Thrombocytopenia and Bacterial Sepsis in Neonates

S. H. Arif · I. Ahmad · S. M. Ali · H. M. Khan

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Abstract Thrombocytopenia is one of the common hematological problems encountered in the neonatal period particularly in the sick newborns, premature babies and neonates admitted in neonatal intensive care units and usually indicate an underlying pathologic process. Thrombocytopenia is reported in neonates with bacterial, fungal, rickettsial, protozoal and viral infection. Some patients with bacterial septicemia may develop coagulopathy associated with DIC. The presence of thrombocytopenia is seen frequently in early sepsis with or without laboratory evidence of overt DIC. This study was conducted on 85 neonates admitted in NICU with clinical diagnosis of septicemia and 50 age and weight matched neonates served as control. Thrombocytopenia was seen in 83.5% cases where as bacterial culture was positive in only 41.1% cases. Further it was noted that, in gram negative (Gm –ve) septicemia, thrombocytopenia was more severe as compared to gram positive (Gm +ve) septicemia. It is

concluded that thrombocytopenia is early predictor of septicemia but other causes of neonatal thrombocytopenia should also be ruled out.

Keywords Neonatal septicemia · Thrombocytopenia · DIC

Introduction

Thrombocytopenia is one of the common hematological problems encountered in the neonatal period particularly in the sick newborns, premature babies and neonates admitted in neonatal intensive care units and usually indicate an underlying pathologic process. It is a significant cause of morbidity and mortality in the sick pre and full term infant and accounts up to 20 to 40% of the newborns admitted to Neonatal Intensive Care Unit (NICU). Most of the ill and premature infants have low platelet count [1].

It is also reported that not only bacteria, fungal, protozoal, rickettsial and viral etiology may also cause neonatal septicemia. Apart from infection due to any of the above agents there are many other causes of thrombocytopenia such as immune mediated, chromosomal anomalies and genetic disorders [2].

Some patients with bacterial septicemia may develop a coagulopathy associated with DIC. The presence of thrombocytopenia is seen frequently in early sepsis with or without laboratory evidence of overt DIC [3, 4].

Various studies are carried out in developed countries and in India show vast difference in growth of isolates. It is noted that in western worlds, Gram positive (Gm +ve) bacteria such as Group B Streptococci and coagulase negative staphylococci (CONS) are usual isolates whereas in India Gram negative (Gm –ve) bacteria form majority

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S. H. Arif (✉)
Department of Pathology, J. N. Medical College, Aligarh
Muslim University, Aligarh 202002, India
e-mail: s_h_arif@yahoo.com

I. Ahmad
Lady Harding Medical College, New Delhi, India

S. M. Ali
Department of Pediatrics, J. N. Medical College, Aligarh Muslim
University, Aligarh, India

H. M. Khan
Department of Microbiology, J. N. Medical College, Aligarh
Muslim University, Aligarh, India

of isolates. *Klebsiella* species is noted to be the commonest organism along with *E. coli*, *Staph. aureus*, *Staph. epidermidis*, Group B Streptococci, *Enterococcus faecalis*, *Enterobacter* sp., *Acinetobacter* sp., *Pseudomonas* sp., *Proteus* sp., *Citrobacter* seen in developing countries [2, 5–8].

Thrombocytopenia is usually seen with Gm +ve septicemia as compared to Gm –ve septicemia [9] and low platelet is usually seen even before the pathogens are cultured from the blood. Therefore, thrombocytopenia may be considered as an important and early tool in diagnosis of septicemia in neonates [10].

Mechanism of Thrombocytopenia in Septicemia

The mechanism of thrombocytopenia in patients with septicemia, with no evidence of disseminated intravascular coagulation (DIC) is uncertain. On the other hand thrombocytopenia in DIC is considered mainly due to consumption of certain coagulation factors and circulating platelet. There is evidence of an activated fibrinolytic mechanism by the presence of FDP in serum and low levels of factor II, V, VIII, and fibrinogen. All these observations indicate consumption of circulating platelet as a usual cause of thrombocytopenia [3, 11].

Bacteria or bacterial products may cause endothelial damage leading to platelet adhesion and aggregation or may bind directly to platelets leading to aggregation and accelerated clearance from circulation [12, 13]. There may be a possibility of immune mechanism for development of thrombocytopenia in septicemia as there is presence of circulating immune complex in septicemic patients and reduced number of complement complex in patients with septicemic shock [14, 15].

Materials and Methods

This study was conducted in Department of Pathology in collaboration with Neonatology section of Department of Pediatrics and Department of Microbiology of J. N. Medical College, AMU Aligarh.

A total of 135 full term neonates of >2.5 kg weight were included in the study, 85 of them were with clinical diagnosis of septicemia and admitted to NICU, the other 50 neonates were considered as control. Blood was collected with all septic precaution in BD Vacutainer EDTA tube and in blood culture bottle and transported to respective laboratory for platelet count and blood culture quickly.

Platelet count was performed by automatic hematological cell counter as a part of complete blood count and blood culture was done after inoculation of blood into blood culture bottle containing BHI with 0.025% sodium

polyanethol sulfonate, it was incubated at 37°C for 7 days, subculture was done on blood agar and MacConkey's agar media after 24 h and 7 days of incubation. Blood culture was reported sterile if no growth was seen on subculture after 7 days of incubation. If turbidity or hemolysis was seen earlier, subculture was done on that day.

Identification of the Isolation

After incubation of the plate over night, isolated bacterial organisms were identified according to the criteria laid down in Collee et al. [16]. Colony characteristics on sheep blood agar and MacConkey's agar were studied and organism was identified by examination of Gram stain smear, motility and using the various biochemical tests.

Statistical Analysis

Statistical analysis was performed applying Pearson Chi-square test using SPSS software.

Observations

This study comprised of 135 neonates out of which 85 neonates had clinical diagnosis of septicemia and 50 age and weight matched full term neonates served as control. Out of 50 neonates 31 were male and 19 were female (M:F ratio 1.6:1), average platelet count was 195×10^3 and blood cultures were negative. None of the neonates in the control group has thrombocytopenia (Table 1).

Table 2 shows that thrombocytopenia was recorded in 71 cases (83.5%), although blood culture was positive in only 24 cases (33.8%) whereas in 47 cases (66.1%) blood culture were negative. 14 cases showed normal platelet count, out of which 11 cases (41.1%) showed positive blood culture whereas in three cases (21.4%) blood culture were negative. It is shown in the Table 2 that 50 controls which were selected for study had normal platelet count and none of them showed blood culture positive.

These results were statistically analyzed using Pearson Chi-square test by SPSS software. The value obtained as follows–

Table 1 Control group

	Number of control	Avg platelet count (μl^{-1})	Blood culture
Male	31	190×10^3	Negative
Female	19	200×10^3	Negative
	M:F ratio = 1.6:1	195×10^3	Negative

P value was 0.02 which was $<0.05\%$ and significant at 5% level of significance. The result was significance at 5%. The interpretation of this analysis is that there is a significant relation in platelet count and septicemia, indicating that platelet count is invariably low in cases of septicemia irrespective of blood culture as 71 cases (83.5%) showed low platelet count, out of which only 24 cases (33.8%) showed positive blood culture.

Table 3 shows that neonatal septicemia was observed commonly due to Gm +ve bacteria ($n = 20$, 57.1%), mainly coagulase negative *Staphylococcus* ($n = 12$, 60%) and *Staphylococcus aureus* ($n = 6$, 30%) whereas in only 42.9% cases ($n = 15$) Gm –ve septicemia was reported and out of 15 cases, in 11 cases (73.3%) *Klebsiella* was isolated. Table 3 also showed the range of platelet count in Gm +ve and Gm –ve cases and it was seen that thrombocytopenia is very severe in Gm –ve cases as platelet count was as low as $33 \times 10^3/\mu\text{l}$ in a case (range— 33×10^3 – $87 \times 10^3/\mu\text{l}$).

Out Come of the Patients

Five cases (5.8%) of the study group developed DIC. The bacteria, which were isolated in these cases, were *Klebsiella*. Specific antibiotic after sensitivity along with FFP and

platelet transfusion were given in these cases. Out of these five cases, three cases (3.5%) were recovered and platelet counts were increased and reached to normal limit in 14–16 days. Two cases (2.3%) were died of the disease. The cause of death was DIC and patients were developed petechiae and bleeding from various sites including internal bleeding. The platelet count reduced to $5,000/\mu\text{l}$.

Broad spectrum antibiotic was gives in those cases where blood cultures were negative and specific antibiotic was gives after sensitivity in those cases where blood cultures were positive in rest of the cases. Patients were responded; platelet counts were increased and reached to normal with in 10–14 days with improvement of clinical condition of neonates. All 83 neonates were discharged from the hospital after recovery.

Discussion

Thrombocytopenia is a problem frequently encountered in the neonatal period and it is a significant cause of morbidity and mortality in the sick, pre and full term babies [17]. The frequency of neonatal thrombocytopenia has been estimated to range from 20 to 40% of the newborns admitted to NICU in different studies [1, 18]. Thrombocytopenia is

Table 2 Comparison of thrombocytopenia and blood culture in diagnosis of neonatal septicemia ($n = 85$)

Figures in *parenthesis* show percentage of total of cases with thrombocytopenia and cases without thrombocytopenia

Cases				Controls		
	Blood culture positive	Blood culture negative	Total	Blood culture positive	Blood culture negative	Total
Thrombocytopenia	24 (33.8%)	47 (66.1%)	71 (83.5%)	Nil	Nil	Nil
No thrombocytopenia	11 (78.5%)	3 (21.4%)	14 (16.5%)	Nil	50	50
Total	35 (41.1%)	50 (58.8%)	85 (100%)	Nil	50	50

Table 3 Bacteriological profile of septicemia with and without thrombocytopenia

Causative organism		Total	Thrombocytopenia	No thrombocytopenia	Platelet count range in cases of thrombocytopenia (μl)
Gm +ve bacteria $N = 20$ (57.1%)	Coagulase-negative staphylococcus	12 (60%)	3 (25%)	9 (75%)	95×10^3 – 115×10^3
	<i>Streptococcus viridan</i>	1 (5%)	1	–	115×10^3
	<i>Staphylococcus aureus</i>	6 (30%)	4 (66.7%)	2 (33.3%)	110×10^3 – 115×10^3
	<i>Streptococcus-B</i>	1 (5%)	1	–	125×10^3
	Total	20 (57.1%)	9 (45%)	11 (55%)	95×10^3 – 125×10^3
Gm –ve bacteria $N = 15$ (42.9%)	<i>E. coli</i>	1 (6%)	1 (6%)	–	68×10^3
	<i>Acinetobacter</i>	1 (6%)	1 (6%)	–	45×10^3
	<i>Pseudomonas</i>	1 (6%)	1 (6%)	–	33×10^3
	<i>Klabsiella</i> sps	11 (73.3%)	11 (73.3%)	–	35×10^3 – 99×10^3
	<i>Citrobacter</i>	1 (6%)	1 (6%)	–	87×10^3
	Total	15 (42.9%)	15 (100%)	Nil	33×10^3 – 87×10^3

also a common manifestation of bacterial septicemia. Neonates are particularly vulnerable to infection because of weak immune barrier. Moreover, several risk factors have been identified both in the neonates and in the mothers who make them susceptible to infections. Neonatal septicemia requires rapid, accurate diagnosis and treatment for better prognosis. Thrombocytopenia occurs in early course of septicemia, therefore platelet count may be considered as early predictor for the diagnosis of septicemia [19, 20]. It was seen in the present study that 71 (83.5%) neonates had low platelet count ($<150 \times 10^3/\mu\text{l}$), indicating that thrombocytopenia is an important finding in sick neonates admitted in NICU. Out of these 71 cases only 24 (33.8%) cases showed positive blood culture. These findings indicate that low platelet count is important finding in bacterial septicemia. Further it was also observed that thrombocytopenia was noted in majority of cases in which blood culture was negative [21]. Therefore, it was observed from the study that platelet count is an important indicator of septicemia and not related with blood culture, although not specific.

It was observed from the study that Gm +ve bacteria is commonly isolated as compared to Gm -ve in neonates with septicemia which is similar to study of Jack et al. [22] although various Indian studies showed isolation of Gm -ve bacteria [23, 24]. But thrombocytopenia was more severe in Gm -ve infections as compared to Gm +ve infection as all cases with Gm -ve septicemia showed low platelet count and platelet count had gone down to $33 \times 10^3/\mu\text{l}$ (range— 33×10^3 – $87 \times 10^3/\mu\text{l}$) in the present study. Further it was noted from the study that five neonates who developed DIC during the course of illness were also suffering with Gm -ve septicemia. Riedler et al. [10] reported 80% cases of thrombocytopenia with Gm -ve septicemia whereas with Gm +ve septicemia only 65% cases showed low platelet count.

There are wide ranges of growth of organism in various studies. Coagulase negative staphylococcus is considered to be the commonest organism causing neonatal septicemia ranging from 4.46 to 90% in various studies [25, 26]. 34.4% (12/35) cases in our study showed growth of CONS. This bacterium was earlier not considered as a pathogen and frequently discarded from the isolates as majority of the neonates admitted in NICU become infected with CONS [26] but owing to development of thrombocytopenia and other complications it was considered as pathogenic organism [27].

Klebsiella is also considered as one of the important organisms grows in neonates admitted to NICU. The ability of Klebsiella sps to cause outbreak of serious infection in NICU may be related to its virulence, its ability to colonize neonates, to survive in the inanimate environment and ability to acquire antibiotic resistance [28]. Another factor

that is related to the prevalence of Klebsiella is that neonates are born without a normal flora and is acquired over first few weeks of life. Premature neonates acquire intestinal flora in which Klebsiella are highly prevalent. This acts as a reservoir for infecting the colonized neonates and for colonizing and infecting others in neonatal unit. Isolation of Acinetobacter, Citrobacter and Pseudomonas were considered as incidental finding although these bacteria are considered as outbreak of nosocomial infection but as only one case each of these organism were isolated over a period of study this may not be considered as outbreak. Even then samples from all the articles used in NICU and health workers were taken for bacterial culture and all the samples were negative for all these pathogens [29–31].

Mortality is reported to be common in preterm and low birth weight neonates, but as this study includes only full term and normal weight neonates low mortality was noted as only two cases (2.3%) were died. The most virulent organism causing mortality was Klebsiella [3]. DIC is a fatal complication of septicemia and need intensive treatment. Thrombocytopenia is an important finding in DIC and usually due to consumptive coagulopathy [2]. Similar finding is also noted in present study. But in majority of cases thrombocytopenia is noted without any evidence of DIC [32].

Fungal, rickettsial, protozoal and viral infections also cause thrombocytopenia [6]. As this study was done only for bacterial isolate, there might be other cause as mentioned above may be associated with thrombocytopenia which did not show bacterial growth.

Conclusion

It is concluded from the study that thrombocytopenia is a common finding in septicemic neonates. Coagulase +ve staphylococcus is the common isolates, although, thrombocytopenia is commonly seen with Gm -ve septicemia. A good number of cases with no bacterial growth also showed thrombocytopenia. This indicates that thrombocytopenia may be considered an early but nonspecific indicator of septicemia but other causes of neonatal septicemia should also be ruled out.

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