

Etiological study of neonatal septicaemia

Dhanjal G.S.¹, Agrawal S.², Sharda P.³

¹Dr. Gurdeep S Dhanjal, Professor of Pediatrics, Maharishi Markandeshwar Institute of Medical Science and Research, Mullana, Ambala, Hararyana. ²Dr. Sonam Agrawal, Senior Resident, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, Ambala, Hararyana. ³Dr. Prateek Sharda, Senior Resident, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, Ambala, Hararyana. India.

Corresponding Author: Dr. Sonam Agrawal, Senior Resident, MMIMSR, Mullana Ambala. **E-mail:** dr.sonamagrwal@gmail.com

Abstract

Introduction: Neonatal sepsis is a clinical syndrome of bacteremia characterized by systemic signs and symptoms of infection in the first month of life. Our aim was to study the etiological profile of neonatal septicaemia and their sensitivity pattern. **Methodology:** The study was conducted over a period of one and half years in the Neonatal Intensive Care Unit (NICU), MMIMSR, Mullana, Ambala, Haryana. A total of 100 cases with positive sepsis screen were identified with standard biochemical tests and these newborns were subjected to blood culture and antimicrobial susceptibility test. **Results:** There were 66 male babies and 34 female babies with male: female ratio of 1.9:1. In the study 94% cases were early onset neonatal sepsis and 6% were late onset neonatal sepsis. The blood cultures of 54 babies showed growth, out of these 16 (29.6%) cases were gram positive bacteria, 34 (62.9%) were gram negative bacteria and 4 (7.4%) showed fungal growth (i.e. candida albicans). Acinetobacter species were most common among gram negative organisms i.e. 64.7%. Staphylococcus aureus along with coagulase-negative staphylococci (CONS) were common organisms seen among gram positive bacteria. Among gram negative isolates 62.9% were extended spectrum beta lactamase (ESBL) producers. **Conclusion:** Neonatal septicaemia is a life-threatening emergency. The study of etiological profile and their antibiotic sensitivity pattern plays a significant role in decreasing the neonatal mortality rate. The rational use of antibiotics will reduce infection rate ensuring better therapeutic success and reduce the resistance of the organism to available antibiotics.

Keywords: Neonatal septicaemia, Blood culture, Sepsis, Antimicrobial susceptibility

Introduction

Neonatal sepsis is a clinical syndrome of bacteremia characterized by systemic signs and symptoms of infection in the first month of life [1]. It comprises meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections [2]. This excludes local infection of newborn such as omphalitis, pyoderma and conjunctivitis [3]. It is estimated that 20% of neonates develop sepsis and approximately 1% deaths are related to sepsis [4].

Neonatal septicemia is by far the most fatal sequelae of such infections [5]. For epidemiological and therapeutic purpose, neonatal septicaemia is categorized into early onset septicaemia (presents within the first 72 hours of life) and late onset septicaemia (presents after 72 hours of life) [2].

The gold standard for the diagnosis of neonatal sepsis is a positive blood culture [2,6]. Definitive culture results take at least 48-72hrs resulting in treatment delays.

But with improved bacteriological techniques such as BACTEC and BACT/ALERT blood culture system can detect bacteria at a concentration of 1-2 colony forming unit (cfu)/ ml [2,7]. Early diagnosis and treatment with appropriate antibiotics would minimize the risk of severe morbidity and mortality besides reducing the emergence of multiple resistant organisms in nurseries by rational antibiotics use. Periodic evaluation of organisms causing neonatal sepsis is essential for the appropriate management of neonates.

Therefore, the present study was undertaken to determine the etiological profile and the antibiotic sensitivity pattern of the microbial isolates in a tertiary level hospital.

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Material and Methods

The study was a prospective observational study conducted in neonatal intensive care unit (NICU) of Maharishi Markandeshwar institute of medical sciences and research (MMIMSR), Mullana, Ambala over a period of one and half year (November 2013 to May 2015). Institutional ethical clearance was taken for the study.

Neonates admitted in the hospital with maternal risk factors were subjected to a battery of test collectively called the "sepsis screen". Parameters included in the sepsis screen and their abnormal values were total leukocyte count (TLC) < 5000/cumm, absolute neutrophil count (ANC) < 1800/cumm, immature to mature neutrophil ratio > 0.2, micro ESR > 15mm in 1st hour and C-reactive protein (CRP) > 1mg/dl. Sepsis screen was considered positive when two or more parameters were abnormal.

Neonates with positive sepsis screen were included in the study. Babies whose parent did not give consent were excluded from the study. 100 newborns satisfying the inclusion criteria were enrolled in the study.

With all aseptic precautions 2 ml blood was taken in EDTA vacutainer and was processed for TLC, ANC, peripheral blood smear (PBS) for I:T ratio and micro ESR. Also 1 ml blood sample was taken for estimation of a qualitative CRP result.

Auto hematology analyzer (3 parts) was used to analyze the sample. TLC was calculated by direct counting of leukocytes in an improved Neubauer's chamber. ANC was calculated on PBS stained by Leishman's stain.

Results

In the present study, total of 100 cases with significant sepsis score were studied and blood culture were performed. Among these 90 cases (i.e. 90%) were of early onset neonatal septicaemia and 10 (i.e. 10%) cases were of late onset sepsis. In the present study the ratio of male: female is 1.9 : 1. Out of 100 babies 62% babies were preterm and 38% were term with preterm to term ratio of 1:1.6.

Out of 100 neonates, blood culture was positive in 54 (54%) cases where as in 46 (46%) cases blood culture was negative. Among the 54 blood culture positive cases gram negative bacteria were present in 34 (63%) cases followed by gram positive bacteria in 16 (29.6%) cases and fungal growth in 4 (7.4%) cases (TABLE 1)

Acinetobacter was the commonest pathogen found in blood culture followed by Citrobacter. Only 37.5% isolates of Staphylococcus aureus and Staphylococcus epidermidis respectively were sensitive to penicillin and oxacillin. Methicillin resistance was seen in 25% cases of Staphylococcus aureus.

All gram-positive cocci were sensitive to vancomycin. The enterobacteriaceae family showed high resistance to various antimicrobial agents like ampicillin, gentamycin, cefazolin, cefoperazone, cefotaxime and ceftriaxone. Most of them had good sensitivity to amikacin, ciprofloxacin, ceftazidime and piperacillin tazobactam.

Neutrophils were classified as band forms when there are no nuclear segmentation or when the width of the nucleus at any constriction was not less than one-third the width at its widest portion. Band forms together with less mature cell forms were classified as immature polymorphonuclear (PMN) leukocytes [8]. Immature to total neutrophil ratio was calculated on PBS stained by Leishman's stain. Micro ESR was performed using standard preheparinised micro hematocrit tubes (75mm in length with internal diameter 1.1mm and outer diameter of 1.5mm) and by recording the fall of erythrocyte column after 1 hour. [8].

Another 2 ml blood collected with all aseptic precautions was inoculated into BACT/ALERT RPF (Biomérieux, INC. Durhams, NC 27704) containing 20ml of broth. The blood and broth were mixed gently and bottles were transported to laboratory for incubation in BACT/ALERT 3D system and further processing was done as per manufacturer's guidelines.

Interpretation: Those blood culture indicated positive or negative by BACT/ALERT 3D system were sub cultured on sheep blood agar and Mac Conkey agar. The sheep blood agar and Mac Conkey's medium were incubated at 35 + 2° Celsius for 18-24 hours in aerobic conditions. Various organisms were identified on the basis of colony morphology and standard biochemical tests. Those blood culture bottles which were negative for 5 days (as setting of BACT/ALERT 3D system) were reported as "no growth". The isolates were subjected to antimicrobial susceptibility testing by Kirby Bauer disk diffusion method as per clinical and laboratory standard institute (CLSI) Guidelines 2011 (9).

Pseudomonas aeruginosa and *acinetobacter* species showed high resistance to various antimicrobial agents like cefepime, cefoperazone, cefotaxime and ceftazidime. Most of the gram negative organism had moderate sensitivity to amikacin, piperacillin, tazobactam and good sensitivity to meropenem and imipenem.

We observed that meropenem and imipenem were highly effective antibiotic for infections with multidrug resistant gram-negative bacilli. All the four cases with positive candida growth were sensitive to fluconazole.

Table-1: Microbial profile of neonatal septicemia.

Organisms	Cases (54/100) (blood culture positive)	Drug sensitivity
Gram-positive : 16 cases		
Staph aureus	6 (37.5%)	Penicillin, oxacillin, vancomycin, linezolid
MRSA	4 (25%)	Vancomycin, linezolid
CONS (<i>S. epidermidis</i>)	6 (37.5%)	Penicillin, oxacillin, vancomycin, linezolid
Gram-negative: 34 cases		
Acinetobacter	22 (64.7%)	amikacin, ciprofloxacin, piperacillin, tazobactam, meropenem, imipenem
Citrobacter	8 (23.5%)	amikacin, ciprofloxacin, ceftazidime, piperacillin, tazobactam, meropenem & imipenem
Pseudomonas	4 (11.7%)	Piperacillin, tazobactam, Meropenem and imipenem
Fungi: 4 cases		
<i>Candida albicans</i>	4	Fluconazole

Discussion

The advancement in neonatal intensive care medicine is a double edge sword, with neonatal survival improvement on one side and increased rate of long term morbidity on another. The microorganism pattern of neonatal sepsis is different at different hospitals and its pattern changes with time hence periodic reevaluation of the etiological agent is useful in the management of neonatal sepsis. Despite all efforts, a rapid sensitive diagnostic tool for neonatal septicemia is yet to be found.

Number of studies have been done on the risk factors, etiology, haematological parameters and on the clinical profile of neonatal septicemia. Blood culture is the gold standard for definitive diagnosis of neonatal septicemia, but it has its own limitations as it requires a well equipped laboratory, has a success rate of 40%, very time consuming, and may give spurious positive results. The results of blood culture may take about a week, necessitating initial empirical treatment of suspected septicemia. Overall incidence of culture proven sepsis varies between 1-8 cases per 1000 live births with almost equal distribution of early onset and late onset cases.

This present study was undertaken to study the etiological profile in newborn with positive sepsis screen so that prompt therapy be instituted to reduce morbidity and mortality. The most common agents causing neonatal sepsis are bacteria and only a proportion of the blood culture from cases with clinical sepsis will show growth of organism.

In our study the male to female ratio was 1.9:1 which is similar to the findings by Jain NK et al (2:1) [10] and by Jia-hong Jiang et al (1.4:1) [11]. Probably the ratio in favour of male could be because of the priority given to the male babies for medical care in our society.

In our study 90% of the cases were of early onset neonatal sepsis whereas late onset sepsis was seen in 10% cases. The large number of cases of EONS may be because our tertiary care hospital has a well-established maternity wards and caters rural population which do not have good health services especially NICU leading to early referral to our institute.

In the present study the blood culture positivity rate was 54 %, which is similar with other studies [12,13]. The

frequency of isolation of gram positive and gram negative bacteria from blood culture in our study was 29.6% and 63% of culture positive cases respectively. This finding is almost similar to that of other studies done by Ahmed et al [14] and Kapoor et al [15] who found gram-negative bacilli in 70% and 62% of culture positive neonates respectively. In the study done by Simiyu et al [16] 66.6% of culture positive cases were gram-negative organisms and 33.4% were gram-positive. In the study by Agnihotri et al [17] 58.5% of positive culture showed growth of gram-negative organisms.

Acinetobacter and *Citrobacter* (gram negative organisms) were the two most common bacteria isolated from culture in our study. Most of the studies done earlier also shows predominance of gram negative organisms. The predominance of *Acinetobacter* as the causative agent of neonatal sepsis may be due to the selective pressure of antimicrobial agents so that resistant organisms tend to colonize and proliferate in the neonates.

In the present study majority of gram-negative bacilli i.e. *Acinetobacter* and *Citrobacter* isolates were resistant to all the commonly used antibiotics except meropenem, imipenem, piperacillin, tazobactam and amikacin. In another study from North India, 30-80% of the gram negative isolates were resistant to third-generation cephalosporins [18]. As amikacin shows good activity against gram-negative bacteria it should preferably be included in empirical regimen while third generation cephalosporins should not be used alone.

Conclusion

Neonatal septicaemia is a life-threatening emergency. The study of etiological profile and their antibiotic sensitivity pattern plays a significant role in decreasing the neonatal mortality rate. Blood culture results takes at least 48-72 hrs which result in treatment delays causing an increase in neonatal mortality.

Hence knowing the changing pattern of microorganism with studies done at intervals would help to decrease the neonatal mortality with appropriate use of antibiotics along with good infection control practices.

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