



Bacteriological Profile and C Reactive Protein Level of Neonatal Septicemia

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Abstract

Neonatal Sepsis is a life threatening emergency, and any delay in the treatment may cause death. Because of the importance of this problem, the objective of the present study was to determine the etiological agents and C Reactive protein level of neonatal septicemia. Two seventy three consecutive neonates with risk factors and clinical features suggestive of sepsis were selected as per operational definition and fulfilling the inclusion and exclusion criteria. Detailed physical examination was carried out. Blood samples for culture and CRP were taken from all the patients. Results of blood culture and CRP were noted down in the Performa. Culture positivity rate was 33%. Comparing with culture positivity, sensitivity of CRP was 67% and specificity was 60% in diagnosis of neonatal septicemia. Gram negative organisms constituted 64% of the total bacterial isolates. Klebsiella pneumoniae(40%) was the most common organism in early and late onset septicemia. This was followed by Staphylococcus aureus (19%), CONS (13%), Escherichia coli (10%), Enterobacter spp (7%), Pseudomonas aeruginosa (5%), Aceinetobacter (2%) and streptococci (2%). Escherichia coli and streptococci were more prevalent in early onset septicemia than late onset septicemia. Single CRP level measured at the onset of infection lacks sufficient sensitivity to be useful in identifying neonate with septicemia. To reduce neonatal mortality due to infections, we must expand the research activities on diagnosis, etiology, and optimal management of neonatal sepsis at all levels of the healthcare system

Keywords: Neonatal Sepsis, C reactive protein, blood culture.

Introduction

World Health Organization (WHO) estimates that there are about 5 million neonatal deaths occur globally per year. Ninety eight percent of them are occurring in developing countries. The most common causes of death in neonatal period is infection (32%) followed by birth asphyxia (29%) and prematurity (24%). Neonatal infections

currently cause 1.6 million deaths in developing countries.

Neonatal septicemia can be classified into two relatively distinct illnesses based on the postnatal age at onset. Early-onset neonatal septicemia (EOS) occurs in the first 7 days of life. Late-onset septicemia occurs (LOS) within 7-28 days of life^{[1], [2]}. The microbial etiology of

neonatal septicemia is variable and often changes temporally^[3]. In developed countries Group B *Streptococci* (GBS) and Coagulase Negative *Staphylococci* (CONS) are the most common etiologic agents for early onset and late onset septicemia respectively. In developing countries *Escherichia coli*, *Enterobacter spp*, *Klebsiella spp* and *Acinetobacter* precede GBS and CONS in causing EOS. *Klebsiella spp*, *Pseudomonas spp*, *Salmonella spp*, and *Serratia* precede CONS and *Staphylococcus aureus* in causation of LOS. The source of infection in EOS is generally the maternal genital tract. Infants with EOS usually present with respiratory distress and pneumonia. The source of infection in LOS is either nosocomial or community acquired and the neonates usually present with pneumonia or meningitis^[4]. Blood cultures are considered as the gold standard for diagnosis of neonatal septicemia. Nevertheless, their positivity varies widely (50 to 87%) and the results are not available rapidly for therapeutic management. For this reason, other, faster, laboratory tests like white blood cell count (WBC) and other biological markers as interleukin-8, C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- α) and procalcitonin are used^[5]. These biological markers, combined with clinical assessment, increase the probability of correct diagnosis and offer the physicians' greater confidence in promptly initiating antimicrobial therapy, in parallel with supportive care⁶. On the other hand, they can also avoid the indiscriminate use of antibiotics thereby reducing the risk of developing multidrug resistant pathogens.

Material and methods

Approval was obtained from the ethical committee prior to conduct of this study. Informed consent was obtained from the parents of neonates. Inclusion criteria was Age < 28 days, Full term babies, Presence of three or more clinical symptoms and /or signs of septicemia like lethargy, poor feeding, irritability, fever,

vomiting, abdominal distension, jaundice, respiratory distress, hypothermia, cyanosis and convulsions. Exclusion criteria was extreme prematurity < 30 wks of gestation, neonates with gross congenital anomalies and underlying surgical conditions. The name, age, sex, address, date of admission, inpatient number and detailed clinical history of the patients were noted. A thorough head to foot general examination, systemic examination and Respiratory Distress Syndrome (RDS) scoring were carried out by the pediatricians.

Blood samples for culture and CRP were collected from 273 clinically suspected septicemia neonates admitted to Neonatal Intensive Care Unit, over a period of 14 months. For blood culture 2 ml of samples were inoculated in to brain heart infusion broth (HiMedia,). The broth was incubated aerobically at 37°C. A subculture was done after 18 hours; if no growth was obtained, the bottles were tested for seven consecutive days. Any sign of growth was followed-up by subculture. Media used for sub culturing included chocolate agar, blood agar and MacConkey agar (HiMedia). Isolates were identified by using standard biochemical test. Antimicrobial susceptibility tests were performed by Kirby Bauer disc diffusion method as per CLSI guidelines

C reactive protein level was determined qualitatively and semi quantitatively by using CRP latex kit (Mediclone biotech pvt ltd). Raised C reactive protein was defined as level of C reactive protein more than 6 mg/L in serum. Blood culture result was taken as gold standard for diagnosis of neonatal sepsis. Diagnostic accuracy of CRP was measured in terms of sensitivity and specificity, which was calculated by using True Positive (T P): If C reactive protein raised and blood culture comes positive. True Negative (T N): If C reactive protein not raised with negative blood culture result, False Positive (F P): If C reactive protein raised while the blood culture result comes negative, False Negative (FN): If C reactive protein is not raised but blood culture comes positive. Sensitivity, specificity,

positive predictive value and negative predictive value for C RP were calculated using following formula: Sensitivity = $T P / (T P + FN) \times 100$, Specificity = $T N / (T N + FP) \times 100$, Positive predictive value = $T P / (T P + FP)$, Negative predictive value = $T N / (T N + FN)$ [6].

Results

The blood culture isolation rate was only 33% (n=91). Out of this, culture positivity was more in EOS cases 60 % (n=55) than the LOS cases 40 % (n=36). Gram negative organisms constituted 64 % (n=59) of the total bacterial isolates whereas Gram positive organisms constituted 36 % (n=32). *Klebsiella pneumoniae* (40%) was the most common organism among the GNB isolates followed by *Escherichia coli* (10%), *Enterobacter spp* (7%), *Pseudomonas aeruginosa* (5%), and *Acinetobacter spp* (2%). In case of GPC isolates, *Staphylococcus aureus* constituted (19%), followed by CONS (13%) and streptococci sp (2%). The most common organism isolated from both early and late onset septicemia was *Klebsiella pneumoniae* followed by Staph. Aureus

Table 5: Pathogens isolated in neonatal septicemia

| Name of the organisms | EOS NO (%) | LOS NO (%) | Total NO (%) |
|-------------------------------|------------|------------|--------------|
| <i>Klebsiella pneumoniae</i> | 21 (38%) | 15 (42%) | 36 (40%) |
| <i>Staphylococcus aureus</i> | 11 (20%) | 6 (16%) | 17 (19%) |
| CONS | 7 (13%) | 6 (16%) | 13 (15%) |
| <i>Escherichia coli</i> | 7 (13%) | 2 (6%) | 9 (10%) |
| <i>Enterobacter spp</i> | 4 (7%) | 3 (8%) | 7 (7%) |
| <i>Pseudomonas aeruginosa</i> | 3 (5%) | 2 (6%) | 5 (5%) |
| Streptococci spp | 2 (4%) | - | 2 (2%) |
| <i>Acinetobacter spp</i> | - | 2 (6%) | 2 (2%) |

The serum sample from all the suspected neonatal septicemic cases were tested for CRP using Latex agglutination test. It was found to be positive in 131 cases, of which only 64 cases showed culture positivity. The sensitivity of CRP was 49% and specificity was 80%. Negative predictive value and positive predictive value were 63% and 70% respectively.

Table 2: Correlation of C Reactive protein level

| Culture | CRP positive | CRP negative | Total |
|------------------|--------------|--------------|-------|
| Culture positive | 64 | 27 | 91 |
| Culture negative | 67 | 115 | 182 |
| Total | 131 | 142 | 273 |

Discussion

Deterioration in the condition of a neonate can occur in many conditions. It is always difficult to establish a definite cause of deterioration. Of various causes, bacterial infection is usually at the top. The gold standard of identifying bacterial infections in blood culture has got a low yield. Therefore the paediatricians suggest certain surrogate tests to identify neonatal sepsis.

Two seventy three suspected septicemic neonates were investigated for septicemia. Among which 91 were culture positive. The positivity rate of Blood cultures was 33%. This culture positivity rate (33%) is comparable to that reported by E Malakan Rad and N Momtazmanesh et al (2004) [7] (30%), Mahabatra et al (2002) [8] (40%) and by Bhattacharjee et al (2008) [9] (48%). In contrast S.Mahmood et al (2008) [10] reported 11% of culture positivity rate: this discrepancy might have arisen due to the administration of antibiotics before blood collection either to the mother or to the baby or the possibility of infection with viruses, fungi or anaerobes. In the present study, early onset septicemia was observed in 61% and late onset septicemia in 39% of the neonates. Early onset septicemia was more common than late onset septicemia which is compatible with the reports from Choudry habibur rasul et al (2007) (70.7%-EOS, 30% -LOS), A.H.Movahedion et al (2006) [11] (77.5 % -EOS, 22.5 % -LOS) and Vinod kumar et al (2008) [12] (55%-EOS, 47%-LOS).

In the present study sensitivity of CRP was 49%. Specificity was 80%. Negative predictive value and positive predictive value were 63% and 70% respectively. Similar results were obtained in a

study by Garland Suzane et al (2003) ^[13]. In contrast Khassawneh et al (2007) ^[14], reported that CRP had a sensitivity of (95%) and negative predictive value of (98%) in screening neonatal septicemia. This difference could be due to the fact that these studies measured CRP quantitatively with different cut off points and different times from the onset of the signs of infection. However, it can be emphasized that a single CRP level measured at the onset of infection lacks sufficient sensitivity to be useful in identifying neonate with septicemia. In addition CRP cannot be recommended as a sole indicator of neonatal sepsis, but it may be used as part of a sepsis workup and in combination with other laboratory tests.

The pathogens most often implicated in neonatal septicemia in developing countries differ from those seen in developed countries. In developed countries GBS and CONS are the more common etiological agents of neonatal septicemia. But in developing countries Gram negative organisms are more common and are mainly represented by *Klebsiella pneumoniae* followed by *Escherichia coli* and *Pseudomonas aeruginosa*. It is not known whether these differences reflect the true differences in pathogens across the world, reflecting the epidemiological transition in some countries or whether it reflects an epidemiological bias.

In the present study, Gram negative organisms constituted 64% of the total bacterial isolates causing neonatal septicemia whereas Gram positive organisms constituted 36%. This distribution pattern correlates well with the results published by Nalini Agnihotri et al (2004) ^[15] from Chandigarh who reported that Gram-negative organisms accounted for 59% of all positive cultures. Another study conducted by Mahapatra et al (2002)⁸ in Orissa also reported that Gram-negative bacilli were isolated in maximum percentage (88.45%) of cases whereas gram-positive bacteria in 11.6% of culture. In contrast S.mahmood et al (2008)¹⁰ from Iran reported that Gram positive bacteria constituted

the major group of isolates (62.1%), 68% respectively.

In the present study, among the Gram negative organisms *Klebsiella pneumoniae* (40%) was the most common organism followed by *Escherichia coli* (10%), *Enterobacter spp* (7%), *Pseudomonas aeruginosa* (5%), and *Acinetobacter spp* (2%). Among the Gram positive organisms *Staphylococcus aureus* constituted (19%), followed by CONS (13%) and *streptococci sp* (2%). This distribution pattern correlates well with the results published by Vinodkumar CS et al (2008)¹² who reported that *Klebsiella* (26.9%) was the most common infective organism followed by *Staphylococcus aureus* (20%), CONS (13.4%), *E.coli*(10.5%) and *Acinetobacter spp* (6.1%). In contrast Malakan rod et al (2004)⁷ reported that *Pseudomonas aeruginosa* as the most common causative organism of neonatal septicemia.

There appears to be a wide variety of bacteria causing early onset septicemia and late onset septicemia in developing countries. This variation may be true, but important confounders may include different definitions of early onset septicemia and late onset septicemia, different inclusion criteria for studies, inability to culture certain organism, small numbers and short period of surveillance.

Conclusion

Klebsiella pneumoniae (40%) was the most common organism in early and late onset septicemia. CRP cannot be recommended as a sole indicator of neonatal sepsis, but it may be used as part of a sepsis workup and in combination with other laboratory tests. Further research, which better understands the neonatal inflammatory response to sepsis, may result in the identification of sensitive and specific markers of inflammation or the development of pathogen-specific rapid diagnostic tests for early detection of neonatal sepsis.

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