Is Thrombocytopenia Consistent with Specific Bacterial/Fungal Neonatal Sepsis?

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Abstract

Background
Neonatal sepsis is a clinical syndrome with presence of both infection and systemic inflammatory response syndrome. Thrombocytopenia is a marker of neonatal sepsis. Present study was conducted to determine association of thrombocytopenia with any specific infection.

Methods
A total of 120 neonates were included in this cross sectional study conducted in the neonatal intensive care unit (NICU) of the Children's Hospital, Lahore from January to July 2015. All neonates with presumed sepsis and positive blood cultures were included. The neonates who had received platelet/ blood transfusion or drugs known to cause thrombocytopenia were excluded. Data on age, gender, platelet count, microbiological culture and clinical outcome was collected on a proforma. Statistical analysis was performed using SPSS 20 to look for association between thrombocytopenia and different organisms (Gram positive, Gram negative or fungal) causing sepsis. Chi-square test and logistic regression methods were used for calculating significance. P-value of <0.05 was taken as significant.

Results
A total of 120 newborns were included with 81 males (68%) and 39 females (32%). Their mean age was 140.28 hours (±130.71 SD). Thrombocytopenia was detected in 29% (n=35, p=0.90). Out of these septic & thrombocytopenic babies, Gram negative organisms were found in 69% (n = 24, p =0.31) and Gram positive organisms in 31% (n = 11, p=0.44). No case of fungal sepsis had thrombocytopenia. Considering the individual microorganism, only *Klebsiella pneumoniae* (p=0.027) had a significant association with thrombocytopenia.

Conclusions
In neonatal sepsis, presence of thrombocytopenia may be an indicator of Klebsiella infection.

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Key words
Neonatal sepsis, thrombocytopenia, blood culture, microorganism

Introduction
Neonatal sepsis is defined as the clinical syndrome with presence of both infection and systemic inflammatory response syndrome.[1] Thrombocytopenia i.e. a platelet count of <100,000 is being used as a marker of neonatal sepsis.[2]

The incidence of thrombocytopenia in well newborns is 1-5% while it is observed in 22-35% of neonates admitted in NICUs.[3] According to a study 59.5 % of septic neonates have been found to have low platelet count.[4]

The underlying causes of thrombocytopenia include Infections, prematurity, birth asphyxia, IUGR, neonatal alloimmune / autoimmune thrombocytopenia and rare disorders like congenital amegakaryoctic thrombocytopenia.[5,6] Bacterial, Viral and Fungal Infections can result in low platelet count through a number of mechanisms resulting in suppression of the bone marrow, immune-mediated destruction, DIC and platelet aggregation due to bacterial products adhesion to platelet membrane,[7] structural changes in platelet membranes intravascular platelet aggregation, and decreased production from degeneration of platelet precursors in bone marrow.[7]

Clinical manifestation of low platelet count can be prolonged bleeding from skin, bleeding from lungs, GIT or intraventricular hemorrhage while treatment options include management of underlying cause with platelet transfusion in some cases.[8]

As sepsis is seen in one third of NICU cases and according to a study 59.5% of septic neonates have been found to have low platelet count, whether there is an association between low platelet count and type of microorganism responsible for sepsis is a debatable issue .Previous studies have shown conflicting results in this regard.[4,9] Some suggesting thrombocytopenia as an early marker of Gram negative and fungal infections while other contradicting it.[3,8] The aim of study was to determine potential role of platelets as an early marker for specific type of infection, thus modifying the choice of empirical antibiotics. This may help reduce the irrational use of the antibiotics, prevent emergence of resistance, check loss of finances, save the patient from

antibiotics associated side effects and result in early recovery.

Materials & Methods

This prospective cross sectional study was conducted from 1st January to 31st July 2015 in Neonatology Unit, the Children's Hospital & the Institute of Child Health, Lahore. The study was initiated after obtaining permission from the IRB. Informed consent was obtained from the parents or guardians. A total of 120 neonates of either gender admitted in neonatal unit through neonatal OPD/ emergency room who presented with clinical sepsis or developed sepsis in hospital and their blood culture was found to be positive were included in the study. Detailed history and examination were carried out in all patients presenting with sepsis. Patients who had already received antibiotics known to cause thrombocytopenia or received platelet transfusion before blood culture or with incomplete data were excluded from the study.

Three milliliters of blood was collected under aseptic conditions in EDTA test tube (Improvacuator), while 1.5cc blood was collected in blood culture bottles containing 15 ml of BHI under sterile precautions. Samples were transported to respective laboratories for processing of platelet count and blood cultures. Platelet count was performed by automatic hematological cell counter (Sysmex KX-21) as part of complete blood count. Blood culture was performed after inoculation of sampled blood into blood culture bottles containing TSB with 0.025% sodium polyanethol sulfonate. Blood culture vials were incubated at 37°C for 7-days, during which subcultures were done on solid media - blood agar and MacConkey's agar at 24 hours, 48hours and 6th day of incubation. Blood culture was reported sterile if no growth was seen on subculture after 6 days of incubation. If turbidity or hemolysis was seen earlier, subculture was done on respective day. Isolated bacterial organisms were identified according to the criteria laid down in Collee *et al*.[10] Colony characteristics on blood and MacConkey's agar were identified by examination of Gram stained smears and using the various biochemical tests including API.

Data on age, gender, platelet count, microbiological culture and clinical outcome was collected on a proforma. Statistical analysis was performed using SPSS v20 to look for association between thrombocytopenia and different (Gram positive, Gram negative or fungal) organisms causing sepsis. Chi-square test and logistic regression methods were used for calculating significance. P-value of ≤0.05 was taken as significant.

Results

A total of 127 babies were initially included. Out of these 07 were excluded and data of total of 120 babies was used for analysis. There were 81 male (68%) and 39 females (32%), with male: female of 2:1. The mean age of admission was 140 ± 131 hours. Mean birth weight was 2.44+ 0.71 kg (0.8-4.5 kg) (Table 1).

A total of 47 cases (39%) of early onset sepsis (EOS) and 73 cases (61%) of late onset sepsis (LOS) were observed. Thrombocytopenia was observed in 35 (29%) cases (Table 2). Out of these septic thrombocytopenic babies EOS was present in (n=14, 29%, p=0.904) and LOS (n=21, 29%, p=0.90) both being statistically insignificant (Fig.1).

Table 1: Demographic data

Gender	Male	81
	Female	39
Age	<72 hr	47
	<72 hr	73
Birht Weight	<1 kg	3
	<1.1 - 1.5 kg	14
	<1.6 - 2.5 kg	25
	>2.5 kg	78

Table 2: Association table

Parameters	Thrombocytopeni		P- value
	Yes	No	
Gram +ve	11	33	0.31
Gram -ve	24	50	0.44
EOS	14	33	0.90
LOS	21	52	0.90

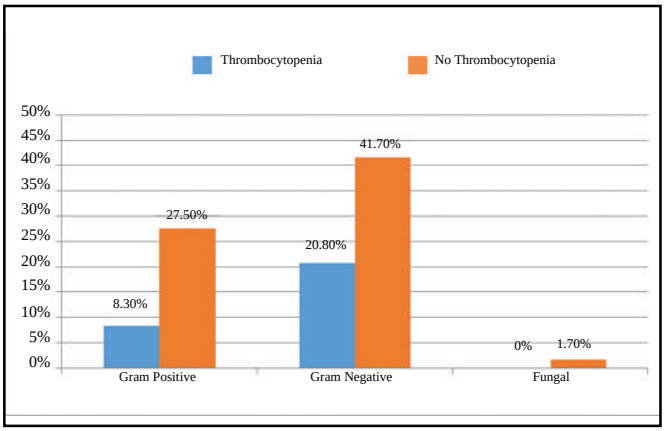

Fig. 1. Frequency of Thrombocytopenia in Bacterial/Fungal neonatal sepsis

Out of the thrombocytopenic babies, Gram negative organisms were found in 69% (n=24, p =0.31) and Gram positive organisms in 31% (n=11, p=0.44). No case of fungal sepsis had thrombocytopenia (Fig.2).

Considering individual microorganism, neonatal sepsis because of *Klebsiella pneumoniae* (p=0.027) had a significant association with thrombocytopenia. Other organisms found during study did not show significant association with thrombocytopenia (Fig.3).

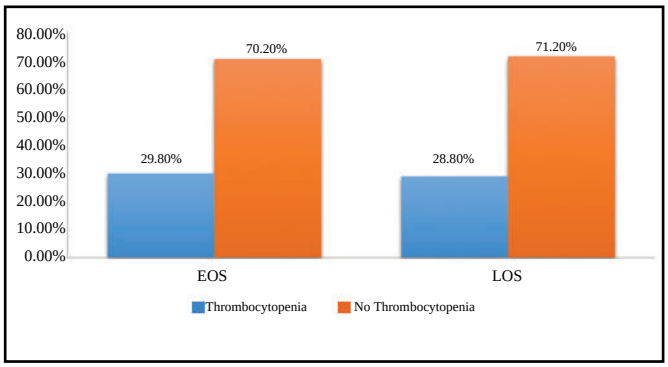

Fig. 2. Association of EOS and LOS with Thrombocytopenia

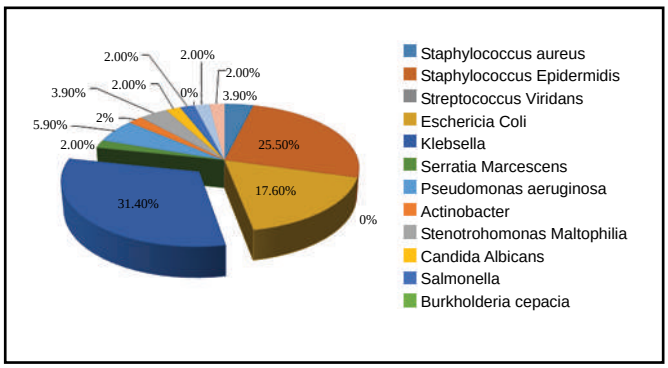

Fig. 3. Frequency of thrombocytopenia in microorganisms responsible for neonatal sepsis

Discussion

Thrombocytopenia is accepted as a non-specific marker of neonatal sepsis.[1] Various studies done in different places aimed to find its association with specific causative organisms have shown variable results.[4,9] Current study was aimed to find association of thrombocytopenia with a specific organism.

The male predilection of neonatal sepsis is a common observation. Hubacek observed an immunological difference between males and females claiming that the polymorphism in genes for Lysosomal Binding Protein is responsible for it.[11] Marriot has shown that males on account of cell surface receptors like TLR 4 trigger a different response than females in terms of inflammatory cytokines and acute phase reactants thus making males more prone to sepsis.[12] Muhammad, Sheikh and Tayeb have demonstrated in their studies that males are more susceptible to neonatal sepsis than females.[13,14,15] Above studies support the results of our study that has revealed similar results with male to female distribution of 2:1.

A study conducted by Najeeb in Peshawar found LOS to be more common than EOS which coincides with results of our study.[16] The similarity may be because of same population and organisms' common in our set-up. Haque has also described matching situation in his study done to find the pattern of culture-proven neonatal sepsis in the United Kingdom.[17] The similarity may be because of same definitions of types of neonatal sepsis and cut-off limit i.e. 72 hours used in our studies. On the contrary the results of a study conducted by Ahmad in local population found EOS to be more common than LOS.[18] The difference may be due to different cut-off limits i.e. 7-days to categorize EOS or LOS.

The studies performed by Sheikh *et al*, Waseem, Muhammad *et al* and Basheer *et al* found that Gram negative bacteria were more common than Gram positive in neonatal sepsis.[14,19,20] We had similar observation which may be due to similar pattern of infections in our population. Benjamen in his study found a frequency of fungal sepsis in neonates to be 7% in western population.[21] However, Basheer and Charoo found a frequency of 2% and 3% respectively in our population which is consistent with our results of 1.78%.[4,20] The difference with study conducted by Benjamen can be because his study was conducted in a different population.

Jeremiah found that 63.7% of septic neonates had thrombocytopenia while Arif calculated a percentage of 33.8 in culture positive neonatal sepsis.[2,7] Percentage of thrombocytopenia in neonatal sepsis was 29.2 in our study which is similar to study by Arif. Jermiahs study focused on a cohort of only 22 septic Nigerian newborns. Small sample size and different study population may be the cause of variation in results. On the other hand, Arif selected similar sample size, study population and lab method for diagnosis of neonatal sepsis, as we did in our study, which explains similarity in our results.

Guida in his study conducted on very low birth weight and preterm babies revealed an association between thrombocytopenia with gram negative and fungal sepsis.[21,22] The results are not in conformity with our study which may be because of different study population as our study included both preterm as well as term babies. However, in the study conducted by Alshorman, with similar objectives, no association was found between thrombocytopenia and bacterial/fungal organisms. These results coincide with our study as both the studies included both term and preterm neonates.[23]

Many studies conducted in tertiary centers in developing world found that *Klebsiella* infection is significantly associated with

thrombocytopenia.[4,9,24] The results are in conformity with our study that may be because these studies have similar study design and have been performed in similar set-ups.

Conclusions

In neonatal sepsis, presence of thrombocytopenia may be taken as indicator of *Klebsiella* infection.

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