Prevalence and course of Thrombocytopenia in culture positive and culture negative Neonatal Sepsis

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Abstract

Introduction: Neonatal Sepsis is commonly associated with thrombocytopenia. Objective: To assess the prevalence and course of thrombocytopenia in culture positive and culture negative neonatal sepsis in comparison to normal newborns. Methods: This is a retrospective case analysis of 533 neonates between January-2012 to December-2014. The parameters examined were Baseline Platelet Count, Change In Platelet Count, (Baseline Platelet Count- Change In Platelet Count)/Baseline Platelet Count, Platelet Nadir, Incidence, Duration & Severity of Thrombocytopenia. Statistical Analysis: All data were collected in validated preformatted proforma sheet & analysed using appropriate statistical methods. Results: Among 533 neonates, 21.2% had Culture negative sepsis, 9.75% had culture positive sepsis & 69.04% had no sepsis. The prevalence of early onset sepsis was 17.44% & late onset sepsis was 13.50%. About 24.76% babies had thrombocytopenia; 9.56% had mild thrombocytopenia, 10.13% had moderate & 5.06% had severe thrombocytopenia. Late onset sepsis was associated with significant thrombocytopenia. Culture positive sepsis had significant drop in platelets with lower platelet nadir, higher incidence, more severe & prolonged thrombocytopenia compared to culture negative sepsis & normal neonates. Conclusion: There are quantitative differences in the platelet response to neonatal sepsis, particularly to culture positive sepsis. Hematological changes in platelet count induced by culture proven and culture negative neonatal sepsis can be used to make an early diagnosis and prompt management of neonatal sepsis.

Key words: Thrombocytopenia, Culture Positive Sepsis, Culture Negative Sepsis

Introduction

Neonatal Sepsis is a clinical syndrome characterised by signs and symptoms of infection with or without accompanying bacteremia in the first month of life [1]. It is extremely important to make an early diagnosis of neonatal sepsis for the prompt institution of anti-microbial therapy, which improves the outcomes [2]. CRP & Platelet count are diagnostic markers of neonatal sepsis [3,4].

We have conducted this study to assess the prevalence and course of thrombocytopenia in culture positive and culture negative neonatal sepsis in comparison to normal newborns.

Methodology

This is a retrospective case analysis between January-2012 to December-2014 in a single centre. Blood samples of all the patients included in this study were obtained for CBC and CRP levels. Blood culture was done when indicated. Name, date of admission, age, platelet count, CRP levels, blood culture reports were recorded on a data form. We enrolled 533 eligible neonates. The data was analysed for differences in platelet count in terms of Culture positive, Culture negative sepsis & no sepsis. All patients included in this study received appropriate antibiotics.

Unit Protocol for Investigation of Neonatal Sepsis: Soon after admission two ml blood sample was taken in EDTA vacutainer and was processed for Platelet count. Another 2 ml blood sample was taken for conventional blood culture. Also 1 ml blood sample was taken for estimation of a qualitative CRP result. Direct counting of platelets in an improved Neubauer’s Chamber was done. Platelet count less than 1.5 Lakh / cumm was considered abnormal. CRP in serum was estimated by CRP Turbi Latex Kit using Latex turbimetry. Test showed positivity when CRP value was more than 6 mg/ L. Blood was collected for blood culture in BD BACTEC bottles &
cultured in Sabouraud’s Dextrose agar & Brain Heart Infusion Broth and colony growth was observed. Smears were made from peripheral blood and stained by Leishmans stain and examined to confirm thrombocytopenia. The platelet count & CRP used for this study was the one obtained at the same time as the positive blood culture or the one closest to the time the blood culture was drawn in cases of neonatal sepsis and the admission values in those babies with no sepsis.

**Interventions**: Management of neonatal sepsis as per standard unit protocol. The study was approved by the Hospital Research and Ethics Committee.

**Definition of Parameters**: The parameters that were examined in this study were

**Baseline Platelet Count**: Platelet count obtained at least 24 hours before the time that the blood culture was obtained in cases of neonatal sepsis or the admission values in those babies with no sepsis.

**Change In Platelet Count**: Platelet count at the time of onset of sepsis in cases of neonatal sepsis or the second CBC values in those babies with no sepsis.

**Platelet Nadir**: Lowest platelet count obtained during a 20-day period starting from the time the initial blood culture was drawn in cases of neonatal sepsis or during a 20-day period since admission in those babies with no sepsis.

**Incidence of Thrombocytopenia**: Number of episodes with a platelet nadir of less than 1,50,000/mm$^3$ during a 20-day period starting from the time the initial blood culture was drawn in cases of neonatal sepsis or during a 20-day period since admission in those babies with no sepsis.

**Duration of Thrombocytopenia**: Number of continuous days that the platelet count remained less than 1,50,000/mm$^3$. If the neonate had no Thrombocytopenia at the time of sepsis, the duration was considered to be zero.

**Severity of Thrombocytopenia**: [5]

| Category               | Description                                      |
|------------------------|--------------------------------------------------|
| No Thrombocytopenia    | Platelet count more than 1,50,000/mm$^3$         |
| Mild Thrombocytopenia  | Platelet count between 1,00,000 to 1,50,000/mm$^3$|
| Moderate Thrombocytopenia | Platelet count between 50,000 to 1,00,000/mm$^3$ |
| Severe Thrombocytopenia | Platelet count less than 50,000/mm$^3$          |

**Culture Negative Sepsis**: was defined as those with clinical signs and symptoms of sepsis, without growth of any pathogen from blood, but with presence of CRP > 6mg/L [6].

**Statistical Analysis**

All the data were collected in validated preformatted proforma sheet and analysed using software Statistical Package for Social Sciences. Categorical variables were analyzed using Chi-square analysis with Yates correction. Student’s t’ test was used to compare the means. A p-value of < 0.05 was considered significant. Analysis of variance was used to compare groups, and data are expressed as mean ± standard deviation. Scheffe test was used for pairwise comparisons.

**Results**

533 babies were studied. About 113 babies (21.2%) had Culture negative sepsis, 52 babies (9.75%) had culture positive sepsis & 368 babies (69.04%) had no sepsis. The prevalence of sepsis in our study was 30.95%. The prevalence of early onset sepsis was 17.44% & late onset sepsis was 13.50%. 72 babies (63.71%) had early onset sepsis & 41 (36.28%) had late onset sepsis among culture negative sepsis group. 21 babies (40.38%) had early onset sepsis & 31 (59.61%) had late onset sepsis among culture positive sepsis group, (Chi square p <0.0001, significant). Totally 132 babies (24.76%) had thrombocytopenia. 51 babies (9.56%) had mild thrombocytopenia, 54 babies (10.13%) had moderate & 27 babies (5.06%) had severe thrombocytopenia. Analysing Prevalence of thrombocytopenia among the groups, 48 babies (42.47%) among 113 babies with culture negative sepsis had thrombocytopenia, 38 babies (73.07%) among 52 babies with culture positive sepsis had thrombocytopenia, 46 babies (12.50%) among 368 babies with no sepsis had thrombocytopenia, (Chi square p <0.0001 , significant). Prevalence of thrombocytopenia was more in culture positive sepsis. Analysing Prevalence of thrombocytopenia among the type of sepsis, 45 babies (48.38%) among 93 babies with early sepsis had thrombocytopenia, 41 babies (56.94%) among 72 babies with late onset sepsis had thrombocytopenia, 46 babies (12.5%) among 368 babies with no sepsis had thrombocytopenia, (Chi square p <0.0001 , significant). Prevalence of thrombocytopenia was more in late onset sepsis.
Table 1: Platelet Variations among no sepsis, culture negative & culture positive sepsis

| FACTOR            | Levene’s p | ANOVA p | NO SEPSIS MEAN | NO SEPSIS SD | CN SEPSIS MEAN | CN SEPSIS SD | CP SEPSIS MEAN | CP SEPSIS SD |
|-------------------|------------|---------|----------------|--------------|----------------|--------------|----------------|--------------|
| AGE (DAYS)        | p < 0.001  | p < 0.001 | 2.4457         | 3.9572       | 1.7699         | 2.5355       | 5.5769         | 7.8699       |
| GESTATION (WEEKS) | p < 0.001  | p < 0.001 | 36.269         | 2.0084       | 36.2035        | 2.7844       | 34.9038        | 3.1577       |
| WEIGHT (KG)       | p < 0.001  | p < 0.001 | 2.4583         | 0.5724       | 2.5573         | 0.7911       | 2.132          | 0.8492       |
| BASELINE COUNT    | p = 0.015  | p = 0.434 | 2.3402         | 0.7388       | 2.245          | 0.9923       | 2.2417         | 0.6871       |
| CHANGE IN COUNT   | p = 0.885  | p < 0.001 | 2.1618         | 0.8442       | 1.7008         | 0.8763       | 1.2487         | 0.8092       |
| NADIR             | p < 0.001  | p < 0.001 | 2.2099         | 0.7818       | 1.7477         | 1.0244       | 1.1154         | 0.8652       |
| SEVERITY          | p < 0.001  | p < 0.001 | 0.1875         | 0.5116       | 0.7434         | 1.0069       | 1.7115         | 1.21         |
| INCIDENCE         | p < 0.001  | p < 0.001 | 0.2201         | 0.6627       | 1.2389         | 1.9239       | 2.2308         | 1.843        |
| DURATION          | p < 0.001  | p < 0.001 | 0.3886         | 1.2324       | 2.3717         | 3.4877       | 5.1346         | 3.8757       |

Analysis of variance was used to compare the groups, and data was expressed as mean ± standard deviation. There was a significant fall in platelet counts when compared with baseline values in culture positive patients. Results were statistically significant when compared with culture negative and normal neonates. Similar findings were noticed for platelet nadir. Culture positive sepsis had higher incidence & more prolonged thrombocytopenia along with more severity when compared to culture negative sepsis and normal neonates.

Table 2: Platelet variations among no sepsis, early onset & late onset sepsis

| FACTOR          | Levene’s p | ANOVA p | NO SEPSIS MEAN | NO SEPSIS SD | EOS MEAN | EOS SD | LOS MEAN | LOS SD |
|-----------------|------------|---------|----------------|--------------|----------|-------|----------|-------|
| BASELINE COUNT  | p = 0.107  | p = 0.032 | 2.3402         | 0.7388       | 2.1199   | 0.8371 | 2.4043   | 0.9686 |
| CHANGE           | p = 0.354  | p < 0.001 | 2.1618         | 0.8442       | 1.6285   | 0.8124 | 1.409    | 0.9465 |
| NADIR           | p < 0.001  | p < 0.001 | 2.2099         | 0.7818       | 1.5995   | 0.852  | 1.4825   | 1.2022 |
| SEVERITY        | p < 0.001  | p < 0.001 | 0.1875         | 0.5116       | 0.9032   | 1.0841 | 1.2361   | 1.239  |
| INCIDENCE       | p < 0.001  | p < 0.001 | 0.2201         | 0.6627       | 1.4301   | 2.0451 | 1.7083   | 1.8188 |
| DURATION        | p < 0.001  | p < 0.001 | 0.3886         | 1.2324       | 2.3717   | 3.4877 | 5.1346   | 3.8757 |

There was a significant fall in platelet counts when compared with baseline values in babies with late onset sepsis. Results were statistically significant when compared with early onset sepsis. Similar findings were noticed for platelet nadir. Late onset sepsis had higher incidence & more prolonged thrombocytopenia along with more severity when compared to early onset sepsis.

Discussion

Prevalence of Sepsis: In our study, 21.2% babies had Culture negative sepsis & 9.75% had culture positive sepsis. The overall prevalence of sepsis in our study was 30.95%. Prevalence between 20-30% was noted in studies by Khaleesi N et al. [7], Roberts I et al.[8], Beiner ME et al.[9], Bolat F et al.[10]. However lower prevalence of 16% was noted by Jack D,Guida et al.[11]. The prevalence of early onset sepsis in our study was 17.44%. The prevalence of early onset sepsis was between 20-30% in studies by Khaleesi N et al.[7], Roberts I et al.[8], Beiner ME et al.[9], Bolat F et al.[10]. The prevalence of late onset sepsis in our study was 13.50%. However Bizzarro MJ et al.,[12] & Van den Hoogen etal.[13] observed that late-onset sepsis was more common in their studies.

Prevalence of Culture Positivity: The culture positivity in our study was 31.51%. Wide variations in culture positivity have been noted in various studies. It was between 20-30% in studies by Sharma et al.[14], Ramesh Bhat Y et al.[15], Ahmed et al.[16], Baltimore et al.[17], Gladstone et al.[18], d Haens et al.[19], Qazi Iqbal et al.[20]. About 34.45% babies had culture positivity in a study by Anil K Gupta et al.[22]. It was between 40-50% in other studies[23-25]. Deepa et al.[26] & Buch et al.[27] noted culture positivity in a study by Anil K Gupta et al.[22].

Prevalence of Thrombocytopenia: Totally 24.76% of our babies had thrombocytopenia in this study. The
prevalence of thrombocytopenia among hospitalized neonates was between 22 - 35% in other studies[32-39]. It was between 15-20% in studies by Khaled N et al.[7], Gupta et al.[40], Anil K Gupta et al.[22], Qazi Ishqala b et al.[20]. However a higher prevalence of 69.9% was noted by Kyoung Hee Choi et al.[41] & 53% by Zaccheaus A Jeremiah et al.[5]. Overall the prevalence of thrombocytopenia was 52.12% among babies with sepsis. It was between 50-60 % in studies by Guida et al.[11], Zaccheaus A Jeremiah et al.[5], Mannan et al.[42], Torkaman M et al.[43] & Tiago de Oliveira Boechat et al.,[44]. Lower prevalence of 24.1% by Khaled N et al.[7] & 24.7% by Charoo BA et al.[45] were noted.

Prevalence of Thrombocytopenia Among Culture Positive Sepsis: There was a significant high prevalence of thrombocytopenia (73.07%) among babies with culture positive sepsis. Results were statistically significant when compared with culture negative and normal neonates (Chi square p <0.0001, significant). Guida JD et al [11] & Mannan MA et al.[42] noted that the prevalence was 50% among babies with culture positive sepsis.

Prevalence of Thrombocytopenia among Early & Late Onset Sepsis: Significantly higher prevalence of thrombocytopenia (56.94%) was noted among late onset sepsis when compared with early onset sepsis (48.38%) (Chi square p <0.0001, significant). Khaled N et al.[7] & Zaccheaus A Jeremiah et al.[5] noted the prevalence of thrombocytopenia among early onset sepsis as 67.7% & 84.84% respectively in their studies. Charro et al.[45] noted the prevalence of thrombocytopenia among late onset sepsis as 59.5% which was comparable to our study.

Platelet Variations among No Sepsis, Culture Negative & Culture Positive Sepsis: There was a significant fall in platelet counts when compared with baseline values in culture positive patients. Results were statistically significant when compared with culture negative and normal neonates. Similar findings were noticed for platelet nadir. Culture positive sepsis had higher incidence & more prolonged thrombocytopenia along with more severity when compared to culture negative sepsis and normal neonates. Deepa S et al.[26] also noted lower platelet counts in culture positive sepsis as compared to normal babies.

Platelet Variations among No Sepsis, Early Onset & Late Onset Sepsis: We noted a significant fall in platelet counts when compared with baseline values in babies with late onset sepsis. Results were statistically significant when compared with early onset sepsis. Similar findings were noticed for platelet nadir. Late onset sepsis had higher incidence & more prolonged thrombocytopenia along with more severity when compared to early onset sepsis.

Severity of Thrombocytopenia: In our study, 9.56% babies had mild thrombocytopenia,10.13% had moderate & 5.06% had severe thrombocytopenia. Zaccheaus A Jeremiah et al.[5] noted that 39.4% had mild thrombocytopenia, 12.1% had moderate & 1.5% had severe thrombocytopenia. Anil K Gupta et al. [22] noted that 8% had mild thrombocytopenia, 4.5% had moderate & 4.2% had severe thrombocytopenia. Severe thrombocytopenia was found to be present in 5.4%, 2.4%, 2.4% & 6% in studies by Khaled N et al.[7], Roberts I et al.[33] , Christensen et al.[46] & Murray NA et al.[47] respectively. Sainio S et al.[48], De Moerloose P et al.[49], Burrows RF et al.[50], Uhrynowska M, et al.[51] & Dreyfus M et al.[52] observed that severe thrombocytopenia occurs in 0.1–0.5% of neonatal sepsis.

Summary: We noted significant drop in platelet count among late onset sepsis .Significant variations of all platelet indices were noted among lower gestational age babies. Culture positive sepsis was associated with significant drop in platelets with lower platelet nadir, higher incidence, more severe & prolonged thrombocytopenia when compared to culture negative sepsis & normal neonates.

Conclusion

There are quantitative differences in the platelet response to neonatal sepsis, particularly to culture positive sepsis. Hematological changes in platelet count induced by culture proven and culture negative neonatal sepsis can be used to make an early diagnosis and prompt management of neonatal sepsis.

Key words: Thrombocytopenia, Culture Positive Sepsis, Culture Negative Sepsis

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