

Research article

Detection and evaluation of positive acute phase reactant protein markers in neonates and adults with sepsisAzhar Mahdi Abed Alamer¹, Israa Saeed Abbas², Riadh Abd ALrasul Hnewa³¹ College of Applied Medical Sciences, Department of Clinical Laboratories, University of Karbala, Iraq² College of Medicine, University of Karbala, Clinical Laboratories³ College of Medicine, College of Applied Medical Sciences, University of Karbala, Hay-Almodafien, Iraq

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Corresponding author: **Azhar Mahdi Abed Alamer**. Email: azhar_mahdi92@yahoo.com**ABSTRACT**

Introduction and Aim: Septicemia is a clinical condition caused due to bacterial infection of the blood. Septicemia leading to sepsis could be life-threatening leading to tissue damage, organ failure, and death. The current study aimed to determine the impact of various biomarkers on patients suffering from sepsis, including Serum amyloid A, C-reactive protein, HS-Troponin, fibrinogen, haptoglobin and α_1 -antitrypsin, as well as identify the common bacterial types associated in septic patients.

Materials and Methods: This case-control study conducted during October 2022 to May 2023 involved 100 participants that were divided into groups which included patients diagnosed with septicemia due to bacteria (Group A), patients with septicemia with non-bacterial infection (Group B) and healthy individuals as controls (Group C).

Results: Serum amyloid A, C-reactive protein, HS-Troponin, Fibrinogen, Haptoglobin, and Alpha-1 antitrypsin levels in neonates and adults with sepsis were found to be elevated when compared to healthy individuals. No significant difference ($p < 0.05$) was observed for these parameters between Group A patients (positive for blood culture) and Group B patients (negative for blood culture) among neonates and adult sepsis patients.

Conclusion: Neonates and adult sepsis patients exhibited higher levels of serum amyloid A, C-reactive protein, HS-Troponin, fibrinogen, hemoglobin, and Alpha-1 antitrypsin in comparison to healthy controls. Hence, these biochemical parameters could be used as biomarkers to assess a patient's level of sepsis for further therapeutic action.

Keywords: Sepsis; CRP; serum amyloid A; HS-Troponin; Fibrinogen; Haptoglobin; Alpha-1 antitrypsin

INTRODUCTION

Sepsis is a potentially fatal immunological response that arises when the body injures itself while defending itself against an infection (1). Sepsis is caused by a variety of species, including viruses, bacteria, and fungus (2). Frequent indications and symptoms include disorientation, fever, raised heart rate, and increased breathing rate. In order to stop sepsis from progressing to severe sepsis and septic shock, which is able to result in organ failure and death, early identification is essential (3,4). The gold standards for identifying microbe's species in the body is blood cultures, yet test results are positive in about 30–40% of individuals with septic shock or severe sepsis (5-7). In neonates, sepsis is a clinically prevalent condition with a higher incidence in premature and low birth weight infants (8).

Acute phase reactants (APR) are indicators of inflammation that exhibit dramatic fluctuations in blood concentration during an inflammatory response. These APRs produced by the liver in both acute and long-term inflammatory states, may be further divided into categories that are either positively or negatively regulated based on the serum concentrations during the time of inflammation. During inflammation, positive APRs are upregulated wherein their concentrations rise, while negative APRs are lessened in concentration and

downregulated. Negative APRs include antithrombin, retinol-binding protein, albumin, prealbumin, and transferrin, whereas positive APRs included ferritin, procalcitonin, fibrinogen, C-reactive protein, serum amyloid-A and hepcidin (6).

A more complicated reaction to local or systemic inflammation (sepsis), known as the acute-phase response, is in charge of the development of increased acute-phase protein levels in body fluids such as blood. This response is characterized by changes in hormone levels, reoriented iron metabolism, and decreased albumin production by hepatocytes. Subclinical inflammation and chronic inflammatory disorders are also associated with these alterations (9). Additionally, sepsis has been classified as positive and negative according to the systemic inflammatory response syndrome (SIRS) criteria based on baseline characteristics exhibited by sepsis patients (10). Studies on biomarkers in sepsis patients on admission has been shown to represent the combined effects from the day the disease first appeared, in addition to its severity in cases associated with trauma, burns, or cardiac arrest (11). The goal of this study was to evaluate and compare the positive biomarkers of inflammation in neonates and adults with and without infection who were clinically diagnosed with sepsis.

MATERIALS AND METHODS

Study design and setting

This case-control study was conducted from October 2022 to May 2022 on patients clinically diagnosed with septicemia at the Imam Hussein hospital in the center of intensive care units and Imam Zain Alabdeen hospital in Karbala city, Iraq. The patients (n=50) which consisted of both neonates and adults were further divided into 2 groups. Group A included patients (2 neonates and 25 adults) who were positive for blood culture. Group B included patients (6 neonates and 17 adults) who were negative for blood culture. The study also consisted of a control group (Group C) that included 10 neonates and 40 adults, that were randomly chosen from the general population and seemed to be in good health. The average age of neonates was ≤ 30 days, while for adults it ranged between 18-73 years.

Ethical consideration

The study adhered to the recommendations set forth by the Department of Clinical Laboratories at the University of Karbala's College of Applied Medical Sciences for dealing with biological materials and harmful microbes. After receiving the required consent from the patients and hospital management, The patients' samples used in this study were obtained from the Karbala Health Directorate's Imam Hussein Center Intensive care units and Imam Zian Alabden hospital.

Biochemical assay

Serum amyloid A and CRP concentrations were measured by nephelometric immunoassay (10) using an automated device Mindray BS-430 (MINDRAY, China). The serum levels of HS-Troponin and Fibrinogen was measured based on an Immunofluorescent assay using the Troponin kit (Biomerieux, France) and Fibrinogen kit (HMG, China) respectively with the fluorescence measured in an automated machine MINI-VIDAS (Biomerieux, France). Haptoglobin and alpha 1 antitrypsin was measured using an automated electrophoresis analyzer (MiniPhor 08, Italy), following an earlier protocol (13).

Statistical analysis

The quantitative data are expressed as mean \pm standard deviation. The Student t-test was utilized to compare data amongst patients that were discharged favorably and those required ICU admission. Binomial data are presented as percentages of frequency and analyzed by Chi square test. Characteristics of operation of receiver (ROC) curve was used to evaluate the predictive value for all markers that had a significant variation between the two groups at admission in predicting ICU admission. All data were analyzed with SPSS for

windows, v.25.0; IBM Corp, Armonk, New York, USA.

RESULTS

A comparison of the biochemical parameters assayed and the values obtained for neonates and adults in this study is presented in Table 1. As seen all the biochemical parameters (Serum amyloid A, C-reactive protein, HS-Troponin, Fibrinogen, Haptoglobin and Alpha-1 antitrypsin) assayed were significantly higher (p value ≤ 0.05) in septicemia patients (neonates and adults) in comparison to healthy controls (Table 1). However, for these measures, there was no significant difference between neonates and adults (p <0.05) (Table 1).

DISCUSSION

According to the SIRS criteria, sepsis has been classified as sepsis, severe sepsis and septic shock (10). Study of biomarkers of sepsis has been shown to improve screening, sepsis risk and enhance the process of diagnosing and making treatment decisions in patients at high risk (14). In this research we assayed the positive biomarkers of sepsis in neonates and adults with sepsis. Results in this study indicated the mean levels of serum Amyloid in neonate and adult septic patients to be significantly higher than healthy individuals which is consistent with an earlier study (15), which also reported the serum Amyloid A levels to gradually rise and peak 3–4 days following infection in individuals infected with respiratory viruses. A study of clinical features in coronavirus-infected patients revealed a significant quantity of IL-1, IFN-, IP-10, and MCP to be released during infection, resulting in activation of the inflammatory factor serum Amyloid A, reflecting the body's response to infection (16). Similarly, an increase in serum Amyloid A levels during influenza A virus infection has been shown to be vital for the early control of infection by this virus (17). We presume the elevated levels for serum amyloid observed in this study have significance and could be used as a biomarker for onset of sepsis in neonates and adults as reported in previous studies (18, 19). Also found CRP as a sensitive marker of sepsis which is consistent with other studies (19,20).

A study by Hesamuddin *et al.*, (21) demonstrated that CRP had an overall diagnostic accuracy of 70.07% for the diagnosis of acute neonatal sepsis, with sensitivity and specificity of 76.92% and 53.49%, respectively. The risk of sepsis related mortality appears to be increased with increasing values. Therefore, in patients suffering from severe sepsis in intensive care units, CRP has been suggested as a useful marker in predicting mortality (22).

Table 1: Comparison of biochemical parameters (serum amyloid A, CRP, HS-Troponin, fibrinogen, Haptoglobin, alpha 1 antitrypsin) among neonates and adults in the three groups studied

Biochemical parameter	Groups	Neonates (Mean ± SD)	Adults (Mean ± SD)	P value(t-test)
Serum amyloid A	Group A	33.91 ± 17.00 ^a	28.35 ± 23.97 ^a	0.608 NS
	Group B	39.5 ± 27.76 ^a	27.76 ± 20.62 ^a	0.488 NS
	Group C	5.38 ± 5.12 ^b	5.9 ± 6.48 ^b	0.683 NS
	P value (post Hoc test)	0.001* S	*S	
CRP	Group A	93.16 ± 80.44 ^a	88.7 ± 74.54 ^a	0.925 NS
	Group B	25.5 ± 9.19 ^b	52.7 ± 26.59 ^b	0.169 NS
	Group C	4.1 ± 1.2 ^c	3.6 ± 1.55 ^b	0.492 NS
	P value (post Hoc test)	0.001*	0.005*	
HS-Troponin	Group A	4.9±1.3	6.11±42.26 ^a	0.445 NS
	Group B	7.6±1.4	5.20±38.67 ^a	0.094 NS
	Group C	0.95±0.3	1.1±0.08 ^b	0.671 NS
	P value (post Hoc test)	0.007	0.003	
Fibrinogen	Group A	413.50 ± 98.42 ^a	434.35 ± 168.11	0.779 NS
	Group B	489.00 ± 76.36 ^a	440.76 ± 154.95	0.671 NS
	Group C	334.97 ± 102.38 ^b	306.31 ± 136.99	0.203 NS
	P value (post Hoc test)	0.043 * S	0.015 S	
Haptoglobin	Group A	445.83± 132.97 ^a	400.35 ± 139.45	0.495 NS
	Group B	298.5 ± 282.13 ^a	412± 132.98	0.287 NS
	Group C	112.79 ± 94.88 ^a	119.87± 101.82	0.946 NS
	P value (post Hoc test)	<0.001* S	<0.001* S	
Alpha-1 antitrypsin	Group A	7.60± 3.73	9.36 ± 4.6	0.421 NS
	Group B	6.45 ± 2.05	9.52± 4.4	0.345 NS
	Group C	3.42 ± 2.42	3.6± 3.2	0.998 NS
	P value (post Hoc test)	0.020* S	0.017* S	

CRP: C-reactive protein, HS-Troponin: High sensitivity troponin. Group A: Patients +ve for blood culture; Group B: Patients -ve for blood culture; C: Control group. t- test for significance between neonates and adults; Post Hoc test for significance between groups. NS: no significance *: significance. (a,b) The design used was ANOVA (one-way) design, and the means of the study groups were compared by using the least significant difference, (^a) its mean significance, (^b)its mean non-significance

The current study also showed the HS-troponin levels to be elevated in neonate and adult sepsis patients. Associations between cardiovascular sepsis complications and troponin levels have been shown (23) with elevated cardiac troponin levels in intensive care unit sepsis patients being suggested to be released due to myocardial damage caused by infection or inflammation (24). A subgroup of sepsis patients with elevated troponin are identified as having a greater mortality risk. Hence, troponin higher levels in neonates and adults could be used as a biomarker of sepsis progress for how to handle and care for septic patients in the right way. Fibrinogen is another useful prognostic biomarker for sepsis. Plasma fibrinogen is considered a valuable tool for assessing neonatal outcomes (25) wherein it was shown that increased coagulant activity and decreased fibrinolysis induced by inflammation lead to fibrin deposition in the microcirculation, resulting in ultimately organ dysfunction (25). The current study observed increased levels of haptoglobin in neonate and adult patients with sepsis. This assumes significance as elevated serum concentration of haptoglobin has been associated with improved outcome of patients with septic shock (26) and lowered mortality in patients with sepsis (27). Research suggests that haptoglobin

is an endogenous scavenger of cell-free hemoglobin, thus playing a protective role in patients with sepsis rather than just being an acute-phase reactant (27). Similarly, the alpha 1-antitrypsin levels were also seen to be elevated in sepsis patients in this study. Alpha 1-antitrypsin is an inflammation-sensitive protein with antiprotease activity, deficiency of which could lead to mortality (28). As with haptoglobin, elevated levels of Alpha 1-antitrypsin is associated with protecting cells from various stresses thereby increasing the therapeutic efficacy (29).

CONCLUSION

Serum amyloid A, C-reactive protein, HS-Troponin, Fibrinogen, Haptoglobin and Alpha-1 antitrypsin levels assayed were observed to be elevated in neonates and adult sepsis patients. Since no significant difference was seen in the levels of these parameters among neonates and adults these biochemical parameters could be used as biomarkers in assessing the severity of sepsis in patients.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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