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Original Research Article

Role of procalcitonin in early diagnosis of neonatal sepsis

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ABSTRACT

Background: Neonatal sepsis is the most common cause for neonatal mortality and morbidity in India, Therefore it is essential that we diagnose early onset sepsis using clinical signs and symptoms and rapid diagnostic techniques and start appropriate treatment without any delay. Various diagnostic tests that differentiate infected and non-infected neonates particularly in the first few days of life can potentially make significant impact on the neonatal care.
Methods: This was a hospital based clinical prospective study, done in the NICU department of pediatrics at Yenepoya medical college hospital, from January 2013 to December 2013. Sample size in this study was 50. All consecutive neonates fulfilling the inclusion and exclusion criteria were subjected to investigations like serum Procalcitonin, CRP, Total count, Gastric aspirate, Peripheral smear and Blood culture before starting treatment with antibiotics. Positive blood culture was taken as proven sepsis. The results obtained from our study parameters were statistically compared with cases of proven sepsis.
Results: In our study 16(32%) out of 50 neonates had proven sepsis. In comparison to the other markers of neonatal sepsis Elevated levels of serum Procalcitonin was found to be the most sensitive test with sensitivity of 100%, specificity of 50%, positive predictive value of 48.5%and negative predictive value of 100% and with a very highly significant p value of <0.001.Among the levels of PCT moderately elevated (2-10ng/dl) had sensitivity of 100%, specificity of 84.5%, PPV of 62.5% and NPV of 100% and highly elevated (>10ng/dl) had sensitivity of 100%, specificity of 80%, PPV of 75% and NPV of 100%.
Conclusions: The use of procalcitonin in the diagnosis of neonatal sepsis has proved to be very useful compared to other regular sepsis markers. Procalcitonin performs better than CRP in the diagnosis of neonatal infection. Serum Procalcitonin levels >2 ng/dl has got a better sensitivity; PPV and NPV thus help us not only in the early diagnosis and also in the prognosis of the treatment and helps us in guiding in reducing the unwanted usage on antibiotics.

Keywords: CRP, NICU, PCT, PPV, Total count

INTRODUCTION

Early diagnosis, rational antimicrobial therapy and aggressive supportive care can prevent sepsis related mortality and morbidity.[1,2] The incidence of neonatal sepsis varies from 25. 3 per 1000 live births in Asia, of which 22.2 per 1000 live birth is early onset sepsis and 2.9 per 1000 is late onset sepsis. Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first month of life. It encompasses various systemic infections of the newborn such as septicemia meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infection.[1,2]

PCT as a marker of neonatal sepsis

Normal PCT plasma concentrations are found to be below 0.05ng/ml and it can increase up to 1000ng/ml in patients with sepsis. Concentrations of PCT exceeding 0.5ng/ml are interpreted as abnormal values suggestive of a sepsis syndrome. Concentrations above 10 ng/ml are found in patients with severe sepsis. PCT serum concentration will increase within 2-3 hours of beginning of infection peaking by 6-12 hours and return to normal concentration in 2 days. Half-life of PCT is 20-24 hours and this enables not only rapid detection but also response to treatment. PCT has been shown to be useful not only in the diagnosis but also monitoring the prognosis and response to treatment of patients with neonatal sepsis.[3]

The return to baseline is usually rapid and the second peak of PCT is interpreted as development of a new episode. Estimation of PCT levels has been reported to be useful for critically ill patients with severe systemic inflammatory response.[3] PCT has the highest sensitivity and specificity for differentiating SIRS from sepsis. The advantages are:

- Earlier rise in infection
- Better negative predictive value
- Better correlation with outcome
- Early onset sepsis can manifest as asymptomatic bacteremia, generalized sepsis, pneumonia or meningitis. Clinical signs are apparent in the first few hours of life
- Premature and sick infants are more prone to sepsis and can have non-specific initial presentations.[4]

Laboratory evaluation of a symptomatic neonate with EONS includes complete blood count (CBC), with differential count, immature - total neutrophil ratio, absolute neutrophil count (ANC) and blood culture. CBC, ANC and I: T ratio does not have high sensitivity if done early and isolation of the causative organisms from microbial culture takes up to 72 hrs. And this doesn't diagnose most infected patients in view of low culture yield.[3,4]

Therefore a number of investigations have been evaluated for their ability to predict which high risk patients will develop symptomatic or culture proven sepsis. Apart from CBC and blood culture this includes the acute phase reactants like CRP and procalcitonin. Procalcitonin is another marker applied for the diagnosis of neonatal sepsis. Its relation with sepsis is still a question as with the other markers as it can be elevated in situations other than sepsis also. The sensitivity of this test varies in different studies probably due to the heterogeneous methods used in different studies, including subject of wide ranges, varied definition of sepsis, as well as different threshold levels used for PCT.[3,4]

The fetal inflammatory response and fetal stress may have distinct roles in nrbc production into the peripheral circulation. Clinical signs of sepsis are non-specific and there are no reliable early laboratory indicators. Since outcomes and prognosis depend on early and efficient antibiotic therapy, there is a need for sensitive and specific indicators for sepsis at the earliest stage of the disease.[5]

METHODS

A prospective hospital based clinical study was done at Neonatal intensive care unit of Yenepoya Medical College, Yenepoya University, Deralakatte, Mangalore. Study was conducted over a period of one year from January 2013 to December 2013. Elevated procalcitonin is an early marker in neonatal sepsis. 50 Neonates with suspected sepsis within the study period.

Alternate hypothesis

Serum procalcitonin can be physiologically elevated in first 48 hours due to other factors.

All consecutive neonates fulfilling the inclusion and exclusion criteria are subjected to investigations like serum procalcitonin, CRP, total count, gastric aspirate, peripheral smear and blood culture before starting treatment with antibiotics.

Inclusion criteria

All neonates with risk factors and clinical features of sepsis

Major risk factors

• PROM>18hrs
• Maternal fever >38˚c within 15 days
• Foul smelling liquor
• Fetal distress

Minor risk factors

• Low birth weight < 1500gms
• Prematurity < 34 wks
• Birth asphyxia (APGAR <5)
• Maternal WBC > 15000
• Vaginal swab positive for GBS

Clinical signs and symptoms

• Sclerema
• Lethargy
• Apnea
• Hypotonia
• Poor cry
• Breathlessness
• Irritability
• Grunting
• Poor feeding
• Vomiting
• Loose stools

• Temperature instability
• Mottling.

Exclusion criteria

- New-born babies with gestational age < 28 weeks
- Neonates with birth weight <1000 gms
- Neonates with obvious malformation/congenital anomalies
- Outside born babies who have received any treatment.

Written and valid informed consent was taken from the parent of the subject included in the study.

A study proforma was designed and accordingly the study subject underwent detailed history, clinical examination and laboratory investigations . Maternal history was elicited and risk factors were noted in the proforma. Birth details were recorded as per babies' case sheet details. Birth weight was recorded using electronic weighing scale at birth. Gestational assessment was done using modified Ballard's assessment scale. At the admission baby's vital signs were recorded followed by systemic clinical examination was done and findings were recorded in the proforma. All neonates admitted to our NICU fitting into the inclusion and exclusion criteria were screened for sepsis.

Complete blood count was done using automated analyzer. Total count differential count platelet count and morphology were reconfirmed by peripheral Smear study. Nucleated red cell count and band cells to calculate immature to total neutrophil count were also done in peripheral study. Gastric aspirate was examined for polymorph cells. Blood culture was done using BACT-Alert method. Blood culture was taken as negative if no growth after 3 consecutive subcultures. Gastric aspirate - > 5 /HPF polymorphs was considered positive Peripheral smear for band cells or abnormal cells >20% was considered positive Total count > 20000/cumm or < 5000/cumm was considered positive

Procalcitonin

PCT level analysis was done using enzyme linked immunofluorescence assay by using Brahms Pct Semi Quantitative Assay Kit manufactured by Biomeriux India (P) Ltd.

Methods of estimation of PCT

This immunoassay kit allows the specific measurement of human procalcitonin (PCT) concentrations in cell culture supernates, serum and plasma.

PCT levels

• PCT< 0.5 ng/ml - normal

Systemic infection is not likely. Low risk for progression to severe systemic infection.

• PCT >0.5 < 2 ng/ml - MILD

Systemic infection is possible. Moderate risk for progression to severe systemic infection.

• PCT >2 <10 ng/ml - MODERATE

Systemic infection is likely. High risk for progression to severe systemic infection.

• PCT > 10 ng/ml - HIGH

Possible systemic inflammatory response almost exclusively due to severe bacterial sepsis. High likelihood of severe sepsis. In our study PCT levels more than 0.5 ng/ml were considered as elevated levels of PCT.

C-reactive protein

CRP analysis was done using immunoturbidometry method, Erba Mannhein which is a quantitative method for analyzing CRP level.

Latex micro particles coated with anti - CRP mouse monoclonal antibodies react with the CRP in the sample to form an antigen-antibody complex.

Following agglutination, CRP level was measured turbidometrrically, with the minimal detectable concentration being 1 mg/l and > 8 mg/l was considered positive. For the purpose of study, neonates were divided into 2 groups

• *Proven sepsis*

Neonates with signs and symptoms of suggested sepsis with positive blood culture

• *Probable sepsis*

Two or more signs suggestive of sepsis with at least one abnormal laboratory parameter.

One or more signs suggestive of sepsis with two or more abnormal laboratory parameters.

Chi-square test

It has been used to study the significance of study parameters on categorical scale between groups.

Significant figures

+ Suggestive significance (p value: 0.05 <p<0.10)

*Moderately significant (p value: 0.01 <p = 0.05)

**Strongly significant (p value: p = 0.01)

Statistical software

The statistical software namely SPSS 20.0, Stata 8.0, Med Calc 9.0.1 were used for the Analysis of the data and Microsoft word and excel have been used to generate graph, table etc.

RESULTS

This is a prospective hospital based clinical study.

- 50 Neonates were included in the study.
- 52% were Males and remaining 48% were Females.
- 58% were Term babies and remaining 42 % were Preterm babies.

Table 1: Distribution of variables in relation to proven sepsis.

Variables		n = 50	Proven sepsis	P-value
Sex	Male	26 (52%)	7 (26.5%	0.200
	Female	24 (48%)	9 (37.5%)	
Gestation	Preterm	20 (40%)	9 (45%)	0.108
	Term	30 (60%)	7 (23%)	
Type of delivery	LSCS	16 (32%)	4 (25%)	0.204
	NVD	34 (685)	12 (35%)	
Birth weight	Low birth weight	22 (44%)	10 (45%)	0.157
	Normal	28 (56%)	6 (21%)	

Table 2: Distribution of study parameters.

	Frequency	
Investigation	**Positive**	**Negative**
CRP	24	26
Peripheral smear	12	38
Total count	28	22
Polymorphs	10	40
Procalcitonin	33	17

- 68% were born by normal vaginal delivery and remaining 32% were LSCS.
- 44% were low birth weight with birth weight of <2.5 kgs and remaining 56% were normal birth weight.
- 32% were diagnosed as proven sepsis and 68% were probable sepsis.
- Among the positive blood cultures Rapid blood culture methods had better yield with 32% positive cases compared to 12% in conventional method.
- Among risk factors PROM and fetal distress were statistically significant with a p value of 0.009 respectively.
- Among the clinical features 80% of the neonates with mottling and grunting were proven sepsis which were statistically significant with a p-value of 0.015 respectively.
- Serum procalcitonin levels levels of >10ng/ml had the highest sensitivity, specificity, positive predictive value and negative predictive value of 100%, 80%, 75% and 100% respectively when compared to the other serum levels.

Table 3: Comparison of levels of serum procalcitonin with blood culture.

Procalcitonin	Blood culture		Total
	Negative	**Positive**	
Normal-<0.5ng/ml	16	0	16
	48.5%	0.0%	32.7%
Mild-0.5-<2ng/ml	10	3	13
	30.3%	18.8%	26.5%
Moderate-2-<10ng/ml	3	5	8
	9.1%	31.2%	16.3%
Highly->10ng/ml	4	8	12
	12.1%	50.0%	24.5%
Total	**33**	**16**	**49**
	100.0%	**100.0%**	**100.0%**

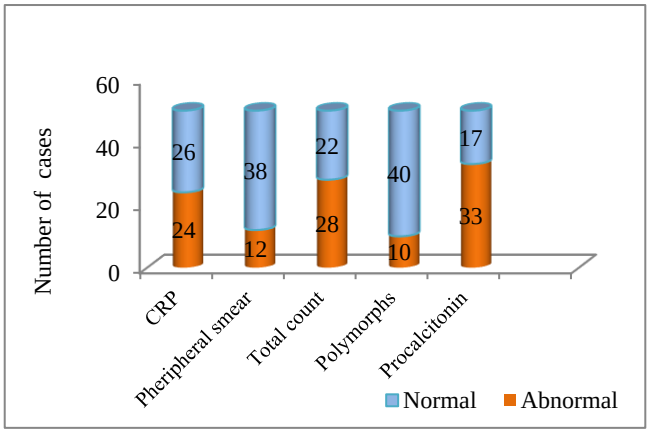

Figure 1: Distribution of study parameters.

Table 4: Comparison of elvated levels of procalcitonin with blood culture.

			Blood culture		Total
			Positive	Negative	
Elevated levels of PCT	Positive	Count	16	17	33
		%	100.0%	50.0%	66.0%
	Negative	Count	0	17	17
		%	0.0%	50.0%	34.0%
Total		**Count**	**16**	**34**	**50**
		%	**100.0%**	**100.0%**	**100.0%**

Table 5: Comparison of CRP with blood culture.

			Blood culture		Total
			Positive	Negative	
CRP	Positive	Count	8	16	24
		%	50.0%	47.1%	48.0%
	Negative	Count	8	18	26
		%	50.0%	52.9%	52.0%
Total		**Count**	**16**	**34**	**50**
		%	**100.0%**	**100.0%**	**100.0%**

Table 6: Comparison of peripheral smear with blood culture.

Peripheral smear		Blood culture		Total
		Positive	Negative	
Positive	Count	5	7	12
	%	31.2%	20.6%	24.0%
Negative	Count	11	27	38
	%	68.8%	79.4%	76.0%
Total	**Count**	**16**	**34**	**50**
	%	**100.0%**	**100.0%**	**100.0%**

Table 7: Comparison of total count with blood culture.

Total count		Blood culture		Total
		Positive	Negative	
Positive	Count	7	21	28
	%	43.8%	61.8%	56.0%
Negative	Count	9	13	22
	%	56.2%	38.2%	44.0%
Total	**Count**	**16**	**34**	**50**
	%	**100.0%**	**100.0%**	**100.0%**

Table 8: Comparison of polymorphs cells with blood culture

Polymorphs		Blood culture		Total
		Positive	Negative	
Positive	Count	4	6	10
	%	25.0%	17.6%	20.0%
Negative	Count	12	28	40
	%	75.0%	82.4%	80.0%
Total	**Count**	**16**	**34**	**50**
	%	**100.0%**	**100.0%**	**100.0%**

- Elevated levels of procalcitonin (>0.5 ng/ml) as a marker of neonatal sepsis had a sensitivity, specificity, positive predictive value and negative predictive value of 100%, 50%, 48.5% and 100% respectively. The p value was calculated to be <0.001, thus statistically significant.

DISCUSSION

Neonatal sepsis is a common disease of newborn with non-specific symptomatology and difficulty in the diagnosis. Early and prompt detection and appropriate treatment of neonatal sepsis can significantly reduce the morbidity and mortality.[1,2] This hospital based prospective 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. 3Preterm are more susceptible to infection due to inherent defensive mechanism. In this study male neonates with proven sepsis was 26.5% of the 26 and females neonates were 37.5% out of 24 were proven sepsis. In the present study 40% neonates were with birth weight less than <2.5 kgs6,7 And our study showed 44% of 22 neonates with low birth were diagnosed with proven Sepsis compared to 21.4% of 28 with normal birth weight were proven sepsis. Anderson et al also showed increased risk of neonatal sepsis with decrease in birth weight.[11] Results in our study were almost comparable with other studies based on risk factors in the study group (n = 50).

In our study 55% of the 9 (18%) with maternal fever, 50% of the 14 (28%) with prom, 50% of 24 (48%) neonates with fetal distress, 45% of 22 (44%) neonates with low birth weight,45% of 20 (40%) preterm, 41% of the 12 (24%) with foul smelling liquor,45% of 20 (40%) had meconium stained liquor had proven sepsis and they were in significant in number.

28% of the 7 (10%) with birth asphyxia, 25% of the 16(32%) with maternal WBC >15000 were diagnosed with proven sepsis and were less in number comparatively. Among the risk factors PROM and fetal distress were statistically significant with a p value of 0.009 respectively. The observation in our study is very similar to the Harikumar et al.[13] In our study PROM was 50% which was higher compared to 26% in Kuruvilla et al foul smelling liquor was 41% in which was more than that observed in various studies but had closer results when compared to Raghavan et al, the variation in the occurrence of intrapartum risk factors probably reflects differences in the rates of occurrence of the predisposing risk factors in various other studies.[11,12]

Based on markers of sepsis (n = 50)

In our study 32% out of 50 neonates had proven sepsis which was very similar to Nowshad et al.[10] In our study comparison of the markers such as serum Procalcitonin, total count, peripheral smear, CRP, polymorphs>5hpf with blood culture.[11] Elevated levels of serum Procalcitonin was found to be the most sensitive test with sensitivity of 100%, specificity of 50%, positive predictive value of 48.5% and negative predictive value of 100% and with a very highly significant p value of <0.001. This observation was very similar to other studies. Among the levels of PCT moderately elevates (2->10 ng/dl) had sensitivity of 100%, specificity of 84.5%, PPV of 62.5% and NPV of 100% and highly elevated (>10ng/dl) had sensitively of 100%, specificity of 80%, PPV of 75% and NPV of 100% .So serum Procalcitonin levels with a cut off >2ng/dl found to be a more sensitive test and similar results have been observed other studies.

CRP had a sensitivity of 50%, specificity of 52.9%, and PPV of 33.3% and NPV of 100% with a p value of 0.846 In the present study CRP with a sensitivity of 50% and specificity of 52.9% was very close compared to Harikumar et al peripheral smear has a sensitivity of 31.2%, specificity of 79.4% PPV of 41.7% and NPV of 71.6% with a p value of 0.410 which is not significant.[13]

Total count has a sensitivity of 43.8%, specificity of 38.2%, PPV of 25%, NPV of 59.1%, with a p value of 0.231 which is not significant. Polymorphs cells has got a sensitivity of 25%, specificity of 82.4%, PPV of 40%, NPV of 70%, with a p value of 0.544 which is not significant.

These results are very similar compared to other studies. The use of procalcitonin in the diagnosis of neonatal sepsis has proved to be very useful compared to other regular sepsis markers. There are many pitfalls but still procalcitonin performs better than CRP in the diagnosis of neonatal infection. All previous studies reported a higher sensitivity for procalcitonin than CRP and other regular markers in early onset neonatal sepsis.[11-13]

It has been proved that there can be a physiological increase in serum procalcitonin up to 48 hours and postnatal administration of antibiotics will decrease procalcitonin more rapidly than CRP.[12,13,15]

Serum procalcitonin levels >2ng/dl has got a good PPV and NPV thus help us not only in the diagnosis and also in the prognosis of the treatment and helps us in guiding in reducing the unwanted usage on antibiotics.[14]

CONCLUSION

Blood culture is the gold standard for diagnosing neonatal sepsis but it requires 48-72 hours and percentage of positivity is better in rapid culture method when compared to conventional culture method. Procalcitonin in comparison to CRP, total count, peripheral smear and gastric, aspirate has better sensitivity and specificity and hence can detect most cases of neonatal sepsis as early as 6-12 hours from the onset of infection.

Procalcitonin has got better positive predictive value and negative predictive value compared to the other markers which will also help to decrease in the number of patients treated unnecessarily and Rapid diagnosis by using procalcitonin gives a reasonable degree of accuracy in early diagnosis of neonatal sepsis which will also help to initiate early antibiotic therapy.

Limitation of the study was that it was single centric study, small sample size, serial sampling was not possible, it was not possible to have a control group.

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