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Original Research Article

A CROSS SECTIONAL STUDY ON EARLY DIAGNOSIS OF NEONATAL SEPSIS USING PROCALCITONIN AT A TERTIARY CARE TEACHING HOSPITAL, MANDYA

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ABSTRACT

BACKGROUND

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection accompanied by bacteraemia, within the first four weeks of life (28 days). Neonatal sepsis is the most common cause of morbidity and mortality in neonatal period. The gold standard method for diagnosis of neonatal sepsis is the isolation of microorganism from blood. Blood culture takes more than three days for complete result and requires well equipped laboratory and trained personnel. The outcome and prognosis of neonatal sepsis depends on early diagnosis and timely initiation of antibiotic therapy. Therefore, our study was conducted with the aim to evaluate the diagnostic accuracy of Procalcitonin in early diagnosis of neonatal sepsis in comparison with the gold standard Blood culture.

METHODS

A hospital based Cross sectional study was carried out at NICU, Department of Paediatrics, Mandya Institute of Medical Sciences, Mandya. A total of 166 neonates admitted with clinical suspicion of sepsis during the study period of one year were enrolled, and were screened for sepsis by ordering Complete blood counts (CBC), CRP, Procalcitonin(PCT) levels, along with the Blood culture before initiation of any antibiotic therapy.

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RESULTS

Among the study cohort of 166 neonates 56.6% were Male and 51.8% were in the age group of > 72hrs of life. A majority of, 140 (84.3%) were term neonates, 122(73.5%) weighing ≥ 2.5kg and 100(60.2%) delivered vaginally. Positivity of the Blood culture was 36.1%., with E coli and Klebsiella being the most common pathogens isolated. CRP was positive among 56.02% of study subjects and had a sensitivity of 83.3% and negative predictive value of 86.3% in diagnosing neonatal sepsis. Procalcitonin was positive among 63.85% of study subjects and had a sensitivity of 90%, negative predictive value of 90% in diagnosing neonatal sepsis. Area under the ROC curve for Procalcitonin (PCT) with 95% Confidence interval was found to be the best at 0.810 to 0.930, with a significant p value of <0.005, compared to other parameters like CRP, TLC, ANC, IT ratio and Platelet count.

CONCLUSION

Early recognition and diagnosis of neonatal sepsis is important since timely initiation of antimicrobial therapy improves the outcome. The findings of the present study align with previous research and emphasize that serum levels of Procalcitonin could be used as an early diagnostic marker of neonatal sepsis.

Key wors: NICU, Neonatal sepsis, PCT, Sensitivity, CRP

INTRODUCTION

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection, accompanied by bacteraemia within the first four weeks of life (28 days). Neonatal sepsis is the most common cause of morbidity and mortality in neonatal period[1]. Every year 135 million babies are born alive worldwide. Statistical data in 2011 estimated 3.0 million of these died during the first four weeks of life[1]. Neonatal sepsis is classified into early onset neonatal sepsis (EONS) and late onset neonatal sepsis (LONS) according to time of onset of signs and symptoms. EONS, this occurs within the first 72 hours of life, usually presents with respiratory distress and pneumonia. LONS usually presents after 72 hours of age and can either be nosocomial (hospital-acquired) or community-acquired infections. These neonates are mainly diagnosed to have septicemia, pneumonia or meningitis[2,3].

Initial diagnosis of neonatal sepsis is based on the clinical signs and symptoms which are non-specific, as other non-infective condition like aspiration, asphyxia and metabolic disorders may also present with similar signs mimicking sepsis[4]. The false positivity in diagnosing sepsis based on the clinical features results in unwarranted initiation of empirical antibiotic therapy may lead to development of drug resistance, prolonged hospital stays, increased treatment cost and the separation of the neonates from their mothers[5].

The gold standard method for diagnosis of neonatal sepsis is isolation of microorganism from blood. Blood culture takes more than three days for complete result, requires a well equipped laboratory and a trained personnel for better results[6]. Increased neonatal mortality in neonatal sepsis scenario necessitates the need of a rapid and an effective diagnostic test. Commonly used diagnostic hematological markers such as total leucocyte

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count(TLC), immature to total neutrophil ratio(IT Ratio), platelet count and absolute neutrophil count(ANC) are less sensitive and specific for diagnosing neonatal sepsis[7,8,9].

In recent days screening of serological markers such as C-reactive protein (CRP), Procalcitonin (PCT) and various cytokines have been suggested as being more sensitive indicators for early diagnosis of sepsis in neonates. The biomarkers are classified into early phase marker (Interleukin-6, Interleukin-8, Tumor Necrosis factor-α and Interferon-γ) mid phase marker (Procalcitonin) and late phase marker (C-reactive protein) [10,11].

Procalcitonin is a precursor in the synthesis of calcitonin (CT), secreted by the C cells of thyroid gland in physiological situation, but its levels may increase during septicaemia, meningitis, pneumonia, and urinary tract infection. . In sepsis, macrophages and the monocytic cells of the liver are involved in the synthesis of PCT. Serum PCT concentration raises 2-4 hours after endotoxin injection, reaches its peak level right after 6 hours, maintains a plateau through 8 to 24 hours and decreases to its normal level if the infection stimulus stops[12,13,14].

The outcome and prognosis of neonatal sepsis depends on early diagnosis and timely initiation of antibiotic therapy. This study was conducted with the aim to evaluate the diagnostic accuracy of Procalcitonin in early diagnosis of neonatal sepsis in comparison with the Blood culture.

MATERIALM AND METHODS

A Hospital based Cross sectional study was carried out at the Neonatal Intensive care unit(NICU), Department of Paediatrics, Mandya Institute of Medical Sciences, Mandya, over a period of one year between January 2020 to December 2020. Institutional ethical committee and Scientific committee approval was obtained.

Sample size- Due to the pandemic of COVID-19 during the study period, only 166 neonates who fulfilled the inclusion and exclusion criteria were included.

Sampling method- Purposive sampling

Inclusion criteria

Neonates admitted in NICU with clinical features of Sepsis during the study period.

Clinical features indicative of neonatal sepsis was enlisted as follows:

1. General: fever, temperature instability, jaundice and poor feeding.
2. Gastrointestinal system dysfunction: abdominal distension, Hepatomegaly
3. Respiratory system dysfunction: apnoea, tachypnoea, chest retractions, grunting, cyanosis
4. Cardiovascular system dysfunction: mottling, tachycardia, bradycardia
5. Central nervous system dysfunction: irritability, lethargy, bulging anterior fontanelle, abnormal moro’s reflex, seizures, hypotonia
6. Haematological system dysfunction: petechiae, Splenomegaly, pallor.

Neonates with two or more of the above-mentioned clinical features were considered clinically septic.

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Exclision criteria

Neonates with Birth Asphyxia, < 28wks gestational age, major congenital anomalies, aspiration syndromes, liver disease, inborn error of metabolism and who had received antibiotics or vaccine or blood transfusion prior to sampling were excluded from the study.

Method of data collection

A Written informed consent was obtained from all the parents prior to inclusion of neonates. All the details including sociodemographic data, maternal history, delivery details, clinical findings of neonates at admission and laboratory investigations performed were recorded on a predesigned study proforma. Birth weight was recorded using an electronic weighing scale. Gestational age assessment was done using the modified Ballard's assessment scale. All the neonates enrolled in the study were screened for sepsis at admission by sending Complete blood counts (CBC), CRP and PCT levels, along with the Blood Culture at the Central Diagnostic Laboratory (CDL) of Mandya institute of Medical Sciences. CBC was done using the Sysmex automated analyser. Total leukocyte count(TLC), differential count, platelet count(PC) and cell morphology were reconfirmed by peripheral smear study. Nucleated red cell count and band cells to calculate immature to total neutrophil count (IT Ratio) was also done by the peripheral smear study. TLC of 5,000- 20,000 cells/cmm, platelet count of 1,50,000- 4,50,000cells/cmm, and IT ratio of < 0.2 was considered normal. For analysis of ANC, values were plotted on the Manroe 's chart[10].

Blood culture was done by using conventional blood culture method. Blood culture was taken as negative if no growth after 3 subcultures. CRP analysis was done by using Immune turbidimetric method. CRP range <0.5mg/dl or <5mg/l was considered normal with measuring range of the instrument at CDL, MIMS being 0.02mg/dl to 32mg/dl. Procalcitonin assay was done by Chemiluminescent Micro Particle Assay (CMIA). PCT levels of <0.5ng/ml was considered normal.

Based on the blood culture result the neonates enrolled were assigned into the following 2 groups, for the study purpose.

1. Culture proven sepsis group: Included neonates with clinical features of sepsis and positive blood culture.
2. Clinical sepsis group: Included neonates with clinical features of sepsis with negative blood culture.

Statistical analysis

Data was analysed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Continuous data was represented as mean and standard deviation. Chi-square test was used as test of significance for qualitative data and independent t test was used as test of significance to identify the mean difference between two quantitative variables. Furthermore, sensitivity, specificity, positive predictive value(PPV) and negative predictive value(NPV) were used for diagnostic efficiency. Receiver operating characteristic (ROC) curves and the area under the curve were used to compare the sensitivity and specificity of the diagnostic procedures.

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RESULT

A total of 166 neonates satisfying the inclusion and exclusion criteria were included in the study. Among the study cohort included 56.6% were Male and 51.8% were in the age group of > 72hrs of life. A majority of, 140 (84.3%) were term neonates, 122(73.5%) weighing ≥ 2.5kg and 100(60.2%) delivered vaginally (Table-1) The common presenting clinical feature was poor feeding (20.5%) followed by seizure (19.3%).

Positivity of the Blood culture in our study was 36.1%(n=60), with E coli (41.7%) and Klebsiella (41.7%) being the most common pathogens isolated in Blood culture (Table-2). Procalcitonin was positive among 63.85% of study subjects and had a sensitivity of 90%, negative predictive value of 90% in diagnosing neonatal sepsis (Table-3&6). Mean procalcitonin levels among the study subjects with culture proven sepsis was 24.44 ± 29.18 and among the study subjects with clinical sepsis or no blood culture growth was 2.01 ± 4.66 (Table-4). There was significant association between Procalcitonin levels and Blood culture result ($p<0.001$). CRP was positive among 56.02% of study subjects and had a sensitivity of 83.3% and negative predictive value of 86.3% in diagnosing neonatal sepsis (Table-5). TLC, ANC, IT ratio and Platelet count did not show a good sensitivity in diagnosing neonatal sepsis (Graph -1). Area under the ROC curve for procalcitonin (PCT) with 95% Confidence interval was found to be the best at 0.810 to 0.930 and with a significant p value of <0.005 , compared to other parameters like CRP, TLC, ANC, IT ratio and Platelet count (Table -7)

Variables		**Number**	**Percentage**
Sex	Male	94	56.60
	Female	72	43.40
Gestational age	Preterm	26	15.70
	Term	140	84.30
Birth weight	≥ 2.5 kg	122	73.50
	< 2.5kg	44	26.50
Mode of delivery	Vaginally	100	60.20
	LSCS	66	39.80
Age at admission	≤ 72 hrs	80	48.20
	> 72 hrs	86	51.80

Table 1: Distribution of study subjects according to variables (n=166)

Culture	**Number**	**Percentage**
E coli	25	41.7
Klebsiella	25	41.7
Staphylococcus	5	8.3
Pseudomonas	3	5.0
Stap Epidermidis	1	1.7
Enterococcus	1	1.7

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Total	**60**	**100**

Table 2: Distribution of study subjects according to Organism isolated in Blood Culture (n=60)

		Blood Culture		**Total**
		Positive (Culture proven sepsis)	**Negative (Clinical sepsis group)**	
Procalcitonin	≥0.5ng/ml	54	52	106
	<0.5ng/ml	6	54	60
Total		**60**	**106**	**166**

Table 3: Comparison of Procalcitonin with Blood Culture (n=166).

		Pro Calcitonin			**P value**
		Mean	**SD**	**Median**	
Blood Culture	Positive	24.44	29.18	14	< 0.001*
	Negative	2.01	4.66	0.45	

Table 4:Mean value of PCT in relation to Blood Culture

		Blood Culture		**Total**
		Positive	**Negative**	
CRP	≥0.5 mg/dl	50	43	93
	<0.5 mg/dl	10	63	73
Total		**60**	**106**	**166**

Table 5: Comparison of CRP with Blood Culture (n=166).

Variable	**PCT**	**CRP**	**TLC**	**ANC**	**IT ratio**	**PC**
Sensitivity	90%	83.3%	18.3%	10.0%	10.0%	41.07%
Specificity	50.94%	59.43%	85.8%	94.3%	62.6%	50.49%

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PPV	49.05%	53.76%	42.31%	50.0%	13.04%	31.08%
NPV	90%	86.3%	65.0%	64.0%	55.0%	61.18%

Table 6: Comparison of validity of various laboratory parameters with Blood Culture

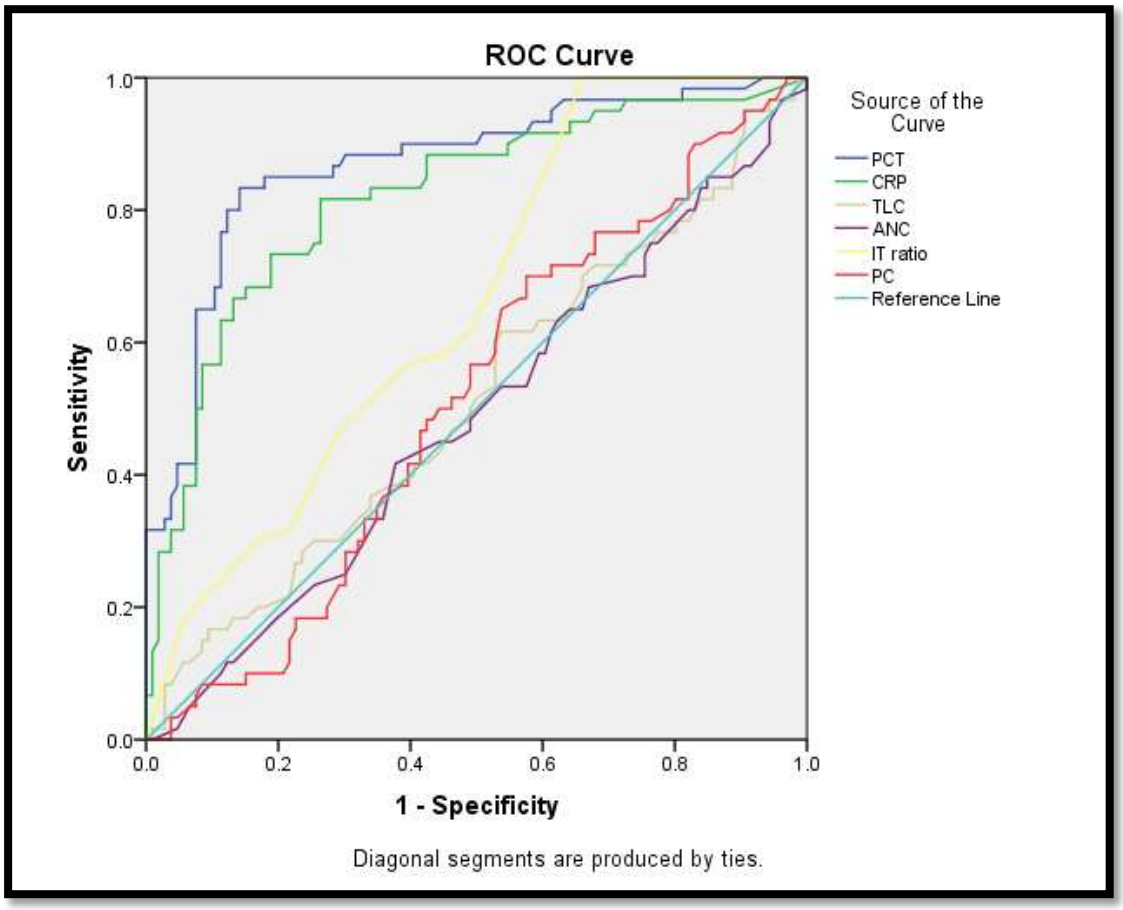

Figure 1: ROC curve showing sensitivity and specificity of various laboratory parameters

Area Under the Curve					
Test Result Variable(s)	**Area**	**Std. Error**	**P value**	**Asymptotic 95% Confidence Interval**	
				Lower Bound	**Upper Bound**
PCT	0.870	0.031	0.000	0.810	0.930
CRP	0.820	0.035	0.000	0.751	0.890
IT ratio	0.661	0.042	0.001	0.579	0.743
TLC	0.515	0.047	0.749	0.422	0.608
ANC	0.485	0.047	0.747	0.393	0.577

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PC	0.515	0.046	0.749	0.426	0.604
a. Under the nonparametric assumption					
b. Null hypothesis: true area = 0.5					

Table 7: Area under the ROC curve

DISCUSSION

Neonatal sepsis is a common disease of neonatal period with non-specific symptomatology and difficulty in diagnosis. Early detection and appropriate treatment of neonatal sepsis can significantly reduce the morbidity and mortality[1,2] . This hospital based cross sectional study has observed and confirmed some known facts. The higher proportion of term neonates compared to the preterm neonates in our study probably reflects difference in the population characteristics and the occurrence of the predisposing factors among them.

In our study positive blood culture was evident in 36.1% which was contrary to the study done by Pravin Charles M V et al[15] (12%), Hasan F et al[16] (32%) and study done by Aqueela Ayub et al[17] (44%), the difference among the studies might be due to difference in sampling method, administration of prior antibiotics from referral centers, infection with anaerobes, incidence of hospital acquired infections and different mode of test employed for culture. In our study the most common organisms were E Coli(41.7%) and Klebsiella (41.7%) similar to study done by Pravin Charles M V et al [15] & Abdel H Hakeem et al [18].

The present study investigated the serum levels of PCT as an early marker of neonatal sepsis in comparison with CRP, TLC, ANC, IT ratio and PC, with blood culture as the gold standard. Procalcitonin level was positive in 66.25% of suspected sepsis babies in our study, similar to study done by Hasan F et al [96] (66%). In our study the mean value of PCT in the culture proven sepsis group was higher (24.4ng/ml) than the that of the clinical sepsis group ($p < 0.0001$). in comparison, study done by Ho park et al[19] had a mean PCT value of 56.27ng/ml among the culture positive sepsis group.

ROC curves for our studied sepsis markers revealed that serum PCT levels with cutoff value/> 0.5 ng/ml showed a highest sensitivity of 90%, a specificity of 50%, a positive predictive value 49.05%, and a good negative predictive value of 90%. Sensitivity and negative predictive value are comparable with study done by Hasan F et al [16] (100%,100%), study done by Ramesh Vazzelwar et al [20] (97%,92%) whereas specificity and positive predictive value were less compared to study done by Hasan F et al[16] (84.4% and 62.5%) and study done by Ramesh Vazzelwar et al[20] (80%,92%) probably due to higher cut off level in later studies.

CONCLUSION

Early recognition and diagnosis of neonatal sepsis is difficult due to variable non-specific clinical features. It is important to make an early diagnosis of neonatal sepsis since timely initiation of the antimicrobial therapy improves the outcome. The findings of the present study confirm that the serum level of Procalcitonin could be used as an early diagnostic marker of neonatal sepsis.

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