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Original Article

Potential Diagnostic Value of Serum Amyloid-A in Preterm Neonates with Late-onset Sepsis. A Prospective Case-Control Study

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Abstract

Background: Neonatal sepsis is a serious infection that continues to puzzle neonatologists with its variable presentation, with great difficulty in distinguishing it from other diseases or even normal birth processes. Discrimination of neonatal sepsis depends mainly on investigations. Blood culture is the cornerstone of the investigations. Several biomarkers are used to provide early diagnosis and follow-up. SAA is a promising biomarker among the available ones. **Objective:** To evaluate the utility of serum Amyloid-A (SAA) in diagnosis of late-onset sepsis (LOS) in preterm neonates. **Methods:** This is a prospective case-control study that was conducted on 46 preterm neonates; 23 cases with neonatal sepsis and 23 healthy age and sex-matched control group. The septic group presented with neonatal sepsis diagnosed clinically and laboratory using blood cultures, complete blood count, and serum C-reactive protein (CRP). The optimal cut-off level of SAA was determined by receiver-operating characteristics curve (ROC) analysis. **Results** Forty-six neonates (33.78±1.7 weeks) were evaluated "23 as cases and 23 as control". The level of SSA in the septic group was significantly higher compared to the control group with P= <0.001. The sensitivity, specificity, PPV, and NPV of serum Amyloid-A for the detection of sepsis were 100%, 95%, 100%, and 95% respectively at a cut-off of 7.6 µg/ml. **Conclusion:** Serum Amyloid-A (SAA) can be used as a valuable biomarker for diagnosing late-onset sepsis in preterm neonates..

Keywords: Neonatal sepsis, Serum Amyloid A, late-onset sepsis, preterm neonates, CRP.

Introduction

In 2010 worldwide, 7.6 million children less than 5 years old died, predominantly due to infectious causes including sepsis; neonatal deaths (in the first 28 days of life), accounted for 40% of the total lives lost [1]

Neonatal sepsis has been classified as either early-onset or late-onset depending on the age of onset and timing of the sepsis episode. Clinical manifestations of early-onset infections usually appear within the first 72 h of life [2].

Neonatal sepsis remains a substantial cause of morbidity and mortality in the nursery setting[3]. Sepsis in neonates and young infants is challenging to diagnose, because infants manifest nonspecific clinical signs in response to sepsis (eg, respiratory distress, hypotension, apnea) that could indicate noninfectious conditions. Furthermore, time to antibiotics affects neonatal sepsis outcome; therefore, there are both clinical and compliance motivations for identifying and treating neonates with sepsis expeditiously [4 , 5]. As a result, clinicians commonly use serum biomarkers to measure inflammation and infection and assess the infant's risk of sepsis [6].

Distinguishing the individual components of sepsis from a dysregulated response to the infection is challenging. The 3 primary issues in neonatal sepsis diagnosis are [1] the myriad of clinical findings that mimic sepsis rather than represent it, and, as a direct result, [2] concern for falsely negative bacterial cultures, also known as culture-negative sepsis, and [3] the need to treat empirically for a minimum of 24 to 48 hours while cultures incubate. A further complication is that the initiation of antimicrobial therapy before cultures are obtained can sterilize subsequent cultures and decrease the opportunity for accurate diagnosis of sterile site infections [6].

Serum Amyloid A (SAA) is an acute phase reactant synthesized by hepatocytes, monocytes, endothelial and smooth muscle cells in 8–24 h after

bacterial exposure and is regulated by proinflammatory cytokines. SAA levels increase with age, with the lowest levels seen in umbilical cord blood and highest levels seen in the old age[7].

The role of SAA protein as a diagnostic or follow-up marker of neonatal sepsis has not been clarified yet, as relevant studies have shown contradictory results [8 -10]. In a published meta-analysis, SAA showed moderate accuracy, which was better than that of C-reactive protein (CRP), in the diagnosis of neonatal sepsis [11]. However, the number of studies included in the meta-analysis was rather small and there was also significant heterogeneity among studies. Thus, it was proposed that further investigation of the diagnostic accuracy of SAA in neonatal sepsis and correlation with other biomarkers is required [11 , 12].

The aim of the present study is to evaluate the diagnostic utility and accuracy of SAA in preterm neonates with late-onset sepsis.

Patients and Methods

Patients: This study is a case control study conducted on 46 neonates admitted to the Neonatal intensive care unit at Minia university hospital, Minia University in the period from (June 2022- October 2022).

Neonates included in this study were grouped as follows: Septic group

This group comprised 23 preterm neonates with late-onset neonatal sepsis diagnosed clinically and confirmed laboratory by blood culture or serum C-reactive protein or Complete blood count.

Control group

This group comprised 23 healthy normal age-matched preterm neonates.

Samples collection

From all included preterm neonates (cases and controls), 4 ml of venous blood were collected under completely sterile conditions for hematological, and biochemical laboratory tests: 1 ml of blood in EDTA tube for CBC and 3 ml of blood into plain tube were collected and allowed to clot then centrifuged and analyzed for serum CRP and serum

amyloid A. For cultures (only for sepsis groups), another 1 ml of blood was inoculated into blood culture bottles with specific media. Blood samples from neonates suspected of sepsis were withdrawn at the time of clinical diagnosis of sepsis, before initiation of antibiotic therapy.

Laboratory methods

CBCs of all patients were evaluated by an automated cell counter (Celltac G, Nihon Kohden Corporation Automated Hematology Analyzer, Japan). CRP was measured by NycoCard Reader II. CRP levels less than 6 mg/dl were considered normal. Blood culture: 1 ml of blood was inoculated aseptically into the blood culture media after which the bottles were incubated at 37°C for 5 – 7 days. Positive blood cultures were subsequently sub-cultured on blood agar. The isolated microorganisms were identified by standard bacteriological methods.). Serum amyloid A was measured by ELISA kit (BT LAB ″Bioassay Technology Laboratory″, Cat. No E1225Hu, China). The optical density was determined using a microplate reader (Huma reader 3700, Germany).

Ethical consent

The study was explained in details to the parents of the participant neonates and written consents were taken from them. The study was designed respecting the expected ethical aspects. It was performed according to the Declaration of Helsinki 1975, as revised in 2008 and approved by the Institutional Review Board and Medical Ethics Committee of Minia University (Approval number: 208:1/2022, Date of approval: 24 January 2022).

Statistical analysis

Statistical Package for the Social Sciences (SPSS) program for Windows, version 22 was used. Quantitative results were presented as the mean±SD while qualitative data were presented by frequency distribution as percent (%). Student's sample t test was used to compare between two means and $\chi 2$ test was used to compare proportions.

Correlations were performed by using Pearson's correlation coefficient (r) and Spearman's test. Receiver operating characteristic (ROC) curve analysis was performed to determine: the optimal cutoff values, the detective performance of different studied markers and scores, and their sensitivities and specificities for the detection of late onset neonatal sepsis. less than 0.05 was used as a cutoff point for all significant tests.

Results

Out of 23 septic cases 11 had positive blood culture. The most common organism isolated from blood cultures was Klebsiella pneumoniae (6/11), followed by MRSA (3/11), lastly, Acinetobacter baumannii (2/11).

A significant moderately positive correlation between SAA and serum CRP (table 2).

The level of SSA as well as serum CRP in septic group was significantly higher compared to control group(table 3).

The ROC curve was constructed to determine the cut-off level of SAA with sensitivity, specificity, PPV and NPV of SAA for detection of sepsis as follows 100%, 95%, 100%, 95% respectively at a cut-off of 7.6 µg/ml (Fig.1)

Table 1: Descriptive data of cases and controls.

Parameter	(n=46)
Gestational age, weeks, Mean ±SD	33.74±1.63
<32 weeks GA, n(%)	4 (8.7%)
32-33 weeks GA, n (%)	14(30.5%)
34-36 weeks GA, n (%)	28(60.8%)
Birth weight, Mean ±SD	1.63±0.39
1000-1499 g, n (%)	16 (34.8%)
1500-2499 g, n (%)	30 (65.2%)
Sex	
Males, n (%)	22 (47.8%)
Female, n(%)	24 (52.2%)
LOS (>72 h), n (%)	23 (50%)
Central line, n (%)	32 (69.6%)
Mechanical ventilation, n (%)	9 (19.5%)

Mean±SD: mean ± standard deviation

Table 2: Correlation between serum CRP and serum amyloid A

Correlation	Serum CRP (mg/l)	
SAA (µg/ml)	R	P value
	0.7	0.001*

**Correlation is significant: at the 0.05 level(2-tailed).*

Table 3: SAA in septic and control group

Item	Cases (n=23)	Control (n=23)	P value
SAA (µg/ml)			
Mean ± SD	24.6±13.7	3.1±0.7	<0.001*
Median (range)	19(10.9:49.5)	3(1.7:4.7)	
Serum CRP (mg/l)			
Mean ± SD	47 ±29.5	0.85 ±2.1	<0.001*
Median (range)	48(0:96)	0(0:6)	

***Mean±SD**: mean ± standard deviation ***P value** <0.01= significant*

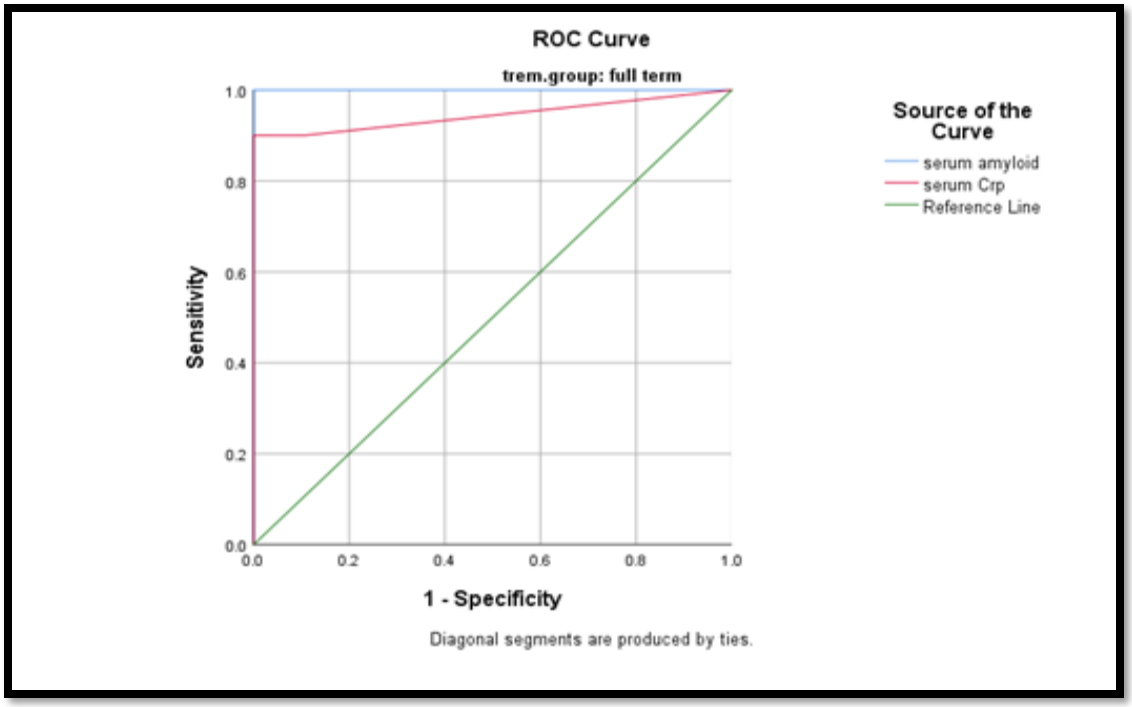

Figure 1: The ROC curve was constructed to determine the cut-off level of SAA with sensitivity, specificity, PPV and NPV of SAA for detection of sepsis

Discussion

The current study assessed levels of SAA in a group of preterm neonates with late-onset neonatal sepsis compared to healthy age-matched control group. Also, the diagnostic accuracy of SAA was assessed. In the present study, culture proven sepsis and clinical sepsis comprised 47.8% and 52.2% of the septic group respectively. With the most common organism is klebsiella pneuomoniae. These results are in agreement with Hashim et al. **[13]**and Elmashad et al. **[14]**.

The correlation between SAA and Serum CRP in this study was moderately positive with r=0.7. Agreeing with our results Mohsen et al. [15] found the correlation between SSA and serum CRP is (r = 0.483, p= <0.01).

Our study showed that the level of SSA in septic group was significantly higher compared to control group with P= <0.001. This finding was congruent with Malle and De Beer, **[16]**which found that SAA levels show an early increase (even as much as 2 days before the onset of clinical signs), reach a peak and then, in absence of inflammatory stimuli, revert to normal in a space of few days. In agreement with our results, Bengnér et al. **[17]**found that SAA displays the greatest difference between the septic group and the control group. Also, Arnon et al (2005) **[18]** reported that SAA could be used as a reliable

marker for early detection of LOS in preterm infants which is in agreement with our study results establishing that SAA had higher levels in LOS. To the contrary of our results, Cetinkaya et al. **[19]**, found that in spite SAA protein increased in septic neonates at onset of sepsis, in comparison with CRP, yet that increase was insignificant. Ucar et al. **[9]**also found that the production of SAA is not adequately stimulated in newborns due to defects in IL-1β production.

Diagnostic accuracy of SAA was assessed by ROC curve. The results as follows; sensitivity, specificity, PPV and NPV of SAA for detection of sepsis were 100%, 95%, 100%, 95% respectively at a cut-off of 7.6 µg/ml. In agreement with El mashad et al.**[14]**, in which results showed that the SAA protein was the most sensitive marker (91.42%, AUC = 0.99, cut-off point 10 µg/ml). In concordance with our results Fathy et al. **[20]**, found that cut-off value of SAA of at least 10 µg/ml with sensitivity 96%, specificity 95%, PPV 85%, and NPV 99% at onset of sepsis. Whereas, the study of Arnon et al. **[21]**, found that the PPV of SAA was 96% and Arnon et al.[18], found that the PPV of SAA was 87% in comparison with the PPV of CRP (86%). However, the most powerful evidence regarding the diagnostic value of SAA in neonatal sepsis derives from the meta-analysis of Yuan et al**[11]**. In which a total of only 9 studies were included in the meta-analysis, with the number of septic neonates in each study ranging from 20 to 123. Nine studies from included papers evaluated the use of the SAA test at the first suspicion of sepsis. The sensitivity ranged from 23% to 100% (pooled sensitivity: 0.84, 95% CI 80%–87%), whereas specificity ranged from 44% to 100% (pooled sensitivity: 0.89, 95% CI 86%–92%). Significant heterogeneity among studies was attributed to differences in the diagnostic cutoff point (ranging from 1.0 to 75.2 mg/L), SAA assay and age of included neonates, among others. Pooled sensitivity, specificity and diagnostic accuracy were 84%, 89% and 90%, respectively; interestingly, the diagnostic accuracy of SAA was slightly better than that of CRP**[11]**.

Conclusions

Serum Amyloid A (SAA) can be used as a reliable marker for late-onset sepsis in preterm neonates.

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Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors shared equally in this work. All the authors write, reviewed, and approved the final manuscript; and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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