ISSN: 0975-1556

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2021; 13(3); 123-130

Original Research Article

Platelet Parameters in Neonatal Bacterial Sepsis with Different Isolated Organisms: Prospective Observational Study

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Received: 22-04-2021 / Revised: 07-05-2021 / Accepted: 18-06-2021
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Conflict of interest: Nil

Abstract

Aim: The aim of the present study to determine the Platelet Parameters with Different Isolated Organism in Neonatal Bacterial Sepsis. **Methods:** This cross sectional prospective observational was done the Department of Paediatrics, Government Medical College, Bettiah, West Champaran, Bihar, India for 12 months. Neonatal with presence of more than 2 clinical features suggestive of sepsis with positive C - reactive protein (CRP). 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. 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. **Results:** Total 120 babies with culture positive sepsis were analyzed. Mean gestational age of study group was 34.78 ± 4.02 weeks; mean birth weight was 1.912 ± 0.85 kg; male: female ratio was 1.4: 1. Mode of delivery was normal vaginal delivery (NVD) in 66.67% neonates. Mean APGAR score of study group was 7.91 ± 1.43. Our study observed early onset sepsis in 70% babies and Gram-negative sepsis in 75% babies. Overall thrombocytopenia was observed in 87.5% babies with 54.17% having moderate to severe degree of thrombocytopenia. The proportion of severe degree of thrombocytopenia(20% Vs 4%), higher MPV (76.67%) Vs (56.67%) and longer duration of thrombocytopenia (3.98 ± 0.75Vs 3.22 ± 0.81) 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. The proportion of isolated organisms was: 30.83% klebsiella, 26.67% pseudomonas, 19.17% Coagulase- negative Staphylococci (CONS), 12.5% Citrobacter, 5.83% S. Aureus & 6.67% Acinetobacter. **Conclusion:** Thrombocytopenia is a frequent occurrence in neonates with sepsis especially with Gramnegative organism.

Keywords: sepsis, microorganism, platelet

Introduction

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection and bacteremia during the first 28 days of life. Sepsis continues to be the most important cause of neonatal mortality, especially in very low birth weight (VLBW) babies, and clinical presentation in these tiny babies can be subtle and nonspecific[1-3]. Incidence of neonatal sepsis in Asia is 7.1 to 38 per 1000 live births[4], in Bangladesh it is 19.6 per 1000 live births[5]. The pattern of infection by various organisms varies from one institution to another and even from year to year in the same institution[6]. The common organisms responsible for neonatal sepsis are Klebsiella pneumoniae, Acinetobacter, Escherichia coli (E.coli), Pseudomonas aeruginosa, Salmonella, Haemophilus influenzae, Proteus, Coagulase negative staphylococci, Staphylococcus aureus, Streptococcus, Pneumococcus, Flavobacterium freundi, Candida etc[7]. Blood culture is the gold standard test to diagnosis neonatal sepsis. But it could be positive only in 30-40% cases[8]. Obtaining blood cultures from neonates can be difficult, sample volumes are small, and a substantial number are negative or contaminated[9]. Estimation of cytokines and C Reactive Protein (CRP) levels are potentially useful in this respect[10]. Complete blood count including platelet count is a good predictor of sepsis in newborns[11]. Thrombocytopenia is a major indicator of sepsis in neonates[11]. It has been shown that 75% of culture positive neonates had thrombocytopenia[12]. Majority of newborn developed thrombocytopenia by 36-48 hours after developing neonatal sepsis and average duration of thrombocytopenia persisted around 6 days[13]. There is a positive association of Gram negative infection and thrombocytopenia[14]. Fungal infection is also associated with a greater degree of thrombocytopenia[15]. Congenital viral infections like cytomegalovirus (CMV), toxoplasma, rubella and herpes simplex are important causes of thrombocytopenia in neonatal period and early infancy.

Material and Methods

This cross sectional prospective observational was done the Department of Paediatrics, Government Medical College, Bettiah, West Champaran, Bihar, India for 12 months, after taking the approval of the protocol review committee and institutional ethics committee.

Inclusion criteria

- 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.

Exclusion criteria

- Neonates with major congenital malformation, chromosomal anomalies.
- Neonates with maternal thrombocytopenia.
- Maternal history of pregnancy inducedhypertension (PIH)

Procedure

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.

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.

Results

Total 120 babies with culture positive sepsis were analyzed. Mean gestational age of study group was 34.78 ± 4.02 weeks; mean birth weight was 1.912 ± 0.85 kg; male: female ratio was 1.4: 1. Mode of delivery was normal vaginal delivery (NVD) in 66.67% neonates. Mean APGAR score of study group was 7.91 ± 1.43. Our study observed early onset sepsis in 70% babies and Gram-negative sepsis in 75% babies.

Table 3. Overall thrombocytopenia was observed in 87.5% babies with 54.17% having moderate to severe degree of thrombocytopenia. The proportion of severe degree of thrombocytopenia (20% Vs 4%), higher MPV (76.67%) Vs (56.67%) and longer duration of thrombocytopenia (3.98 ± 0.75Vs 3.22 ± 0.81) 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 120 neonates with culture positive sepsis, Gm negative sepsis was observed in (90) 75% and Gm positive sepsis was observed in (30)25%. The proportion of isolated organisms was: 30.83% klebsiella, 26.67% pseudomonas, 19.17% Coagulase- negative Staphylococci (CONS), 12.5% Citrobacter, 5.83% S. Aureus & 6.67% Acinetobacter.

Thrombocytopenia was observed in 94.59% babies with Klebsiella and S. Aureus sepsis 85.71% followed by 93.33% pseudomonas and 75% Acinetobacter sepsis.

Severe thrombocytopenia was observed in 33.33% babies with Acinetobacter sepsis, 31.43% Klebsiella sepsis, 21.43% Pseudomonas sepsis and 5.88% 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 (33.33%) of babies with severe thrombocytopenia, 50% had high MPV value with mean MPV 10.77±0.69 which is higher than other isolates, had longer duration of thrombocytopenia (4.63 ± 0.49days) and maximum platelet nadir.

Klebsiella sepsis had 1st highest proportion (31.43%) of severe thrombocytopenia, had greater fall in platelet count after 48 hours of onset of sepsis, 78.38% babies having higher MPV with mean MPV 10.87±0.88. The duration of thrombocytopenia (4.61 ± 0.42 days) was longer (2nd in order) and platelet nadir was 3rd in order.

Pseudomonas sepsis had 21.43% babies havingsevere thrombocytopenia, 53.33% had high MPV with mean MPV value 10.87±0.77. Platelet nadir and duration of thrombocytopenia was less as compared to Acinetobacter & Klebsiellasepsis.

Among Gram positive organism S. Aureus sepsis observed thrombocytopenia in 6 (85.71%) babies. The other platelet parameter like severity of thrombocytopenia, duration of thrombocytopenia,platelet nadir was less affected as compared to other organism.

Table 1: Baseline characteristics: Demographic profile

Neonatal characteristics	**N = 120**	**(%)**
Gestational age		
< 28 weeks	7	5.83
28– 32 weeks	30	25
33– 37 weeks	30	25
> 37 weeks	53	44.17
Mean gestational age = 34.98 ± 3.55 Birth weight		
< 1 Kg	7	5.83
1 – 2 Kg	59	49.17
2 – 3 Kg	48	40
3 Kg	6	5
Mean Birth weight = 1.899 ± 0.72 Gender		
Male	70	58.33
Female	50	41.67
APGAR score <5 at 5 minute	13	10.83
Mean APGAR score at 5 minutes	7.91 ± 1.43	
Mode of Delivery		
NVD	80	66.67
LSCS	40	33.33

Table 2: Baseline characteristics: Type of sepsis

Onset of sepsis	**N = 120**	**%**
Early onset sepsis	84	70
Late onset sepsis	36	30
Gram stainbased sepsis		
Gram positive	30	25
Gram negative	90	75

Table 3: Incidence & degree of thrombocytopenia

Platelet parameters	Overall (N=120)	Gram positive (n=30)	Gram negative (n=90)	P value
Thrombocytopenia	105 (87.5)	25 (80)	80 (93.33)	0.13
Degree of thrombocytopenia				
Mild	40 (38.10)	10 (40)	34 (42.5)	0.17
Moderate	45 (42.86)	14 (56)	30 (37.5)	
Severe	20 (19.05)	1 (4)	16 (20)	
High MPV	72 (60)	17 (56.67)	69 (76.67)	0.56
Mean duration of thrombocytopenia (in days)	3.46 ± 0.87	3.22 ± 0.81	3.98 ± 0.75	0.75
Lowest platelet count (in lacs)	0.27± 0.19	0.29 ± 0.18	0.24 ± 0.13	0.0001

Table 4: Comparison of platelet parameters among different organisms

	Klebsiella N =37	**Pseudomonas N = 30**	**Citrobacter N = 15**	**CONS N= 23**	**Staph. Aureus N =7**	**Acinetobacter N = 8**
	37 (30.83%)	**(26.67%)**	**(12.5%)**	**(19.17%)**	**7(5.83%)**	**(6.67%)**
Thrombocytopenia (occurrence)=105	35 (94.59)	28 (93.33)	13 (86.67)	17 (73.91)	6 (85.71)	6 (75)
Degree of thrombocytopenia						
Mild=40	10 (28.57)	7 (40)	11 (84.62)	6(35.29)	4 (66.67)	2 (33.33)
Moderate=45	14 (40)	15 (53.57)	2 (15.38)	10 (58.82)	2(33.33)	2(33.33)
Severe=20	11 (31.43)	6 (21.43)	0	1 (5.88)	0	2 (33.33)
Platelet count						
Baseline	1.77±0.68	1.98±0.91	2.31±084	2.52±1.46	2.92±0.62	1.93±1.17
At onset of sepsis	1.57 ±0.85	1.41±0.92	1.83±0.51	1.63±0.68	1.65±0.55	1.59±1.57
After 48 hours of onset of sepsis	1.27 ±0.45	1.19±0.83	1.27±0.45	1.35±0.66	1.51 ± 0.32	1.13±0.45
MPV						
Normal	8 (21.62)	14 (46.67)	11 (73.33)	13 (56.52)	4 (57.14)	4 (50)
High	29 (78.38)	16 (53.33)	4 (26.67)	10 (43.48)	3 (42.86)	4 (50)
Mean MPV	10.87±0.88	10.87±0.77	10.22 ±0.43	10.53±0.88	10.35±1.11	10.77±0.69
Duration of thrombocytopenia (In days)	4.61 ±0.42	3.27± 0.17	2.62 ±0.93	3.5 ±0.12	2.7±0.11	4.63± 0.49
Platelet Nadir	0.27±0.17	0.29±0.17	0.18±0.13	0.23±0.18	0.28±0.28	0.18±0.2

Discussion

Thrombocytopenia is one of the most common hematological manifestations in neonatal sepsis.[16] 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[17,18]. The present study showed Gram negative sepsis in 75% and Gram-positive sepsis in 25% neonates. The predominance of Gram-negative sepsis is consistent with other Indian studies[19]. Among Gram negative organisms Klebsiella was the

predominant isolates which is also comparable with earlier reports from India[20,21].

The observed 88% proportion of thrombocytopenia with Gram negative sepsis in our study is consistent with observation by Bhat et al.[22] (70%), Sartaj et al. (66%) and Ree IMC et al.[24] (69%). The proportion of severe thrombocytopenia was 20% 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.[25], R Bhat et al.[22] Ree IMC et al.[24] 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.[26] 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 observe significant difference in platelet parameters when clustering for sepsis caused by Gram positive and Gram-negative organism's done[26].

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.[27], 73% by S Arif et al.[28] and 43% by Sartaj et al.[23] We found 94.59% neonates having thrombocytopenia with Klebsiella sepsis. The effect of Klebsiella organism to various platelet parameters is explained by variation in genetic makeup of O antigen between Klebsiella pneumonia and other Gram negative organism[23].

The isolation rate of Acinetobacter organism in Indian literature ranges from 8.3% to 15.2%[17,23,28]. We found Acinetobacter isolates in 6.67% of babies with sepsis. Thrombocytopenia with Acinetobacter sepsis was seen in 66% neonates by Bhat et al.[22] In our study we found 75% rate of thrombocytopenia in case of Acinetobacter sepsis. Sartaj et al.[23] found 11% and S Arif[28] 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 93.33% rate of thrombocytopenia in our study which is comparable to rate of 67% seen by Bhat R et al.[22].

Among Gram positive organism we found proportion of thrombocytopenia at the rate of 85.71% with S. Aureus and 73.91% with CONS sepsis. S Arif had 67% rate of thrombocytopenia with S.Aureus and 25% with CONS sepsis [27].

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.

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