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Research Article

CORRELATION OF C-REACTIVE PROTEIN AND BLOOD CULTURE IN NEONATAL SEPSIS AT INTEGRAL INSTITUTE OF MEDICAL SCIENCES AND RESEARCH, HOSPITAL LUCKNOW (UTTAR PRADESH)

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ABSTRACT

Objective: Neonatal sepsis is a clinical condition of bacteremia characterized by systemic signs and symptoms of infection in children under 4 weeks of age. It involves systemic infections in newborns, including septicemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections. C-reactive protein (CRP) is an abnormal β-globulin produced by the liver during any inflammatory process. The gold standard method to diagnose neonatal sepsis is blood culture; however, it is time-consuming and requires a well-equipped laboratory and trained personnel. To study the correlation between CRP and blood culture in neonatal sepsis.

Methods: The present study was a retrospective study conducted at the Integral Institute of Medical Sciences and Research, Hospital Lucknow. (UP) All the indoor neonates attending at the Integral Institute of Medical Science and Research Hospital who's both parameters, CRP as well as blood culture were noted in the microbiology department register during my study period. In the blood culture, samples were collected aseptically and processed by either conventional or automated blood culture methods. CRP estimation was done by a latex agglutination card test. CRP was reported as positive if agglutination particles were seen.

Results: CRP positivity rate: out of 235 samples, 72 (30.64%) were positive and 163 (69.36%) were negative. Blood culture positivity rate: out of 118 samples, 71 (60.17%) cases were culture positive and 47 (39.83%) were negative. After comparison of CRP samples with blood culture samples, 90 samples were tested for both blood culture and CRP, and the babies ages were under 4 weeks.

Conclusion: So from our study, we are concluding that blood culture is the gold standard method for the diagnosis of neonatal sepsis, and although we can use CRP as the screening method, this test is not specific enough to be relied upon as the only indicator.

Keywords: C-reactive protein, Neonatal sepsis, Blood culture.

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INTRODUCTION

Neonatal sepsis is a clinical condition of bacteremia characterized by systemic signs and symptoms of infection in children under 4 weeks of age. It involves systemic infections in newborns, including septicemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections [1]. An overall prevalence of blood culture confirmed sepsis of 1–8 cases/1000 live births in developed countries and 4.8–20.7/1000 live births in India has been reported [2]. The clinical manifestation of sepsis in newborn neonates is usually non-specific. Because of the high morbidity and mortality which is allied with neonatal sepsis. An antibiotic therapy is commenced soon after the onset of the symptoms before the diagnosis is definite by blood culture [3].

Neonatal sepsis may be classified according to the time of onset of the disease: early-onset sepsis (EOS) and late-onset sepsis (LOS). The difference has clinical relevance, as EOS disease is mainly caused by bacteria acquired before or during delivery, and LOS disease due to bacteria acquired after delivery (from nosocomial or community sources). EOS generally occurs within the first 72 h of life, and LOS typically presents after 72 h of age. Infections are more common in low-birth-weight and preterm babies [4]. The microorganisms most commonly associated with early-onset infection include Group B Streptococcus, *Escherichia coli*, coagulase-negative *Staphylococcus*, *Haemophilus* influenza, and *Listeria* monocytogenes [5]. The case fatality rate in early-onset neonatal sepsis ranges from 16.7% to 19.4% [6]. LOS develops 10–30 days after birth, and the cause of infection is the care-giving environment. Organisms that have been implicated in causing late-onset sepsis include coagulase-negative *Staphylococcus*, *Staphylococcus aureus*, *E. coli*, *Klebsiella*, *Pseudomonas*, *Enterobacter*, *Candida*, Group B streptococcus, *Serratia*, *Acinetobacter,* and anaerobic bacteria [7].

Bacterial infections are the commonest source of morbidity and mortality during the neonatal stage [8]. The gold standard method to diagnose neonatal sepsis is blood culture; however, it is time-consuming and requires a well-equipped laboratory and trained personnel. It can be a false negative due to the low amount of blood drawn from neonates, the prenatal antibiotic used, or a low level of bacteremia. In the last few years, various biomarkers like white blood cell count, C-reactive protein (CRP), procalcitonin, and interleukin-6 triggered by the host immune system have been targeted as potential indicators for diagnostic and prognostic purposes in the early diagnosis of neonatal sepsis [9].

CRP is an abnormal β-globulin produced by the liver during any inflammatory process. Inflammation may be bacterial, malignant or cause tissue destruction. During the onset of infection, CRP levels increase after 10–12 h, and this is a marker of bacterial infection. After 24 h, the CRP value is very helpful, and this test also has prognostic value as the levels strongly reduce when the patient is responding to treatment. The test can be performed by the passive agglutination method, in which latex particles are coated with anti-CRP antibodies (commercially available kits). Other methods of detection of CRP accurately are measured by turbilatex, ELISA, and nephelometer [10].

The aim of the present study is to study the correlation between CRP and blood culture in neonatal sepsis and determine the predictive value of CRP as an indicator of neonatal sepsis in comparison with blood culture.

METHODS

Types of study
Retrospective study.

Study population
Inclusion criteria

All the indoor neonates attending the Integral Institute of Medical Science and Research hospital whose both parameters, CRP as well as blood culture, were noted in the microbiology department register during my study period will be included.

Exclusion criteria

Neonates whose data were incomplete will be excluded.

Study period

From May 2018 to April 2020.

Data collection
The data will be retrieved from the records maintained at the microbiology department of the Integral Institute of Medical Sciences and Research, Lucknow.

Lab diagnosis
Sample collection

A sample was collected aseptically, and it was processed by either conventional or automated blood culture methods.

Conventional blood culture method

- 1–2 mL of blood collected aseptically was inoculated into blood culture bottles containing 5 mL of brain and heart infusion broth.
- Blood culture bottles were incubated at 37°C aerobically.
- After overnight incubation, blood culture bottles were examined for indicators of growth like turbidity, gas production, and hemolysis.
- If any of these present subcultures were done on blood agar or MacConkey's agar.
- If indicators of growth were not present, subculture was done after 48 h of incubation on blood agar and MacConkey's agar.
- If no growth occurred on plates after overnight incubation, bottles were incubated further and observed daily for indicators of growth until 7 days.
- A final subculture was done at the end of day 6.
- The colonies growth on blood and MacConkey's agar was identified by conventional methods to the standard laboratory protocol, including colony morphology, Gram staining, and biochemical reactions.

Automated blood culture method

A blood specimen was collected aseptically from neonates, and inoculated in blood culture bottles, and incubated in an automated blood culture detection system (BACTEC9050).

CRP estimation

CRP estimation was done by a latex agglutination card test. CRP was reported as positive if agglutination particles were seen [10].

Qualitative method

- Pipette one drop of test specimen (serum) on the slide using the disposable pipette provided with the kit.
- Add one drop of RHELAX"-CRP latex reagent to the drop of the test specimen on the slide. Do not let the dropper tip touch the liquid on the slide.
- Using a mixing stick, mix the test specimen and RHELAX-CRP latex reagent uniformly over the entire circle. Immediately start a stopwatch. Rock the slide gently back and forth, observing for agglutination macroscopically at 2 min.

Semi-quantitative method

- Using isotonic saline, prepare serial dilutions of the test specimen positive in the qualitative method: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and so on.
- Pipette each dilution of the test specimen onto separate reaction circles,
- Add one drop of RHELAX-CRP latex reagent to the drop of test specimen on the slide. Do not let the dropper tip touch the liquid on the slide.
- Using a mixing stick, mix the test specimen and the latex reagent uniformly over the total circle [10].
- Instantaneously start a stopwatch. Rock the slide gently, back and forth, observing for agglutination macroscopically at 2 min.

Interpretation of results
Qualitative method

- Agglutination is a positive test result and indicates the presence of detectable levels of CRP in the test specimen.
- No agglutination is a negative test result and indicates the absence of detectable levels of CRP in the test specimen.

Semi-quantitative method

Agglutination in the highest serum dilution corresponds to the approximate amount of CRP in mg/dl present in the test specimen.

The concentration of CRP can be calculated as follows:

CRP (mg/dl) = S × D

Where, S=Sensitivity of the reagent, i.e., 0.6 mg/dl.
D= Highest dilution of serum showing agglutination [10].

RESULTS

Out of 235 samples 72 (30.64%) samples were positive and 163 (69.36%) were negative as shown in Table 1 and Fig. 1.

Blood culture positivity rate
Out of 118 samples 71 (60.17%) cases were culture positive and 47 (39.83%) were negative as shown in Table 2 and Fig. 2.

Correlation of CRP samples and blood culture samples
After a comparison of CRP samples with blood culture samples, 90 samples were tested for both blood culture and CRP, as shown in Table 3. And, the babies ages were under 4 weeks.

As shown in Table 3, 32 neonates had sepsis with a positive blood culture and a positive CRP level. 17 neonates had clinical signs of sepsis, but their blood culture was negative and their CRP level was positive. Culture-positive but CRP-negative samples are 19; and CRP-negative and culture-negative samples are 22. The Chi-square test is

Table 1: CRP positivity rate

Total no. of CRP sample=235	
Neonates with CRP positive	72 (30.64%)
Neonates with CRP negative	163 (69.36%)

CRP: C-reactive protein

Table 2: Outcome of neonates with respect to their blood culture status

Total no of blood culture sample=118	
Neonates with blood culture positive	71 (60.17%)
Neonates with blood culture negative	47 (39.83%)

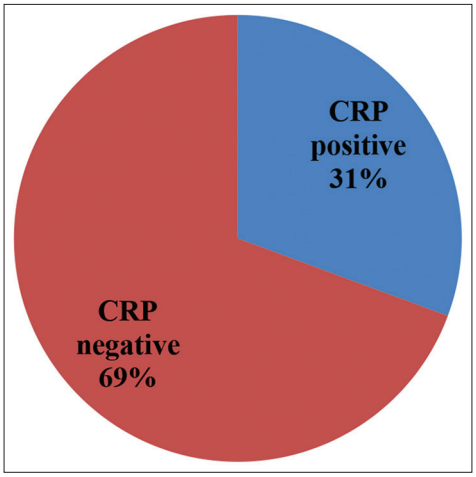

Fig. 1: C-reactive protein positivity rate

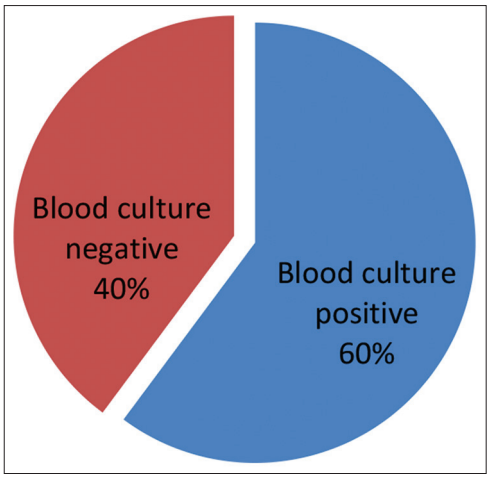

Fig. 2: Percentage of Blood Culture in neonatal sepsis

used to evaluate whether the results of these tests vary significantly from each other. It is observed that results of tests are statistically significant from each other, with X^2=3.26 and p=0.07. There is a statistically significant difference between blood culture and CRP. Hence, blood culture is the gold standard in the diagnosis of neonatal sepsis.

Out of 90 patient who were included in our study with 46 (51.11%) were males and 44 (48.89%) were female as shown in Table 5 and Fig. 3.

The distribution of organisms in blood cultures is shown in Table 6. The most common organisms isolated were *Acinetobacter* (25.49%), *Klebsiella* (23.53%), *E. coli* (5.88%), *S. aureus* (19.61%), coagulase-negative *Staphylococcus* (15.69%), *Streptococcus* (3.92%), and *Candida* (5.88%).

DISCUSSION

Neonatal sepsis is one of the leading causes of morbidity and mortality among newborns in developing countries. It is a life-threatening clinical emergency that demands urgent diagnosis and treatment. In this study, a total of 235 CRP samples and 118 blood culture samples were retrieved from the data available in the microbiology department.

Out of 235 CRP samples, 72 (30.63%) were positive for CRP. Out of 118 blood culture samples, 71 (60.16%) showed growth on blood culture. Among these samples, 90 have both data for CRP and blood culture. In which 46 (51.11%) were male and 44 (48.89%) were female neonates.

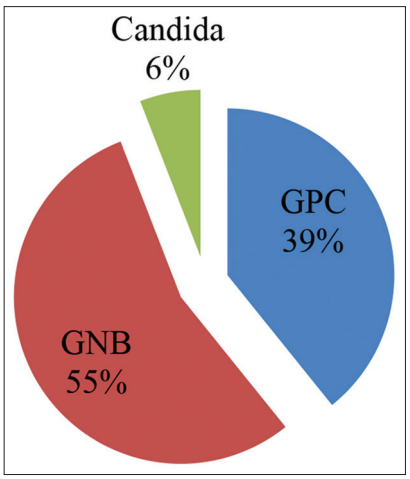

Fig. 3: Percentage of microorganism isolated from blood culture

Table 3: Patient who had requisition for both test (CRP+blood culture)

Variable	Blood culture positive	Blood culture negative	Total	p value
CRP positive	32	17	49	Chi square=3.26 p=0.07
CRP negative	19	22	41	
Total	51	39	90	

CRP: C-reactive protein

Table 4: Predictive value of CRP in patient with neonatal sepsis

Sensitivity	62.75%
Specificity	56.41%
PPV	65.31%
NPV	53.66%
Diagnostic accuracy	60%

PPV: Positive predicative value, NPV: Negative predicative value

Table 5: Distribution of patient according to gender

Gender	Total no of patient	Percentage
Male	46	51.11
Female	44	48.89
Total	90	100

Table 6: Distribution of micro-organism isolated in blood culture

Micro-organism	Number	Percentage
Staphylococcus aureus	10	19.61
Coagulase negative *Staphylococcus*	08	15.69
Streptococcus spp.	02	3.92
Acinetobacter spp.	13	25.49
Klebsiella spp.	12	23.53
Escherichia coli spp.	03	5.88
Candida spp.	03	5.88
	51	100

In the present study, 51 (56.67%) neonates had sepsis with a positive blood culture. Our finding is similar to that of Kheir *et al*. [1], with 41.4%, and Sonawane *et al.* [11], with 60% blood culture positivity.

In our study, we found 54.44% of neonates CRP positive out of 90, and my result corresponded to the study done by Lamichhane and Mishra [12], which revealed 68.57% CRP positive. Other studies have reported a

high prevalence rate of CRP positivity, like Sonawane *et al.* [11], with 77.5%, and Monga *et al.* [9], with 70% CRP positivity.

The demographic profile was analyzed in this study. It was found that 46 (51.11%) were male and 44 (48.89%) were female. Out of 51 blood cultures that showed growth, 28 (54.90%) were male neonates, while 23 (45.09%) were female neonates.

The gender distribution was similar across the groups. This finding is supported by Khanal *et al.* [13], with (52.17%) male and 47.82% female; Singel *et al.* [14], with 53% male and 43% female neonatal sepsis; and Gupta *et al.* [15], with male 78 (53%) and female 70 (47%) neonatal septicemia.

Most studies have reported a high prevalence rate of neonatal septicemia in males, like Monga *et al.* [9], who reported male neonates (62%) and female neonates (38%); Hassan *et al*. revealed (63.4%) males and (36.6%) females; and Kheir *et al.* [1], who reported 43 (62.1%) males and 27 (37.9%) females. Among 51 blood culture samples that showed growth, 32 were CRP positive and 19 were CRP negative, so the sensitivity of the CRP test to detect neonatal septicemia was 62.75% and the specificity was 56.41%. This study correlates with the study done by Kheir *et al*. [1]. With a sensitivity of 63% and specificity of 85%, Hisamuddin *et al.* [16] revealed 76.92% sensitivity and 53.49% specificity. Higher sensitivity and specificity have also been reported by Lamichhane and Mishra [12]. With (88.5%) sensitivity and (46.1%) specificity. Bhatia *et al*. showed 81.25% sensitivity and 42.86% specificity; Younis *et al.* [17], found 100% sensitivity and 94% specificity. In our study, the positive and negative predictive values (NPV) were 65.31% and 53.66%, respectively. This finding correlates with the study done by Bunduki *et al*. [18]. Who revealed positive predictive value (PPV) of 70.2% and NPV of 97.8%, Sonawane *et al.* [11], with PPV of 51% and NPV of 66.67%.

In our study, we found that Gram-negative organisms (55%) caused most neonatal septicemia; this finding corresponds to the finding of Siddhartha *et al*. [19]. 67% are Gram-negative organisms. In contrast, Thakur *et al*. [20] revealed 60% of Gram-positive isolates.

CONCLUSION

Hence from our study, we are concluding that blood culture is the gold standard method for the diagnosis of neonatal sepsis, and although we can use CRP as the screening method, this test is not specific enough to be relied upon as the only indicator. Considering the high morbidity and mortality associated with it, clinical criteria along with other hematological parameters and diagnostic markers, along with serial CRP, should be considered in evaluating a neonate for sepsis.

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AUTHORS' CONTRIBUTIONS

All authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

COMPETING INTERESTS

No conflicts of Interests.

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