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Haematological profile in clinically suspected cases of neonatal Sepsis

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ABSTRACT

Neonatal septicemia is one of the major factors contributing to the high perinatal and neonatal mortality and morbidity in newborns and is recognized as global health challenge. Study was aimed the changes in hematological profile along with blood culture and C-reactive proteins in clinically suspected cases of neonatal sepsis. The present study were included 108 neonates clinically suspicious to have sepsis and admitted in NICU. Avacutainer, Glass slides, Leishman's stain, Automated Hematology analyzer, Staining Kit was used. Maximum number of neonates i.e. 80 (74%) were less than 2 days old. 70 (64.82%) were males and 38 (35.18%) were females. Maximum number (53.70%) were preterm, respiratory distress seen in (76.11%) Premature rupture of membranes was observed in 39 (36.11%). The clinical suspicion of sepsis, 24 (22.22%) had proven sepsis. Rodwell's hematological score of > 3 identified 23 out of 24 (95.83%) proven sepsis group neonates, 24 (88.89%) probable sepsis group neonates. Of the 108 neonates with clinical suspicion of sepsis, 24 (22.22%) had positive blood cultures. The most common pathogen isolated in the blood culture was Klebsiella pneumonia in 12 (50%). Leucocytosis was seen in 4 (16.6%). The total WBC count has low sensitivity (37%) but a high specificity (96%) as indicator of sepsis. Elevated immature PMN count was observed (79.2%) Elevated. Thrombocytopenia was noted in 9 cases (37.5%) proven sepsis. Rodwell's hematological scoring framework is a straightforward, speedy, financially savvy instrument which can be utilized as screening test for early conclusion of neonatal sepsis.

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INTRODUCTION

Neonatal septicemia is a crucial factor contributing to the high perinatal and neonatal mortality and morbidity (Sharma *et al.*, 2008) in newborns and is recognised as a global health challenge. Worldwide neonatal Sepsis is the most critical cause of neonatal deaths and with the risk of neurodevelopmental impairment seen in survivors. 99% of them occur in developing countries like India (Darmstadt *et al.*, 2009). Most instances of Neonatal Sepsis in the network are brought about by Escherichia coli and Staphylococcus aureus. In hospitals, Klebsiella pneumonia is the most frequent cause of Sepsis, followed by Staphylococcus aureus (Tripathi and Malik, 2010).

The ongoing advancements in the administration of neonatal Sepsis include the utilisation of progressively powerful anti-toxins and a variety of complex biomarkers to analyse Sepsis, convenient determination on a successive and testing issue in the

administration (Commins *et al.*, 2010). If diagnosed early and treated aggressively with proper antibiotics and good supportive care, it can be cured, thus reducing the morbidity and shortening the hospital stay. The early diagnosis also minimises the risk of emergence of resistant organisms due to misuse of antibiotics (Haque, 2010). Unfortunately, factors that delay the diagnosis and initiation of therapy include lack of specific clinical features. The neonates are often presented with subtle and non-specific clinical signs and symptoms. Thus, there is a need of high file of doubt, required for early finding. The complete analysis of septicemia is made by positive blood culture, and it is viewed as the best quality level, which requires a base time of 48-72 hours and yield of a positive blood culture ranges from 8-73 % as appeared in different examinations. Moreover, the technique of blood culture is time-consuming and demands a well-equipped laboratory, which is not available in many of the community hospitals (Khair *et al.*, 2011).

Early, the exact and fast finding of neonatal Sepsis remains a significant symptomatic test in neonatology. The current examination was attempted to set up the convenience of fringe smear discoveries and Rodwell's haematological scoring framework for early determination of Neonatal Sepsis. The Rodwell's haematological scoring framework (HSS) is straightforward, fast, practical and promptly accessible apparatus for the initial finding of neonatal Sepsis (Rodwell *et al.*, 1988). In this way, there is a requirement for a test that is readily performed with speedy accessibility of reports. Ongoing data has proposed that the analytic precision of white platelet tally (WBC). The supreme neutrophil check (ANC) and juvenile to add up to PMN proportion (I/T) parameters may better foresee Sepsis utilising age-explicit proportion nomograms as opposed to fixed ordinary reaches, and it is quick. These can be performed in an hour, or two and antibiotics can then be administered judiciously, thereby reducing the incidence of drug resistance and improving the survival rate in septicemia (Ghosh *et al.*, 2001).

Aim

To study the changes in the haematological profile along with blood culture and C-reactive proteins in clinically suspected cases of neonatal Sepsis.

Objectives

1. To study the haematological profile, including the various changes seen in the peripheral smears of neonates clinically suspicious of having Sepsis.
2. To analyse the haematological findings, using Rodwell's Haematological scoring system in neonates who were clinically suspected of Sepsis.
3. To correlate these haematological parameters with other tests like blood culture and C-reactive proteins.

MATERIALS AND METHODS

The present study was a two-year observational study, carried out in the department of Pathology of tertiary care hospital.

The parents of the neonates included in the study gave written informed consent.

The study included an investigation of blood samples of 108 neonates who were clinically presented with symptoms of Sepsis and were admitted in NICU.

Inclusion Criteria

All suspected cases of neonatal Sepsis admitted in NICU of a tertiary care hospital during the study period were included in the study.

Exclusion Criteria

Neonates of mothers with pregnancy-induced hypertension, neonates with a history of birth asphyxia and congenital anomalies were excluded.

Equipment

EDTAvacutainer, Glassslides, Leishman's stain, Automated Haematology analyser (Sysmex – XT 1800-i), Staining Kit

Method

The study was conducted for 24 months. 2 ml blood samples from neonates suspicious of Sepsis was collected in EDTA vacutainer. The blood was collected by peripheral venipuncture using aseptic precautions. In the Pathology Department, the blood samples were processed within half an hour. The blood samples were analysed for routine haematological parameters viz., haemoglobin, haematocrit, red blood cell indices (MCV, MCH and MCHC), total WBC count, differential count and platelet count. These investigations were performed on Automated Haematology analyser Sysmex– XT 1800-i. For every sample, a peripheral smear was made, and the blood film was stained with Leishman's stain.

Quality control

As per the standard guidelines given by National Accreditation Board for Laboratories (NABL), internal quality control by Sysmex (Transasia) is followed by our laboratory and laboratory participates in External Quality Assurance Services (EQAS) by BIO-RAD.

Analysis of the Data

The data collected were statistically analysed by using SPSS software. The analysis ascertained the performance of individual haematological findings, haematological scoring system, blood culture & CRP in neonates with clinical suspicion of Sepsis using the chi-square test. They were also analysed to find out sensitivity, specificity, positive predictive value and negative predictive value of Rodwell's Haematological scores in neonates with clinical suspicion of Sepsis.

RESULTS AND DISCUSSION

The age of the neonates presented in the study ranged from a newborn to a 26-day old neonate. 74% neonates in the study were less than two days old. The mean age of the neonates was 3.41+2.15 days. In the present study, out of 108 cases studied 70(64.82%) were males, and 38(35.18%) were females. Male: female ratio was (1.8) : (1) In the present study out of 108 cases studied 58 (53.70%) neonates belong to preterm gestation and 50 (46.30%) neonates from full-term gestation.

The onset of symptoms out of 108 cases studied 80(74%) neonates show early-onset sepsis and 28 (26%) neonates showing late-onset Sepsis—many of the neonates presented with more than one symptom. The most typical symptoms were respiratory distress (76.6%), followed by lethargy/poor feeding (70.4%). Premature rupture of membranes and meconium aspiration were seen more common maternal risk factor for developing Sepsis. In the study, peripheral smear findings revealed morphological changes as most common finding present in 42 cases out of 108 followed by thrombocytopenia and increased PMN count seen in 27 cases each. Leucocytosis and leucopenia were seen in 11 and 10 cases, respectively. Decreased PMN count was viewed in 09 cases. The blood culture was positive in 24 (22.22%) cases and negative in 84(77.78%) cases the most prevalent pathogen isolated in the blood culture in the present study was Klebsiella pneumonia in 12 (50%) cases followed by E.coli in 5(20.8%) cases.

Haemoglobin and haematocrit

In the present study 7 cases out of 24 (29.1%) proven sepsis group neonates, five instances out of 27 (18.52%) probable sepsis group neonates and 6 cases out of 57(10.5%) no sepsis group neonates were anaemic. They showed low levels of haemoglobin and haematocrit. 62.5% of the proven sepsis group, 63% probable sepsis group and 96.5% of the no sepsis group had WBC count within the reference range. 20.9% of the proven sepsis group neonates showed leucopenia compared to 1.75% cases of no sepsis. 20.9% of neonates with proven Sepsis had decreased PMN count as compared to 11.1 % cases with probable Sepsis. Only 1.75% of no sepsis neonates had a decreased PMN count. Elevated immature PMN (band forms) count was observed in 19 out of 24 (79.2%) proven sepsis group neonates, 21 out of 27(77.8%) Probable sepsis group neonates and 17 out of 57(29.8%) no sepsis group neonates.

Morphologic changes in PMN-75% of cases of proven Sepsis showed morphologic changes in PMN either as toxic granulation, vacuolisation or both.

Lymphocytes and monocytes

10 of the 24 neonates (41.6%) with proven Sepsis, 14 out of 27 probable sepsis and 6 of 57 no sepsis neonates (10.5%) had decreased lymphocyte count. The monocytes ranged from 0 to $1\times$ 103 /μL. In 2 cases the monocyte count was raised and was higher than 1.9×103 /μL.

Platelet count

Neonates with platelet count less than 150 $\times$ 103 /μL were identified as having thrombocytopenia.

C- Reactive Proteins (CRP)

C-reactive proteins levels were measured by turbidimetric method, and result tabulated. Normal reference range of C-reactive protein being < 10 μg/ml. C-reactive protein levels were raised in 87.5% proven Sepsis, 85.2% probable sepsis and 22.8% no sepsis group neonates. Immature: Total PMN ratio (91%) was highly sensitive, followed by Immature: Matureratio (87%) in identifying neonates with Sepsis. Total leucocyte count (96%) followed by a morphological change in PMN (91%) were highly specific test helpful in diagnosing Sepsis. The positive predictive value was high for a morphological change in PMN(78%) followed by total PMN count(68%), which helped identify neonates who had proven Sepsis. The negative predictive value was high in Immature: Total PMN ratio (94%) along with Immature: Mature ratio (93%), which indicated that the neonates had no sepsis.

Discussion

Neonatal Sepsis is a clinical syndrome characterised by signs and symptoms of infection with or without accompanying bacteremia in the first month of life. Sepsis is the most frequent cause of neonatal mortality and is probably responsible for 30-50% of total neonatal deaths each year in developing countries. The neonatologist had to face one of the most challenging tasks. That was to differentiate between

septicemia and nonsepticemic cases clinically. This challenge is because several other conditions have clinical features similar to septicemia. A few same clinical features to that of septicemia may be birth asphyxia, hypoglycemia, and hypothermia, prematurity and intracranial haemorrhage.

The gold standard for the diagnosis of neonatal Sepsis is positive blood culture. However, the procedure is time-consuming requires a minimum period of 48-72 hours. It yields a positive result in 8-73% of cases only, and the facilities for the test might not be available in many laboratories. Hence there is a need for an exact test for bacteremia's that is easily performed, quick, cost-effective and straightforward with maximum sensitivity and specificity. In recent years, various investigations have evaluated some inflammatory markers (e.g. procalcitonin, haptoglobins, interleukins etc.)To diagnose Neonatal Sepsis. Different cost-effective but reliable laboratory test have assessed for the diagnosis of systemic infection in neonates. The complete blood count with various neutrophil parameters and C-reactive protein are the most frequently used.

Age incidence

In the present study, the age of the neonates ranged from newborn to 26 days old neonate. 74% neonates in the study were less than two days old. The mean age of the neonate was 3.41 + 2.15 days. Saleem (2014) found 52.4% neonates in the age group of 07 days. (Makkar *et al.*, 2013) found 51.8% neonates within 24 hours of life.

Sex wise distribution of cases of clinically suspected sepsis

In the present study out of the 108 neonates, 64.82% were males, and 35.18% were females. Male neonates are 2-6 times more likely to develop perinatal Sepsis than females. The present study was in concordance with research done by Supreetha MS et al. 72, Saleem (2014) and Khair *et al.* (2011) which also found male preponderance in their study.

Gestational age

In the present study, 53.70% of clinically suspected neonates of Sepsis were preterm, and 46.30% were full-term. Inherent in the preterm neonates are deficiencies in the immune system. Hence the risk of Sepsis is increased, as seen in several studies. The present study was in conjunction with the research done by Makkar *et al.* (2013) and Saleem (2014).

Onset of symptoms

74% of the neonates in the study presented with the early onset type of Sepsis and 26% neonates showing late-onset Sepsis. Our study findings match with the study done by Supreetha *et al.* (2015); Khair *et al.* (2011)and Tallur *et al.* (2000).

Clinical presentation

In the present study, the most common presenting features of neonatal Sepsis were respiratory distress followed by poor feeding and lethargy. In the case of Rodwell's study (Rodwell *et al.*, 1988), the common symptoms were lethargy, respiratory distress and hypoperfusion with shock. Cardiorespiratory signs are known to be the standard form of presentation, as seen by Ghosh *et al.* (2001); Tallur *et al.* (2000) and Gluck *et al.* (1966).

Other clinical features were seizures, jaundice and abdominal distension. The different clinical features noted by Chandna *et al.* (1988) were lethargy, jaundice, sluggish reflexes, diarrhoea and poor feeding.

Maternal risk factors and perinatal complications

In the present study Premature rupture of membranes (PROM) was seen in 36.11% of cases which was in concordance with the study done by Saleem (2014) observed 39.4% cases had maternal risk factor PROM.

Abnormalities in peripheral smear findings in clinically suspected cases of neonatal sepsis

In the present study, peripheral smear findings revealed morphological changes in PMN as the most common finding present in 42 cases out of 108. This detection was followed by thrombocytopenia in 27 out of 108 cases. These exposures were not in concordance with other studies which showed thrombocytopenia as most common findings.

Blood culture results – organisms isolated

The most prevalent pathogen isolated in the blood cultures in the present study was Klebsiella pneumonia, followed by E. coli. Our findings were in concordance with the study conducted by Krishna *et al.* (2000) and Kumhar *et al.* (2002) Klebsiella was the most typical organism identified.

In contrast to the developed world where Group-B Streptococcus continues to be the most common bacterial pathogen, studies from developing countries have identified Gram-negative organisms as the more frequent infective agent. GBS and E. coli account for about 60% of cases in North America and Europe. Even in studies conducted by Rodwell *et al.* (1988) and Philip and Hewitt (1980), GBS was the most common organism isolated. In the present study, out of the 108 neonates evaluated, 22.20% showed positive blood culture. Our study findings were in concordance with Ghosh *et al.* (2001); Makkar *et al.* (2013) and Supreetha *et al.*

(2015) as shown in the above tables.

Performance of Individual Haematologic Findings

Total WBC Count

Of the 24 neonates with proven Sepsis, 16.6% had leucocytosis, 20.9% had leucopenia, and 62.5% had values within the reference range. 1.75% of the no sepsis neonates had leucopenia. Leucocytosis and leucopenia, as discussed before, are unreliable indicators of neonatal Sepsis as the reference ranges for these parameters changes daily, even hourly in the first few days of life. The WBC count had low sensitivity but a high specificity as seen by the above table. This analysis was also observed in the study conducted by Rodwell *et al.* (1988) and Ghosh *et al.* (2001).

Leucopenia is a more specific indicator of Sepsis. This indicator was seen in the current study and was described in Rodwell *et al.* (1988) and Ghosh *et al.* (2001) as well.

Total polymorphonuclear (PMN) count

The total PMN count was abnormal in 62.7% of cases of proven Sepsis and 51.8% of cases of probable Sepsis and 11.85% no sepsis group of neonates. Anwer and Mustafa (2000) found abnormal PMN counts in 62% of proven Sepsis and 48% of probable and no sepsis group. When compared to the WBC count, the total PMN count is more sensitive. However, according to a study conducted by Anwer and Mustafa (2000), it was found to be the most specific test

Decreased PMN count is more sensitive and specific as an indicator for neonatal Sepsis. In the present study, 20.9% of neonates with proven Sepsis had decreased PMN count compared to none in no sepsis group. Funke *et al.* (2000) studied 168 neonates with Sepsis and found 38% of them to be decreased PMN count.

Decreased PMN count is a useful predictor of Sepsis, particularly in the early-onset type.

Total immature polymorphonuclear leucocytes counts (Band forms)

79.2% of neonates with proven Sepsis, 77.8% neonates with probable Sepsis and 29.8% of neonates with the no sepsis group had elevated band forms counts.

The reference range for band form counts is also known to change rapidly in the first days of life like the leucocyte and PMN count Cornbleet (2002). The present study, along with that conducted by Ghosh *et al.* (2001) shows the band forms count to be a sensitive test. Rodwell *et al.* (1988) and Ghosh *et al.* (2001) found the band cell count to be more specific than responsive, unlike the current study.

Platelet count

Platelet count <150 x103/mL in clinically suspected cases of neonatal Sepsis

37.5% of neonates with proven Sepsis,37.1% of neonates with probable Sepsis and 12.3% of the no sepsis group had thrombocytopenia. While thrombocytopenia is commonly noted in sepsis neonates, it has reduced sensitivity (22-38%), but the specificity and negative predictive value of the platelet count are both known to be >90%32. The present study showed that this test had a sensitivity of 37% with the specificity and negative predictive value is 87% & 76% respectively which is in concordance with Supreetha *et al.* (2015).

Haematological scoring system (HSS) as a sepsis screen

Using Rodwell's haematological scoring system and taking into account the abnormal WBC count, abnormal total PMN count, elevated band forms count, increased I: T and I: M ratio, morphological changes in PMN and decreased platelet counts, the neonates were given scores and then categorised accordingly. The present study was compared to Rodwell's original study (Rodwell *et al.*, 1988). The tables below show the relative value of the various scores for predicting Sepsis.

C-Reactive Proteins

C-reactive protein levels were raised in 87.5% of neonates with proven Sepsis and 85.2% of the neonates with probable Sepsis. 22.8% of the neonates who were no sepsis showed an elevated C-reactive protein level.

The CRP test showed a sensitivity of 87% and specificity of 77% in the present study, with a positive predictive value of 61%. Manucha *et al.* (2002) had reported elevated CRP levels of in76% cases of neonatal Sepsis.

CONCLUSION

Rodwell's haematological scoring framework is a straightforward, speedy, financially savvy instrument which can be utilised as a screening test for early conclusion of Neonatal Sepsis. This investigation stresses the significance of the relationship between clinical data with research facility discoveries.

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The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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