Original Article

The Association between Neonatal Sepsis and C-Reactive protein: A Cross-Sectional Study at Tertiary Care Hospital CAIMS, Karimnagar, T.S

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ABSTRACT

Background and Aim: All neonates treated for suspected bacterial infection were prospectively evaluated using a standardized clinical pathway. Infants were proven sepsis by clinical and laboratory findings (bacteria isolated from blood, cerebrospinal fluid and urine cultures, with consideration of serum C-reactive protein (CRP) levels. The aim of study was to evaluate association between neonatal sepsis and CRP levels for diagnosis of neonatal infection.

Materials and Methods: A cross sectional hospital based study conducted at regional intensive care nursery unit in Tertiary care hospital Karimnagar, Telangana State. Infants whose blood cultures yielded skin flora but who demonstrated no other signs of bacterial infection has not considered having sepsis.

Results: Sepsis was suspected in 262 infants and among them 145 (55.2%) blood culture positive. 114(78.5%) infants had early-onset of sepsis < 72 hours after birth as compared 31 Neonates (21.5%) had late onset septicaemia. 56.75% Gram negative organisms were causative agents as compared to 43.24% gram positive organism in study group. Increased CRP level is a marker in neonatal septicaemia but still blood culture is gold standard for diagnosis of septicaemia. Proven or probable sepsis 39.4% sensitivities and 64.6% for and 35.0% and 61.5% for proven sepsis in early-onset and late-onset episodes, respectively.

Conclusion: Neonatal septicemia is still a leading cause of mortality and morbidity in developing countries like India. In view of the changing spectrum of the causative agents of neonatal septicemia and their antibiotic susceptibility patterns from time to time and from one hospital to another, a positive blood culture and the antibiotic susceptibility testing of the isolates are the best guide to the antimicrobial therapy. Blood culture is still the "Gold standard" for the diagnosis of septicemia in neonates and should be done in all cases of suspected septicemia prior to starting the antibiotics.

Keywords: Neonate, septicaemia, premature, C-Reactive protein.

INTRODUCTION

Neonatal septicaemia refers to generalized bacterial infection documented by positive blood culture in the first four weeks of life. [1] Neonatal septicaemia is one of four leading causes of neonatal mortality and morbidity in India. [2,3,4] According to WHO there are 5million neonatal deaths a year in developing countries [5] and 40% Neonatal deaths attributable to sepsis. [6]

Neonates are at the highest risk for bacterial sepsis with the prevalence of1-10/1000 live births worldwide. [7] But in contrast, according to National Neonatal Perinatal database in India, the incidence of neonatal septicaemia is 24/1000 livebirths. [8] Neonatal septicaemia can broadly be classified into 2 groups: 1. Early Onset Sepsis (<72hrs): Caused by microorganisms acquired from the Mother before or during birth. 2. Late Onset Sepsis (>72hrs-28days): Caused by microorganisms acquired from the

environment. National Neonatology Forum of India defines Neonatal sepsis as follows: Proven sepsis: The baby presents with clinical picture of sepsis and isolation of pathogens from blood, CSF, urine or other body fluids or autopsy evidence of sepsis. [9]

Probable sepsis: New born with clinical picture suggestive of sepsis with one or more of the following criteria: 1) Evidence of predisposing factors like maternal fever, foul smelling liquor 2) Positive septic screen Absolute neutrophil count <1800/mm3 C-Reactive protein >1mg/dl and micro-ESR >15mm in 1st hour 3) Radiological evidence of pneumonia (Klebsiella pneumonia was again the commonest organism isolated followed by Staphylococcus aureus). [8] The risk factors for Neonatal septicaemia include premature rupture of membranes, prolonged rupture of membranes, prematurity, UTI, poor maternal nutrition, LBW, birth asphyxia and congenital anomalies. [10,11, 12]

It is important that supportive and antimicrobial therapy of a neonate with sepsis is instituted quickly. Hence only a minimum of essential investigations should be undertaken.[12] Blood culture is the gold standard for diagnosis of septicaemia and would be performed in all cases of suspected sepsis prior to starting antibiotics.

Aims and Objectives:

1) To isolate and identify the causative organism in neonatal septicaemia.
2) To evaluate the levels of C- reactive protein in neonatal septicaemia
3) To assess the drug sensitivity for appropriate drug use.

MATERIALS AND METHODS

This study was a cross sectional hospital based study conducted with co-operation of Paediatric NSU and the Department of Microbiology, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar for a period of 1year from February 2018 to January 2019. Blood samples from 262 clinically suspected neonatal septicaemia cases are subjected to aerobic culture. From 145 neonates the culture isolates obtained are tested for antibiotic susceptibility pattern. Serum obtained from 145 cases are subjected to CRP evaluation.

Inclusion criteria:

Neonates with at least three of the following risk factors were included. Febrile illness in mother during or within two weeks of delivery (more than 380C, oral temperature).

1. More than 3 vaginal examinations during labour.
2. Prolonged rupture of membranes (more than 12 hours).
3. Foul smelling or meconium stained liquor.
4. Preterm baby or LBW baby.
5. Birth asphyxia and difficult resuscitation.
6. Pathological evidences of funisitis.
7. In addition, neonates who presented with symptoms of septicaemia like refusal of feeds, decreased activity, lethargy, respiratory distress, fever, hypothermia, sclerema, abdominal distension, seizures and shock were all included.

Exclusion criteria:

1. Age>28 days
2. Neonates with lethal congenital anomalies
3. Babies who were referred to our hospital and who had received antibiotics prior to their admission.
4. Severe hepatic and renal dysfunction

Blood Culture:

Approximately 5 cm over the vein puncture site was disinfected with 70% alcohol gently and allowed to dry. Application of Providence Iodine in concentric circles over the site and allowed to dry for at least 1minute. About 1 ml of blood was drawn aseptically and inoculated into a blood culture bottle containing 5ml of Brain Heart Infusion broth, thus making a dilution of 1 in 5 to nullify the natural bacteriostatic/bactericidal activity of blood. After inoculation, the blood culture bottles will be incubated at 37°C under aerobic conditions in the incubator for 7 days. The first subculture will be done after 24 hours of incubation, the second on the third day and a final on the seventh day. Subcultures will be done onto 5% sheep blood agar and Mac-Conkey agar plates.

According to AIIMS –NICU protocol 2008, the growth will be identified by colony characteristics, Gram's stain, hanging drop method for motility and standard biochemical tests described in Mackie and McCartney, Practical Medical Microbiology[13], and Bailey and Scott's Diagnostic Microbiology.[14] Cultures which did not yield any growth following three subcultures were reported negative at the end of 7 days.

Antibiotic Susceptibility Testing:

Antibiotic susceptibility testing will be done for all the isolates on Muller Hinton agar using commercially available discs (Hi media), by Kirby Bauer disc diffusion technique as per the CLSI guidelines (2010). The plates were incubated at 370C. After 16-18 hrs incubation the diameter of each zone was measured with a scale, recorded in mm and interpreted as sensitive or resistant, in accordance to the indications of the disc manufacturer. The results were recorded and interpreted as per CLSI recommendations.

Detection of C-reactive protein:

CRP was measured for 262 clinically suspected neonatal septicaemia cases by immune-turbidi metric assay using Turbodynev CRP by Tulip Diagnostics Pvt Limited.

Presence of CRP in the test specimen results in the formation of an insoluble complex producing a turbidity, which is measured at ~ 650 nm wavelength. The increase in turbidity corresponds to the concentration of CRP in the test specimen.

Measuring range of Turbodyne CRP:

The range is 0.6 – 10mg/dl. Detection limit: 0.6mg/dl.

STATISTICAL ANALYSIS

Collected data enter in the master chart using Microsoft excel 2010 and for qualitative data were analysed by descriptive statistics.

Quantitative data analysed by using inferential statistics, difference between the population was analysed by chi square goodness of fit test at 5% level of significance with the help of statistical application software SPSS version 25.

RESULTS

In the present study period (from February 2018 to January 2019), 262 cases were included considering inclusion and exclusion criteria.

Out of 262 cases, blood culture was positive in 145 (55.3%) cases.

Table 1: Distribution of Blood Culture

Blood Culture (N=262)	Number of Patient	Percentage (%)
Positive	145	55.3
Negative	117	44.6
Total	262	145
	p-value = 0.08	

Table 2: Neonates Sex Vs. Blood Culture Reports

Study group	Suspected sepsis (262)	Blood Culture Positives (145)	Blood Culture Negatives(117)
Male neonates	151(58%) clinical	77 (52.8%)	55(47.2%)
	septicemia		
Female neonates	111(42%) clinical	68(47.2%)	62 (52.8%)
	septicemia		
P. Value= 0.32			

Total cases 262 clinically suspected neonatal septicemia. Blood culture positive 145 (55.3%) 117(44.7) blood culture negative.

Out of 262 cases of clinical sepsis 58% were male neonates 42% were female neonates. Male neonates with clinical sepsis were admitted more frequently than female neonates which is statistically significant. (p-value 0.001).

Among 145 cases of proven sepsis 77 (52.85%) were male neonates and 68 (47.14%) were females Neonates. There was no sex difference in blood culture positive sepsis (p-value 0.32)

Table 3: Gestational age distribution among clinical and blood culture positive sepsis comparing with mode of delivery.

Gestational age	Clinical sepsis	P-value	Positive Sepsis	P-value
Preterm	117 (44.6%)		68(47.2%)	
Term	145 (55.4%)	0.03	77(52.8%)	0.35
Total	262		145(55.34%)	

In neonates with clinical sepsis, term neonates were 55.4% and pre term neonates were 44.6% which is statistically significant (0.03). However, Blood culture positivity was comparable in both preterm 47.2% and term 52.8% neonates (p-value 0.35).

Table 4 : Distribution of cases according to onset of symptoms

Clinical Diagnosis	Number of Culture Positive	Bacterial Isolates	
		Gram Positive (%)	Gram Negative (%)
Early Onset sepsis	116(80%)	60(41.4%)	85(58.6%)
Late Onset Sepsis	29(20%)		
Total	145	60	85

Of the total 145 culture positive cases, Early onset septicemia was seen in 80% cases and 20% cases showed late onset septicemia, indicating majority of culture positive cases were seen among early onset septicemia. Out of 262 clinical sepsis 145 (55.2%) were blood culture positive. Among blood culture positives, 60 (41.4%) were gram positive and 85(58.6%) were gram negative.

K.pneu – Klebsiellapneumoniae
E.coli – Escherichia coli
S.aureu – Staphylococcus aureus
CoNS – Coagulase Negative Staphylococci.

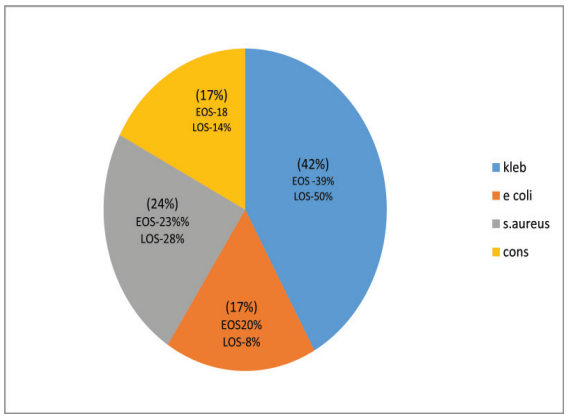

Figure 1: Spectrum of isolates in blood culture positives In EOS and LOS

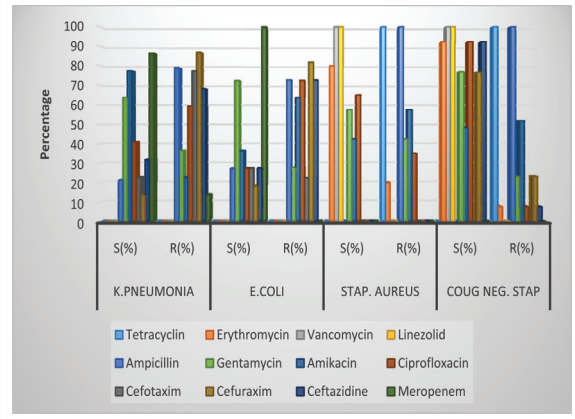

Figure 2: Antibiotic susceptibility pattern for isolated organisms.

Klebsiella (41.42%) was the most common organism in our study followed by Coagulase positive Staphylococcus(24.28%) and CONS(17.14%). In gram positive organisms most common are coagulase positive Staphylococcus(24.28%), CONS(17.14%). In Gram negative organisms most common are Klebsiella (41.42%), followed by E.coli (17.14%).

Table 5: Correlation of CRP with positive blood culture cases

Total number of cases	Positive CRP cases	Negative CRP cases
262	171 (65.26%)	91 (34.74%)
Positive blood culture cases	Positive CRP Cases	Negative CRP cases
145 (55.2%)	132 (91.42%)	13 (8.58%)

Out of 145 positive blood culture cases, 91.42%were CRP positive and 8.57% were CRP negative.

Klebsiella pneumonia, E.coli – Escherichia coliS.aureu – Staphylococcus aureus CoNS – Coagulase Negative Staphylococcus

In the present study majority of isolates were Gram negative accounting for 58.57% of the total cases, susceptible to Meropene 86.36%, Amikacin72.27% and Ceftazidime 31.81%. Most of the isolates were resistant to Gentamicin and Ciprofloxacin..

In Gram positive organisms common isolate was S.aureus (24.28%), 100% sensitive to Vancomycin and Linezolid, followed by Erythromycin and Ciprofloxacin 66.6% sensitivity. E.coli (17.14%) were 100% susceptible to Meropenem, followed by Gentamicin 77.27%. Majority were resistant to Amikacin, Ciprofloxacin, and Cefotaxime.

DISCUSSION

Recommendations from developed countries suggest that presence of >2 risk factors should be Considered an indication for starting antibiotics.[15] The source of infection is either nosocomial or community-acquired and neonates usually present with septicaemia, pneumonia or meningitis. [16,17] Various factors that predispose to an increased risk of nosocomial sepsis include NICU admissions, low birth weight, prematurity, invasive procedures, parenteral fluid therapy, ventilation and use of stock solutions, include poor hygiene, poor cord care, bottle-feeding and pre-lacteal feeds.

Roy et al in their study blood culture positive were 47.5% in 2002. [18] In our present study group of 262 clinically suspected neonatal septicemia. Blood culture positive were 55.3%.in 2011 according to Misra et al, study has shown increased to 65.2%[20] Effects of septicemia shows the higher in total suspects of septicemia neonates in the study of Kavyange. N et al (2010). 58% male neonate and 42.7% female neonates. [19] In our present study among suspected septicemia cases (262), male neonates 58% and 42% female neonates. (p-value0.001) (Table 1).

In the study of Shah A et al (2011) 55% in male neonates as compared to female neonates 45%. [20] In our present study among blood positive (145) 52.8% in male neonates as compared with females Neonates 47.14%). There was no sex difference in blood culture positive sepsis (p-value 0.42) (Table 2).

About the gestational age of pre-term 46.7% and term neonates 53.29% In the cohort study of neonates with clinical sepsis, Bhat YR et al(2011). [21] In our study shows term neonates were 55.4% and pre term neonates were 44.6% which is statistically significant (0.03) (Table 3).

In the study of Jyothi et al (2010) shows on early onset of septicemia 74.8% and Late Onset of Septicemia 25.2%. [22] Another study of Roy et al shows 71.8% on Early Onset of Septicemia and 28.7% in Late Onset of septicemiain. [23] The present study shows early onset septicemia was seen in 80% cases and 20% cases showed late onset septicemia, indicating majority of culture positive cases were seen among early onset septicemia (Table 4).

Among different isolated organism were showed klebsiella pneumonia in the study of Vrishali Avinashand Miley et al [24] 35.4% and in Kavyanga N et al [19] showed 37%. As compared with our study Klebsiella 41.42%. S. aureos was 22.9 % in the study of Vrishali Avinash Miley et al. in our present study S.aureos 24.28%. In the study of Vrishali Avinash Miley et al [24] E.coli was 16.7% and in our study shows 17.4%. In the study of Monjur et al Coagulase Negative Staph. Aureos (CoNS) shows 27% and in our present study Coagulase Negative Staph Aureos17.83% (Figure: Pie Chart). [25]

Out of 145 positive blood culture cases, 91.42% were CRP positive and 8.57% were CRP negative (Table 5).

In study about susceptibility pattern of isolated organisms Roy et al (2002) in their study showed 100% sensitive to Ampicillin, 83.3% sensitive to Gentamycine, cefotaxim 62.6%. [23] Kavyanga.N etal (2010) shows sensitive to ampicillin 58.3%, Ciprofloxin 90.7%, marapenem 100%. [19]

In the present study majority of isolates were Gram negative accounting for 58.57% of the total cases, susceptible to Meropene 86.36%, Amikacin 72.27% and Ceftazidime 31.81%. Most of the isolates were resistant to Gentamicin and Ciprofloxacin.

In Gram positive organisms common isolate was S.aureus (24.28%), 100% sensitive to Vancomycin and Linezolid, followed by Erythromycin and Ciprofloxacin 66.6% sensitivity. E.coli (17.14%) were 100% susceptible to Meropenem, followed by Gentamicin 77.27%. Majority were resistant to Amikacin, Ciprofloxacin (Figure 2).

CONCLUSION

Neonatal septicemia is still a leading cause of mortality and morbidity in developing countries like India. In view of the changing spectrum of the causative agents of neonatal septicemia and their antibiotic susceptibility patterns from time to time and from one hospital to another, a positive blood culture and the antibiotic susceptibility testing of the isolates are the best guide to the antimicrobial therapy. Blood culture is still the "Gold standard" for the diagnosis of septicemia in neonates and should be done in all cases of suspected septicemia prior to starting the antibiotics.

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CONFLICT OF INTEREST:

The authors declared no conflict of interest.

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