DETECTION AND POTENTIAL UTITITY OF SALIVARY C-REACTIVE PROTEIN FOR DIAGNOSIS OF NEONATAL SEPSIS

By

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ABSTRACT

Objective: *To evaluate salivary CRP as a diagnostic and prognostic biomarker in neonatal sepsis.*

Subjects and Methods: *This case-control study included 64 neonates were selected from Ain Sham University Hospital during the period from May 2022 and March 2023 by simple random method, assigned to two equal groups; septic group and a control group. Clinical findings and routine laboratory data including blood culture and complete blood picture results were documented. According to hospital protocol, Serum CRP was measured while salivary CRP levels were measured using enzyme-linked immunosorbent assay.*

Results: *The median salivary CRP was significantly higher in septic neonates compared to controls ($p < 0.001$). For salivary CRP, the optimum cut-off value for sepsis diagnosis was found to be 1.27 mg/L with sensitivity, specificity, positive and negative predictive values of 96.9%, 96.9%, 96.9%, and 100%, respectively. strong positive correlation was found between the levels of salivary and serum CRP ($r = .962$, $p = 0.001$).*

Conclusion: *Salivary CRP, at a cut-off value of 1.27 mg/L, exhibited a high specificity and positive predictive value in sepsis diagnosis and showed positive correlation with serum CRP.*

Key words: *neonatal sepsis, salivary CRP, serum CRP.*

INTRODUCTION

According to **Zhang et al. (2019)**, newborn sepsis is a major cause of morbidity and mortality in neonatal intensive care units (NICU). it is described as dysregulated host response due to

infectious pathogens (bacterial, viral, fungal) causing life-threatening organ malfunction **(Singer et al., 2016)**. Early onset sepsis affects newborns in their first three days of life, while late onset sepsis affects newborns from their fourth day up to their 28th day of life **(Puopolo et al., 2018)**.

Diagnosis of sepsis is suggested by multiple laboratory tests including CBC with differential count, Monocyte Distribution Width (MDW), C-reactive protein (CRP) and interleukin-6 (IL-6), urine analysis and Procalcitonin (PCT) **(Rashwan et al., 2018)**, and confirmed by blood culture **(Dong and Speer, 2015)**.

CRP is an acute phase reactant and a member of the pentraxin family which plays an important role in innate and adaptive immunity **(Bray et al., 2016)**. it is the most often utilized biomarker for monitoring sepsis **(McWilliam and Riordan, 2010)**.

Serum CRP is withdrawn twice weekly or up to every 48hours with the subsequent risk of recurrent sampling such as infection, anemia and cardiorespiratory instability especially in preterm **(Ranger et al., 2013)**. Thus, a non-invasive test is needed to avoid such complications and improve neonatal health. Our study investigated, salivary CRP as a novel, improved non-invasive test for early diagnosis of newborn sepsis, with prior studies suggesting that it may serve as a measure of systemic inflammation **(Omran et al., 2018)**.

AIM OF THE WORK

The objective of this study is: To evaluate salivary CRP as a diagnostic and prognostic biomarker in neonatal sepsis.

PATIENTS AND METHODS

Patients: This case control study conducted at the neonatal intensive care units (NICU) of Ain Shams University Hospital, Cairo, Egypt, during the period from May 2022 and March 2023 were enrolled to the study.

Ethical Consideration:

- The study was authorized by the Ain Shams University ethical committee, and a written informed consent was obtained from the parents of all patients. Ethical approval number: MS 123/2022.
- The data of the study are confidential, and the care giver has the right to keep it.
- The care giver has the right to refuse and withdrew from the study.

- The researcher explained to the caregiver the aim of the study.
- There is no conflict of interest regarding the study or the publication.
- The authors report no financial fund or support regarding the study or the publication.

Sample size:

By using G power program for Sample Size Calculation setting alpha error at 5% and power at 80% and assuming medium effect size difference (0.3) in serum CRP and Salivary CRP levels between neonates with sepsis and neonates without sepsis; based on that, a sample size of at least 64 neonates (32 septic and 32 without sepsis) will be sufficient to achieve study objective.

Inclusion criteria:

Groups I: Any neonate with clinical symptoms and signs or lab. data of neonatal sepsis according to Tollner and hematological sepsis score.

Groups II: Non septic neonates with matched age and sex.

Exclusion criteria:

Neonates with meconium Aspiration syndrome, or undergoing surgery and neonates with Suspected inborn error of metabolism were excluded from the study.

Study Design: The study was conducted on 64 neonates who were admitted in our NICU.

They were classified into:

Group I (septic neonates):

- It includes 32 neonates diagnosed to have clinically neonatal sepsis based on clinical findings and laboratory tests CBC, CRP and Blood culture.

Group II (control neonates):

- Including 32 age and gender matched non septic neonates were taken as a control group.

Initial assessment:

All the included neonates were subjected to:

- Complete history taking with stress on symptoms suggesting sepsis like poor feeding, jaundice, convulsion and cold extremities.
- Meticulous clinical examination with stress on sepsis signs as mottled skin, hyper or hypothermia, bulging ant.fontanel, respiratory distress, poor perfusion and shock.

Laboratory evaluation:

All neonates will follow NICU. protocol and the following were done:

- CBC by automated cell counters (sample 2cm).
- Serum CRP by immunoturbidimetry on admission and follow up sample as indicated by NICU protocol.
- Salivary CRP by ELISA within 4-12 hour after serum CRP.
- Salivary sample precaution and technique: samples were collected with a special collecting tube in salivary kits, it was placed under the neonates' tongues and in their gingival crevices, where saliva was known to pool. Suctioning occurred for approximately 10–15s. using low wall suction <20mmHg. After collection, samples were placed in polypropylene tubes. Then placed on ice and brought back to the laboratory where it stored in -20° C until use **(Iyengar et al., 2014)**.
- ABG by POCT device.
- chest x-ray as needed.
- Blood Culture on the same time of salivary and serum CRP.

Statistical Analysis:

Using the statistical tool for social science (SPSS 25 for Windows), the acquired data were edited, coded, tabulated, and introduced. Test of normality One-Sample Kolmogorov-Smirnov Test was done.

Quantitative parametric data are reported as mean and standard deviation (SD).

For quantitative nonparametric data, the median and interquartile range (IQR).

When analyzing quantitative data, Student T test or Mann-Whitney test are utilized, whereas chi square test and fisher exact test are used to assess qualitative data.

The link between serum and salivary CRP was measured using Pearson correlation.

The optimum salivary CRP cut off point for predicting newborn sepsis with the greatest sensitivity, specificity, PPV, and NPV was evaluated using a quantitative Receiver Operating Characteristic Curve (ROC) analysis.

The allowed margin of error was set at 5% with a 95% confidence interval. In light of the above, the p-value was considered significant:

- P-value > 0.05 indicates non-significance.

- Significant if P 0.05.
- Highly significant P-value: 0.01.

RESULTS

The results of our study will be demonstrated in the following tables and figures:

Table (1): Demographic data of all studied groups

		CONTROL GROUP (N=32)					SEPSIS GROUP (N=32)					P-value
		Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	
Gestational age (weeks)		35.34	37.00	3.41	28.00	40.00	35.71	37.00	2.90	29.00	39.00	0.434*
Birth weight (kg)		2.25	2.50	0.77	1.00	3.80	2.41	2.50	0.74	1.10	4.00	0.784*
weight on admission (kg)		2.28	2.50	0.81	1.00	3.80	2.36	2.30	0.77	1.10	3.80	0.784*
		N (%)					N (%)					P-value
Mode of delivery	NVD	11 (34.4%)					9 (28.1%)					0.788**
	CS	21 (65.6%)					23 (71.9%)					
Sex N (%)	Male	14 (43.8%)					19 (59.4%)					0.317**
	Female	18 (56.3%)					13 (40.6%)					
PROM N (%)	No	27 (84.4%)					24 (75.0%)					0.536**
	Yes	5 (15.6%)										

This table shows that patient and control were comparable regarding gestational age and sex distribution and they had no significant difference in weight, Mode of delivery or Presence of PROM prior to delivery.

Table (2): Comparison between the two groups regarding Laboratory finding

	CONTROL GROUP (N=32)					SEPSIS GROUP (N=32)					P-value
	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	
TLC (x103/cm3)	8.99	9.2	1.58	5.2	11	12.4	11.7	5.6	2	25.3	0.002*
HB (mg/dl)	14.10	13.15	2.78	9.00	19.80	12.54	12.50	2.77	6.30	17.60	0.058*
Plt (x103/ l) Mean± (25^{th}-75^{th} IQR)	292.9± (217.7-376.7)					189.3± (96.2–248.5)					<0.001*

This table shows that TLC was significantly higher and platelets significantly lower in sepsis group compared to control.

Hb. shows that no significant difference between two groups.

Table (3): Comparison between salivary and serum CRP in both groups

	CONTROL GROUP (N=32)					SEPSIS GROUP (N=32)					P-value
	Mean	Median	SD	Min.	Max.	Mean	Median	SD	Min.	Max.	
Serum CRP	2.39	1.50	2.09	0.10	<6	51.38	33.75	43.72	8.70	174.00	<0.001*
Salivary CRP	0.73	0.73	0.29	0.33	1.28	2.18	2.13	0.62	1.27	3.84	<0.001*

This table shows that septic neonates had significant higher serum and salivary CRP levels compared to controls.

Table (4): Results of blood culture in septic group (n= 32)

	Culture	Frequency	Percent
Sepsis group	**No growth**	**9**	**28.1%**
	klebsiella	**11**	**34.4%**
	CONS	**5**	**15.6%**
	pseudomonas	**3**	**9.4%%**
	Normal flora	**2**	**6.3%**
	candida non albicans	**1**	**3.15%**
	MRSA	**1**	**3.15%**
	Total	**32**	

This table shows that the most frequently encountered organism was Klebsiella followed by CONS in septic group.

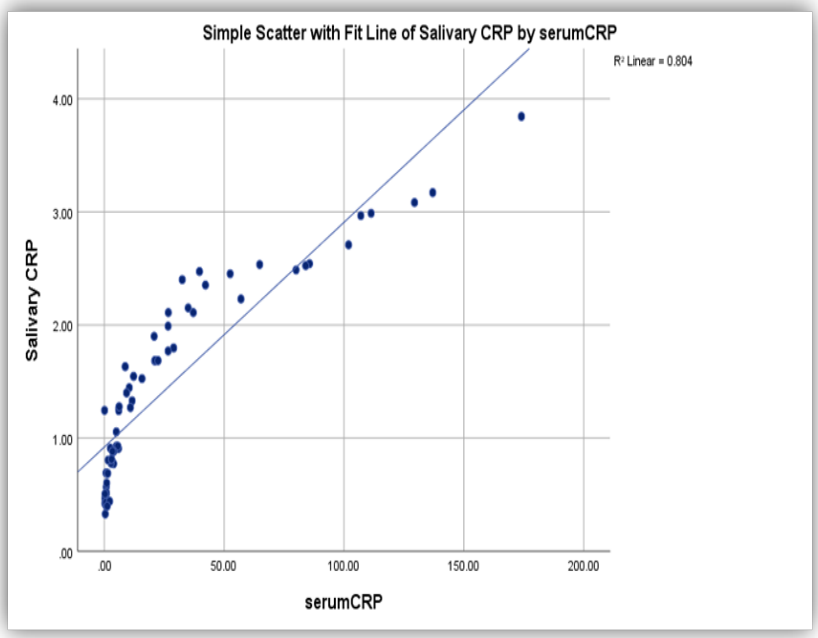
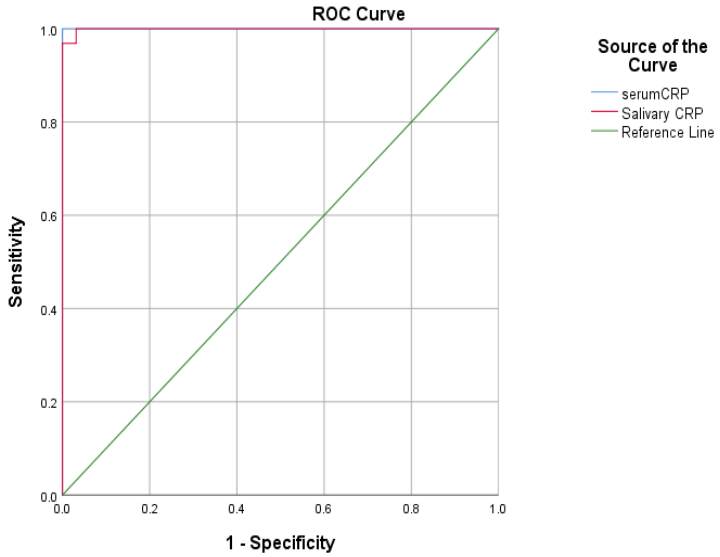


Figure (1): Correlation between serum and salivary CRP

There was a strong statistically significant positive correlation between serum and salivary CRP among cases and control.

ROC curve analysis for ability of CRP (serum vs salivary) to differentiate between cases and controls (diagnostic ability):



Test Result Variable(s)	AUC	Sig	Best cut off value	Sensitivity	Specificity	PPV	NPV
Serum CRP	**1.000**	**<0.001**	**>6.1**	**100%**	**96.9%**	**96.9%**	**100%**
Salivary CRP	**0.999**	**<0.001**	**>1.2740**	**96.9%**	**96.9%**	**96.9%**	**100%**

Using ROC curve, the AUC for salivary CRP to predict neonatal sepsis was 0.99, indicating excellent predictive validity.
Optimal salivary CRP cut off point to diagnose sepsis neonatorum was chosen to be >1.2740 with highest sensitivity and specificity.

DISCUSSION

Neonatal sepsis is the 3rd cause of death in neonatal period after prematurity and intrapartum-associated complications **(Liu et al., 2012)**. About 2.6 million neonates die annually and three-quarters of these deaths within their first week of life **(Wang et al., 2013)**. Symptoms and signs of sepsis ranging from vague non-specific up to focal or systemic organ affection. The most accurate diagnostic technique is blood culture but it needs longtime with limited sensitivity and false negative results **(Dong and Speer., 2015)**. In this study, we recruited 64 neonates. They were divided during the study into two

comparable groups; septic group and control group. In the present study, there were no statistically significant differences between the two groups in terms of the demographic variables of gestational age, gender, mode of delivery and premature rupture of membrane (PROM).

WBCs were significantly higher in septic group compared to control group, as nearly all control neonates had normal WBCs, while half of septic group had leukocytosis which might be due to secretion of cytokines and growth factors that accelerate bone marrow production especially in severe cases, while newborns with viral infection often had normal or slightly decreased WBC counts **(Ognean et al., 2017)**, while leucopenia was seen in 9.3% of our patients and is explained as a result of the effect of infection on bone marrow as it causes its inhibition from producing more neutrophils, depletion of neutrophils and increases neutrophil adhesion to the altered endothelial cells **(Bhardwaj et al., 2016)**.

Similarly, Saleh et al found that WBCs count was higher in septic group compared to normal controls as leukocytosis was seen in (48%) and leucopenia was seen in (4%) **(Saleh et al., 2017)**.

In contrary to our result, Ashour et al. showed that the mean of WBCs was higher in control group, as most of septic neonate developed leucopenia and neutropenia **(Ashour et al. et al., 2022)**.

low platelet count was seen in more than one third of our septic group, that was in line with an Ethiopian report conducted by worku et al in 2022, revealed that mean platelet count in the septic group was lower than in the control group. Sepsis related-thrombocytopenia occurred due to the effect of microbial products and endotoxins that cause platelet clumping and adherence, leading to platelet destruction **(Garg et al., 2015)**.

Among septic group, Gram Negative Bacteria (GNB) was the predominant isolate with klebsiella being the most frequently found organism.

Gram Positive Bacteria (GPB) was less commonly isolated and coagulase negative staphylococci (CONS) was the most common GPB isolate. Less commonly isolated micro-organisms were Pseudomonas, candida non albicans and MRSA were about. In accordance to these results were those discovered by the Indian research which showed that GNB were the predominant isolate and

most common isolate was Klebsiella pneumoniae **(Pay and Shaw., 2019)**.

Negative blood culture was recorded in 28% of our cases that was attributed to use of maternal antibiotics before delivery or during labor which cause false negative results or because of low level bacteremia in some neonates **(Klingenberg et al., 2018)**.

The mean serum CRP in sepsis was 51.38 mg/L which is significantly higher compared to control, it had 100% sensitivity and 96% specificity with a cut-off value of >6.1mg/L in detection of sepsis. Similarly, omran et al reported that the mean serum CRP was 43.7 (±27.7) mg/L with 74.3% sensitivity and 60% specificity in the diseased group, at a cut-off value of 10 mg/L in detection of pneumonia **(Omran et al., 2018)**.

Salivary and Serum CRP were positively correlated between two groups, we found a significant difference in salivary CRP between sepsis and control with a high sensitivity and specificity at cut off point 1.27 ng/L. It had a sensitivity 96%, specificity 96.9%.

Hence, our study showed that salivary CRP at a cutoff point 1.72 may be used as an indicator for sepsis in neonates with serum CRP > 6.1, also salivary CRP showed a strong positive correlation with serum CRP (r =.962, p = 0.001).

This goes with DATLA et al who performed a similar study in which all neonates with suspected sepsis or with perinatal risk factors for sepsis and less than 28 days of life were enrolled, the study showed that a cut off value for salivary CRP to predict serum CRP ≥10 mg/L was 0.6 ng/mL which had 77% sensitivity, 94% specificity.

In contrast with our result, Tosson et al found that median salivary CRP among clinically septic patient was 0.42 mg/L, while it was 0.23 mg/L in control group, yet, it didn't reach statistical significance.

The various gestational ages of the enrolled newborns may provide an explanation for these differences, the timing of sepsis onset, concentration of CRP was affected by oral environment as localized oral infection in addition to the absence of an established method for collecting salivary samples **(Pay and Shaw., 2019)**.

CONCLUSIONS

We concluded that salivary CRP can be used as a diagnostic test for neonatal sepsis as long as with hematological parameters. Salivary CRP could detect sepsis at a cutoff point >1.27ng/ml.

Further studies including large number of neonates are needed to validate the cut off value of salivary CRP.

RECOMMENDATION

- Salivary CRP can be used as predictor for sepsis instead of frequent blood sampling from neonates especially preterm.
- Introduction of Salivary CRP in the NICU work up.
- Training neonatologist and medical staff on sample collection can help to reduce invasive sampling side effects as well as having the same diagnostic and follow up value as serum CRP.
- Further studies on larger scale are needed to confirm salivary CRP as predictor of neonatal sepsis and also as a prognostic tool to assess when to stop antibiotic treatment.

LIMITATIONS

- Limited number of patients.
- Long-time needed for samples collection as we trained two personnel to properly collect samples.

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