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Original Article

Diagnostic Utility of Serum Amyloid A and Salivary C-Reactive Protein in Diagnosis of Late Onset Sepsis in Neonates. A Prospective Case Control Study

Nagwa M. Sabry Mahmoud[1*], Gamal Baheeg Mohamed[1], Nagwa Ismail Okaily[2], Manar Anwar Abd-Elaziz[1], Nageh Shehata Ismail[1]

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***Correspondence**: Department of pediatrics, Faculty of Medicine, Minia University, Egypt
Email: dr_nagwa163@ mu.edu.eg
Full list of author information is available at the end of the article

Abstract

Background: Neonatal sepsis is a global mortality risk, necessitating early identification and use of reliable biomarkers, despite ongoing controversy surrounding their use. **Objectives:** The study aimed to evaluate the values of salivary C-reactive protein (CRP) and serum amyloid A (SAA) for the diagnosis of neonatal sepsis. **Patients and methods:** The study involved 80 neonates with late-onset sepsis (LOS) and 40 healthy controls from June 2022 to October 2023. They underwent a detailed perinatal history, physical examination, complete blood count (CBC), blood culture, salivary CRP, SAA, and comprehensive metabolic panel (CMP) included an electrolyte panel, renal and liver function tests, glucose and calcium. We evaluated salivary CRP and SAA before initiating antibiotic therapy. A positive blood culture confirmed the diagnosis of sepsis. We determined the biomarker diagnostic values using receiver operating characteristic curve (ROC curve) analysis and assessed their sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and diagnostic accuracy. **Results:** Significant differences between cases and controls regarding salivary CRP, serum CRP, and SAA levels (p value <0.001) were present. Cases had higher mean salivary CRP (1.6) and SAA (26.4) levels than controls (0.16) and (3.1) respectively. Additionally, cases had a significantly higher mean serum CRP level (53.4) compared to controls (0.9). A moderately positive correlation was found between salivary CRP, serum CRP, and SAA (r = 0.70), with a significant p value <0.001. **Conclusion:** The study suggests salivary CRP can be a valuable noninvasive biomarker for late-onset neonatal sepsis diagnosis, with comparable results to SAA and serum CRP.

Key words: Salivary CRP, serum amyloid A, neonatal sepsis, biomarkers, newborn.

Introduction

Neonatal sepsis is a bloodstream bacterial infection causing severe clinical symptoms, often leading to death or long-term deficits. Death can occur in 3–4% and up to 24% of neonates born in industrialized countries. [1] and in the developing world respectively [2] with long-term neurodevelopmental issues such as cerebral palsy and vision impairment among survivors.[3] Neonatal sepsis is classified as early-onset sepsis (EOS) if diagnosed within 72 hours after birth or late-onset sepsis (LOS) if diagnosed after this period. [4] EOS is primarily caused by vertical transmission of Escherichia coli and Group B Streptococcus from women with chorioamnionitis, prolonged membrane rupture, and GBS colonization. Pathogens acquired nosocomially often cause LOS in neonates at risk due to prematurity, invasive instrumentation, parenteral nutrition, and mechanical ventilation. [5,6] In developed countries, the incidence of EOS and LOS is no lower than 0.3–0.8 cases per 1000 live births, and in developing countries, it is about 6 cases per 1000 live births. [7-9] Factors such as birth weight and gestational age also influence the risk of neonatal sepsis development. [10,11] However, studies have revealed that only 53% of enrolled infants identify EOS, leaving many documented cases of neonatal sepsis undetected specially in preterm neonates. [12,13] Neonates have an immature immune system, increasing their risk of infection, as well as varying inflammatory and clinical responses to infectious agents. This explains why the criteria used to define sepsis in adults do not apply to neonates. [14] Early-stage symptoms of sepsis in neonates are subtle and non-specific, often common to other conditions; regardless of, whether they are infectious, metabolic, or traumatic. [15,16] Therefore, the definition of sepsis in neonates is still lacking, and several scientific institutions have suggested specific criteria for

proper identification. We consider these parameters and child conditions to create a risk calculator that identifies which children require treatment. A risk calculators has been created by considering many parameters and conditions of the neonates to determine the need for treatment of neonatal sepsis . [17,18] Antibiotics are the primary treatment for sepsis, but their overuse in neonates has led to various problems, including the emergence of antimicrobial resistance and the promotion of dysbiosis. [19-23] Despite the introduction of stewardship programmes, antibiotic overuse in neonates persists and can lead to the development of life-long health problems. This overuse can lead to life-long health issues such as obesity, type I diabetes, asthma, autism spectrum disorders, necrotizing enterocolitis, and earlier death. [24-26] Early diagnosis of neonatal sepsis can improve outcomes and reduce antibiotic use. An ideal marker should have high sensitivity, specificity, and predictive values, provide reliable antibiotic therapy information, reduce overuse, prevent bacterial resistance, and be simple to perform, cost-effective, and comparable across laboratories.[27] Neonatal sepsis diagnosis traditionally relies on positive blood cultures, but these have long turnaround times and low sensitivity, leading to inappropriate antibiotic therapy. Invasive procedures and inoculant volume condition blood cultures, leading to negative results. [28,29] Advances in molecular methods like polymerase chain reaction (PCR), real-time PCR, pyrosequencing, and microfluidic technology have improved sensitivity and specificity but require specialised biology laboratories and equipment, which are not available in many hospital settings, particularly in the third world. [30,31] A lot of research has looked at white blood cell count (WBC), absolute neutrophil count (ANC), immature-to-total neutrophil ratio (I/T), and platelet count as possible signs of sepsis in newborns. [32-38] The

widespread use of these tests stems from their simplicity, cost-effectiveness, and the absence of advanced laboratory equipment. However, most studies have limitations in design, sample size, and sepsis case definition, limiting the reliability of the results. [36] Maternal and neonatal factors can significantly modify these indices, making differentiation between infected and non-infected babies difficult. [36] Identifying neonatal sepsis markers could improve early diagnosis, improve outcomes, and reduce antibiotic use. Researchers have developed several cytokines and molecular biomarkers for this purpose over the past 30 years, but their widespread use is unlikely due to their high cost and limited benefits. Developing biomarkers could improve the long-term outcomes of sepsis cases. [37,38] CRP is a widely recognized biomarker that is extensively used for diagnosing and monitoring newborn sepsis. Identifying sepsis in its early stages, however, is challenging due to constraints such as low specificity and a minimum length of 3 days to rise. [39] Salivary CRP is a recently studied measure for diagnosing sepsis. Although research on this marker is still in its early stages, it directly correlates with serum levels of CRP, which is a well-established sign of systemic inflammation. [40] Consequently, researchers are conducting ongoing research to identify reliable indicators for diagnosing newborn sepsis. serum amyloid A (SAA), is an apolipoprotein, produces by the liver under the precise control of IL-1, IL-6, and TNF-a in response to inflammation. [41] In healthy newborns, the usual range for SAA is between 3.2 and 3.4 mg/dL. However, in cases of NS, the concentration is significantly higher, at 44.4±57.3 mg/dL.[42] Multiple studies have suggested that SAA can serve as a biomarker for EOS, demonstrating promising diagnostic capabilities for risk stratification. [43] The study aims to evaluate the diagnostic utility of salivary

CRP as a noninvasive biomarker in neonatal sepsis, its potential use for follow-up, and its accuracy in diagnosing and predicting sepsis. It also assesses the level of SAA in neonates with sepsis, as well as its value in diagnosing sepsis and predicting culture positivity in cases diagnosed with sepsis.

Patients and Methods

This was a case-control study that included 80 neonates (both preterm and full term), 40 of whom had a diagnosis of neonatal sepsis (late-onset sepsis) admitted to the neonatal intensive care unit (NICU) at Minia University Hospital as cases, and 40 apparently healthy, age- and sex-matched neonates as controls . 40 cases were full-term, and 40 cases were preterm neonates. The study took place between June 2022 and October 2023. History, clinical findings, laboratory findings, and blood culture determined the diagnosis of neonatal sepsis. This study included 40 neonates, divided into cases and control groups. There were 20 full-term and 20 preterm neonates, all with late-onset sepsis . The control group consisted of 40 healthy neonates age-matched with cases who were chosen as any normal healthy newborn, of both genders, of any mode of delivery who attended the hospital for regular follow-up in the well-baby clinic or were admitted to the nursery postnatally with their mother for routine care. In the study, the Minia University neonatal intensive care unit admitted neonates born after 27 weeks gestation and less than 28 days old for sepsis, or those who developed sepsis within the first 28 days of life. The study excluded individuals with known immune system problems in the mother, mothers on immune-modulating drugs, high doses of corticosteroids, or chemotherapy, newborns with potential cancer, oral infections, hypoxia, oral ulcers, metabolic disease, mechanical ventilation, post-surgery cases, and parents who preferred not to have their child in the hospital. The case group had a complete perinatal history, physical

examination, and investigations related to sepsis, such as a blood count, serum CRP, blood culture, salivary CRP, and SAA. They also had routine blood chemistry and renal function tests. on tests. The control group will undergo a comprehensive perinatal history, physical examination, salivary CRP, and SAA.

Sampling:

Blood samples: To prevent frequent sampling, we collected blood samples during routine investigations. For a complete blood count, we collected one mL in tubes containing EDTA as an anticoagulant. We collected three mL samples for serum CRP, renal function, electrolyte, and SAA in plain tubes, centrifuged them, and stored them at -20 °C.

Salivary samples: To avoid contamination with milk, the study collected salivary samples from neonates. The process involved tilting the head forward to draw saliva into the mouth, as well as connecting a syringe to low-wall suction. We collected saliva from gingival crevices and under tongues, which took 30–45 seconds. Reterm neonates required longer collection times. We placed the samples in Eppendorf tubes to prevent contamination. We centrifuged the samples at 3000 rpm for 15 minutes after collection and stored the supernatant at -20 °C until batch analysis.

Methodology: The study involved a blood count using Celltac G, a Japanese hemocytometer, and a microscopic examination of a blood film. We performed blood cultures using Bactec and analysed serum C-reactive protein using GENRUI. We measured serum amyloid A using ELISA.

Assay procedure for SAA The BT LAB prepared reagents, standard solutions, and samples for an assay. We stored the strips at a temperature of 2–8 °C, then added the standard to the standard wells, samples to the sample wells, anti-SAA antibody to the sample wells, and streptavidin-HRP to both wells. We incubated the plate for 60 minutes at

37°C, washed it five times, and soaked it for 30 seconds to 1 minute. We added substrate solutions A and B to each well.

BT LAB in China measured salivary CRP using ELISA. The assay involved preparing reagents, standard solutions, and samples and incubating them at 37 °C for 60 minutes. We added a standard and sample first, then added an anti-CRP antibody and streptavidin-HRP after that. We washed, soaked, and blotted the plate. SAfter adding substrate solutions A and B, we incubated the plate in the dark for 10 minutes. top solution was added, and the optical density (OD value) was determined using a microplate reader set to 450 nm.

Ethical consent

The study involved neonates' parents, who provided written consent. Minia University's Institutional Review Board and Medical Ethics Committee approved it with approval number 208:1/2022, adhering to the revised Declaration of Helsinki 1975. We conducted the study in accordance with ethical standards.

Data management and analysis:

We collected, tabulated, and analysed the data using the SPSS-26 programme for Windows. The Shapiro Walk test was used for normal distribution, and quantitative results were presented as mean±SD. We presented qualitative data as numbers and percents. We used the Student's t test for comparison, and employed Spearman's test and Pearson's correlation coefficient for correlations. We performed ROC curve analysis for the prediction of neonatal sepsis.

Results

As demonstrated in Table 1, The study involved 62.6% females and 37.5% Was male with a mean gestational age of 36±2.3. Most cases (82.5%) improved, and 17.5% died. All septic cases had late-onset sepsis, with clinical signs including fever, tachycardia, decreased sucking, apnea, and oxygen need. 32.5% of patients had post-mechanical ventilation, and 45% had a central venous catheter (CVC) via internal jugular vein.

Table 2 The cases had a higher mean salivary CRP (1.6 ng/ml), higher serum amyloid levels (26.4 µg/ml), and higher mean serum CRP levels (53.4 mg/L) compared to the control 0.16 ng/ml , 3.1 µg/ml and 0.9 mg/L recpectively.

The cases had a higher mean TLC (15.8 ×103 / µl)) than the control (11.0 ×103 / µl)) and lower mean levels of hemoglobin and platelets (13.2 g/dl and 166 x103/ µl , respectively) than the control (16.7(g/dl and 264, x103/ µl respectively). Table 3 shows the correlation between serum CRP and both salivary CRP and SAA ,there is a moderate positive correlation between salivary CRP and serum CRP (r =0.50); also, a moderate positive correlation was found between serum CRP and SAA (r = 0.70), with a significant p value (0.001). As demonstrated in Table 4, there are statistically significant difference between culture positive cases, clinical septic cases and control regarding salivary CRP, serum CRP, SAA and outcome (p value <0.001). In Table (5) Figures (1,2,3): sensitivity and specificity of salivary CRP for prediction of neonatal sepsis was 100% and 97.5% respectively when level was more than 0.31ng/ml, with significant p value <0.05, also sensitivity and specificity of serum CRP for prediction of neonatal sepsis was 92.5% and 95% respectively when level was more than 9mg/L. When cut off value of serum amyloid more than 4.6µg/ml, it will have a sensitivity and specificity of 100% and 95% respectively for prediction of neonatal sepsis. With significant p value<0.001.

Table 6 and Figure 4 demonstrate that salivary CRP with level >0.65 has a sensitivity and specificity of 97% and 95% respectively for prediction if serum CRP >10.

Table 7 and Figures 5 and 6, showed that salivary and serum CRP can accurately predict culture-positive cases 90% of the time when serum amyloid levels are above 0.96 mg/L and 80% of the time when they are above 18 mg/L, with a p value less than 0.05 .

Table 8 demonstrates that, there are non-statistically significant difference between preterm babies and full term babies regarding salivary CRP, serum CRP and serum amyloid (p value>0.05) as mean salivary CRP was (1.4ng/ml) on preterm neonates compared to (1.7ng/ml) in full term neonates, also mean serum amyloid and serum CRP was lower in preterm (24.5µg/ml and 47mg/L respectively) than in full term babies (28.3 µg/ml and 60mg/L respectively).

As shown in Table 9: The study revealed that 50% of septic patients had no growth, while the remaining 50% had positive blood cultures, predominantly gramme-negative (37.5%). Klebsiella pneumoniae (30%) was the most common organism, followed by MRSA and Acinetobacter. Only two cases tested positive for Candida albicans and CONS.

Table (1): Demographic data of the septic group

Demographic data. (N=40)		**Descriptive statistics(N-%)**
Gestational Age	Mean ± SD	36 ± 2.3
	Median (Range)	36.5 (30:40)
	>37 weeks GA, n (%)	20 (50%)
	34-36 weeks GA, n (%)	13 (32.5%)
	32-33weeks GA, n (%)	5 (12.5%)
	<32 weeks GA, n (%)	2 (%)
Sex	Male	15 (37.5%)
	Female	25 (62.6%)
Birth weight	Mean ± SD	2± 0.65
	(Range)	1.1:3.60
	>2500	13 (25%)
	1500-2499	19 (47.5%)
	1000-1499	8 (20%)
Delivery type	NVD	27 (67.5%)
	CS	13 (32.5%)
Post natal age at which test performed	Mean ± SD	11.1 ± 3.6
	Median (Range)	10 (6:20)
Sepsis	Late onset	40(100%)
	Other	0
Outcome	Died	7 (17.5%)
	Improved	33 (82.5%)
Post mechanical ventilation	Yes	13 (32.5%)
	No	27 (67.5%)
CVC	Yes	18 (45%)
	No	22 (55%)
Fever	Yes	14 (35%)
	No	26 (65%)
Tachycardia	Yes	12 (30%)
	No	28 (70%)
Decreased sucking	Yes	25 (62.5%)
	No	15 (37.5%)
Apnea	Yes	16 (40%)
	No	24 (60%)
Need for oxygen	Yes	23 (57.5%)
	No	17 (42.5%)

CVC: central venous catheter, NVD: Normal vaginal delivery, CS: Caesarean section, SD: standard deviation.

Table (2): Comparison between cases and control as regard SAA, salivary CRP, serum CRP, HB, platelet and TLC.

Item	**Cases (n=40)**	**Control (n=40)**	**P value**
Salivary CRP (ng/ml)			
▪ Mean ± SD	1.6 ±0.7	0.16±0.6	<0.001*
▪ Median (Range)	1.6(0.6:3)	0.15 (0.1:0.32)	
SAA (µg/ml)			
▪ Mean ± SD	26.4 ±11.2	3.1±0.8	<0.001*
▪ Median (Range)	26.7 (10.9 :49.5)	3 (1.5:4.8)	
Serum CRP (mg/L)			
▪ Mean ± SD	53.4 ±32	0.9±2.5	<0.001*
▪ Median (Range)	48 (0:96)	0 (0:12)	
Hb (g/dl)			
▪ Mean ± SD	13.2±3.1	16.7±1.5	<0.001*
▪ Median (Range)	12.8 (5.3:21)	16.8 (12.4:19.4)	
TLC (×103 / µl)			
▪ Mean ± SD	15.8±9300	11.0±2500	0.003*
▪ Median (Range)	14.0 (2800:47000)	11.0(6600:16300)	
Platelet (x103/ µl)			
▪ Mean ± SD	166±100	264.3±56.5	<0.001*
▪ Median (Range)	160 (11:400)	259 (182:410)	

Significant at p value<0.05, SD:Standard deviation, SAA: Serum Amyloid A, Hb : Hemoglobin
TLC: Total Leukocytic Count

Table (3): Correlation between serum CRP and both serum amyloid A and salivary CRP in septic group.

Correlation	**Serum CRP (mg/L)**	
	R	**P value**
Salivary CRP (ng/ml)	0.50	0.001*
SAA (µg/ml)	0.70	0.001*

* Significant at p value<0.05 - CRP: C-reactive protein

Table (4): Comparison between culture positive (n=20), clinical sepsis (n=20), and control (n=40)] as regard SAA, salivary CRP, serum CRP and outcome.

Item	**Culture positive (n=20)**	**Clinical sepsis (n=20)**	**Control (n=40)**	**P value**		
Salivary CRP				<0.001*		
				P1	**P2**	**P3**
(ng/ml)	1.8 ±0.5	1.3 ±0.7	0.16±0.6	0.01*	<0.001*	<0.001*
Mean ± SD	1.9 (0.6:2.8)	1 (0.7:3)	0.15(0.1:0.3)			
Median (Range)						
SAA (µg/ml)				<0.001*		
Mean ± SD	33.7 ±7.8	19.2 ±9.2	3.1±0.8	**P1**	**P2**	**P3**
Median (Range)	33.2(16 :49.5)	14.5(10.9:46)	3(1.5:4.8)	<0.001*	<0.001*	<0.001*
Serum CRP				<0.001*		
(mg/L)	52.2 ±35	54.6 ± 40	0.9±2.5	**P1**	**P2**	**P3**
Mean ± SD	48(0:96)	48(12:96)	0(0:12)	0.94	<0.001*	<0.001*
Median (Range)						
Outcome						
Died	4(20%)	3(15%)	0	0.01*		
Improved	16(80%)	17(85%)	40(100%)			

* significant at p value<0.05.
P1 mean p value between culture positive group and clinical sepsis.
P2 mean p value between culture positive group and control.
P3 mean p value between clinical sepsis and control.
SD: standard deviationSAA: Serum Amyloid A, CRP: C-reactive protein .

Table (5): ROC curve analysis of salivary CRP, serum CRP and SAA for prediction of neonatal sepsis.

Item	**Salivary CRP (ng/ml)**	**Serum CRP (mg/L)**	**Serum amyloid (µg/ml)**
Optimal cut off point	>0.31	>9	>4.6
AUC	1	0.95	1
P value	<0.001*	<0.001*	<0.001*
Sensitivity	100%	92.5%	100%
Specificity	97.5%	95%	95%
PPV	100%	92.5%	100%
NPV	97.5%	97.5%	92.5%

* Significant at p value<0.05, ROC curve: receiver operating characteristic curve, AUC: area under curve, CRP: C-reactive protein, PPV: positive predictive value, NPV: negative predictive value.

Table (6): ROC curve analysis of salivary CRP for prediction if serum CRP ≥10 mg/L.

Item	Salivary CRP (ng/ml)
Optimal cut off point	>0.65
AUC	0.94
P value	<0.001*
Sensitivity	97%
Specificity	95%
PPV	97%
NPV	95%

ROC curve: receiver operating characteristic curve, AUC: area under curve, PPV: positive predictive value, NPV: negative predictive value, CRP: C-reactive protein

Table (7): ROC curve analysis of salivary CRP, serum CRP and SAA for prediction of culture positive neonatal sepsis.

Item	Salivary CRP (ng/ml)	Serum CRP (mg/L)	SAA (µg/ml)
Optimal cut off point	>0.96	>18	>4.4
AUC	0.90	0.77	0.96
P-value	<0.001*	<0.001*	<0.001*
Sensitivity	90%	85%	100%
Specificity	80%	69%	60%
PPV	90%	85%	100%
NPV	80%	69%	60%

* Significant at p value<0.05, ROC curve: receiver operating characteristic curve, AUC: area under curve, PPV: positive predictive value , NPV: negative predictive value, SAA: Serum Amyloid A , CRP : C-reactive protein

Table (8): Comparison between full term and preterm neonates regarding salivary CRP, SAA and serum CRP

Item	Preterm group (n=20)	Full term group (n=20)	P value
Salivary CRP (ng/ml)			
Mean ± SD	1.4 ±0.6	1.7 ±0.7	0.26
Median (Range)	1.25 (0.72:2.8)	1.9 (0.6:3)	
SAA (µg/ml)			
Mean ± SD	24.5 ±13.7	28.3 ±7.8	0.22
Median (Range)	19 (10.9 :49.5)	30.5 (12:40.5)	
Serum CRP (mg/L)			
Mean ± SD	47 ±29.5	60 ± 34.3	0.23
Median (Range)	48 (0:96)	48 (0:96)	

SAA: Serum Amyloid A , CRP : C-reactive protein

Table (9): Causative organisms in positive blood culture group

Blood culture (N=40)	Descriptive statistics (N%)
Gram negative n, (%)	15 (37.5%)
Klebsiella pneumoniae	12 (30%)
Acinetobacter	3 (7.5%)
Gram positive n, (%)	4 (10%)
MRSA	3 (7.5%)
CONS	1 (2.5%)
Candida Albicans	1 (2.5%)
No growth	20 (50%)

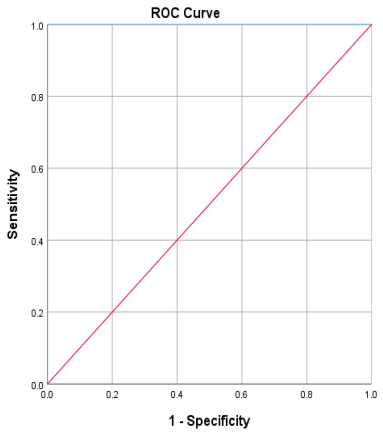

Figure (1): ROC curve analysis of salivary CRP for prediction of neonatal sepsis.

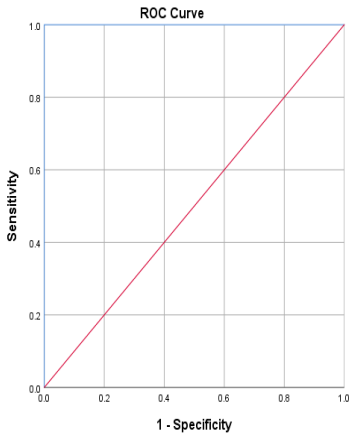

Figure (2): ROC curve analysis of serum amyloid for prediction of neonatal sepsis.

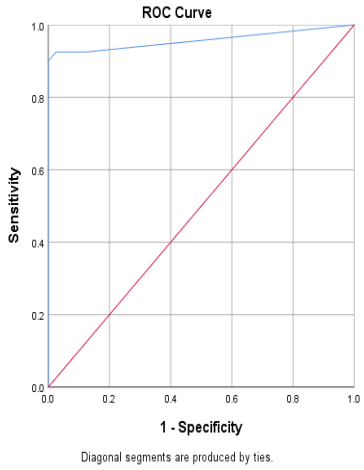

Figure (3): ROC curve analysis of serum CRP for prediction of neonatal sepsis.

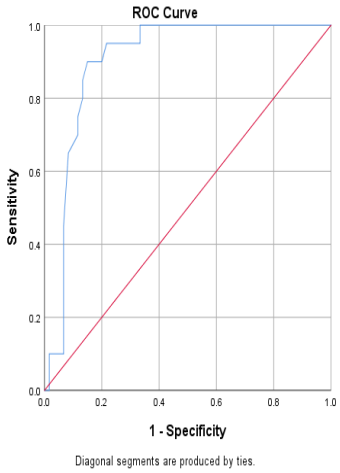

Figure (4): ROC curve analysis of salivary CRP for prediction of positive culture sepsis.

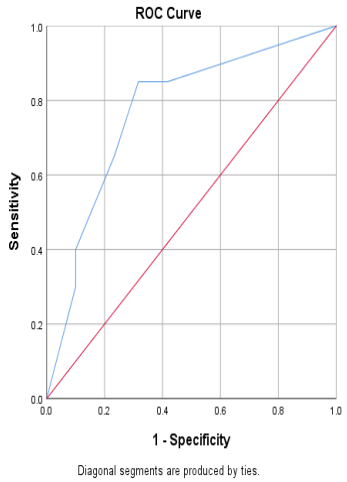

Figure (5): ROC curve analysis of serum CRP for prediction of positive culture sepsis.

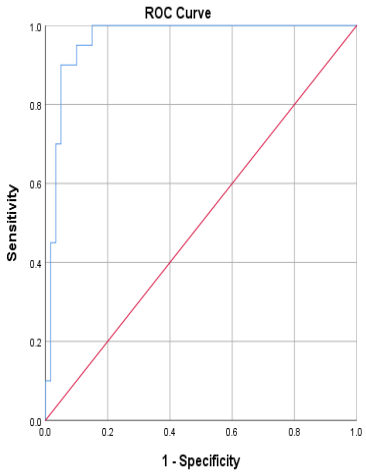

Figure (6): ROC curve analysis of serum amyloid for prediction of positive culture sepsis.

Discussion

The identification of biomarkers for early neonatal sepsis could improve immediate and long-term outcomes, reduce the use of prophelaxis antibiotics, and reduce the risk of infection, pain, and anaemia in sick hospitalised neonates, who often require frequent blood sampling. Our study involved 80 neonates, with a mean gestational age of 36±2.3 and a birth weight of 2± 0.65 kg, with 62.6% being females, and Forty healthy sex and age-matched babies selected as a control group.The study found that all cases had late-onset sepsis, with 35% experiencing fever, 12% having tachycardia, 25% having decreased sucking, 16% having apnea, and 57.5% needing oxygen. The majority of cases improved (82.5%), while only 17.5% died. Salivary CRP is detectable in neonates, and there is a moderate positive correlation with serum CRP, making it a valuable biomarker for diagnosing neonatal sepsis and detecting abnormal serum CRP levels. The study In culture-positive septic groups, there was a significant difference in the amount of CRP in the saliva of cases compared to controls. Klebsiella pneumoniae was the most common organism, followed by MRSA and Acinetobacter. The cases tested positive

for Candida albicans and CONS. The study found a statistically significant difference regarding salivary CRP levels in septic and control neonates, similar to previous studies by Omran et al. **[**44] and Iyengar et al. [40]**.** However, Tosson et al. [45] found no significant difference in salivary CRP values between septic and controls, possibly due to the different kits used and population, as their study enrolled only full-term neonates. The study found a moderately positive correlation between salivary CRP and serum CRP ($r = 0.78$; $p<0.001$), consistent with previous studies by Datla et al.,[46] Omran et al**.[**44] and Iyengar et al. [40] However, Tosson et al. [45] did not find a statistically significant correlation, possibly due to the different methodology and study population. The study found that the median salivary CRP levels were significantly different between the control, culture-positive cases, and clinical sepsis groups. This difference was statistically significant ($P<0.001$). Inding aligns with previous studies by Ramavath et al. [47] and Datla et al. [46]**.** However, Tosson et al. [45] did not find a significant difference between the three groups, possibly due to differences in the studied population and isolated organisms. Only a few studies have evaluated the predictive value of salivary CRP in neonatal sepsis, with favorable results and variable AUC values. This study found very significant predictability with an AUC of 0.9 for culture-positive sepsis, consistent with Ramavath et al.'s [47] findings. The cut-off values of salivary CRP vary among studies due to different ELISA methods and measuring units. Previous studies predicted serum CRP levels of 10 mg/mL or higher. The time between serum sample collection and salivary sample collection also differs. In our study, we collected salivary samples within 12 hours of collecting serum CRP samples. Methodological differences and time intervals contribute to the different cutoff values reported. We need further large-scale studies to determine the

optimal cut-off value. Currently, there is no published normative data on salivary CRP in healthy neonates. Salivary CRP levels above 0.96 ng/mL and serum CRP levels above 18 mg/L were the best ways to tell if someone had culture-positive sepsis, according to the study. The AUC on the ROC curve analysis was 0.9. Ramavath et al.'s [47] study showed cut-off scores for culture-positive sepsis The use of Hs-CRP kits, which are more accurate in detecting CRP, could explain why salivary CRP is a reliable predictor of positive blood culture sensitivity, specificity, and PPV in neonates. CRP detection is accurate. It seems that salivary CRP is a good way to tell if someone has culture-positive sepsis, which is in line with what Ramavath et al**.** [47] found, even though they used different salivary CRP kits. With an AUC of 0.94 and a sensitivity of 97%, salivary CRP was a good way to diagnose CRP levels above 10 mg/L in serum. This aligns with a 2014 study by Iyengar et al. [40] **,** which found similar accuracy at a cut-off point of 4.84 ng/mL. Results may vary due to population characteristics. The study compared the results of two previous studies, Iyengar et al. [40] and Omran et al. (44) which focused on post-operative neonates with sepsis and full-term neonates, respectively. We can attribute the differences to various pre-processing techniques and assay methods. The incidence of positive blood cultures was 50%, with LOS occurring in 100% of cases. The study found that gramme-negative bacteria, predominantly Klebsiella pneumoniae, were the most common isolates, accounting for 30% of the sample. This aligns with previous studies, such as Ramavath et al. [47] Hashim et al. [48] and Elmashad et al. [49] However, Tosson et al. [45] found MRSA to be the most common organism isolated in the septic group. Hammoud et al. [50] found CoNs to be the most common pathogen in LOS. The study's sensitivity and specificity for serum CRP cut-off levels were 92.5% and 95%,

respectively. Similarly, Tosson et al.[45] discovered that a serum CRP cut-off level could diagnose LOS 91% of the time and 100% of the time. Our study had a 92.5% PPV and a 97.5% NPV, indicating that serum CRP has a PPV of 100% and an NPV of 85.7%, similar to Tosson et al.'s findings. [45] However, Brown et al. [51] found that serum CRP is insufficient for accurately diagnosing LOS or guiding antibiotic treatment. The study also compared SAA with other biochemical markers in neonatal sepsis. The correlation between SAA and serum CRP in this study was moderately positive, with r = 0.50. Agreeing with our results, Mohsen et al. [52] found the correlation between SSA and serum CRP to be significant (r = 0.483, p = <0.01). The study found that the level of SSA in cases was significantly higher than controls (P<0.001), consistent with previous research. This finding aligns with Malle and De Beer's [53] study, which found an increase in SAA levels as an acute-phase reactant in clinical practice. Bengnér et al. [54] also found a significant difference in SAA levels between septic cases and controls. Arnon et al. [55] reported that preterm neonates could use SAA for early detection of late-onset sepsis. According to Cetinkaya et al. [42] , the increase in SAA in septic neonates at the onset of sepsis was insignificant. We used ROC curves to test how well SAA could diagnose neonatal sepsis. At a cut-off level of 4.6 μg/ml, the test had 100% sensitivity, 95% specificity, 100% PPV, and 92.5% NPV. This aligns with El Mashad et al. [49] study, which found SAA protein as the most sensitive marker. According to Arnon et al. [56] and the PPV of serum CRP was 87%, while the PPV of SAA was 96%, and the PPV of serum CRP in our study was 92.5%. The study found that the diagnostic utility of serum albumin (SAA) for distinguishing between culture-positive and clinical sepsis was better than serum CRP. This finding is consistent with Bourika et al.'s [57] findings, which found similar

diagnostic utility in serum CRP. However, the most significant data on SAA's diagnostic utility in neonatal sepsis comes from a meta-analysis by Yuan et al. [58], which included nine studies with varying cases of neonatal sepsis. The studies assessed the use of SAA for diagnosis in the first suspicion, with sensitivity and specificity ranging from 23%–100% and 44%–100%, respectively. The study found that the diagnostic accuracy of SAA was slightly better than that of CRP, with a pooled sensitivity, specificity, and diagnostic accuracy of 84%, 89%, and 90%, respectively, indicating significant variability among studies. Yuan et al. [58] The study found that cases had significantly lower platelet counts compared to controls ($p<0.001$), consistent with Omran et al. [59] findings. However, Tosson et al. [45] did not find a significant difference in platelet count between cases and controls, indicating a potential difference in hematological parameters. The study found a significant increase in TLC in cases compared to controls ($P = 0.003$), consistent with Mubaraki et al. [60] findings**.** However, Tosson et al. [45] and Omran et al. [44] did not find a statistically significant difference.

The study had limitations, including the time required to collect a viable sample from preterm neonates, the exclusion of neonates with oral disease as candida, and the exclusion of neonates on mechanical ventilation due to increased respiratory secretions.

Conclusions

The study suggests that salivary CRP can be a valuable noninvasive biomarker for late-onset neonatal sepsis diagnosis, with comparable results to SAA and serum CRP. However, we need to conduct further research on sample collection methods, normative values, cost effectiveness, and standardization of assay procedures.

Data Availability: The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author's contributions

Study concept and design: Nagwa Mohamed Sabry Mahmoud, Gamal Baheeg, Manar Anwar Abd-Elaziz, Nageh Shehata Ismail. Data collection : Manar Anwar Abd-Elaziz. Analysis and interpretation of data: Nagwa Mohamed Sabry Mahmoud, Gamal Baheeg, Manar Anwar Abd-Elaziz, Nageh Shehata Ismail. Drafting of the manuscript: Nagwa Mohamed Sabry Mahmoud. Critical revision of the manuscript for important intellectual content: Nagwa Mohamed Sabry Mahmoud, Nagwa Ismail Okaily. Statistical analysis: Nagwa Mohamed Sabry Mahmoud, Gamal Baheeg, Manar Anwar Abd-Elaziz, Nageh Shehata Ismail. laboratory data interpretations, Nagwa Ismail Okaily. All authors read and approved the final manuscript

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Conflict of interest

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Author's details

[1]Pediatric department, Faculty of Medicine, Minia University, Egypt

[2]Clinical pathology department, Faculty of Medicine, Minia University, Egypt

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