Research article

Assessment of C-reactive protein, procalcitonin and interleukin-6 as diagnostic aid for neonatal infections at a tertiary care centerJayesh Pandey¹, Dakshina Bisht², Mahima Mittal³, Amresh Kumar Singh⁴¹Research Scholar, ²Professor, Department of Microbiology, Santosh Medical College, Santosh Deemed to be University, Ghaziabad 201009, Uttar Pradesh, India³Professor & Head, Department of Paediatrics, All India Institute of Medical Sciences, Gorakhpur, 273008, Uttar Pradesh, India⁴Assistant Professor & Head, Department of Microbiology, Baba Raghav Das Medical College, Gorakhpur 273013, Uttar Pradesh, India

(Received: August 2021

Revised: November 2021

Accepted: December 2021)

Corresponding author: **Dakshina Bisht**. Email: dakshinabisht@gmail.com**ABSTRACT**

Introduction and Aim: Neonatal infections are the leading cause of mortality among neonates after prematurity. The importance determining biological markers to be used as a diagnostic test to detect neonatal infections in the early stage of the disease is a challenge. The purpose of this study was to evaluate the usefulness & sensitivity of various serological markers such as serum Procalcitonin, C-reactive protein and chemokine IL-6 for diagnosis of neonatal infections leading to sepsis in new born infants.

Materials and Methods: This cross-sectional study was carried out among newborns admitted in neonatal intensive care unit (NICU) and meeting the selection criteria. Samples were collected for blood culture and ELISA was performed for detection of CRP, PCT & IL-6.

Results: A total of 300 newborns were included in this study from NICU of which 132 (44%) neonates were found to be blood culture positive. The most frequently isolated organisms were *Klebsiella pneumoniae* (26.5%), followed by *Candida albicans* (18.1%). In case of confirmed neonatal sepsis, significant higher levels of CRP, PCT and IL-6 were detected than in cases of probable sepsis. Serum procalcitonin levels exhibit highest sensitivity and specificity as 65.91% and 91.67% respectively.

Conclusion: Serum procalcitonin has better diagnostic utility in terms of biological marker for the diagnosis of neonatal infections than C-reactive protein and Interleukin-6.

Keywords: Neonatal sepsis; biomarkers; procalcitonin; C-reactive protein; IL-6.

INTRODUCTION

The first four weeks of life (neonatal period) is considered the most vulnerable time for a child. Globally, 2.4 million children die in the first month of their life. A recent data from the year 2019 shows approximately 6,700 neonatal deaths every day with about a third of all neonatal deaths occurring within the first day after birth and close to three-quarters occurring within the first week of life. About 0.75 million neonates die every year in India, the highest for any country in the world (1,2).

In India beside prematurity, neonatal infections are the leading cause of mortality among neonates (2). Early identification of source and nature of neonatal infection is very important—for appropriate etiological treatment, avoidance of unnecessary antimicrobial therapy and to reduce the morbidity, mortality, risk and also the costs to therapy. The traditional diagnostic methods, such as C-reactive protein (CRP) and leukocyte count are not specific enough for differentiating bacterial infections from viral infections and systemic inflammation (3).

Till date blood culture is considered as the gold standard test for the diagnosis of neonatal infection

leading to sepsis – but it is a time-consuming method (take at least 24 - 48 hours) and often gives a false negative result. Also, it has some other limitations such as the inability to give specific information on host response. Blood cultures show definite growth in only about 30 - 40 % of cases. Due to use of maternal antibiotics sometimes false-negative results are obtained from blood culture and even some time false positive results due to contamination so it has low sensitivity (3,4). Biological markers are components of human blood that increase in response to infection. Several studies have shown improved diagnosis of neonatal infection by use of biological markers such as interleukin-6, procalcitonin and C-reactive protein (5).

An acute-phase protein which is synthesized in human liver, usually undetectable but their concentration in blood changes significantly in response to any infection or inflammation is known as C-reactive protein (CRP) (6). Procalcitonin (PCT) is a prohormone of calcitonin produced by parafollicular cells of thyroid glands. Usually, PCT concentration in blood increases in response to lipo-polysaccharide of bacteria and during injury. It rises earlier than CRP

levels but then normalizes more rapidly, enabling faster detection and better monitoring. In more severe infections the level may rise up to several thousand-folds (7). Interleukin-6 a cytokine, is recently being analyzed in several studies as a biomarker for neonatal sepsis being paramount cytokine which increases primarily during the inflammatory process and hence IL-6 is called a pro- inflammatory. It is mostly required for the persistence of the plasma cells which secrete antibodies and intensify cytotoxic T-cells differentiation. It is also a hepatocyte stimulating factor which promotes rise of the acute-phase responses including CRP. The concentration of IL-6 starts rising just 4-6 hours after bacterial stimulation which would be marvelous quality for any diagnostic biomarker and having a very short half-life lowers the chance of detection as routine investigations are not time bound in hospital settings (8,9).

Although several literature reviews show serum PCT, IL-6 and CRP are sensitive markers for neonatal sepsis however the results are conflicting. This study was done to assess the involvement of biological markers as CRP, PCT and IL-6 for prompt diagnosis of neonatal infections.

MATERIALS AND METHODS

This study was performed in the Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh and B.R.D. Medical College, Gorakhpur, Uttar Pradesh and was conducted on the neonates admitted in the neonatal intensive care unit (NICU) of associated Nehru Hospital of B.R.D. Medical College, Gorakhpur U.P. from January to December 2019. Medical records of all neonates admitted were obtained from bedside tickets.

Inclusion criteria

All neonates fulfilling any of the following criteria were included in this study: maternal risk factor such as fever, prolonged rupture of amniotic membrane >24 hours, neonatal history: low birth weight (< 2500 grams), premature birth (<37 weeks), poor feeding, excessive / delayed cry, hypothermia, hyperthermia, neonatal jaundice, vomiting, abdominal distension, tachypnoea and grunting, convulsions, diarrhea, cyanosis, bulged fontanelle, DIC/ bleeding, perfusion/ shock, apnea (9)

Exclusion criteria

Any type of congenital anomalies e.g., tracheoesophageal fistula, malrotation of the gut, lobar agenesis, complex heart diseases, microcephaly, anencephaly (10,11). Hydrops, neonates with administration of antibiotic at admission, refusal of parental consent and samples from LAMA / abscond patients were excluded from the study.

After receiving authorization from the Institutional Human Ethical Committees and obtaining written informed consent from parents / guardian of every

enrolled neonate and samples were collected for estimation of biomarkers.

Methods

For blood culture 1-2 ml of peripheral venous blood was collected by trained staff ensuring all the necessary aseptic precautions. Samples were inoculated immediately bedside in the pediatric blood culture bottle (Hi-Media), which were aerobically incubated at 37°C up to 7 days. Subculture on Blood agar and MacConkey agar were done every 48 hours to observe any significant growth. The isolated microorganisms were identified and antimicrobial susceptibility testing was performed using standard microbiological techniques and (CLSI 2019) guidelines (12,13).

For CRP, PCT and IL-6 (2-3 ml) whole blood samples were collected following strict aseptic precautions by venepuncture from neonates. It was clotted, centrifuged within 30 minutes of collection and serum was separated to perform estimation by commercially available Human CRP, PCT & IL-6 ELISA Kits from Elabscience ® (Catalog no: E-EL-H0043-96T, E-E-EL-H0102-96T and E-EL-H1492-96T). Rest of the samples were further stored at 2-8°C for up to 3 days or frozen at -20°C for longer period if required.

Statistical analysis

The relevant data was collected and entered into a spreadsheet computer programme (Excel 2016; Microsoft, US). Mean and standard deviation were calculated and compared by Z test & Student's t-test and also for significance of difference in proportions (rates), Chi-square test for presence of association between affecting factors. $p\text{-value} < 0.05$ was considered statistically significant and $p\text{-value} < 0.01$ was considered statistically highly significant. Chi-square test was used for statistical evaluation of presence of association between factors affecting the results of the present study. Medcalc program was used to calculate sensitivity and specificity and receiver operating characteristic.

RESULTS

Of the 300 neonates 132 (44%) neonates were found to be blood culture positive of which 87 (65.90%) were male and 44 (33.33%) were female. Among male neonates a mortality rate of (24.29%) was observed and the mean $\pm$ SD age of these neonates were 6.426 ± 5.494 days. Among the female neonates the mortality rate was (19.51%) and mean $\pm$ SD age of these neonates were 5.606 ± 4.545 days. The overall mortality rate in combined group of both males and females was found to be 22.33%. The differences between mean $\pm$ SD ages of male and females in the studied cases were statistically not significant. The distribution of blood culture positive cases according to various etiological characteristics in blood culture is shown in Fig. 1.

Fig. 1: Frequency of isolated microorganisms in blood culture

Table 1: Mean $\pm$ SD of Levels of the Biological markers According to Blood Culture

Study biomarker	Study group = Proven sepsis (N=132)		t – Test p value
	Early onset sepsis (N=86)	Late onset sepsis (N=46)	
CRP (mg/L)	21.09 $\pm$ 9.43	17.10 $\pm$ 9.65	2.98 (< 0.023)
PCT (pg/ml)	1347.45 $\pm$ 802.63	1027.95 $\pm$ 841.40	2.143 (<0.034)
IL-6 (pg/ml)	273.08 $\pm$ 142.61	250.30 $\pm$ 156.03	0.846 (0.399)

The maximum frequency of isolates among pathogens was observed in *Klebsiella pneumoniae* (26.51%) followed by *Candida albicans* (18.18%), CoNS (15.90 %), *Staphylococcus aureus* (12.12%) and the least isolated organism was *Streptococcus agalactiae* (3.01%).

The mean $\pm$ SD of serum biomarker levels among neonates was observed and it was analyzed that there

were statistically significant differences in the levels of PCT, CRP and IL-6 among the three groups ($p < 0.001$) as shown in Table 1.

The mean $\pm$ SD of serum levels of biomarkers in blood culture proven sepsis is described in Table 2 which shows statistically significant differences in the levels of PCT and CRP.

Table 2: Mean $\pm$ SD of levels of biological markers in outcome of blood culture

Study biomarker	Study group		t – Test p value
	Death (N=67)	Survivor (N=233)	
CRP (mg/L)	18.80 $\pm$ 9.94	13.98 $\pm$ 9.29	6.136 (< 0.0001)
PCT (pg/ml)	1484.25 $\pm$ 741.61	556.83 $\pm$ 567.97	4.25 (< 0.0001)
IL-6 (pg/ml)	298.19 $\pm$ 152.95	188.25 $\pm$ 127.05	5.953 (< 0.0001)

The maximum number of male neonates (42.53%) as well as female neonates (31.82 %) were blood culture positive within CRP level 16.40 - 24.60 mg/l. There was no significant difference between male and female mortality rates for significance of difference in

proportions the mean $\pm$ SD of CRP levels of overall neonates 15.74 $\pm$ 9.02 mg/l and 18.80 $\pm$ 9.94 mg/ml in neonates those succumbed was statistically significantly ($p < 0.05$) as shown in Table 3.

Table 3: Mean $\pm$ SD of levels of the assessed biological markers

Study biomarker	Study group		t-Test p value
	Probable sepsis (N=168)	Probable sepsis (N=168)	
CRP (mg/L)	11.42 $\pm$ 7.93	19.70 $\pm$ 9.66	8.1 (< 0.001)
PCT (pg/ml)	392.98 $\pm$ 286.16	1236.11 $\pm$ 827.37	12.30 (< 0.001)
IL-6 (pg/ml)	171.68 $\pm$ 120.70	265.14 $\pm$ 147.18	6.04 (< 0.001)

Among the total studied neonates on comparing the linear coefficient of correlation between CRP, PCT and IL-6 the highest correlation was found between PCT & IL-6. Among the neonatal deaths comparing

the linear coefficient of correlation between CRP, PCT and IL-6 was found to have highest correlation between CRP and PCT as shown in Table 4.

Table 4: Comparison of linear correlation coefficient in studied biomarkers

Total studied cases			Mortality cases		
Linear Correlation Coefficient between			Linear Correlation Coefficient between		
CRP & PCT	PCT & IL-6	CRP & IL-6	CRP & PCT	PCT & IL-6	CRP & IL-6
r = +0.3762	r = + 0. 4637	r = + 0.2950	r = +0.4338	r = +0.3767	r = +0.3956

Fig. 2: Receiver Operating Characteristic (ROC) curve of C-reactive protein (CRP), procalcitonin (PCT), interleukin-6 (IL-6) in diagnosing neonates with sepsis. CRP vs. PCT (P=0.16), CRP vs. IL-6 (P=0.07) and PCT vs. IL-6 (P<0.05).

Receiver operating characteristic (ROC) curve for studied biomarkers depicts PCT, CRP & IL-6 has areas under the curve of 0.746, 0.802 And 0.682

respectively when compared with blood culture as shown in Figure 2. The sensitivity and specificity of the studied biomarkers are illustrated in Table 5.

Table 5: Area under the curves (AUC) of the receiver operating characteristic (ROC) for C-reactive protein (CRP), procalcitonin (PCT) and interleukin-6 (IL-6).

Biomarkers	AUC (95%CI)	Sensitivity (%)	Specificity (%)	Youden Index J
CRP (mg/L)	0.746 (0.693-.0795)	63.64	79.17	0.428
PCT (pg/ml)	0.802(0.753-0.846)	65.91	91.67	0.802
IL-6 (pg/ml)	0.682(0.626-0.734)	53.79	76.79	0.305

DISCUSSION

Sepsis in neonates is often life threatening and are considered as the second most common reason for neonatal mortality and the cause for sepsis differs from patient to patient and depends on many internal and external factors leading to the infections as there are many underlying risk factors to blame, microbial infections is one of them, early diagnosis and root cause of the infection is crucial in the management but often is most challenging. Till date blood culture is known to be gold standard for diagnosing neonatal sepsis. As there is always a need for an early, sensitive and specific laboratory test to that would be helpful for clinicians to assess the severity of infection and to guide when to start and stop the empirical antibiotics.

In our study, *Klebsiella pneumoniae* was found to be the most frequently isolated microorganism (95%) followed by *Candida albicans* (18.32%). Among Gram positive isolates, Coagulase Negative Staphylococci (CoNS) were found to the predominant

pathogen these findings are similar with Rath *et al.*, (14) and Rashwan *et al.*, (15).

In our study, when analyzing the differences in markers between proven and probable cases of neonatal sepsis, the mean $\pm$ SD serum level of procalcitonin was 1236.11 ± 827.37 pg/ml in proven sepsis when compared with mean serum level 392.98 ± 286.16 pg/ml of probable sepsis, and the difference was found to be highly significant ($p \leq 0.001$). These observations are in coherent with a similar study (16). In case of CRP the mean $\pm$ SD serum level in proven sepsis was 19.70 ± 9.66 mg/l when compared with mean serum level 11.42 ± 7.93 mg/l among probable sepsis, and the difference was found highly significant ($p \leq 0.001$). Similar findings have been found in study by Chowdhary *et al.*, (17) and Morad *et al.*, (18). IL-6 also showed a significant difference between proven sepsis and probable sepsis cases ($p \leq 0.001$), with mean $\pm$ SD serum level of 265.14 ± 147.18 pg/ml in the proven cases versus mean $\pm$ SD serum level of

171.68 ± 120.70 pg/ml in case of probable sepsis similar results were reported by Chowdhary *et al.*, (17) and Noor *et al.*, (19).

Highest mortality rate was observed within the CRP levels of 24.60-32.80 mg/L these observations are similar with the study done by Adib *et al.*, (20) and Kocabaş *et al.*, (21). The estimation of CRP when compared to blood culture in terms of sensitivity and specificity was found to be 66%, and 92% respectively as shown in Figure 2 and Table 5 and such similar findings has been reported by Chowdhary *et al.*, (17) and Qu *et al.*, (22).

In our study when PCT was compared to blood culture in terms of sensitivity and specificity it was found to be 64% and 71% respectively, similar findings have been reported by Qu *et al.*, (22) and Morad *et al.*, (18). Lastly in case of IL-6, when compared to blood culture in terms of sensitivity and specificity it was found to be 54% and 77% respectively and similar findings have been reported by Al-Zahrani *et al.*, (23) and Rashwan *et al.*, (15)

On analyzing the observations in this study, it was found that there is a higher positive correlation and regression coefficients in PCT level and CRP level among those neonates that died during this study which indicates that there is progressive association between the two markers as observed in overall cases wherever CRP level was found to be increased, the PCT level in body is also elevated more significantly these findings that can be attributed with observations by Hasan *et al.*, (24) and Rashwan *et al.*, (15). Similar findings were observed by Sharma *et al.*, (25) where IL-6 and CRP levels were correlated with the high PCT levels in blood culture positive cases (p value <0.05). In our study it was observed that procalcitonin is a better biological maker than CRP and IL-6 when prediction of infection is assessed in neonates, these findings are in accordance with Rashwan *et al.*, (15) and Morad *et al.*, (18).

However, early diagnosis in neonates is based solely upon availability of resources and in terms of cost, effectiveness with easy approach towards diagnostic testing. In a developing country like India, diagnosis made by biomarkers is yet to be indulge as routine testing in hospital-based settings. Procalcitonin as a diagnostic test is expensive and economically unapproachable by majority of population.

CONCLUSION

To conclude for a suitable biological marker for diagnosis of the neonatal infections, it needs sufficient data, imparting knowledge about the sensitivity, specificity and timely detection of that compound in neonates. In our study, procalcitonin comes out be that suitable biomarker, better than CRP and IL-6. Incorporating rationale use of PCT as diagnostic aid in neonatal sepsis will be useful for clinicians as a guide for starting the empirical treatment as promptly as

possible and curb the gratuitous use of antibiotics. This would be advantageous in curbing the number of NICU admissions, reducing hospital duration, which would increase the accessibility of neonatal beds in hospital settings, as it is a very notable issue, especially in progressive countries with meagre facilities.

ACKNOWLEDGEMENT

Authors are thankful to clinicians and nursing staff of Department of Paediatrics, BRD Medical College, Gorakhpur and Staff of Department of Microbiology, Santosh Medical College for their kind assistance that helped us to measure analytics.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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