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Thrombocytopenia and Prolonged Prothrombin Time in Neonatal Septicemia

Authors

Shittu Bashirat T[1], Shittu Mujeeb O[2], Oluremi Adeolu S[3], Orisadare Olayiwola P[4] Jikeme Osemeke[5] Bello Lateef A[6]

[1]Department of Medical Laboratory Services
Hematology and Blood Group Serology Unit, Ladoke Akintola University of Technology
Osogbo, Osun State, Nigeria
[2]Department of Medical Laboratory Services
Chemical Pathology unit, Ladoke Akintola University of Technology Teaching Hospital
Ogbomoso, Oyo State, Nigeria
[3]Department of Biomedical Sciences
Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria
[4]Department of Biomedical Sciences
University of Greenwich, London, England
[5]Department of Pathology, Biochemistry Unit
Stepping Hill Hospital, United Kingdom
[6]Department of Medical Laboratory Services
Outreach Children's Hospital, Festac Town, Lagos, Nigeria

Corresponding Author
Shittu MO.
Department of Medical Laboratory Services
Chemical Pathology unit, Ladoke Akintola University of Technology Teaching Hospital
Ogbomoso, Oyo State, Nigeria
P.O Box 3363, Osogbo, Osun State, Nigeria
E-mail: *shifemjay1@gmail.com*
Tel: +2348038572374

Abstract

Septicemia in neonates refers to generalized bacterial infection documented by positive blood culture in the first 28days of life and is one of the leading causes of neonatal mortality in sub-Sahara Africa. Thrombocytopenia in newborns is a result of increased platelet consumption; sepsis was found to be the most common risk factor. The objective of the study was to determine if there are organism-specific platelet responses among the 2 groups of bacterial agents: Gram-positive and Gram-negative bacteria, and also to examine the association of platelet count and prothrombin time with neonatal septicemia.

232 blood samples were collected for this study. The blood culture was performed using Bactec 9050, an instrumented blood culture system. The platelet count and prothrombin time were performed using Abacus Junior5 hematology analyzer and i-STAT 1 analyzer respectively.

Of the 231 neonates hospitalised with clinical sepsis, blood culture reports were positive in 51 cases (21.4%). Klebsiella spp. (35.3%) and Staphylococcus aureus (27.5%) were the most common Gram-negative and Gram-positive isolates respectively. Thrombocytopenia was observed in 30(58.8%) of the neonates with septicemia. Of the 9(17.6%) patients with severe thrombocytopenia, seven (77.8%) had Klebsiella spp. septicemia. Out of the 21(63.6%) of thrombocytopenia produced by Gram-negative isolate, 17(80.9) had increased prothrombin time. In conclusion, Gram-negative organisms showed the highest cases of severe thrombocytopenia and prolonged PT. This study has helped to establish a disturbance in hemostatic systems in neonates with septicemia. Further studies, however, may be required to assess other hemostasis parameters in order to understand their interaction with the infectious organisms in neonates.

Keywords: Neonates, septicemia, thrombocytopenia, prolonged prothrombin time, platelet count

INTRODUCTION

Thrombocytopenia was defined based on published literatures as having a platelet count of $<150\times10^3$/uL [1],[2]. Neonatal platelet counts of $100\text{-}150\times10^3$/uL represent mild thrombocytopenia, platelet counts of $50\text{-}100\times10^3$/uL are considered moderate thrombocytopenia, and levels less than 50×10^3/uL are categorized as severe thrombocytopenia[3].

Thrombocytopenia in newborns is a result of increased platelet consumption; sepsis was found to be the most common risk factor [3-6].

Thrombocytopenia has been used as an early but nonspecific marker for sepsis in critically ill newborns [7].

The Prothrombin time (PT) is functional determination of the extrinsic (tissue factor) pathway of coagulation. The PT is a widely used laboratory assay for the detection of inherited or acquired coagulation defects related to the extrinsic pathway of coagulation. The reference range for prothrombin time depends on the analytical method used, but is usually around 12-13 seconds [8].

Septicemia in neonates refers to generalized bacterial infection documented by positive blood culture in the first 28days of life and is one of the leading causes of neonatal mortality in sub-Sahara Africa; 44 per 1000 live births, four times more than the rate in Europe (11 per 1000 births) [9-11].

Septicemia is characterized by a complex series of events resulting to a disturbed microcirculation [12]. The activation of several humoral and cellular mediator systems by bacteria toxins is responsible for the pathophysiological consequences of neonatal septicemia [13]. One of these systems is the coagulation system, which when activated, can lead to disseminated intravascular coagulopathy (DIC) [14].

Thrombocytopenia is also a typical feature of DIC, which frequently complicates neonatal sepsis. Activation of coagulation proteins leads to widespread fibrin deposition and consumption of platelets. In these cases, the prothrombin time, activated partial thromboplastin time, and thrombin clotting time are prolonged; fibrinogen concentration is reduced; and fibrin degradation products and D-dimers are present [3].

A recent study showed that fungemia is associated with a greater degree of thrombocytopenia [15]. However, fungemia is not alone in its tendency to affect the platelet count. Jack et al [16] reported that Gram-negative organisms or fungi had significantly lower platelet counts and a higher incidence of thrombocytopenia. In their study however, they concluded that there are quantitative differences in the platelet response to infection. An earlier study also showed evidence of relationship between Gram-negative infections and thrombocytopenia [17].

To date, most studies focus on the organism-specific platelet response in neonatal sepsis. There are limited data about the association of platelet count and prothrombin time with neonatal septicemia.

The objective of the present study was to determine if there are organism-specific platelet responses among the two groups of bacterial agents: Gram-positive and Gram-negative bacteria, and also to examine the association of platelet count and prothrombin time with neonatal septicemia.

PATIENTS AND METHODS

Study Population

The analysis was conducted on 232 newborns (age 0-28days) admitted with clinical symptoms and risk factors suggestive of neonatal septicemia in the Intensive Care Unit of Outreach Children's Hospital, Festac Town, Lagos, Southwest Nigeria, between January 2013- March 2014. Fifty one (51) neonates whose blood culture yielded growth of microorganism were enrolled for this study.

Isolation of Etiologic Agents

Blood culture samples were collected with all aseptic precautions for culture and sensitivity. Ethical approval was obtained from the Institution Ethical Committee. With strict adherence to Helsinki Declaration on research bioethics, the participants' parents were given the option to exclude their babies from participating in the study. 3ml of blood was collected into the vial (bottle) in Bactec 9050, an

instrumented blood culture system. When the Bactec system detects microbial growth, it gives alarm sound that continues until the positive bottle is removed.

Subcultures were done on MacConkey agar, blood agar and chocolate agar. The chocolate agar plates were incubated in candle jar, while the blood agar and MacConkey agar plates were incubated aerobically.

Significant bacteria isolates were identified by Gram staining and confirmed by the pattern of biochemical reactions using the standard technique [18]. Blood culture broths that yielded no microbial growth within seven days were reported as culture negative.

Analysis of Platelet and Prothrombin Time

The platelet count was performed using Abacus Junior5 hematology analyzer. Prothrombin time analysis was performed by filling the PT/INR cartridge of i-STAT 1 analyzer with capillary blood. The cartridge was inserted into i-STAT 1 analyzer; the analyzer automatically controls all functions of the testing cycle including fluid movement within the cartridge.

Data analysis was carried out using Statistical Package for Social Sciences (SPSS) version 19.0 for Windows.

RESULTS

Of the 231 neonates hospitalised with clinical sepsis, blood culture reports were positive in 51 cases (21.4%), 23(45.1%) were male and 28(54.9%) were female. Klebsiella spp. (35.3%) and Staphylococcus aureus (27.5%) were the most common Gram-negative and Gram-positive isolates respectively, while Proteus spp. (5.9%) was the least common isolate causing neonatal septicemia (Table 1).

Thrombocytopenia was observed in 30(58.8%) of the neonates with septicemia. The platelet count was normal in 21(41.2%) neonates. Of the 9(17.6%) patients with severe thrombocytopenia, seven (77.8%) had Klebsiella spp. septicemia. None (0%) of the Gram-positive organisms caused severe thrombocytopenia (Table 2).

30(58.8%) of neonates with septicemia had different degrees (mild-to-severe) of thrombocytopenia. Of the thrombocytopenic neonates, 19(63.3%) showed increased prothrombin time. 21(63.6%) of Gram-negative infection resulted in thrombocytopenia while 9(50.0%) of Gram-positive infection produced thrombocytopenia. Out of the 21(63.6%) of thrombocytopenia produced by Gram-negative isolates, 17(80.9) had increased prothrombin time (Table 3).

Table 1: Frequency of microbial isolates from culture positive neonates (n=51).

Organisms	Frequency of isolation (%)
Klebsiella spp.	18(35.3)
Escherichia coli	8(15.7)
Pseudomonas spp.	4(7.8)
Proteus spp.	3(5.9)
Staphylococci aureus	14(17.5)
Coagulase negative staphylococcus (CONS)	4(7.8)

Table 2: Frequency of microorganisms causing mild to severe thrombocytopenia in neonates

Type of microorganism	No of neonates with thrombocytopenia				
	Normal	Mild	Moderate	Severe	Total
Klebsiella spp	4	2	5	7	18
Escherichia coli	4	-	2	2	8
Pseudomonas spp	2	2	-	-	4
Proteus spp	2	-	1	-	3
Staphylococci aureus	6	5	3	-	14
CONS	3	1	-	-	4
Total	21	10	11	9	51

Table 3: Association between thrombocytopenia and prothrombin time in culture positive neonates

Category of isolates	No of neonates with Thrombocytopenia (%)	No of neonates with Prothrombin time >13secs (%)
Gram-negative (n=33)	21(63.6)	17(51.5)
Gram-positive (n=18)	9(50.0)	2(11.1)
Total	30	19

DISCUSSION

Up to 25% of infants admitted to the neonatal intensive care unit (NICU) have thrombocytopenia. Most cases of thrombocytopenia in the NICU are discovered accidentally when routine studies are completed on infants admitted for non-hematologic conditions [3].

Fewer than 3 per 1000 term infants have been reported to have severe thrombocytopenia (platelet count $<50\times10^9$/L) and most of the causes has been linked to alloimmune thrombocytopenia [19].

In the present study, the prevalence of neonatal thrombocytopenia was 58.8%, which is in concordance with previous studies conducted in both

Iran and Nigeria [20],[21]. Bacterial sepsis causes thrombocytopenia by several mechanisms, including disseminated intravascular coagulation (DIC), endothelial damage, immune-mediated destruction, platelet aggregation due to bacterial products adhering to platelet membrane, and decreased platelet production from infected bone marrow [3],[19]. However, many neonatal complications exacerbate this thrombocytopenic potential and 17.6% of thrombocytopenia in this study are severe. This result is similar to the findings by previous researchers who reported 20% [19].

Gram-negative organisms constituted the major group of isolates (64.7%) from neonatal septicemia cases, which correlates with the findings (67.85%) of Kairavi JD et al [22]. Among this group Klebsiella spp. has been found to be the most prominent pathogen (35.3%), which correlate with the previous finding [22],[23]. In our study population, septic neonates with Gram-negative organisms had a higher incidence of thrombocytopenia (41.1%), this finding is in resonance with the previous findings by Guida JD et al [16]. Specifically, of the Gram-negative isolates, Klebsiella spp. (77.8%) showed the highest incidence of severe thrombocytopenia. None of the Gram-positive organisms caused severe thrombocytopenia as observed in this study.

Coagulation system and platelets are strongly activated in sepsis. In severe sepsis, most of the coagulation factors are depleted, platelet is decreased and global coagulation tests are prolonged, indicating exhaustion of hemostasis [24].

In this study, 63.3% of neonates with thrombocytopenia also had prolonged PT which is more common among Gram-negative organisms (33.3%). Also, 80.9% of neonates with thrombocytopenia caused by Gram-negative organisms had prolonged PT. Therefore, neonates with septicemia due to Gram-negative infection showed significantly marked alterations in hemostatic parameter (PT) than newborns with septicemia due to Gram-positive organisms.

Various organisms may complicate infections by consumption coagulopathy [25]. Septicemic cases with bleeding probably suffered more consumption coagulopathy leading to a state of DIC (11). DIC often accompanies septicemia caused by Gram-negative bacteria possessing endotoxins [26].

Various researchers have reported that exposure to endotoxins induces a procoagulant state characterized by activation of the contact system, induction of tissue factor and inhibition of fibrinolytic activity due to the release of the plasminogen activator inhibitor. This may be attributed to the direct action of the endotoxins on endothelial cells or may be an indirect result of the production of interleukin1 or tumor necrosis factor [27],[28].

CONCLUSION

Gram-negative organisms showed the highest cases of severe thrombocytopenia and also, prolonged PT is more common among Gram-negative infection. Though both Gram-positive and Gram-negative infections caused thrombocytopenia and prolonged

PT. This study assessed PT in neonatal septicemia and the data has helped to establish a disturbance in hemostatic systems in neonates with septicemia. Thus, our findings would assist the physicians in proper management of neonates with septicemia. Though further studies, however, may be required to assess other hemostasis parameters in order to understand their interaction with the infectious organisms in neonates.

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