Section: Paediatrics

Original Research Article

PREDICTION AND DIAGNOSIS OF NEONATAL SEPSIS BY SALIVARY C-REACTIVE PROTEIN

Received : 10/05/2023
Received in revised form : 06/06/2023
Accepted : 15/06/2023

Keywords:
Late-onset neonatal sepsis, Neonatal sepsis, Highly sensitive serum CRP, Salivary CRP.

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DOI: 10.47009/jamp.2023.5.3.430

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (3); 2187-2191

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Abstract

Background: One of the main factors contributing to neonatal morbidity and mortality globally and in India is neonatal sepsis. Thus, the study aimed to utilise salivary c-reactive protein to predict and identify newborn sepsis. **Materials and Methods:** A prospective study was conducted at SNCU of Thanjavur Medical College between January and December 2019. The study recruited 58 term neonates from birth to the 28th day of life and included 28 neonates with suspicion of sepsis (23 with culture-proven and 5 with clinical sepsis) and 26 healthy controls. Clinical sepsis was diagnosed based on clinical criteria and serum CRP levels greater than 10 mg/L. **Results:** Compared to vaginal delivery (46%), Lower Segment Caesarean Section (LSCS) was the mode of delivery used in a higher percentage of patients (54%). There was no significant difference in age and birth weight between groups, but a significant difference was observed in gestational age between groups (p=0.042). Among the 28 sick neonates, 7 (25%) presented with seizures, 10 (35%) of them presented with the refusal of feed with respiratory distress, and the remaining 11 (40%) presented with feed intolerance. A significant difference between groups was seen in haemoglobin (p=0.009), mean platelet volume (p=0.002), Serum C-Reactive Protein, and Salivary C-Reactive Protein. The ROC curve analysis showed that salivary CRP at the cut-off value of 2.6 had a sensitivity of 94% and specificity of 96%. **Conclusion:** Our study demonstrates that salivary CRP is a reliable indicator for quickly diagnosing sepsis and starting therapy without waiting for time-consuming blood culture results.

INTRODUCTION

About three million newborns are affected with neonatal sepsis each year, with a death rate ranging from 9% to 20% in cases of severe sepsis.[1-3] Neonatal sepsis is one of the primary causes of morbidity and mortality in neonates worldwide and in India. Delays in receiving appropriate diagnoses and treatments are most likely to blame for this high fatality rate. The 'gold standard' sepsis diagnostic test is still blood culture. Cultural results can take up to 48 hours to become available. Thus, relying too heavily on them could cause unwarranted treatment delays and serious problems, including death. The acute-phase reactant serum C-reactive protein (CRP) is frequently combined with other indicators in a sepsis screen.[4-6]

Serum CRP assays, whether assessed alone or combined with other related tests and biomarkers, are important in managing treatment results in neonates with suspected sepsis before getting blood culture results.[7,8] However, as CRP and other biomarkers depend on serum or whole blood samples, blood sampling and laboratory assistance are required. The best method to reduce sepsis-related mortality and morbidity is to administer antibiotics immediately. Giving the antibiotic within an hour of having a sepsis suspicion is recommended by the Surviving Sepsis Campaign recommendations for treating sepsis and septic shock.[9]

Serial monitoring is also highly beneficial for understanding the course of the disease or the response to treatment. Therefore, the development and application of biomarkers in biofluid analysis offer a greater potential for early detection of infection risk that may aid in clinical decision-making. Salivary indicators could offer a viable and minimally intrusive method for screening for newborn sepsis and inflammation in this susceptible population.[7-9] The study aimed to use salivary c-reactive protein to predict and identify newborn sepsis.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

MATERIALS AND METHODS

This prospective study was conducted at SNCU of Thanjavur Medical College between January 2019 and December 2019. The study recruited 58 term neonates from birth to the 28th day of life and included 28 neonates with suspicion of sepsis (23 with culture-proven and 5 with clinical sepsis) and 26 healthy controls. Four suspected sepsis neonates were excluded because their CRP levels were below 10 mg/L. The Institutional ethical committee approved the study, and the parents of the newborns provided their informed permission.

Inclusion Criteria

Neonates admitted with temperature instability (hypothermia <36ºC, hyperthermia >38ºC), respiratory abnormality (grunting, intercoastal retraction, apnoea, tachypnoea, cyanosis), cardiovascular abnormality (bradycardia, tachycardia, low perfusion, hypotension), neurologic abnormality (hypotonia, lethargy, seizures), gastrointestinal abnormality (feeding intolerance, abdominal distension) and CRP value > 10 mg/l were included.

Exclusion Criteria

Neonates who did not meet the diagnosis of sepsis, intrauterine growth restriction, CNS malformation (spina bifida, anencephaly, dandy walker syndrome), birth asphyxia, and chromosomal abnormalities were excluded.

Clinical sepsis was diagnosed based on clinical criteria and serum CRP levels greater than 10 mg/L. Before beginning antibiotic treatment, samples from newborns with septic shock were taken within 4 hours of the clinical suspicion. 3ml of peripheral blood was drawn, 1ml of which was used for CBC measurement and the calculation of the neutrophil-lymphocyte ratio, 1ml of which was collected in a plain tube for the measurement of C-reactive protein using an immunoturbidimetric technique, and 1ml of which was directly inoculated into the blood culture medium.

Samples for salivary CRP were taken about one hour before feeding to prevent milk contamination. After tilting the head, a 2ml syringe was used to suck samples from the mouth floor. Salivary CRP was quantified using the immunoturbidimetric method in the laboratory after the sample had been collected and transferred there in an ice-filled, sterile container. After that, the newborns were monitored, and the test findings were examined.

Statistical Analysis

Analyses were performed using IBM SPSS statistics software 23.0 Version. An Unpaired t-test assessed the differences between the groups. The sensitivity, specificity, and optimal salivary CRP cut-off were determined using the receiver operating characteristic curve (ROC). For all statistical calculations, p0.05 was chosen as the significance level.

RESULTS

Table 1: Baseline characteristics among cases and controls

		No of Cases (%)	No of Controls (%)
Gender	Male	16 (59%)	16 (61%)
	Female	12 (41%)	10 (39%)
Mode of Delivery	Vaginal	13 (46%)	15 (57%)
	LSCS	15 (54%)	11 (43%)
Age in Days	≤ 3 days	12 (41%)	9 (34%)
	≥ 3 days	16 (59%)	17 (66%)

Most cases were male (59%) compared to females (41%). Compared to vaginal delivery (46%), Lower Segment Caesarean Section (LSCS) was the mode of delivery used in a greater percentage of patients (54%). When age was taken into account, a higher percentage of cases were ≥ 3 days old (59%) compared to those ≤ 3 days old (41%). Among the controls, similar trends were seen, with a slightly higher percentage of males (61%), a higher proportion of vaginal deliveries (57%), and a higher percentage of individuals ≥ 3 days old (66%) [Table 1].

Table 2: Demographic data of the study

		Frequency	Percent
Groups	Sepsis	28	51.9
	Non-Sepsis	26	48.1
Gender	Male baby	32	59.3
	Female baby	22	40.7
Delivery	AVD	1	1.9
	LSCS	26	48.1
	NVD	27	50.0
Outcome	Dead	6	11.1
	Discharge	48	88.9

Group distribution data indicated that 48.1% did not have sepsis, compared to 51.9%. Among the babies, 40.7% were female, and 59.3% were male. Regarding delivery methods, 1.9% of births were assisted vaginal

ISSN (O): 2687-5365; ISSN (P): 2753-6556

deliveries, 49.1% were lower segment caesarean section (C-section) deliveries, and 50% were normal vaginal deliveries. Among the outcomes, 11.1% of cases resulted in death, while the remaining 88.9% were discharged [Table 2].

Table 3: Comparison of patient characteristics and biochemical parameters

	Sepsis	Non-Sepsis	p-value
Age	4 ± 5	3 ± 1	0.267
Gestational age	37 ± 2	38 ± 2	0.042
Birth weight	2.37 ± 0.73	2.41 ± 0.62	0.816
Haemoglobin	14.99 ± 2.76	12.98 ± 2.66	0.009
White blood cells	9385.71 ± 4231.86	20034.62 ± 48147.23	0.249
Neutrophil-Lymphocyte Ratio	1.75 ± 1.53	2.54 ± 2.15	0.126
Mean platelet volume	9.94 ± 1.16	8.98 ± 0.99	0.002
Platelet	182000 ± 119937.6	198576.9 ± 91030.6	0.572
Serum C-Reactive Protein	24.72 ± 5.96	7.43 ± 1.16	0.0005
Salivary C-Reactive Protein	3.69 ± 0.64	0.72 ± 0.48	0.0005

There is no significant difference in age and birth weight between groups, but a significant difference was observed in gestational age between groups (p=0.042). Among the 28 sick neonates, 7 (25%) presented with seizures, 10 (35%) of them presented with the refusal of feed with respiratory distress, and the remaining 11 (40%) presented with feed intolerance.

In CBC indices, leucocytosis was observed in 8 (28%) septic neonates and 5 (19%) controls. Thrombocytopenia was observed in 11 (39%) sick and two (7.6%) controls. In the septic group, blood culture was positive in 23 (82%) neonates. Klebsiella (58%) was the most frequently isolated organism, followed by CONS (21%), E. coli (14%), and Acinetobacter (7%).

A significant difference between groups was seen in haemoglobin (p=0.009), mean platelet volume (p=0.002), and Serum C-Reactive Protein and Salivary C-Reactive Protein. However, there was no significant difference in white blood cells, neutrophil-Lymphocyte ratio and platelet volume [Table 3].

Table 4: Receiver Operator Characteristic (ROC) curve

Area Under the Curve			
Area	p-value	95% C. I	
		LB	UB
1.000	0.0005 **	1.000	1.000

The ROC curve analysis showed that salivary CRP at the cut-off value of 2.6 had a sensitivity of 94% and specificity of 96% [Table 4].

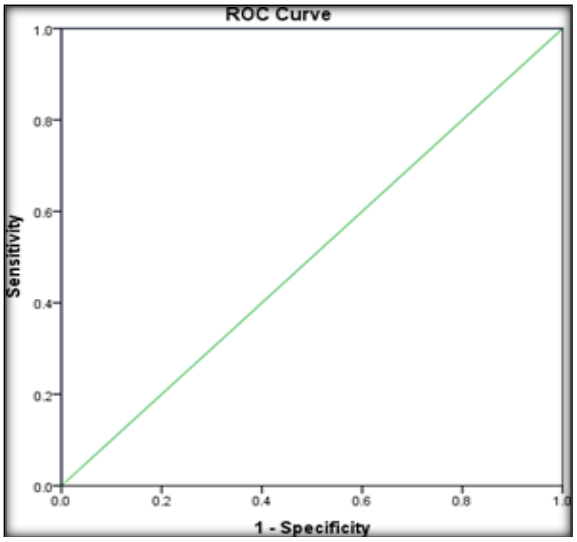

Figure 1: Receiver Operator Characteristic (ROC) curve

Table 5: Analysis of CRP and CBC Parameters

		Septic (28)	Controls (26)	P-value
CRP	Serum	24.72 ± 5.96	7.43 ± 1.61	< 0.01
	Salivary	3.69 ± 0.64	0.72 ± 0.48	<0.01
CBC	MPV	9.94 ± 1.16	8.98 ± 0.99	<0.01
	NLR	1.75 ± 1.53	2.54 ± 2.15	>0.01

ISSN (O): 2687-5365; ISSN (P): 2753-6556

The other blood parameters, including serum CRP and mean platelet volume, showed significant differences between cases and control groups. The neutrophil-Lymphocyte ratio showed no significant statistical difference between the groups [Table 5].

DISCUSSION

Neonatal sepsis has been recognised as a worldwide health challenge since it can be catastrophic and cause high morbidity and mortality in babies. Culture-positive sepsis accounted for 6.2% of India's overall 14.3% sepsis incidence. Sepsis with an early onset (i.e., occurring within the first 72 hours of life) comprised nearly two-thirds of all cases. Therefore, reliable diagnostic biomarkers are crucial for making a precise and timely diagnosis of newborn sepsis. A convenient and suitable biofluid for non-invasive screening of common infant morbidities is saliva. Recently, clinical surveillance and biomarker detection in neonates using saliva analysis has shown tremendous potential for enhancing neonatal healthcare.[8–10]

Serial CRP measures may also help detect neonates with infections, track treatment effectiveness, and calculate the length of antibiotic therapy. However, newborns with serial CRP readings are in danger due to frequent sampling. A more recent technique for diagnosing newborn sepsis involves the measurement of salivary CRP. The first paper regarding the detection and use of salivary CRP in septic newborns was published in 2014 by Iyengar et al.[11] This was the first study to identify, measure, and show that salivary CRP is a better index for clinically meaningful serum CRP thresholds.

In our study, the predominant mode of delivery associated with sepsis was lower Caesarean section delivery (53.6%), followed by normal vaginal delivery (42.9%) and assisted vaginal delivery (3.6%), which was concordant with Omran et al.[12] study. The study population comprised 32 (60%) males and 22 (40%) females. The male: female ratio in the study group was 1.45:1, having male predominance. Fifteen males (23%) and nine females (13.8%) have a positive blood culture.

Omran et al.[12] also suggested male preponderance in the septic group. Late-onset sepsis was diagnosed in 74.3% of the septic neonates, while early-onset sepsis was diagnosed in 25.7%. However, we have seen late-onset sepsis diagnosed in 59% and early-onset sepsis in 46%. Both mean platelet volume and neutrophil-lymphocyte ratio significantly differed between septic and healthy neonates. However, in our study, Mean platelet volume indicated a highly significant difference between septic and healthy groups. Other indices like Neutrophil-Lymphocyte Ratio and platelets with Groups show no significant difference between cases and Groups, which is discordant with Omran et al. study.[12]

Iyengar et al. study.[11] seen that salivary CRP has a sensitivity and specificity of 54% and 95%, respectively, in accurately predicting a serum CRP > 10 mg/L. Omran et al.[12] showed a statistically significant difference between salivary CRP among septic and healthy neonates. Also, it showed a cut-off point of 3.48ng/l; salivary CRP's sensitivity and specificity are 94.3% and 80%. Our study observed that Serum C-Reactive Protein showed a highly significant difference between Serum C-Reactive Protein and Groups. Salivary C-Reactive Protein with Groups showed a highly significant difference between Salivary C-Reactive Protein and Groups. Further, we found the salivary CRP at a cut-off point of 2.6 ng/L had 94% sensitivity and 96% specificity in accurately predicting a serum CRP level of ≥10mg/L, which was concordant with Iyengar et al.[11] and Omran et al.[12] study. The major disadvantages of salivary CRP include the sample should be transported at 4-80 C, and it does not give an antibiogram.

Due to their inherent sensitivity to infection and the intrusive treatments they are subjected to during birth, newborns are particularly susceptible to nosocomial infections. Simple preventive methods to lessen the burden of newborn infection include hand washing, an early discharge policy, reducing the number of invasive procedures, prepping the skin before the procedure, and using the proper sterilisation procedure for equipment. The potential to reduce newborn mortality through effective interventions is greater. The inflammatory cascade, which reflects the host's immunological state and reaction to infection, has numerous infection markers as its constituents, and one of these is salivary CRP.[13-15] The major limitations of the present study were a small number of septic neonates, and we used the same cut-off CRP in both early and late-onset sepsis

CONCLUSION

Our study demonstrates the value of salivary CRP as a reliable indicator for quickly diagnosing sepsis and starting therapy without waiting for time-consuming blood culture results. Leukopenia and an elevated blood c-reactive protein are two laboratory findings connected to sepsis. However, further research with a sizable cohort is required to validate the findings and the function of salivary CRP.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556