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Role of C-reactive protein & etiological profile with antibiotic susceptibility in patients of neonatal sepsis

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Abstract: *Early diagnosis and treatment of neonatal septicaemia is important to reduce the mortality associated with it. In this cross sectional study, 155 clinically suspected cases of neonatal septicaemia were collected for CRP screening & to study their etiological profile with antibiotic sensitivity pattern. Sensitivity of CRP was found to be 93.22%, specificity 84.38%, positive predictive value 78.57 % & negative predictive value 95.29%. Majority 59.32% of the cultures isolated gram negative bacilli, most commonly Klebsiella. Among these bacilli most effective antibiotics were imepenem and amikacin. In gram positive isolates Staphylococcus was commonest with maximum resistance to penicillin & no resistance to vancomycin and linezolid. CRP is a valuable screening test for neonatal sepsis. Based on our antibiogram, empiric therapy in neonatal sepsis can be started with amikacin and ciprofloxacin immediately as soon as CRP report is available.*
Keywords: *CRP, antibiotic sensitivity, etiology, neonatal sepsis*

I. Introduction

Neonatal sepsis is a clinical syndrome characterized by systemic signs of circulatory compromise because of infection in the first month of life.[1]

Neonatal septicaemia even today constitutes a significant cause of neonatal morbidity and mortality in developing countries. Neonates are particularly vulnerable to infections because of weak immune barrier.[2] Clinical signs of sepsis, such as fever, tachycardia, tachypnea and leukocytosis, are quite sensitive but poorly specific for sepsis.[3]

This uncertainty in the clinical decision-making led investigators to look at the biomarkers such as C-reactive protein (CRP) to be used as surrogate marker for sepsis. CRP is an abnormal serum glycoprotein produced by the liver. It is a component of the innate immune system, an acute phase reactant, and increased levels are observed within 24 to 48 hours in response to severe bacterial infection.[4]

The microbial profile of neonatal septicaemia is constantly under change with advances in early diagnosis and treatment. Thus a rational protocol for sepsis management must be based on adequate knowledge of the causative organisms and there antibiotic susceptibility pattern. With this background, we investigated the role of CRP as a septic screen and describe spectrum of isolates in cases of neonatal septicaemia.

II. Material And Methods:

The present cross sectional study was conducted in Department of Microbiology at a tertiary care hospital from October 2014 to March 2015.
A total 155 neonates with clinical diagnosis of neonatal sepsis were included in the study.

a. Preparation of site and collection of blood:

The skin of venepuncture site is disinfected and blood is collected for CRP test and blood culture examination. CRP test is carried out from the patient's serum by using latex agglutination test, Biolab Diagnostic (I) Pvt. Ltd. (Tarapur), which detects serum levels greater than 6 µg/ml of CRP.

Another one ml blood is inoculated aseptically into blood culture bottle having 10 ml Brain Heart Infusion Broth containing 0.05% Sodium Polyanethole Sulfonate (SPS), so that blood is diluted to 1:10 fold. Blood culture bottles are incubated aerobically at 37°C for 7 days. Sub-cultures are done on 2nd, 4th & 7th day on blood agar & MacConkey agar plates. In cases where no growth was obtained after 7 days of incubation, it was considered as a negative blood culture. In culture positive cases colonies were identified by conventional microbiological techniques. All the laboratory procedures were done as per standard protocol & under all aseptic precautions. [5,6]

Antimicrobial susceptibility test was done by Kirby Bauer's Disk Diffusion method[7] on Muller Hinton agar as per CLSI 2014[8] penicillin (P) (10 units), oxacillin (O) (30 µg) erythromycin (E)(15 µg), gentamicin (G)(10 µg), amikacin (AK)(30 µg), tobramycin (TB)(10 µg), vancomycin (VA)(30µg), linezolid (LZ)(30 µg), co-trimoxazole (1·25 µg trimethoprim/23·75 µg sulfamethoxazole) (CO), chloramphenicol

(C)(30µg), ampicillin (10 µg), ciprofloxacin(CF) (5 µg), cefoperazone(CS) (75 µg), cefazolin (CZ) (30µg), imipenem (IP)(10 µg), piperacillin/tazobactam (P/T)(100/10 µg), aztreonam (AZ)(30 µg), cefepime (CPM)(30 µg), cefproxil (CFZ) (30 µg) and. The discs were obtained from Himedia (India) Laboratories.
The data was analyzed using SPSS version 17.0. Chisquare test was used in assessing the associations between variables. A p-value of 0.05 or less was considered statistically significant.

III. Results:

Out of 155 clinically suspected cases of neonatal septicaemia, 59 were culture positive. This shows the prevalence of neonatal sepsis to be 35.48%.

Table 1: Correlation of CRP and Blood culture

CRP	BLOOD CULTURE		TOTAL
	POSITIVE	NEGATIVE	
CRP POSITIVE	55	15	70
CRP NEGATIVE	04	81	85
TOTAL	59	96	155

Chi-square statistics is 88.8401. P-value is 0.000. This value is significant at $p < 0.05$. Sensitivity of CRP is 93.22%, specificity 84.38%, positive predictive value 78.57 % & negative predictive value 95.29%.

Table 2: Distribution of microbial isolates from Blood Culture in patients with sepsis:

Gram negative organisms: 35(59.32%)	**No. of organisms**
1. *Klebsiella pneumoniae*	11
2. *Klebsiella aerogenes*	07
3. *Escherichia coli*	08
4. *Pseudomonas aeruginosa*	05
5. *Acinetobacter baumannii*	03
6. *Citrobacter freundi*	01
Gram positive organisms: 24(40.68%)	**No. of organisms**
1. *Staphylococcus aureus*	12
2. *Staphylococcus epidermidis*	02
3. *Enterococcus faecalis*	01
4. *Candida albicans*	02
5. *Non albicans candida*	07

In present study, gram negative bacilli 59.32% were found to be the commonest cause of neonatal septicemia. *Klebsiella* predominate the gram negative list. Gram positive cocci were found in 25.42% cases. Among gram positive cocci, *Staphylococcus* was predominant. *Candida spp.* was isolated in 15.25% cases. Among candida, non-albican candida species were more than candida albicans.

Table 3: CRP positivity in patients with culture positive and negative results

Category	CRP positivity (%)
Microbiologically documented infection (Culture positive) (59)	55 (93.22)
Gram negative bacilli (35)	34 (97.14)
Gram positive cocci (15)	13 (86.67)
Fungus infection (9)	08 (88.89)
No documented pathogen (96)	15 (15.63)

Patients with a positive culture had significantly higher CRP positivity than those from whom no pathogen was isolated. Among patients with a positive culture, CRP value for Gram negative infection was higher than for Gram positive infections or fungal infection.

Table 4: Antimicrobial resistance of gram positive cocci in neonatal sepsis patients:

Organisms	Antibiotics										
	P	OX	E	G	AK	TB	VA	LZ	C	CF	CO
S. aureus (n=12)	9	9	2	5	2	3	0	0	3	2	4
S.epidermidis (n=2)	2	1	0	1	1	0	0	0	1	1	1
E. faecalis (n=1)	1	-	1	0	-	-	1	1	1	-	-

Table 4 shows Antimicrobial resistance of gram positive cocci isolates from blood culture of neonatal septicemia cases. The antibiotics which were found to show no resistance were vancomycin and linezolid. Also less degree of resistance in gram positive cocci were seen to amikacin, ciprofloxacin and erythromycin. Maximum resistance in gram positive cocci was seen to penicillin and oxacillin.

Table 5: Antimicrobial resistance of gram negative bacilli in Neonatal sepsis patients:

Organisms	Antibiotics														
	A	CZ	CS	CPM	CFZ	CX	CE	CF	PC	P/T	IP	G	TB	AK	AZ
K.pneumoniae n=11	9	11	11	3	3	3	3	2	4	1	0	4	1	1	8
K.aerogenes n=7	5	7	7	4	4	1	2	1	2	1	0	1	2	2	6
E. coli n=8	7	6	6	4	4	2	2	2	3	2	1	2	2	1	2
C.freundi n=1	1	1	1	1	1	1	1	0	`1	0	0	0	0	0	1
P. aeruginosa n=5	1	1-	-	5	5	-	-	1	4	1	0	1	1	1	2
A. baumanni n=3	1	-	-	3	3	-	-	1	2	0	0	1	1	0	2

Table 5 shows that all the isolates of *K. pneumoniae, K. aerogenes* were found to show no resistance to imipenem (100%). Among aminoglycosides, *Klebsiella* spp. showed maximum sensitivity towards amikacin & tobramycin followed by gentamicin. *Klebsiella* isolates in our study showed complete resistance to cefazolin and cefoperazone and, very low sensitivity to ampicillin and aztreonam.

Among 8 isolates of *E.coli* maximum resistance was seen towards cefazolin and cefoperazone followed by ampicillin. Maximum sensitivity was seen to imipenem, amikacin and ciprofloxacin.

Among nonfermenter maximum resistance was seen to cefepime and ceftazidime. Both *P. aeruginosa* and *A. baumanni* were 100% sensitive to imipenem.

IV. Discussion:

Sepsis remains one of the leading causes of death in neonates. All newborns suspected to have neonatal sepsis should have a septic screen to corroborate the diagnosis of sepsis and blood culture to know the spectrum of organism prevalent in hospital.

IV.1. Role of CRP in diagnosis of sepsis:

In our study, we found sensitivity of CRP 93.22%, specificity 84.38%, positive predictive value 78.57 % & negative predictive value 95.29% considering blood culture as gold standard. Our study has good co-relation with study by Jha BK et al [9] and Gandhi TN et al[10]. Jha BK et al[9] found CRP sensitivity 100%, specificity 65.67%, PPV 87.3% and NPV 100%. Gandhi TN et al[10] found CRP sensitivity 100%, specificity 75%, PPV 77 % & NPV 100%. This suggests that CRP can be used to rule out neonatal sepsis.

Patients with a positive culture had significantly higher CRP positivity (93.22%) than those from whom no pathogen was isolated (15.63%). This finding suggests that most of our neonates with positive CRP had septicaemia.

Among patients with a positive culture, CRP positivity for Gram negative infection was higher than for Gram positive bacterial infections or fungal infection. This finding is similar to findings of Chacha F et al[11] 2014 and Alexandraki I et al 2010[12].

IV.2. Etiology and antibiotic susceptibility of neonatal sepsis:

Knowledge of the spectrum of micro-organism is essential because the first antibiotic administered will not wait for the culture results and keeping in mind the high morbidity and mortality associated with neonatal sepsis, a right choice for such empiric therapy is of utmost importance.

In present study, prevalence of neonatal septicaemia confirmed by culture was 38.06% close to finding of 41.6% of Zakaria BP et al 2011[13]. A low blood culture isolation rate could be due to administration of antibiotic before blood collection or the possibility of infection with anaerobes or viruses, identification of which was not performed. Among the blood culture positive cases, incidence of gram negative septicemia was 59.32% and that of gram positive septicemia was 40.68% which is comparable with Jyothi P et al 2013[14] which reported that Gram-negative and Gram-positive organisms to be 55.7% and 44.3% of the septicemia cases, respectively.

We identified *Klebsiella spp.* to be the most frequent offender (30.50%) among gram negative organisms similar to Roy I et al 2002[2]. Among gram positive organism *Staphylococcus aureus* was isolated in 21.82% cases close to the findings of Kumar GD et al 2002[15].

In present study *Candida spp.* was isolated in 15.25% cases. This finding of neonatal candidemia is similar to Kapoor et al 2005[16] (18%) but differs from Kumar GD et al 2002[15] (2.43%). The high incidence of candidemia may be because wide use of broad spectrum antibiotics, length of hospital stay and very importantly low birth weight. Also there is increased incidence of non-albicans candida species in present study which points to the change in spectrum of infections in neonatal sepsis.

Antibiotic susceptibility pattern of all bacterial isolates were studied. Among gram negative organism, enterobacteriacea showed almost complete resistance to cefazolin and cefaperazone similar to Kamble et al [17].

Among all gram negative isolates high degree of resistance was also observed to ampicillin while no resistance was seen with imepenem similar to findings of Jyothi P et al 2013[14]. Among aminoglycosides, amikacin was found to have an edge over gentamicin and tobramycin in Gram-negative septicaemia similar to Vinodkumar C et al 2008[18]. Ciprofloxacin was also found to be quite sensitive in most of the organism.

Among Gram-positive isolates, high resistance was seen to penicillin, oxacillin similar to the observation of Jyothi P et al 2013[14]. All gram positive isolates were sensitive to linezolid and vancomycin as observed by Kamble et al 2015[17].

V. Conclusion:

CRP is a valuable adjunct in screening for neonatal sepsis complementing clinical decision-making. All babies with suspected sepsis should be screened by CRP testing.

Gram negative bacteria remain the major cause of neonatal sepsis. *Klebsiella sp.* and *E. coli* are the frequent offenders. Among gram positive *S. aureus* is tops the list. Isolated pathogens show resistance to most of the routine antibiotics. The study suggests longitudinal surveillance to be carried out at regular interval to describe the varied pathogens causing neonatal sepsis as well as their changing antibiotic susceptibility pattern. Every hospital must have its own local antibiogram mentioning empirical therapy options.

In places where blood culture is not available, neonates with suspected sepsis and positive CRP should urgently be initiated on appropriate sepsis management in order to reduce associated morbidity and mortality. Based on our antibiogram, empiric therapy can be started with amikacin and ciprofloxacin immediately as soon as CRP report is available so as to reduce the morbidity and mortality.

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