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Bacteriological Profile of Neonatal Septicemia, Antibiogram and Correlation with CRP in A Tertiary Care Hospital

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Abstract: Neonatal septicaemia constitutes a significant cause of neonatal mortality in our country. Blood culture which is considered as gold standard for diagnosis is costly and time consuming. CRP is a rapid tool for screening of neonatal septicaemia and a reliable marker in the absence of positive blood cultures. In present study, we emphasize to study the correlation of C reactive protein with blood culture in neonatal septicemia as it is a relatively economical method and it accelerates the diagnostic process. In this prospective study done at Niloufer Hospital, Hyderabad, neonates admitted in NICU with clinical suspicion of sepsis were studied for 3 months from July 2017 to September 2017. Blood was collected from each patient aseptically and inoculated into Trypticase soy broth and subcultures were made on blood agar and Mac conkey agar, and antibiotic susceptibility test was done. Qualitative estimation of CRP was done by rapid CRP latex slide agglutination test. Of 126 neonates studied, 49 had only positive blood culture 13 had only positive CRP, while 52 had both positive CRP and blood culture. Both blood culture and CRP were negative in 12 neonates. The sensitivity, specificity, positive and negative predictive values of CRP were 80%, 19.67%, 51.48% and 48% respectively. CRP is a very good inflammatory marker and also highly sensitive in case of neonatal septicaemia. Blood culture reports are available only after 48-72 hours and so comparing CRP with blood culture reports provides a rapid and economical method to diagnose neonatal sepsis.
Keywords: Neonatal sepsis, Mortality, Blood culture, C-reactive protein, Latex agglutination, Antibiogram.

INTRODUCTION

Neonatal sepsis is the most common cause of neonatal mortality. It is responsible for 30-40% of neonatal deaths in developing countries [1, 2]. The incidence of sepsis is 30 per thousand live births according to the National Neonatal Perinatal Database.

Neonatal sepsis refers to systemic and generalized bacterial infection of the newborn documented by a positive blood culture in the first 4 weeks of life, and is one of the four leading causes of neonatal mortality in India [3].

Neonatal sepsis is caused by a variety of organisms, ranging from Gram-positive bacteria, Gram-negative bacteria and sometimes yeasts [4]. The major cause of neonatal sepsis in the developing countries is Gram-negative bacteria and these organisms have developed increased resistance over the last 20 years, therefore posing a major problem in managing neonates with sepsis [5-7]. It is essential to conduct periodic review of organisms responsible for neonatal septicemia for the appropriate management of neonates.

Proper management and diagnose early of neonatal septicemia could reduce the mortality and morbidity substantially.

The gold standard for diagnosis of septicemia is the isolation of bacterial agent from blood culture [8]. The prevalence of bacterial profile of blood cultures and their susceptibility patterns in an area, provide guidance to start empirical treatment which is the cornerstone in the management of sepsis.

Microbial culture is the specific method for diagnosis of neonatal sepsis but these reports are usually available after 48-72 hours. Diagnosis of neonatal sepsis based on clinical symptoms is not possible. Although isolating its causative microorganisms using blood culture has been the gold

standard for its diagnosis, but its result is ready 24-72 hours after sampling and during this period, it is necessary to treat suspicious infants for sepsis with antibiotics according to clinical symptoms and risk factors.

CRP is synthesized within six to eight hours of exposure to an infective process or tissue damage. It has a half-life of 19 hours and may increase more than 1000-fold during an acute phase response. The ability to early diagnosis or ruling out neonatal sepsis results in limiting inappropriate antibiotic exposure and lowering the cost of therapy.

Against this background, this study was undertaken to study the bacteriological profile and antibiotic susceptibility pattern of the isolates in septicemic neonates and also correlation between sepsis screening by CRP and blood culture in neonate presenting with features of sepsis was done to accelerate the diagnostic process.

MATERIALS & METHODS

A total of 126 neonates up to the age of 28 days, who were admitted in NICU, Niloufer Hospital, Hyderabad from July 2017 to Dec 2017, with clinical suspicion of neonatal sepsis, were included in the study.

They were enrolled based on signs and symptoms like patients with neonatal sepsis lethargy, poor cry, refusal to feed, changes in body temperature (fever and hypothermia), jaundice, apnoea, respiratory distress, tachycardia, tachypnoea, cyanosis, vomiting, distension of abdomen, sclerema, seizures, bulging fontanelle, irritability, grunting etc.

Blood was collected for CRP test and blood culture. CRP test was carried out from the patient's serum by using latex agglutination test, Span Diagnostic Pvt. Ltd , which detects serum levels greater than 6 µg/ml of CRP. Before the study was started, freshly prepared blood culture bottles were sent to Dept of BSQC, Institute of Preventive Medicine, Hyderabad for quality check. One ml blood was inoculated aseptically into blood culture bottle having 10 ml Tryptone Soya Broth (Microxpress) containing 0.05% Sodium Polyanethol Sulfonate (SPS), so that blood is diluted to 1:10 fold.. Blood culture bottles were incubated aerobically at 37°C for 7 days. Sub-cultures were done on 2nd, 4th & 7th day on blood agar & MacConkey agar plates.In cases where no growth was obtained after 7 days of incubation, then it was considered as a negative blood culture. In culture positive cases, smears were prepared and examined after Gram's staining. Simultaneously, sub-cultures were done on blood Agar and MacConkey's agar and the plates were then incubated at 37°C for 18-24 hours. Growth was identified by standard microbiological techniques. Those samples which showed bacterial growth were subjected to antibiotic susceptibility by modified Kirby baur disc diffusion method.CRP value >6 ug/ml was considered as a positive test. All the laboratory procedures were done as per standard protocol & strict aseptic conditions.

RESULTS

A total of 398 babies were enrolled during the study period. Poor feeding, respiratory distress, and hypothermia were the major clinical features associated with both suspected cases of sepsis as well as culture positive cases (Table-1).

Blood culture was positive in 101 cases (80.1%).Gram negative bacteria (50.5%) were more frequently isolated than Gram-positive bacteria (49.5%) *Klebsiellapneumoniae* (37.6%) was the most commonly isolated organism followed by Coagulase negative staphylococcus (CONS) (28.7%), *Staphylococcus aureus* (6.9%) (Tables-2&3).

Table-1: Clinical features

Clinical feature	Suspected cases (%) (126)	Culture positive cases (%) (101)
Poor feeding	109(86.5%)	85(84.1%)
Respiratory distress	62(42.9%)	90((89.1%)
Seizures	15(12%)	7(6.9%)
Jaundice	44(35%)	32(31.6%)
Hypothermia	95(75.3%)	56(55.4%)

Table-2: Blood culture Results

Blood cultures	**Total (n)**	**Total (%)**
Culture positive	101	80.1%
Culture negative	25	19.8%

Table-3: Distribution of organisms isolated from blood cultures

Microorganism	N (%)
Gram positive	50(49.5%)
S.aureus	7(6.9%)
CONS	29(28.7%)
Enterococci	14(13.8%)
Gram negative	51(50.5%)
Klebsiella	38(37.6%)
E.coli	4(3.9%)
Pseudomonas	4(3.9%)
Citrobacter	2(1.9%)
Acinetobacter	2(1.9%)
Enterobacter	1(0.9%)

The isolated Gram positive bacteria showed high resistance to Ampicillin (21.4%) and considerable resistance towards Gentamicin (17.4%). They showed moderate susceptibility to third generation Cephalosporin (Cefotaxime-59%, Cefixime-48%) Ciprofloxacin-57% and levofloxacin (71%). They were highly susceptible to Linezolid (85.7%) and Vancomycin (100%) (Table-4).

Among the Gram negative bacteria, many of them were resistant to ampicillin (25%), gentamicin (25%), third generation cephalosporins (cefipime-26.3%, ceftriaxone-18.4%). They were moderately susceptible to Amikacin (78.9%) and ciprofloxacin (52.6%), but highly susceptible to imipenem (100%) and Meropenem (100%) (Table-5).

Table-4: Antibiotic sensitivity of gram positive isolates

Antimicrobial	S.aureus (7)	CONS(29)	Enterococcus(14)
Ampicillin	3(42.8%)	10(34.4%)	3(21.5%)
Gentamicin	4(57.1%)	5(17.2%)	5(35.7%)
Amoxiclav	4(57.1%)	5(17.2%)	7(50%)
Linezolid	6(85.7%)	20(68.9%)	10(71.4%)
Ciprofloxacin	4(57.1%)	14(48.2%)	7(50%)
Levofloxacin	5(71.4%)	19(65.5%)	9(64.2%)
Clindamycin	6(85.7%)	22(75.8%)	11(78.5%)
Cefixime	2(28.5%)	12(41.3%)	6(42.8%)
Cefotaxime	1(14.2%)	10(34.4%)	-
Vancomycin	7(100%)	25(86.2%)	12(85.7%)
Piperacillin/tazobactum	5(71.4%)	24(82.7%)	11(78.5%)

Table-5: Antibiotic sensitivity of gram negative isolates

Antimicrobial	Klebsiella(38)	E.coli(4)	Pseudomonas(4)	Acinetobacter(2)	Citrobacter(2)
Ampicillin	15(39.4%)	1(25%)	1(25%)	0	0
Cefoperazone/sulbactam	28(73.6%)	3(75%)	3(75%)	1(50%)	2(100%)
Ceftriaxone	7(18.4%)	1(25%)	1(25%)	0	1(50%)
Cefepime	10(26.3%)	1(25%)	1(25%)	0	1(50%)
Imipenem	38(100%)	4(100%)	2(50%)	2(100%)	2(100%)
Meropenem	38(100%)	4(100%)	3(75%)	2(100%)	2(100%)
Ciprofloxacin	20(52.6%)	2(50%)	2(50%)	0	1(50%)
Levofloxacin	22(57.8%)	2(50%)	2(50%)	0	1(50%)
Piptaz	36(94.7%)	3(75%)	3(75%)	1(50%)	2(100%)
Amikacin	30(78.9%)	2(50%)	1(25%)	0	1(50%)
Gentamicin	29(76.3%)	1(25%)	1(25%)	0	0
Trimethoprim/sulfamethoxole	18(47.2%)	1(25%)	0	0	1(50%)

CRP was reactive in 115 cases out of the total 126 cases suspected of septicaemia.

Out of the 115 CRP reactive cases, 15 cases (11.1%) were false positive and 11 cases (8.73%) were negative.

Table-6: Correlation of CRP with blood culture

CRP	BLOOD CULTURE		Total
	Positive	Negative	
Positive	101(80.1%)	14(11.1%)	115(91.26%)
negative	0	11(8.73%)	11(8.73%)
Total	101(80.1%)	25(19.8%)	126(100%)

Table-7: Statistical analysis of CRP

CRP	PERCENTAGE
sensitivity	100%
specificity	44%
Positive predictive value	87.8%
Negative predictive value	100%

DISCUSSION

Neonatal septicemia with its high incidence and its grave prognosis, in spite of adequate treatment with modern antibiotics, has been a challenge for all times. The varying microbiological pattern of neonatal septicemia warrants need for ongoing review of causative organisms [9].

Mortality and morbidity due to sepsis can be prevented with early diagnosis, rational antimicrobial therapy and aggressive supportive care.

Blood culture is considered as gold standard for diagnosis but its drawback is it is costly and time consuming (preliminary results are delayed for at least 48 hrs). Various studies have shown that raised CRP has high sensitivity to detect neonatal sepsis, moreover detection of raised CRP can be done rapidly by cheap and easily available kits.

In the present study a total of 126 neonates with clinical suspicion of sepsis were considered. Presentation of sepsis varies depending on the severity of disease process and the immune status of the baby. Poor feeding, respiratory distress and hypothermia were the major presentations in our study. Satyamurthi *et al*., [10] and Jain *et al*., [9] reported the similar presentation.

Total blood culture positivity rate among neonates with sepsis in our study was 80.1%. Murthy et al reported 52.6% [10], Tallur *et al*., [11] reported 64% whereas Martn *et al.*, [12] reported 95% and Aletayeb *et al.*, [13] a very low rate of 4.1%.

A high isolation rate of our study could be due to the short time period and also the wide range in incidence of culture positive neonatal sepsis could be as a result of lack of standard definition of clinical sepsis across different centres.

The organisms causing neonatal septicemia differ from area to area and also change with respect to time even in the same area, which may be due to different life conditions [14].

Gram negative bacterial isolates (50.5%) were more than Gram positive isolates (49.5%) in our study. This was in concordance with national neonatal perinatal database (NNPD) 2003 [15], Kumar GD *et al*., [16], Roy *et al*., [9], Kayange *et al*., [17], Maimoona *et al*., [18]. Escherichia coli was the second most Gram negative organism followed by Pseudomonas aeruginosa.

Among the Gram positive isolates, coagulase negative Staphylococcus CONS (28.7%) was reported in our study. Similar isolation rate was reported by Sangeeta D. Patel *et al*., (23.3%) [19] and Satyamurthy *et al*., (26%) [10]. This could be due to immature immune system development.This was followed by Staphylococcus aureus which was similar to studies done by UravashiRana *et al.*, [20] Maimoona *et al*., (11.1%) [18], Dar A. K. *et al*., (25%) [3].

Klebsiellapneumoniae and other Gram negative organisms were the common causes of sepsis in the present study as well as other studies from India (Kaistha. N. Mehta) [6]. Klebsiella and CONS were the common etiological agents causing neonatal sepsis in our study. In an epidemiological study performed to observe the long term trends in the agents causing neonatal sepsis, CONS were showing an increasing trend [21].

In this study, Pseudomonas aeruginosa isolates were found to be highly resistant to routinely used antibiotics, followed by Klebsiellapneumoniae and Escherichia coli. This increasing resistance could be due to irrational use of antibiotics [22]. All Gram negative isolates were having considerable sensi-tivity to Amikacin and Ciprofloxacin; but were highly susceptible to meropenem (100%) and imipenem (100%). Our study findings correlated well with the findings of others viz. Aletayeb SMH *et al*., [13] .Maimoona *et al*., [18].

The Gram positive isolates were having better sus-ceptibility to Amikacin, Cephalosporins and Ciprof-loxacin; but were more resistant to Ampicillin and Gentamicin in the present study. They showed

high susceptibility to Linezolid and Vancomycin. Our findings correlated with the studies by Ale- tayeb SMH *et al*., [13], Roy I *et al.*, [9] and Bhat R *et al*., [23].

The utility of CRP test as marker for diagnosis of neonatal septicemia was done in this study. Serum concentration of CRP increases several hundred fold in response to bacterial infection, making it an attractive diagnostic test for neonatal sepsis.

In our study CRP showed 100% sensitivity in all the respected cases at neonatal septicemia. This was in accordance with Anuradha *et al*., (100%), Aijazi *et al*., (76%), B. K. Tha (100%). This shows that CRP increases to significant level in the patient having bacterial septicemia and it has maximum sensitivity.

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