ORIGINAL ARTICLE

# Investigation of salivary C-reactive protein and interleukin-18 for the diagnosis of neonatal sepsis

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**Background:** Neonatal sepsis is a leading cause of death in neonates worldwide. The investigation of biomarkers for the early diagnosis of neonatal sepsis is in progress with controversial outcomes. The current report aims to evaluate the values of salivary C-reactive protein (CRP) and interleukin-18 (IL-18) for the diagnosis of neonatal sepsis. **Materials and Methods:** In this cross-sectional study, 89 neonates, including 49 neonatal septic case and 40 healthy group admitted at the neonatal intensive care unit, were evaluated. The salivary samples of IL-18 and CRP were measured before the antibiotic therapy initiation, as soon as blood samplings. Sepsis diagnosis was confirmed by the positive blood culture. The diagnostic values of the biomarkers were determined using the receiver operating characteristic curve (ROC curve) analysis. Besides, the sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) positive likelihood ratio (LR+), negative likelihood ratio (LR-), and diagnostic accuracy were measured. **Results:** Salivary CRP level was remarkably higher in septic case than healthy group (5.2 ± 4.61 vs. 3.5 ± 1.7; $P = 0.02$), while salivary IL-18 was not different between the groups (0.1 ± 0.29 vs. 0.04 ± 0.19; $P = 0.25$). The ROC curve for IL-18 showed insignificant values ($P = 0.37$). The ROC curve of salivary CRP showed area under the curve of 0.63 (95% confidence interval: 0.51–0.74; $P = 0.03$) with the sensitivity, specificity, PPV, NPV, LR+, LR − and diagnostic accuracy of 44.9% (31.8–58.7), 80% (65.2–89.5), 73.3% (55.5–85.82), 54.2% (41.6–66.3), 60.6% (50.29–70.18), 2.24 (1.57–3.2), and 0.68 (0.63–0.75) at the cutoff of 4.55 ng/L, respectively. **Conclusion:** Based on the findings of the current study, salivary CRP can be considered a biomarker for the early diagnosis of neonatal sepsis, while no statistical values for salivary IL-18 were detected. Due to the significance of neonatal sepsis, further evaluations are strongly recommended.

**Key words:** C-reactive protein, interleukin-18, neonatal sepsis, saliva

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## INTRODUCTION

Neonatal sepsis is a systematic inflammatory response to the bacterial infection within the 1st month of life.[1] Neonatal sepsis is one of the concerning etiologies of morbidity and mortality worldwide estimated to affect 3–5 septic case per 1000 live births worldwide.

Due to the immunological deficiencies in the 1st day of life, neonates cannot respond early and adequately to the infection. Besides, the probable concomitant conditions make it difficult to quickly diagnose neonatal sepsis, and therefore, initiate its management. This challenging condition is better clarified, considering the variations in the clinical manifestations of newborn infections.[2,3] Accordingly, the early concise diagnosis of neonatal sepsis is a significant concern, and a need for a reliable marker to diagnose neonatal sepsis in early courses is making notifying sense.[4]

Although blood culture is known as the most reliable means for the definite diagnosis of infection, it is considerably time-consuming to grow microorganisms, has the potential of being contaminated, and has false-negative outcomes. In this regard, various cytokines and molecular biomarkers have been

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introduced; however, they seem far to be widely used in the near future, considering their cost-beneficence.[5,6]

C-reactive protein (CRP) is a well-known biomarker used extensively for the diagnosis and follow-up of neonatal sepsis. On the other hand, limitations such as low specificity and comprising the least duration of 3 days to increase makes the diagnosis of sepsis in early stages difficult.[7] Salivary CRP is a more novel marker investigated for the diagnosis of sepsis, and despite the studies that are in early stages, it is directly associated with the serum levels of CRP, as a well-established marker for systematic inflammation.[8] Therefore, studies regarding the detection of reliable markers for the diagnosis of neonatal sepsis are in progress.

Interleukine-18 (IL-18), a member of the IL-1 cytokine super-family, has a substantial role in the pathophysiology of sepsis and regulation of immune response. This cytokine has a crucial role in the induction of both types of T-cells in the context of the immune response.[9] There are studies in the literature representing the association of urinary IL-18 with systematic inflammatory conditions such as sepsis in adults, children, and neonates.[10,11]

CRP and IL-18 is the acute-phase reactant and theoretically rise in body liquids as saliva and can predict the inflammatory response of body against sepsis process. Obtaining inflammatory reactants such as CRP and IL-18 through noninvasive methods is preferred to invasive methods such as blood sampling especially in new-borns with lower gestational age because manipulation of new-borns can lead to severe complication such intraventricular hemorrhage and pneumothorax.

In the current study, we are aimed to investigate the values of salivary CRP and salivary IL-18 for the detection of neonatal sepsis.

## METHODS

### Study population

The current cross-sectional study has been conducted on 89 neonates with a gestational age of over 32 weeks admitted at the neonatal intensive care unit (NICU) of hospitals affiliated at Isfahan University of Medical Sciences from April 2017 to November 2018.

All of the neonates with a gestational age of 32 weeks and above admitted at the NICU and their legal guardians represented their willingness for participation in the study were included. Any cardiac or brain anomalies, asphyxia, metabolic or chromosomal disorders, and history of intrauterine gestational retardation were considered as unmet criteria. The impossibility for the follow-up of the patients or over 20% of defect in the medical records was the exclusion criteria of the current study.

The Ethics Committee of Isfahan University of Medical Sciences approved the study protocol. With approval ID: IR.MUI.MED.REC.1397.289. Then, the study protocol was entirely explained for the legal guardians of the patients, and written consent for participation in the study was obtained.

The diseased group was included nonrandomly through convenience sampling until achieving the desired number of the study population. Then, the healthy group who were admitted at NICU due to any reason were entered in the study using a convenience sampling method. The control group was similar to the septic case in terms of inclusion and exclusion criteria except for the diagnosis of sepsis.

### Diagnostic evaluations

The diagnosis of sepsis, whether early-onset sepsis or late-onset sepsis, for the case group, was made based on positive blood cultures sent to the laboratory before the initiation of antibiotic therapy. The blood cultures were reported using a cell counter device, sysmex, Germany. Besides, serum levels of CRP were measured for the diseased group, as well.

The salivary samples of IL-18 and CRP were measured before the antibiotic therapy initiation as soon as possible to the time of blood samplings. In order to maximize the similarity in the sampling, the salivary samples were taken within an hour before the neonates' feeding. Thus a skilled target neonatologist performed the sampling processes by lateral rotating the neonate's head and taking the sample from the sublingual area. One milliliter of salivary secretion was taken sterilely, and for the prevention of contamination, gathered in the polypropylene sample tube. The measurement of IL-18 and CRP was performed immediately following transmission to the laboratory, if possible, or the sample was preserved in −20°C, and the measurements were performed within the next day.

In order to minimize bias, all of the assessments were done in the laboratory of Alzahra Hospital Affiliated at Isfahan University of Medical Sciences using kits made by Hangzhou Eastbiopharm, The United States.

The measurements of salivary CRP and salivary IL-18 were performed for the control group as well.

### Statistical analysis

Data were analyzed using the SPSS software version 20 (IBM Company, Chicago, USA). Mean (standard deviation)

and frequency were used to show the continuous and categorical variables. Independent sample *t*-test and Chi-square tests were used to compare quantitative and qualitative variables between septic and neonate groups. To determine the diagnostic value of serum and salivary CRP and IL-18, indices of sensitivity, specificity, positive and negative diagnostic value (positive predictive value [PPV] and negative predictive value [NPV]), positive likelihood ratio and negative likelihood ratio (LR + and LR−) were used. Furthermore, the receiver operating characteristic curve (ROC curve) and the area under the curve (AUC) with 95% confidence interval (CI) were used to show the accuracy of serum and salivary tests. The level of statistical significance was considered 5%.

## RESULTS

In the current study, 89 participants, including 49 septic case and 40 healthy group, were assessed.

The mean gestational age of the septic case was 34.64 ± 1.43 and healthy group was 38 ± 1.66 ($P < 0.0001$). There was no statistically significant difference between septic case and healthy group in terms of gender distribution ($P = 0.699$). Table 1 represents the demographic information of the studied population in detail.

The comparison of salivary CRP and IL-18 between septic case and healthy group revealed significantly higher levels of salivary CRP in septic neonates ($P = 0.02$), but for IL-18 ($P = 0.25$) [Table 2].

Besides, the comparison of salivary versus blood levels of CRP with paired *t*-test showed higher serum levels than salivary ones in both septic and healthy neonates (4.8 ± 2.66 vs. 4.18 ± 2.76 respectively, $P = 0.05$).

In the neonatal group with sepsis, salivary and serum CRP levels increase with increasing salivary IL-18 levels, but this relationship was not statistically significant for serum CRP levels (respectively, Spearman's rho = 0.335, $P = 0.01$ and 0.115, $P = 0.45$) and there was a statistically significant relationship between serum and salivary CRP levels. (Spearman's rho = 0.657, $P < 0.0001$).

In the following, the ROC curves representing the values of salivary CRP and IL-18 are demonstrated. Based on Figures 1 and 2, the AUC for salivary and serum CRP and salivary IL-18 markers for the diagnosis of neonatal sepsis is given in Table 3. The AUC was statistically significant AUC = 0.630 ± 0.05 (95% CI: 0.51–0.74), and $P = 0.03$ for the salivary, but for IL-18 with AUC = 0.557 (95% CI: 0.43–0.67) and $P = 0.37$ [Table 3].

The diagnostic value of salivary CRP level in determining sepsis in neonates was statistically significant and the best cutoff for it was at the level of 4.55 ng/L so that sensitivity, specificity, PPV, NPV, LR+, LR − and diagnostic accuracy of the test at different levels of this marker are given in Table 4.

## DISCUSSION

Despite the overall progression in the antimicrobial therapy and life support provisions in the NICUs, neonatal sepsis is still a major etiology of morbidity and mortality worldwide.

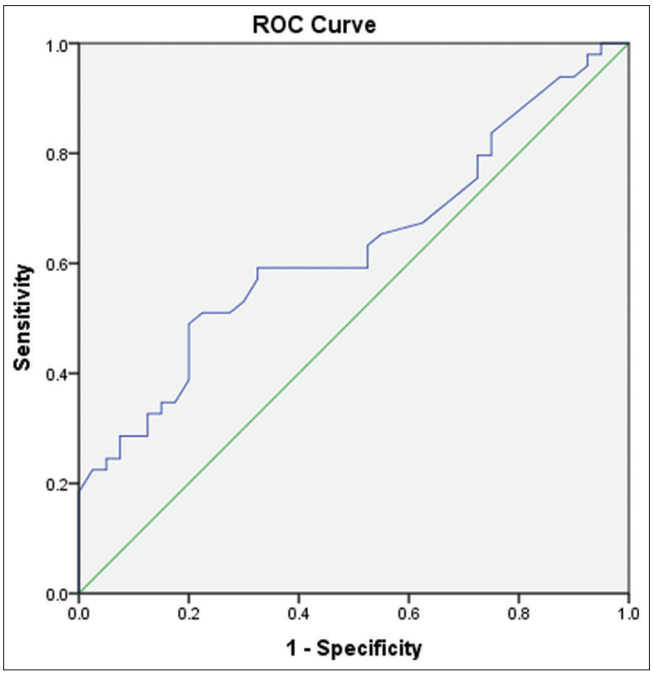


**Figure 1:** Receiver operating characteristic curve of salivary C-reactive protein for the detection of neonatal sepsis; area under the curve = 0.630, 95% confidence interval: 0.51–0.74, $P = 0.035$

**Table 1: Comparison of the demographic characteristics of the healthy and septic neonates**

| | Septic neonates | Healthy neonates | *P* |
|---|---|---|---|
| Gender, *n* (%) | | | |
| Male | 28 (57.2) | 20 (50) | 0.669[a] |
| Female | 21 (43.8) | 20 (50) | |
| Body birth weight (g), mean±SD | 2038.02±404.04 | 3052.5±471.41 | <0.0001[b] |
| Gestational age (week), mean±SD | 34.64±1.43 | 38±1.66 | <0.0001[b] |
| Admission duration (day), mean±SD | 20.72±13.27 | 1.72±0.64 | <0.0001[b] |

[a]Chi-square test, [b]Independent sample *t*-test. SD=Standard deviation

**Table 2: Comparison of C-reactive protein and interleukin -18 between the neonates with sepsis and healthy group**

| | Septic patients | Healthy group | *P** |
|---|---|---|---|
| CRP (ng/L) | 5.2±4.61 | 3.5±1.7 | 0.02 |
| IL-18 (pg/ml) | 0.1±0.29 | 0.04±0.19 | 0.25 |

*Independent *t*-test. CRP=C-reactive protein; IL-18=Interleukine-18

**Table 3: The area under the curve for salivary and serum C-reactive protein and salivary interleukine-18 for the neonatal sepsis diagnosis**

| Diagnostic biomarker | AUC | SE | *P* | 95% CI (lower-upper) |
|---|---|---|---|---|
| Salivary CRP | 0.630 | 0.05 | 0.03 | 0.51-0.74 |
| Serum CRP | 0.144 | 0.05 | 0.22 | 0.04-0.24 |
| IL-18 | 0.557 | 0.06 | 0.36 | 0.43-0.67 |

CI=Confidence interval; CRP=C-reactive protein; IL-18=Interleukine-18; AUC=Area under the curve; SE=Standard error

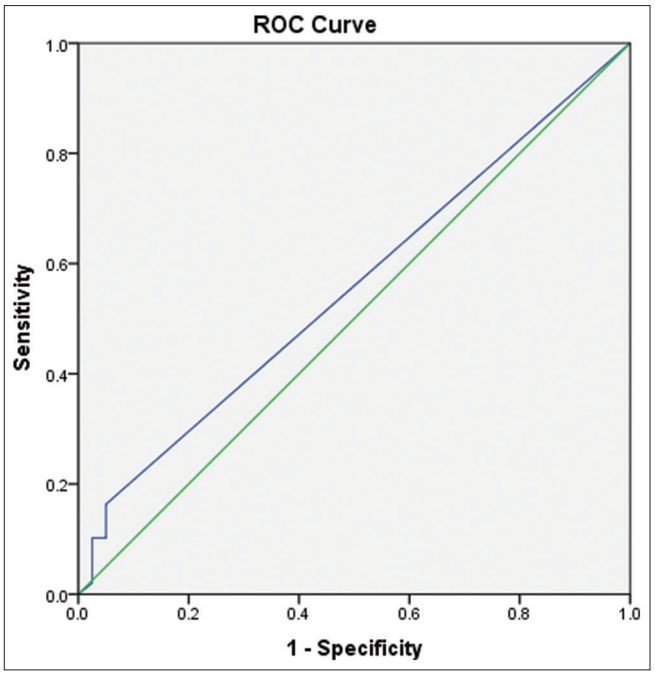


**Figure 2:** Receiver operating characteristic curve of salivary interleukin -18 for the detection of neonatal sepsis; area under the curve = 0.557, 95% confidence interval: 0.43–0.67, *P* = 0.36

The early and definite diagnosis of sepsis plays a substantial role in the prevention of the abundant useless antibiotics administration and also the prevention of morbidities and mortality incidence.[12] On the other hand, sepsis signs and symptoms mimic other neonatal-related complications such as apnea of prematurity, transient tachypnea, meconium aspiration syndrome, respiratory distress syndrome, and acute exacerbation of chronic lung disease that are indistinguishable from early presentations of sepsis. Besides, hesitate to initiate antimicrobial therapy in order to diagnose sepsis definitely may cause deterioration of sepsis, leading to irrecoverable complications such as intravascular coagulation.[13] Accordingly, achieving a reliable biomarker for the early diagnosis of sepsis is crucial; however, yet we have not achieved a precise biomarker for early and definite diagnosis of neonatal sepsis.[14]

To the best of our knowledge, the current presentation is the first one assessing the values of salivary interleukin -18 and among the limited ones assessing salivary CRP for the diagnosis of neonatal sepsis. In this regard, we found significantly higher levels of salivary CRP among the septic neonates as compared to the healthy ones, while the outcomes for IL-18 were not statistically different. Further evaluations showed a significant direct association between the salivary levels of CRP with serum levels of this biomarker, as the most popular marker for the diagnosis of systematic inflammation in the body.[13]

The other investigation of our study revealed insignificant predictive values for IL-18 ($P > 0.05$), while salivary CRP had the significant value for the prediction of sepsis ($P = 0.035$; 95% CI: 0.51–0.74), with sensitivity and specificity of 49% and 80% at the cutoff level of 4.55 ng/L, respectively.

CRP is a positive reactant protein produced by hepatic tissue in response to trauma, cellular damage, tissue injury, and infection. The first traces of CRP increase would appear within 6 h, while its peak levels are detectable within 48–72 h and remain high until infection resolved.[15,16] The statistically higher serum levels of CRP among the septic neonates in comparison to healthy ones have been reported previously;[17] however, there are studies in which normal levels of this biomarker have been represented, while the blood culture was positive in neonates, low birth weight (LBW) ones in particular.[18] In general, studies in terms of serum CRP have demonstrated the wide ranges of sensitivity and specificity, ranging from 35% to 94% and 60%–96%, respectively.[19] The ranges are consistent with our findings; however, they are achieved through blood sampling, an invasive procedure for LBW neonates, while our outcomes are derived from salivary samples.

Similar to our study, the direct association of salivary CRP with serum levels of CRP, as the major inflammatory marker, has been represented in the literature. Iyengar *et al*. reported a slight but significant direct correlation between the measured salivary CRP and the serum levels of CRP among neonates with sepsis, whether new-onset, or late-onset, or in general.[8] This correlation has been shown by Gutiérrez *et al*.,[20] Dillon *et al*.,[21] and Ouellet-Morin *et al*.,[22] as well. This direct correlation of salivary CRP levels with its serum levels on a hand and its rapid elevation in the sepsis only within 6 h better clarifies the value of salivary CRP for the early detection of sepsis.

Similar to our report, there are other studies in the literature representing the notifying values for the salivary CRP to

**Table 4: Assessment of the diagnostic value of different levels of salivary C-reactive protein for the neonatal sepsis diagnosis**

| Diagnostic biomarker | Cut-off (n/L) | Parameter (lower-upper 95% CIs) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Diagnostic accuracy (%) | LR+ | LR− |
| CRP | ≥2.05 | 16.3 (8.51-29.04) | 75 (59.81-85.81) | 44.4 (24.56-66.28) | 42.2 (31.45-53.85) | 42.7 (32.93-53.07) | 0.65 (0.15-2.78) | 1.11 (1.04-1.19) |
| | ≥2.95 | 34.7 (22.92-48.6) | 55 (39.83-69.29) | 48.5 (32.99-64.43) | 40.7 (28.68-54.03) | 43.8 (33.98-54.17) | 0.77 (0.55-1.06) | 1.18 (1.03-1.35) |
| | ≥3.75 | 40.8 (28.22-54.7) | 40 (26.35-55.4) | 45.4 (31.71-59.93) | 35.5 (23.22-50.16) | 40.4 (30.85-50.84) | 0.68 (0.54-0.85) | 1.48 (1.15-1.90) |
| | ≥4.55 | 44.9 (31.8-58.7) | 80 (65.2-89.5) | 73.3 (55.5-85.82) | 54.2 (41.6-66.3) | 60.6 (50.29-70.18) | 2.24 (1.57-3.2) | 0.68 (0.63-0.75) |
| | ≥6.25 | 71.4 (57.59-82.15) | 10 (3.958-23.05) | 49.3 (38-60.66) | 22.2 (9-45.22) | 43.8 (33.98-54.17) | 0.79 (0.73-0.85) | 2.85 (0.03-270.4) |

CI=Confidence interval; CRP=C-reactive protein; PPV=Positive predictive value; NPV=Negative predictive value; LR+=Positive likelihood ratio; LR−=Negative likelihood ratio

detect neonatal sepsis. Iyengar *et al*. conducted a study in order to assess the values of salivary CRP for the diagnosis of sepsis. Therefore, salivary samples were taken from 40 neonates with positive blood cultures. The samples were primarily taken prior to the antibiotic initiation, and they found remarkable sensitivity and specificity of 64% and 94% at cut-off levels of 10 mg/L of salivary CRP levels. In addition, they found a statistically significant association of neonatal sepsis with salivary CRP levels at the cut-off of 5 mg/L with sensitivity and specificity of 54% and 95%, as well.[8] In this term, a similar study was conducted by Omran *et al*. in Egypt in 2018. They assessed 70 full-term neonates, among which 35 ones were healthy, 20 ones had positive blood cultures, and sepsis diagnosis was made clinically for 15 remained ones. In this study, they declared significant specificity and sensitivity of 93.4% and 80% at the cutoff of 3.48 ng/L, respectively.[23]

A few studies have assessed the use of interleukin-18 for the diagnosis of critical illnesses. Besides, most of the researchers have assessed the values of urinary or serum, but salivary ones. These studies have shown the sharper rise in the interleukine-18 levels than CRP, which shows the merit of IL-18 for early detection of sepsis.[4] Wagner *et al*.[24] and Kingsmore *et al*.[25] have separately used serum levels of IL-18 as the marker for the early diagnosis of neonatal sepsis, and unanimously declared the value of this biomarker for the detection of sepsis. Moreover, Cui *et al*. not only represented elevated serum levels of IL-18 among critically ill adults, but they also found elevated plasma miRNA expression of IL-18, as well.[26]

Li *et al*. conducted a study on 111 neonates divided into two groups of septic case versus healthy group. Contrary to our findings, they represented considerably increased levels of elevated urinary interleukine-18 among the septic neonates as compared to healthy group, while the limitation of their study was the small number of septic case as compared with the healthy group.[11]

Higazi *et al*. conducted their study to evaluate the best cut-off for the urinary IL-18 for the early detection of neonatal sepsis. In their study, the cut-off of 8.85 μg/ml of urinary IL-18 had the remarkable sensitivity of 91.2% and specificity of 100%.[13]

In summary, in our investigation, the salivary CRP was significantly higher among septic neonates than healthy ones, the salivary CRP was directly correlated with its serum levels, and eventually, salivary CRP levels at the cutoff of 4.55 ng/L had the prognostic values for early diagnosis of sepsis. Our findings were consistent with a few numbers of studies in this regard that represented statistical cutoffs for salivary CRP at diverse levels. On the other hand, salivary

interleukin -18 was not considerably different among septic case versus healthy ones. Contrasting results were presented by other studies using urinary and serum interleukin -18.

CRP is the acute phase reactant and theoretically rise in body liquids as saliva and can predict the inflammatory response of body against sepsis process.

One of the limitations of this study was related to the sample size, which was too small to be generalizable to the entire community, and further studies should be conducted with larger sample sizes.

Another limitation was the lack of measuring serial of salivary CRP. As the current study was the first one investigating salivary IL-18, further studies are recommended.

## CONCLUSION

Based on the findings of the current study, salivary CRP at the level of 4.55 ng/L has the statistical values for the diagnosis of sepsis in LBW neonates with the sensitivity of 49% and specificity of 80%, while no statistical values of salivary IL-18 for the early diagnosis of sepsis was detected.

This study represents elementary evidence about usefulness of salivary CPR combined with serum CRP in the diagnosis of neonatal sepsis, although these data need to be verificated by another studies with larger numbers of newborn.

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### Conflicts of interest

There are no conflicts of interest.

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