

Bacterial profile of neonatal sepsis and the role of biomarkers

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Abstract

Context: Infections are the single largest cause of neonatal deaths globally. Until now, there is no ideal diagnostic test for detecting sepsis and thus management of possible sepsis cases often depends on clinical algorithm leading to empirical treatment. **Aims:** To evaluate the utility of various laboratory tests (blood culture, C- reactive protein and pro-calcitonin) for the early diagnosis of neonatal sepsis and to determine the bacterial profile of neonatal sepsis with antimicrobial susceptibility pattern. **Settings and Design:** Prospective study in a tertiary care teaching hospital over a period of one year. **Materials and Methods:** Neonates with suspected clinical features of sepsis were included in the study. Maternal and neonatal risk factors were noted. Blood culture was done using BACTEC and antimicrobial susceptibility pattern determined, C- reactive protein levels and serum pro-calcitonin levels were estimated. **Results:** Prematurity was the most common risk factor (63%). Out of 60 cases, blood culture was positive in 42 (70 %), *K. pneumoniae* was the most common organism isolated. Sensitivity and specificity of Pro-calcitonin test was 97.6 % and 88.8 % respectively whereas that of C- reactive protein was 59.5% and 55.6%. **Conclusion:** Prematurity is an important risk factor for neonatal sepsis. *K. pneumoniae*, *S. aureus* and *P. aeruginosa* in decreasing order are the predominant pathogens and initial therapy should cover these organisms. Pro- calcitonin has a good sensitivity and specificity for diagnosis of neonatal sepsis and can be used as an early marker of neonatal sepsis.

Key Words: Neonatal sepsis, Pro calcitonin, C Reactive Protein.

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INTRODUCTION

Infections are the single largest cause of neonatal deaths globally.¹ Neonatal infections annually claim 3.6 million lives worldwide.^{2,3,4} Early diagnosis and treatment of the newborn infants with suspected sepsis are essential to prevent severe and life threatening complications. Diagnosis of neonatal sepsis is difficult because of the variable and nonspecific clinical presentation.⁵ Until now,

there is no ideal diagnostic test for detecting sepsis and thus management of possible sepsis cases often depends on clinical algorithm leading to empirical treatment. Blood culture is currently the gold standard for the diagnosis of sepsis. However, in addition to the fact that culture reports are available only after 48-72 hours, blood cultures are frequently false negative due to the small amount of blood that can be drawn from neonates.⁶ To improve empirical antimicrobial therapy, it is necessary to generate data on the current spectrum and susceptibility profile of associated bacteria. In the last few years, biomarkers, triggered by the host immune system in response to infections, have been targeted as potential indicators for diagnostic and prognostic purposes.⁷ The reliability of most laboratory markers, including white blood cell count (WBC), C-reactive protein (CRP), Procalcitonin (PCT) and IL-6 for the diagnosis of neonatal infection has been assessed in highly diverse groups of ill neonates with a mixture of diagnoses and conditions and has yielded variable results.⁸ This

prospective study was carried out to evaluate the utility of various laboratory tests (blood culture, C- reactive protein and pro-calcitonin) for the early diagnosis of neonatal sepsis and to determine the bacterial profile of neonatal sepsis with antimicrobial susceptibility pattern.

MATERIAL AND METHODS

A prospective study was carried out over a period of one year in a tertiary care teaching hospital after obtaining institutional ethics committee approval. Neonates delivered in the hospital and admitted in NICU, with suspected sepsis or those coming to hospital with signs and symptoms of sepsis up to 28 days of life were enrolled for the study. Neonates having serious congenital malformations like meningomyelocele, microcephaly, heart disease and hydrops were excluded from the study. Babies who were referred to our hospital who had received antibiotics prior to their admission were also excluded. After written informed consent, detailed history, clinical examination findings and laboratory findings were noted on pre-designed proforma. Blood cultures were done on admission following strict aseptic precautions and using BACTEC technique (Becton–Dickinson Diagnostic Systems, USA). Any isolate was further characterized up to species level. Antimicrobial susceptibility testing (AST) was performed by the Kirby Bauer disc diffusion method as per Clinical Laboratory Standards Institute (CLSI) standards⁹ Extended spectrum beta lactamase (ESBL) production in *E. coli* and *Klebsiella spp* was detected as per CLSI guidelines. *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control strains for AST. Serum Procalcitonin was done by using B.R.A.H.M.S. PCT-Q card test, a semi-quantitative rapid test kit. PCT assessment was done for each neonate on admission except in those who presented between 6 to 48 hours of life. In these neonates, PCT assessment was done after 48 hours of life as PCT values physiologically increase during 6 to 48 hours. C- reactive protein levels were determined. Chi-square or Fisher’s exact test was used for categorical variables. P value of <0.05 was considered significant.

RESULT

Sixty neonates were included in the study of which 39 (65%) were males and remaining females (Male: female=1.9:1). 22/60 (36.7%) were full term neonates (> 37 weeks) and 38/60 (63.3%) were preterm. Prematurity was the most common risk factor for neonatal sepsis. (Table 1) Out of 60 cases, blood culture was positive in 42 (70 %), PCT was positive in 43 (71.7%), and 2 cases had positive PCT result with sterile blood culture. Statistical analysis of PCT concentration > 2 ng/ml in our study of 60 cases yielded a sensitivity of 97.6 % and

specificity of 88.8 %. The positive predictive value was 95.4 % and negative predictive value was 94.1 %. There was no statistical difference between results of procalcitonin and blood culture (P = 1) CRP was positive only in 25 (59.52%) out of 42 culture proven cases. 8 cases were showing positive CRP in spite of sterile blood culture. The sensitivity, specificity, PPV and NPV of CRP was 59.5%, 55.6%, 75.8% and 37% respectively. Out of 42 positive blood cultures, 39(93%) yielded bacterial growth while three (7%) cases showed fungal growth. Gram negative organisms accounted for 66.67% (28/42) of all isolates. *K. pneumoniae* was the predominant pathogen (26.2%) followed by *S. aureus* (19.05%) and *P. aeruginosa* (16.7%). (Table 2) All strains of *S. aureus* were susceptible to vancomycin. MRSA accounted for 25% of *S. aureus* strains. 87.5% (7/8) strains were resistant to penicillin. 50% (4/8) of the strains were resistant to ciprofloxacin and co-trimoxazole. The resistance to erythromycin, gentamicin and amikacin was 37.5%, 25% and 12.5% respectively. 85.7% (6/7) strains of *P. aeruginosa* were susceptible to anti-pseudomonal agent ceftazidime and amikacin. 57.1% (4/7) strains were resistant to cefotaxime and ceftriaxone. 42.9% (3/7) of the strains were resistant to netimicin. 27.3% (3/11) isolates of *K. pneumoniae* were resistant to amikacin. The resistance to cefuroxime, gentamicin and ciprofloxacin was 63.6% (7/11) and to ceftriaxone was 54.5% (6/11). Three strains (27.3%) were ESBL producers.

Table 1: Risk Factors for Neonatal Sepsis

Risk Factor	No. of Cases (%)
Low Birth Weight or Preterm	38 (63)
Febrile Illness in Mother	13(21)
*MSAF	17(28.3)
†PROM > 24 hours	18(30)
More than 3 vaginal examinations	14(23.3)
Prolonged or difficult labour	9(15)
Birth asphyxia and resuscitation	19(31.6)
Polymorphs in gastric aspirate	9(15)

*MSAF- Meconium stained amniotic fluid, †PROM- Pre-mature rupture of membranes Other risk factors included home delivery, multiple gestations, and faulty feeding practices

Table 2: Organisms isolated in neonatal sepsis

Organisms	No. Of cases	Percentage
<i>Klebsiella pneumoniae</i>	11	26.2
<i>Staphylococcus aureus</i>	8	19
<i>Pseudomonas aeruginosa</i>	7	16.7
<i>Acinetobacter spp</i>	6	14.3
<i>E. coli</i>	2	4.8
<i>Streptococcus spp</i>	2	4.8
<i>Citrobacter spp</i>	1	2.4
<i>Enterococcus spp</i>	1	2.4
<i>Enterobacter spp</i>	1	2.4
Fungi	3	7.1
Total	42	100

DISCUSSION

Neonatal septicaemia, its high incidence and grave prognosis in spite of all newer antibiotics is a challenge. Blood culture is the gold standard for diagnosis of neonatal sepsis but not many health care centers in India possess the required infrastructure, manpower and money to support a diagnostic microbiology service. Various surrogate markers from hematology and biochemistry like total leucocyte count, C-reactive protein, procalcitonin, presence of toxic granules in the polymorphs have been used as markers of sepsis. The present study was carried out to compare the diagnostic utility of various tests such as blood culture, serum procalcitonin and CRP estimation in neonatal sepsis. In the present study, 65% of neonates were males. Male predominance in neonatal sepsis has also been reported by other studies in literature. 66.7% of neonates were males in a study from Gujarat by Arpita Shah *et al.*¹⁰ Forhad monjur *et al* in their study observed 63.3% of neonates to be males.¹¹ Schlegel *et al* have postulated that genes on the X chromosome regulating thymic development and antibody production may lead to a sepsis gender gap.¹² In the present study prematurity was the most common risk factor present in 63% of the neonates. Ananthkrishnan *et al* in his study on risk factors for neonatal sepsis have also reported prematurity to be the most common underlying factor in babies with neonatal sepsis.¹³ This finding is also supported by many other studies in literature.^{10,14} Prematurity is a known risk factor for septicaemia. Numbers of possible explanations have been proposed.¹⁵

1. Maternal genital tract infection is considered to be an important cause of preterm labour, with an increased risk of vertical transmission to the new born.
2. Frequency of intra-amniotic infection is inversely related to the gestational age.
3. Premature infants have documented immune dysfunction.
4. Premature infants often require prolonged intravenous access or other invasive procedures that provide a portal of entry for the organisms.

The other important risk factors observed were birth asphyxia requiring resuscitation (31.6%), Pre-mature rupture of membranes (PROM) > 24 hours (30%) and Meconium stained amniotic fluid (MSAF) (28.3%). Blood culture positivity in the present study was 70% (42/60). This is higher as compared to other studies.^{13,14} The high positivity may be due the use of BACTEC system for blood culture as the other studies have used conventional blood culture method. In western countries, antibiotics of choice are directed towards group B Streptococcus and *E. coli*. But in tropical areas, neonatal infections may be caused by multiresistant hospital

acquired bacteria which are transmitted during delivery by lack of hygiene. These organisms are usually resistant genera of Enterobacteriaceae family, *Pseudomonas spp.* and Staphylococcus.¹⁶ Predominance of Gram negative organisms was seen (66.7%) in comparison to Gram positive organisms (26.2%) as is reported by other investigators.^{10,11,17,18} In our study, *Klebsiella pneumoniae* (26.2%) was the most common organism isolated as is reported by other studies.^{13,19,20,21} *Klebsiella spp.* are ubiquitous in nature. *K. pneumoniae* is present as a saprophyte in the nasopharynx and in the intestinal tract. The principal reservoirs for transmission of *Klebsiella* in the hospital setting are the gastrointestinal tract of patients and the hands of hospital personnel.²² The ability of this organism to spread rapidly often leads to nosocomial outbreaks, especially in neonatal units.²³ *Klebsiella pneumoniae* is the most commonly reported cause of neonatal sepsis in several studies from developing countries.^{24,25, 26} Zakariya BP *et al* in their study to determine the risk factors for neonatal sepsis caused by locally prevalent *Klebsiella pneumoniae* strains found that this pathogen was significantly associated with sepsis occurring among inborn babies and those with birth weight ≤ 2.5 Kg. As *Klebsiella pneumoniae* is known to cause outbreaks among inborn babies in neonatal units, infection control measures should be strictly practiced. Similarly, adequate care of the low birth weight babies is of utmost importance to prevent infection by *Klebsiella pneumoniae*.²⁷ With increasing survival of smaller, more immunocompromised preterm infants, the incidence of invasive fungal infection is increasing among NICU patients, with high associated morbidity and mortality.²⁸ The vast majority of fungal infections in preterm neonates are due to *Candida spp.*²⁹ In the present study, *Candida spp* made a significant contribution (7.1%). These findings are similar to a study by Arpita *et al.*^{10,30} B-lactam resistance was high in gram negative organisms including pseudomonas spp. , as well as *S. aureus*. This makes them unsuitable to use as empirical antibiotic therapy in intensive care unit settings where right antibiotic can save life. In *S. aureus*, resistance was highest for penicillin. The high resistance to ciprofloxacin and co-trimoxazole may be attributed to their increased usage in the community since these antibiotics are available in oral form. Quinolones are excreted in human sweat. This may explain the rapidity with which quinolone resistance has emerged in staphylococci.³¹ Amikacin had good activity against *Klebsiella*, *Pseudomonas* and *Staphylococci* and can be used for empirical therapy as it has a broad spectrum of activity and resistance is comparatively low even after decades of use. ESBL production was seen in 27.3% of *K. pneumoniae* strains. A Bhattarjee *et al* in their study on

investigation of prevalence of ESBL producing gram negative rods in neonatal sepsis found that 32% of isolates were ESBL producers.¹⁸ This implies that ESBL detection should be routinely done in the laboratory and empirical use of third generation cephalosporins should be discouraged in neonatal sepsis. A number of adjunctive tests, including measurements of serum interleukin-6, IL-8, procalcitonin and CRP levels, have been studied for their ability to predict sepsis in neonates with clinical signs and symptoms of infection. In many studies, CRP has been shown to have higher sensitivity and negative predictive value than leukocyte indices such as the immature/total neutrophil ratio for predicting bacteremia.³² While the finding of a normal CRP level can be reassuring, the significance of an abnormal CRP value in a preterm neonate is less clear. Common conditions such as fetal distress, premature or prolonged rupture of membranes, hyperbilirubinemia, and respiratory distress syndrome have been associated with elevation of serum CRP levels in some studies.^{33,34} As many as 9% of apparently healthy neonates have been reported to have a CRP level greater than 1.0 mg/dl.^{35,36} For these reasons, elevations in CRP levels should not be used as the sole indication for either starting or stopping antibiotic therapy; instead, they should be viewed in the context of blood culture results, clinical findings, and other laboratory studies. PCT, one of the precursors of calcitonin is a 116 amino acid peptide. It is secreted into the blood circulation during infection without increasing calcitonin. PCT is detectable in the plasma as early as 2h after exposure to the bacterial products. Its levels rise for 6-8 hours reaches a plateau after 12h and then decreases to a normal level after 2 to 3 days.³⁷ Assicot M *et al* in 1993 first proposed PCT as an early marker of bacteremia.³⁸ Gendrel *et al* in 1995 found increased levels of PCT in bacterial sepsis. He also reported that neonates with viral infection, bacterial colonization or neonatal distress had normal or slightly raised PCT levels.³⁹ In a study of 183 neonates, Ballot DE in 2004 reported that the PCT predicted 89.5 % of definitive infections with a negative predictive value of 95 %.⁴⁰ B Resch in 2003 determined the reliability of PCT by comparing 41 neonates with positive blood culture and clinical sepsis with 27 uninfected neonates. The study revealed sensitivity and specificity of 77 % and 91 % respectively.⁴¹ The diagnostic accuracy of PCT in differentiating bacterial from non infectious causes of inflammation was 88 % sensitive and 81 % specific in the study by Liliana Simon in 2004.⁴² Ramesh Wazzalwar in 2005 reported that the sensitivity of PCT at cut off value of 0.5 ng/ml was 97%.⁴³ In the same year, Sudin Thayyil *et al*⁴⁴ found out the sensitivity and specificity of PCT at concentration > 2 ng/ml to be 50 % and 85 %

respectively, while in our present study, the sensitivity and specificity at 2 ng/ml concentration was 97% and 88.8 % respectively. CRP and PCT have been compared for their efficacy to diagnose sepsis cases in young infants. Overall studies have reported that the optimum sensitivity and specificity for CRP was obtained during the window of 24-48 hours after the onset of symptoms. On the other hand, PCT was sensitive enough to detect the cases much earlier than CRP.⁷ In the present study, CRP was positive only in 33/60 (55%) of cases. This is more as compared to other studies {C reactive protein was positive (more than 8mg/dl) in 32% (8/25).¹³ In our study, fatal outcome was seen in 12 cases. 9 out of these 12 had PCT concentrations > 10 ng/ml suggesting PCT increases as the severity of sepsis increases. 3 cases in our study were positive for fungal culture. PCT concentration was > 2 ng/ml in 2 of these cases. This is similar to the observation made by Gerard Y *et al* in 1997.⁴⁵ There was no control group in the present study, only clinically suspected cases of neonatal sepsis were included and is a limitation of this study. Prematurity is an important risk factor for neonatal sepsis. To improve empirical antimicrobial therapy, it is necessary to determine the causative organisms and their susceptibility pattern on a regular basis. Gram negative organisms predominate as etiological agents of neonatal sepsis all over the country. *K. pneumoniae*, *S. aureus* and *P. aeruginosa* in decreasing order are the predominant pathogens and initial therapy should cover these organisms. ESBL detection should be carried out routinely in the laboratory for isolates of neonatal sepsis. Resistance to erythromycin, co-trimoxazole and ciprofloxacin has significantly increased in the past few years and these should be avoided unless susceptibility reports are available. PCT has a good sensitivity and specificity for diagnosis of neonatal sepsis and can be used as an early marker of neonatal sepsis.

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