	65 (57)
Female	49 (43)
APGAR score <5 at 5 minute	10 (9.34)
Mean APGAR score at 5 minute	7.68 ± 1.24
Mode of Delivery	
NVD	72 (67)
LSCS	35 (32.7)

Table 2. Baseline characteristics: Type of sepsis

Onset of sepsis	N = 114, n (%)
Early onset sepsis	79 (69)
Late onset sepsis	35 (31)
Gram stain based sepsis	
Gram positive	26 (23)
Gram negative	88 (77)

Thrombocytopenia was observed in 100% babies with Klebsiella and S. Aureus sepsis followed by 88% pseudomonas and 75% Acinetobacter sepsis. Severe thrombocytopenia was observed in 50% babies with Acinetobacter sepsis, 22.8% Klebsiella sepsis, 17.6% Pseudomonas sepsis and 4.7% CONS sepsis. In S. Aureus sepsis none of the babies had severe thrombocytopenia. This observation reflects that proportion of thrombocytopenia and severity of thrombocytopenia was more with Gm negative organism.

Acinetobacter sepsis had highest proportion (50%) of babies with severe thrombocytopenia, 50% had high MPV value with mean MPV 10.77 ± 0.71 which is higher than other isolates, had longer duration of thrombocytopenia (4.52 ± 0.51 days) and maximum platelet nadir.

Klebsiella sepsis had 2nd highest proportion (23%) of severe thrombocytopenia, had greater fall in platelet count after 48 hour of onset of sepsis, 74% babies having higher MPV with mean MPV 10.53±0.71. The duration of thrombocytopenia (4.40 ± 0.45 days) was longer (2nd in order) and platelet nadir was 3rd in order.

Pseudomonas sepsis had 18% babies having severe thrombocytopenia, 59% had high MPV with mean MPV value 10.53 ± 0.67. Platelet nadir and duration of thrombocytopenia was less as compared to Acinetobacter & Klebsiella sepsis.

Among Gram positive organism S. Aureus sepsis observed thrombocytopenia in all (100%) babies. The other platelet parameter like severity of thrombocytopenia, duration of thrombocytopenia, platelet nadir was less affected as compared to other organism.

4. DISCUSSION

Thrombocytopenia is one of the most common hematological manifestations in neonatal sepsis [8]. The cause of thrombocytopenia in sepsis can be due to increased platelet destruction, decreased platelet production or combination of both. Neonates respond to sepsis by up regulation of thrombopoietin (TPO) production; however the degree of up regulation is only modest. The study has found that Gram negative sepsis didn't have the highest degree of up regulation despite more significant level of thrombocytopenia and more severe illness. It was suggested that during severe illness there is down regulation of thrombopoietic response. [11,12].

The present study showed Gram negative sepsis in 77% and Gram positive sepsis in 23% neonates. The predominance of Gram negative sepsis is consistent with other Indian studies [13]. Among Gram negative organisms Klebsiella was the predominant isolates which is also comparable with earlier reports from India [14,15].

The observed 88% proportion of thrombocytopenia with Gram negative sepsis in our study is consistent with observation by Bhat et al. [1] (70%), Sartaj et al. (66%) [6] and Ree IMC et al. [16] (69%). The proportion of severe thrombocytopenia was 18% with Gram negative sepsis as compared to 4% with Gram positive sepsis in our study. Similar observation of severe degree of thrombocytopenia with Gram negative sepsis was found by P Ahmed et al. [4], R Bhat et al. [1]. Ree IMC et al. [16] in their study found that Gram negative sepsis had severe degree of thrombocytopenia, platelet count seems to fall lower and time for platelet to rise to > 100,000/cmm was longer than Gram positive sepsis. In our study we also observed longer duration of thrombocytopenia and maximum platelet nadir with Gram negative sepsis and among different platelet parameters statistical significance was observed only in platelet nadir between Gram negative and Gram positive sepsis. Manzoni P et al. [17] in their study found 17% septic neonates had associated thrombocytopenia and proportion of thrombocytopenia was 19% with fungal sepsis, 16% with bacterial sepsis. In their study they didn't

Table 3. Incidence & degree of thrombocytopenia

Platelet parameters	Overall (N=114)	Gram positive (n=26)	Gram negative (n=88)	P value
Thrombocytopenia	100 (88)	21 (81)	79 (90)	0.1494
Degree of thrombocytopenia				
Mild	41 (36)	9 (35)	32 (36)	0.1628
Moderate	42 (37)	12 (46)	30 (34)	
Severe	17 (15)	1 (4)	16 (18)	
High MPV	68 (60)	14 (54)	54 (61)	0.4712
Mean duration of thrombocytopenia (in days)	3.29 ± 0.50	2.95 ± 0.52	3.63 ± 0.49	0.6847
Lowest platelet count (in lacs)	0.21± 0.15	0.23 ± 0.18	0.20 ± 0.13	0.0001

Table 4. Comparison of platelet parameters among different organisms

Platelet parameter	Organism					
	Klebsiella N = 35(31%)	PseudomonasN = 34 (30%)	Citrobacter N = 15 (13%)	CONS = 21 (18%)	Staph. Aureus N = 5(4%)	Acinetobacter N = 4 (3%)
Thrombocytopenia (occurrence)	35 (100)	30 (88)	10 (66)	17 (80)	5 (100)	3 (75)
Degree of thrombocytopenia						
Mild	13 (37)	9 (26)	9 (60)	6(28)	3 (60)	1 (25)
Moderate	14 (40)	15 (44)	1 (7)	10 (47)	2 (40)	0
Severe	8 (23)	6 (18)	0	1 (5)	0	2 (50)
Platelet count						
Baseline	1.74±0.62	1.84±0.72	2.10±0.64	2.32±1.19	2.74±0.47	1.74±1.03
At onset of sepsis	1.46 ±0.79	1.33±0.76	1.83±0.61	1.45±0.58	1.46±0.53	1.25±1.13
After 48 hour of onset of sepsis	1.01 ±0.60	1.12±0.54	1.16±0.38	1.26±0.58	1.44 ± 0.28	1.01±0.39
MPV						
Normal	9 (26)	14 (41)	9 (60)	10 (52)	2 (40)	2 (50)
High	26 (74)	20 (59)	6 (40)	11 (48)	3 (60)	2 (50)
Mean MPV	10.53±0.71	10.53±0.67	10 ± 0.42	10.19±0.90	10.21±1.03	10.77±0.71
Duration of thrombocytopenia (in days)	4.4 ± 0.45	3.14 ± 0.15	2.46 ± 0.85	3.0 ± 0.9	2.9 ± 0.14	4.52 ± 0.51
Platelet Nadir	0.23±0.17	0.27±0.16	0.18±0.12	0.21±0.16	0.25±0.21	0.14±0.1

observe significant difference in platelet parameters when clustering for sepsis caused by Gram positive and Gram negative organisms done [17].

The organism specific platelet response was studied to some extent by other researchers. Accordingly proportion of thrombocytopenia in Klebsiella sepsis was found to be 60% by Charoo et al. [18], 73% by S Arif et al. [19] and 43% by Sartaj et al. [6].We found 100% neonates having thrombocytopenia with Klebsiella sepsis. The effect of Klebsiella organism to various platelet parameters is explained by variation in genetic make up of O antigen between Klebsiella pneumonia and other Gram negative organism. [6]

The isolation rate of Acinetobacter organism in Indian literature ranges from 8.3% to 15.2%. [6,11,18] We found Acinetobacter isolates in 4% of babies with sepsis. Thrombocytopenia with Acinetobacter sepsis was seen in 66% neonates by Bhat et al. [1]. In our study we found 74% rate of thrombocytopenia in case of Acinetobacter sepsis. Sartaj et al. [6] found 11% and S Arif [19] found 6% rates of thrombocytopenia with Acinetobacter. The platelet parameters were maximally altered in neonates with Acinetobacter sepsis in our study. In a neonate with clinical features of sepsis along with altered platelet parameters, it can be suggested to consider Gram negative organism especially klebsiella or Acinetobacter at our setup. Acinetobacter being a nosocomial infection stresses the need for continuous bacteriological surveillance and implementation as well as adherence to strict infection control policy.

The other Gram negative organism Pseudomonas had 88% rate of thrombocytopenia in our study which is comparable to rate of 67% seen by Bhat R et al. [1].

Among Gram positive organism we found proportion of thrombocytopenia at the rate of 100% with S. Aureus and 66% with CONS sepsis. S Arif had 67% rate of thrombocytopenia with S.Aureus and 25% with CONS sepsis [18].

5. CONCLUSION

Thrombocytopenia is a frequent occurrence in neonates with sepsis especially with Gram negative organism. Sepsis with Acinetobacter, Klebsiella & Pseudomonas organism was associated with prolonged duration, higher MPV and lower platelet count as compared to other isolated organisms.

CONSENT AND ETHICAL APPROVAL

Consent was taken from parents/ guardians of the neonate before enrollment. Ethical approval was obtained from institutional ethical committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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