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PROCALCITONIN: AS AN EARLY DIAGNOSTIC MARKER FOR NEONATAL SEPSIS

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ABSTRACT

Introduction: Early detection of neonatal sepsis is essential to reduce mortality and morbidity associated with it. Though blood culture is the gold standard for diagnosis it takes long time and can be false negative. So, there is a need of a rapid diagnostic test with good sensitivity and specificity.

Aim of the study: To determine the diagnostic performance of serum Procalcitonin (PCT) in neonatal sepsis as compared to C- Reactive protein (CRP) and blood culture.

Methods: 50 neonates with clinical signs of sepsis were included in the study. Blood sample was collected from each of them for culture, serum PCT and CRP level estimation. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of PCT and CRP were determined.

Results: Out of the 50 neonates, 3 were culture confirmed sepsis. PCT was positive in 28 cases with sensitivity, specificity, PPV and NPV as 100%, 46.8%, 10.7% and 100% respectively. CRP was positive in 20 cases with sensitivity, specificity, PPV, NPV as 100%, 69.7%,35%, and 100% respectively. All the culture confirmed cases were PCT and CRP positive.

Conclusion: PCT can be used as an early detection marker in neonatal sepsis along with CRP.

KEYWORDS : Neonatal sepsis, Procalcitonin, CRP

INTRODUCTION:

Sepsis is the most important cause of morbidity and mortality in neonates. Throughout the world, every year around 30 million neonates acquire infection and 1-2 millions of them die[1].

Early diagnosis of neonatal sepsis is crucial to reduce the mortality rate associated with it. As the clinical signs and symptoms of neonatal sepsis are nonspecific, its clinical diagnosis is often difficult and misleading. Though blood or CSF (Cerebrospinal fluid) cultures are the gold standard methods for diagnosis of neonatal sepsis, it takes 24-72 hours to obtain a result and also they have a low sensitivity[2]. So, empirical antibiotic therapy is usually started in neonates with clinical suspicion of infection till culture results are available. However, such indiscriminate use of antibiotics leads to increased antibiotic resistance amongst the circulating pathogens.

Traditional tests used for diagnosis of neonatal sepsis like total leukocyte count, absolute leukocyte count, micro ESR, and immature leukocyte to total leukocyte count ratio (I/T ratio) have less specificity[3].So, there is a need of rapid diagnostic tests which can establish or rule out neonatal infections and help in early termination of antimicrobial therapy also.Different markers are used for diagnosis of neonatal sepsis. These include, CRP, PCT,IL-6, IL-8, IFN-g, TNF-α, CD 64,sICAM (Soluble intercellular adhesion molecules) levels[4].Among these, CRP is commonly used marker but it has less specificity[5,6]. Also, it takes 12-24 hours after infection to increase in level and after certain level it reaches to plateau and does not rapidly come back to normal even after subsidence of infection[7]

The role of serum Procalcitonin (PCT) in early diagnosis of neonatal sepsis has been reported recently[8,9]. Serum concentrations of PCT are increased in severe systemic bacterial, fungal and parasitic infections. PCT is a precursor protein of calcitonin. It is normally synthesized by the C cells of thyroid gland. Its levels are extremely low in healthy persons. Bacterial endotoxin is the potent inducer of PCT release in systemic circulation. Concentration of PCT rises from 3-4 hours after bacterial infection, peak about 6 hours and remains at plateau level for 24 hours[9].

The aim of our study was to determine the diagnostic performance of PCT in neonatal sepsis as compared to CRP and blood culture.

MATERIAL AND METHODS

The present study was carried at Department of Microbiology in a tertiary care hospital, Pune. A total of 50 neonates with clinical signs and symptoms of sepsis like poor feeding, lethargy, hypothermia or fever, sclerema, jaundice, bradycardia or tachycardia, respiratory distress, abdominal distension, vomiting, convulsions, oliguria were included.

Exclusion criteria: Babies who had undergone some surgical procedures Neonates were grouped as follow[10]:

Groups	Criteria
Confirmed sepsis	1. Clinical signs suggestive of sepsis are present 2. At least 2 parameters of sepsis screen must bepositive 3. Blood culture positive
Suspected sepsis	1.Clinical signs suggestive of sepsis are present 2. At least 2 parameters for sepsis screen must be positive 3. Blood culture negative
Clinical sepsis	1.Clinical signs suggestive of sepsis are present 2. Less than 2 parameters for sepsis screen positive 3. Blood culture negative

(Sepsis screening parameters: Total leukocyte count, Absolute neutrophil count, Platelet Count and CRP levels)

Collection of sample: 2 ml of blood sample was collected from each neonate with all aseptic precautions. Of which 1 ml was inoculated in BD BACTEC ™PedsPlus™ / F culture bottles and remaining sample was collected in plain bulb for Procalcitonin and CRP estimation.

Serum PCT: Blood sample was allowed to clot at room temperature for about 30 minutes for clot retraction. Serum was separated by centrifugation at a speed of 3000 rpm for 10minutes. Semiquantitative determination of Procalcitonin was done by PCT-Q Immunochromatographictest(BRAHMS diagnostic, Berlin, Germany). The colour intensity of the band is directly proportional to the PCT concentration of the sample and is interpreted as follows:

Interpretation of PCT values:

<0.5ng/ml:Minor or no significant systemic inflammatory response. Local bacterial infection is possible.

≥0.5ng/ml and <2ng/ml: Systemic infection (sepsis) is possible. Follow up of PCT level recommended (6-24 hours).
≥2ng/ml and <10ng/ml: Systemic infection (sepsis) is likely. High risk for progression to severe systemic infection
≥10ng/ml: Severe bacterial sepsis or septic shock

CRP: Qualitative estimation of CRP was done by IMMUSTAR latex agglutination test. Agglutination visible within two minutes was interpreted as a positive test result corresponding to CRP titre>0.6 mg/dl.

Blood culture: The culture bottles were loaded in BACTEC machine as per manufacturer's instructions. When the machine flashed positive, then the concerned BACTEC bottle was removed and subcultured on Blood agar, Chocolate agar and MacConkey agar**.** All the plates were incubated overnight aerobically at 37^{0}C**.** Blood agar and chocolate agar plates were incubated in 5% -10% carbon dioxide jar**.** Further processing and identification of the organisms was done as per standard microbiological techniques[11].A smear was prepared from and Gram Staining was done so that morphology of any organism seen could be reported immediately.

RESULTS:

Amongst 50 clinically suspected cases of sepsis, PCT was positive in 28 patients (Table 1).CRP was positive in 20 (Table 2) and blood culture was positive in 3 (6%) cases [*K. pneumoniae*(n=2), *Candida parapsilosis*(n=1)]

Table No 1: Results of PCT in neonates with sepsis

PCT concentration (ng/ml)	Confirmed sepsis	Suspected sepsis	Clinical sepsis	Number of cases
<0.5ng/ml	0	0	22	22
≥0.5ng/ml and <2ng/ml (weakly positive)	0	3	3	06
≥2ng/ml and <10ng/ml (Positive)	0	4	2	06
≥10ng/ml (Strongly positive)	3	13	0	16
Total	3	20	27	50

Table No2: Results of CRP in Neonatal sepsis

Type of sepsis	Number of CRP positive cases
Confirmed sepsis	3
Suspected sepsis	4
Clinical sepsis	13
Total	20

Table No 3: Evaluating the results of PCT & CRP in patients with sepsis

	PCT	CRP
Sensitivity	100%	100%
Specificity	46.8%	69.7%
Positive predictive value(PPV)	10.7%	35%
Negative predictive value(NPV)	100%	100%

By considering the blood culture as a gold standard, both PCT and CRP had 100% sensitivity and NPV, while CRP had better specificity and PPV (69.7% &35%) as compared to PCT(46.8% &10.7%).

DISCUSSION:

Neonatal mortality rate is one of the indicators for measuring the health status of a nation. According to World health organization estimates, 4 million newborn die each year worldwide during the neonatal period**.**75% of these deaths occur during the first week of life, and of which 25% to 45% of neonatal deaths occur during the first day of life. Systemic bacterial infection in the newborn creates a significant burden due to its impact on neonatal mortality and long-term morbidity[12].

Diagnosis and treatment of neonatal sepsis is a great challenge for neonatologists. Its early diagnosis helps in reducing mortality and avoidance of unnecessary antibiotic use for uninfected neonates. As blood cultures take long time and have poor sensitivity, rapid tests with better sensitivity, specificity and predictability are necessary for early detection of infections.In the present study PCT was positive in 28 cases, of which 16 cases were strongly positive (≥10ng/ml). All the culture confirmed sepsis cases had strongly positive PCT level. This is comparable with the study by Zahedpasha Y et al[13] and Sucilathangam G et al[14].

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for PCT in our study was 100%, 46.8%, 10.7% and 100% respectively.

Many studies have reported, PCT as a promising marker for early diagnosis of neonatal sepsis[15, 16]. It is more specific and its level increases rapidly after infection and come back to normal rapidly after subsidence of infection[17].It also helps in differentiating bacterial infection from other inflammatory process[18]**.**Other studies have reported a sensitivity of PCT ranging from 88% to 100 % and specificity ranging from 46% to 75% (Table No 4)

Table No 4 : Sensitivity, specificity, PPV and NPV of PCT reported by various studies

Author	Sensitivity	Specificity	PPV	NPV
Sucilathangum G et al14	92.8%	75%	59%	96%
Thayi S etal7	88.8%	75%	61.5%	93.7%
Park IH et al19	88.8%	58.17%	13.2%	98.6%
Present Study	100%	46.8%	10.7%	100%

Variations in the sensitivity, specificity of this test in different studies probably due to the different methods and kits used for PCT level estimation ,varied definition of sepsis and also different threshold levels used.

Though the PCT test has shown a good sensitivity in the current study it may however be raised in certain noninfectious conditions like premature birth, perinatal asphyxia, respiratory distress syndrome and hemodynamic instability and these must be excluded before arriving at a diagnosis of neonatal sepsis[17,18].

In our study, specificity of PCT was lower (46.8%) than that of CRP (69.7%). Similar findings were reported by Park IH et al[19] and Ballot et al[22]. These authors felt that the PCT is not sufficiently reliable as sole marker for neonatal sepsis and is useful when used in combination with other tests.

In our study the blood culture positivity was 6%. Park IH et al[19] and Naher BS[23] et al also reported low blood culture positivity in neonatal sepsis. This could be due to prior antibiotic therapy and insufficient volume of blood. If organisms were present at densities of <4 CFU/ml, blood volumes of 0.5ml or less had a significantly diminished chance of detecting bacteraemia[24]**.**

So, to conclude, PCT can be used as marker for early detection of neonatal sepsis along with CRP as blood cultures take long time and have poor sensitivity. But the only limitation is its high cost.

Limitations of our study were the small sample size and the fact that follow up samples were not taken to estimate whether PCT could be used in diagnosis

In our study serum PCT was shown to be a good indicator of the onset of neonatal sepsis and should be included in the evaluations of all neonates with clinical suspicion of sepsis.

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