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Original Research Article

Effect of neonatal sepsis on platelet counts and their indices

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ABSTRACT

Background: Neonatal septicemia is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first month of life. Objective of the study was to know the effect of sepsis on platelet counts and their indices.
Methods: The study was carried out over a period of one and half year from December 2015 to July 2017 at Sangmeshwar and Basaveshwar hospital attached to M. R. medical college, Kalaburagi. 100 cases were considered for this study after proper screening for complete blood count (CBC), platelet count and their indices like mean platelet volume, platelet distribution width and C-reactive protein (CRP) and blood culture in neonates admitted in our neonatal intensive care unit (NICU) with proven sepsis.
Results: A total of 100 neonates with blood culture positive for bacterial cases were considered for the study. Early onset septicaemia (59%) was more common than late onset septicaemia (41%). Out of 100 cases 57% cases had growth of gram negative organisms, 40% had growth of gram positive organisms and 3% had growth of fungal. Tachypnea (27%), lethargy (20%) and refusal of feeds (8%) were the commonest clinical presentation followed by, fever (6%), convulsions (5%) and jaundice (5%). 60% neonates had thrombocytopenia of varying severity. *Staphylococcus aureus* was the most common organism associated with thrombocytopenia (43.3%). Mean platelet volume (MPV) was high in 85% of cases and platelet distribution width (PDW) was high in 96% of cases.
Conclusions: The present study highlights the association of thrombocytopenia, mean platelet volume and platelet distribution width with causative organism in proven neonatal sepsis. *Staphylococcus aureus* was the most common organism causing thrombocytopenia in our NICU.

Keywords: Neonatal sepsis, Thrombocytopenia, Thrombocytopenia, MPV, *Streptococcus aureus*, MPV, PDW

INTRODUCTION

Sepsis is a common complication in the neonatal intensive care unit and is a major cause of neonatal mortality. It is caused by various organisms invading the blood stream, which may be by bacterial, viral, fungal and protozoal infections.

It is characterized by positive blood culture, thrombocytopenia and elevated C-reactive protein (CRP). Septic shock is the most dangerous complication of septicaemia.[1]

Thrombocytopenia (platelet count <150,000/l) is one of the most common haematological problems in neonatal intensive care units (NICUs), with 18-35% of the NICU patients developing this problem before hospital discharge.

In contrast, only 2% of the neonates are thrombocytopenic at birth with severe thrombocytopenia (platelet count <50,000/l) occurring in less than 3/1000 term infants.[2]

Megakaryopoiesis and thrombopoiesis and platelet physiology in the fetus and neonate.

Platelets are small anucleate fragments that are formed from the cytoplasm of megakaryocytes and have a characteristic discoid shape.[3]

Megakaryopoesis include the production of megakaryocytes from stem cells, while thrombopoiesis is the production of platelets from megakaryocytes.

Platelet production begins in the yolk sac and, like the remainder of hematopoiesis shifts to the fetal liver and then to the marrow at the time of gestation.[4]

The most primitive progenitor cell that gives rise to megakaryocytic lineage cells is the multipotent progenitor, colony forming unit-granulocyte I erythrocyte/monocyte/megakaryocyte (CFU-GEMM).[5]

The most primitive progenitor cell committed exclusively to the megakaryocytic lineage is burst forming unit megakaryocyte (BFU-MK).[5]

Its immediate mature progeny is colony forming unit-megakaryocyte (CFU-MK), which is CD34, CD 41, c-mpl and HLA-DR positive.[4]

All the major megakaryoctyes progenitor and precursor cells have been identified in the fetus and the newborn.[6]

The size of the platelet in the term and premature infant averages 7-9 fl similar to the adult normal range.[3] But one study has reported a greater MPV in term than preterm infants.[3]

METHODS

The study subjects are all neonates admitted in Basaweshwar and Sangameshwar hospital NICU attached to M. R. medical college and has proven sepsis.

Inclusion criteria

All neonates admitted in our NICU with proven sepsis were included in the study.

Exclusion criteria

Patients with thrombocytopenia other than sepsis and neonates whose parents or guardians did not agree to be a part of study.

The prospective hospital based study was carried out over a period of one and half year from December 2015 to July 2017 at Sangmeshwar and Basaveshwar Hospital attached to M. R. Medical College, Kalaburagi.

100 cases were considered for this study after proper screening for complete blood count (CBC), platelet count and their indices like mean platelet volume, platelet distribution width and CRP and blood culture in neonates admitted in our NICU with proven sepsis.

Volume of blood

The chance of growing an organism effectively increases following inoculation of 0.5 ml venous blood in a pediatric blood culture bottle or 1 ml in an adult blood culture bottle (if the pediatric bottle is not available).[7]

The anticoagulant recommended for the blood culture is sodium polyanethol sulfonate (SPS liquid) in concentration of 0.0025% to 0.003%.

Methods of collection of blood

Collecting a blood sample for culture was carried out under strict aseptic conditions to avoid contamination.

Sterile gloves were worn prior to the procedure and a patch of skin approximately 5 cm in diameter over the proposed veni-puncture site was prepared. This area was cleaned thoroughly with alcohol followed by povidine-iodine followed again by alcohol. Application of povidine-iodine was done in concentric circles moving outwards from the centre to avoid contamination. The skin was allowed to dry for at least minute before sample is collected. Once blood was drawn and inoculated in to the appropriate media, it was immediately sent to the microbiology laboratory for incubation. Blood culture bottles or tubes were never inoculated when the medium was cold nor were they refrigerated after inoculation.

This technique measures the CO_2 derived pH changes by a colometric sensor in the bottom of each bottle. The sensor is separated from the broth medium by a membrane that is only permeable to CO_2.

As organisms grow they release CO_2 which diffuses across the membrane and is dissolved in water present in the matrix of the sensor. As CO_2 is dissolved, free hydrogen ions are generated. These freely generated hydrogen ions cause a colour change in the sensor which is read by the instrument.

Culture techniques

BacT/ALERT automated blood culture system is used to determine the growth of the organism.

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Within 6-18 hours of incubation most bacteria responsible for a clinically significant disease are present in numbers large enough to give a positive signal. Quick screening methods like quantitative direct plating (QDP) by placing few drops of blood may be useful where bacteraemia is of high degree or in neonates. Other sophisticated techniques in rapid isolation of organisms are by the use of radio-labelled carbon (14C) and automated techniques are recommended by some. Blood culture reports were declared at 3-5 days of incubation period. Those babies with proven bacterial sepsis were included in the study and platelet counts, bleeding manifestations and causative organisms were noted. 2 ml venous blood samples were taken in ethylenediaminetetraacetic acid (EDTA) bulbs for platelet count analysis using automated analyser.

RESULTS

The study reveals that, most patients 53 (53.0%) presented within 24 hours of age, followed by 11 (11.0%) patients who presented after 48 hours of age. The minimum age of a patient was 1 day (24 hours) and maximum age of a patient was 9 days. The mean and standard deviation (SD) of age of males was 74.51±64.52 hours and females was 71.03±53.49 hours. Overall mean age of all patients was 72.46±57.23. There was no statistically significant difference of age of patients among males and females ($p>0.05$). The sex ratio of male to female in the study was observed to be 1.85:1. The study reveals that, most of the organisms isolated were gram-negative (57%), followed by gram positive (40%) and fungal (3%).

There was no statistically significant difference of mean gestation age, birth weight, hospital stay, Hb% level and total count among gram-positive, gram negative and fungi.

There was statistically significant difference of neonatal age of patients among gram-positive, gram-negative and fungal ($p<0.05$) (Table 1).

The study reveals that, 60 (60.0%) patients had neonatal thrombocytopenia. Out of 60 cases of neonatal thrombocytopenia, most common causative organism was *Staphylococcus aureus* (43.3%) followed by *E. coli* (21.7%), *Klebsiella* (20.0%), *Pseudomonas* (8.3%) and *Candida* (5%) (Table 2).

The mean and SD of platelet count (per µl) of patients with gram positive septicaemia was 164960±68083, gram-negative septicaemia was 212870±103540 and fungal septicaemia was 143667±18625. Overall mean and SD of platelet count (per µl) was 180479±93754. There was statistically significant difference of platelet count (per µl) among gram-positive and negative and fungi patients ($p<0.01$) (Table 3).

The study reveals that lowest platelet count is found in patient in whom *E. coli* isolated was 11000 followed by Staphylococcus aureus (15000), *Klebsiella* (28000), *Candida* (60000) and *Pseudomonas* (104000). Organism causing severe thrombocytopenia was *E. coli* followed by *Staphylococcus aureus* and *Klebsiella*.

In our study mean platelet volume was 13.3 in CoNS, 10.56 in *Klebsiella*, 10.38 in *Staphylococcus aureus*, 10.1 in *Candida*, 9.82 *E. coli* and 9.65 in *Pseudomonas*.

Our study also reveals, platelet distribution width was 16.4 in CoNS, 15.82 in *Staphylococcus aureus*, 15.46 in *Klebsiella*, 14.73 in *E. coli*, 14.51 in *Candida* and 14.3 in *Pseudomonas* (Table 4).

Table 1: Demographic data of neonatal sepsis.

Variables	All patients N=100	Gram- positive (N=40)	Gram- negative (N=57)	Fungal (N=3)	ANOVA test value	P value and significance
	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
Gestation age in weeks	36.56±2.37	36.63±2.39	36.30±2.36	34.61±1.88	F=0.58	P=0.561 NS
Birth weight in kg	2.27±0.60	2.26±0.58	2.29±0.62	1.30±0.0	F=0.33	P=0.764 NS
Neonatal age in hours	72.46±57.23	81.47±62.12	59.74±39.81	56.10±45.23	F=2.39	P=0.019 S
Hospital stay in days	16.34±5.78	16.88±5.32	15.79±6.12	12.12±7.03	F=0.89	P=0.382 NS
Hb% level	14.86±2.64	14.6±2.55	15.17±2.79	13.82±3.19	F=1.73	P=0.353 NS
Total count	23643±38657	23824±40472	23029±34031	16065±44031	F=0.38	P=0.731 NS
Caesarean section (%)	39 (39.0)	14 (35.0)	24 (42.7)	2 (33.3)	X2=1.34	P=0.743 NS

NS=not significant, S=significant

Table 2: Distribution of neonatal thrombocytopenia according to causative organisms.

Variable	Organism	Number	Percentage (%)
Gram-positive 27/60 (45%)	*Staph aureus*	26	43.3
	CoNS	1	1.7
Gram-negative 30/60 (50%)	*E. coli*	13	21.7
	Klebsiella	12	20.0
	Pseudomonas	5	8.3
Fungal 3/60 (5%)	*Candida*	3	5.0
Total	---	60	100.0

Table 3: Platelet count (per µl) at onset of sepsis in the groups.

Variables	Platelet count (per µl) Mean±SD	Test values	P value and significance
Gram-positive	164960±68083	F=3.12	P=0.043 S
Gram-negative	212870±103540		
Fungal	143667±18625		
Total	180479±93754	-	-

Table 4: Effect of different organisms on platelet indices in neonatal sepsis.

Type of organism	Organism	No. of patients	Platelet count at onset of sepsis (per µl)	Lowest platelet count (per µl)	Average MPV (Fl)	MPV range	PDW	PDW range
Gram-positive 27/60 (46.5%)	*Staph aureus*	26	97300	15000	10.38	9.6-13.3	15.82	14.2-17.4
	CoNS	1	148000	60000	13.3	13.3	16.4	16.4
Gram-negative 30/60 (48.8%)	*E. coli*	13	112400	11000	9.82	8.6-12.5	14.73	14.1-16.3
	Klebsiella	12	98250	28000	10.56	8.9-13.7	15.46	14.9-16.8
	Pseudomonas	5	138000	104000	9.65	9.9-11.2	14.3	14.0-15.5
Fungi 3/60 (4.7%)	*Candida*	3	131000	60000	10.1	9.2-13.3	14.51	15.3-16.5
Total	--	60	-	-	-		-	

DISCUSSION

More than 30-80% of neonates with proven infection become thrombocytopenic.[16,17] Bacterial, fungal and viral infections all have been associated with neonatal thrombocytopenia.[18]

Thrombocytopenia occurs in one-third of infants admitted in NICU. Thrombocytopenia is frequently associated with mucosal bleeds and purpura.

Fungal sepsis is associated with greater degree of thrombocytopenia than is seen with gram positive or gram negative organisms and outcome in these neonates is poor.

MPV levels may increase in mild inflammation because of the raise of large platelets, or on the contrary, MPV levels may decrease in severe inflammation owing to the depletion of large platelets in inflammatory area.

Destructive thrombocytopenia known to be associated with high MPV levels while low level of MPV is reported in hypo-proliferative thrombocytopenia.

These observations indicate that MPV may be a negative acute phase reactant as well as a positive acute phase reactant and may show fluctuation in different phases of sepsis.

In our study we made an attempt to see association of platelet count and their indices in neonatal sepsis.

In our study 6 organisms was isolated and all these organisms were associated with some form of thrombocytopenia.

Among them gram negative sepsis (57%) is more common than gram positive sepsis. In gram negative sepsis (57%) most common organism is *Klebsiella pneumonia* (24%),

E. coli (24%) followed by *Pseudomonas* (9%). In gram positive organism (40%), *Staphylococcus aureus* (37%) was the most common organism causing sepsis.

Rajnesh proved in their study that gram negative organisms causing sepsis were 54%. In that most common were *Klebsiella*, followed by *Pseudomonas* then *Acinetobacter* and gram positive were 40%, of which *Staphylococcus* was most common followed by *Enterococcus*.

Platelet count comparison

In our study most common organism causing thrombocytopenia is *Staphylococcus* (43.3%) next in the line here *E. coli* (21.7%), *Klebsiella* (21%), *Pseudomonas* (8.3%), *Candida* (5%) and CONS (1.7%). Gram negative organisms are the most common organisms causing thrombocytopenia (50%) than gram positive organisms (45%) and fungal organisms (5%).

Guida's study, gram negative were 16% whereas grams positive and fungal were 7.6% and 8% respectively.

Bhat et al identified gram negative culture positive in 67.5% and gram positive is 26.3%, remaining were fungal growth.

A study done by Bashir et al showed that *Klebsiella pneumonia* was the most common organism associated with thrombocytopenia (58%).

Another study done by Arif et al showed that *Klebsiella pneumonia* was the most common organism associated with thrombocytopenia (73.3%).

Mean platelet volume

In our study decreased platelet count associated with increase in MPV (85%).

Nelson et al observed platelet consumption associated with increase in MPV in human subjects having acute infection.

Becchi et al suggested that MPV has an important prognostic value of early stage of sepsis.

Guida reported 54% neonates with thrombocytopenia, of which 61% neonates had increased MPV.

Platelet distribution width

In our study there is an increase in PDW in 96% of cases.

Guclu et al found PDW as a significant parameter in neonates with sepsis. Ferhatcatal et al found that there is significant differences between control and sepsis group in terms of platelet count, PDW/MPV ($p<0.005$).

Patrick et al reported that there is significantly increased presence of bacteremia in those neonates with MPV greater than 10.8 fl and/or PDW greater than 19.1%.

CONCLUSION

Neonatal sepsis was common in males. Gram positive organisms were the predominant causative agents of septicaemia 40% as compared to gram negative organisms 57% and fungal sepsis 3%. *Staphylococcus aureus* was the commonest organism responsible for thrombocytopenia. Among thrombocytopenic neonates 43% had mild thrombocytopenia, 13% had moderate thrombocytopenia and 4% had mild thrombocytopenia.

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