Assessment of Presepsin and Calprotectin in Critically ill adult Patients with Sepsis

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Abstract

the goal of the study is to evaluate the diagnostic utility of Presepsin& Calprotectin as biomarkers compared to the traditional used diagnostic methods in addition to investigate bacterial pathogens causes of septic patients. Method: A total of forty blood samples were obtained from clinically diagnosed adult sepsis patients (sepsis group). The samples were collected from both sexes (20 males and 20 females ranging in age from (17 to 72) years' old who were at the ICU at Al-Hussein teaching hospital in Karbala and the Obstetrics hospital in Karbala city/Iraq between (November 2021 and April 2022). Result: calprotectin and presepsin levels in patient group were elevated compared to control group which is significant for calprotectin (P=0.019) but did not reach statistical significance for presepsin. clarified association between Calprotectin & Presepsin serum levels and sofa score in patient's groups, which revealed that calprotectin serum levels mean were (67.99±47.9, 69.23±41.1, 77.69±55.6) from the lowest to highest score (no significant, P =0.929). Whereas mean Presepsin levels were (312.8±448.8, 153.3±89.9, 220.02±125.7) respectively and again non statically significance is obtaine(P=0.452). Conclusions: calprotectin and presepsin levels elevated in adult sepsis, calprotectin & presepsin could be regarded as good biomarkers for diagnosis of sepsis. In addition, both study markers have relevant specificity for diagnosis of sepsis. No association between severity of sepsis and levels of study markers

1. Introduction

sepsis is a common and possibly fatal infection that requires quick and effective antibiotic treatment. Bacterial infections are the most common cause, however individuals with concomitant conditions and immunosuppression can also contract viruses and fungi. [1]. Fever, tachycardia, and tachypnea are classic sepsis symptoms, a scattered inflammatory response produced by microbial infections. At least one organ malfunction has been associated with severe sepsis. When severe sepsis is combined with multiple organ system failures, the condition is known as septic shock[2].

This life-threatening organ failure caused by a dysregulated host response to an infection is a medical emergency for which early detection, suitable, and prompt therapies are critical in reducing mortality and morbidity[3]. Although there are numerous criteria for defining organ failure during sepsis, using 3 guidelines (the third iteration of the international consensus diagnostic definitions of sepsis) of Sepsis-related Organ Failure (SOFA) score to do so. The new concept gives us a better grasp of sepsis pathogenesis and more precise diagnostic criteria[4],[5].

Septic shock is described as sepsis with circulatory collapse, which is the most severe kind of sepsis. "Adequate routine microbiologic cultures (including blood) should be obtained before beginning antimicrobial therapy in patients with suspected sepsis or septic shock when doing so outcomes in no significant delay in the start of antimicrobials," the recommendation for making a definitive diagnosis changed[6].

Presepsin is new biomarkers tested for acute infections with Cluster of differentiation 14 (CD14) is a glycoprotein expressed on the membrane surface of monocytes and macrophages that functions as a receptor for lipopolysaccharides (LPSs) and LPS-binding proteins. It is being tested as a new biomarker for acute infections with different diagnostic and prognostic value (LPBs). In the innate immune response against microorganisms, CD14 serves as a recognition molecule by triggering a proinflammatory signaling cascade upon interaction with infectious pathogens. During inflammation, soluble CD14 (sCD14) fragments are produced by plasma protease activity. One of them, called sCD14 subtype (sCD14-ST), or presepsin, is normally present in very low concentrations in the serum of healthy individuals and has been shown to be increased in response to bacterial infections[7].

CD14 is the receptor of lipopolysaccharide-lipopoly-saccharide binding protein (LPS-LBP) complexes[56]. With the help of thinositol lipid structure, the carboxyl terminus of the molecule anchors in cell membrane and transducts the endotoxin signal through the Toll-like receptor-4, A series of downstream tyrosine protein kinases and mitogen-activated protein kinase are gradually activated including the nuclear transcription factor NF-κB, thus leading to the release of cytokines such as tumor necrosis factor-α, IFN-γ, IL-1β, IL-8 and

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IL-6.Subsequently, the activation of the secondary inflammatory cascade and acquired immunity stimulate mononuclear macrophages, neutrophils and endothelial cells to release more cytokines and cell adhesion molecules[8]. This could trigger intense and excessive systemic inflammatory response and activate the coagulation and fibrinolytic systems, resulting in SIRS, sepsis shock, disseminated intravascular coagulation (DIC), and multiple organ dysfunction syndrome (MODS), LPS, and is mainly expressed on the cell surface of monocytes/macrophages or distributed a little bit on the cell surface of neutrophils[9].

Nutrophil granulocytes react to bacterial infections and are one of the first responders to bacterial infections. When the neutrophil is activated, it releases calprotectin, one of the most abundant proteins in the neutrophil cytosol. Calprotectin consists of two subunits, S100A8 and S100A9. S100A8 has a molecular weight of 10.8 kDa, while S100A9 has a molecular weight of 13.2 kDa. This biomarker increases within hours in response to bacteria or endotoxin. Determination of serum calprotectin has been proposed as a valuable marker of acute appendicitis, rheumatoid arthritis and sepsis. Early release of calprotectin and rapid test turn-around-times suggest that calprotectin can become a useful biomarker with widespread clinical benefits[10].

2. Material and Methods

Before the collection of samples, all research groups' patients were informed, and verbal consent was acquired. The committee on publishing ethics of the college of medicine gave its approval to this work. University of Karbala, Iraq, and in compliance with the Human Rights and Biomedicine Convention. In this case- 40 persons (sepsis patients) were included. Demographic and clinical data and microbiological and laboratory findings, treatments, and outcomes such as ICU and hospital lengths of stay and mortality were all documented. Data were collected until the patient was discharged from the hospital or died.

A total of forty blood samples were obtained from clinically diagnosed adult sepsis patients (sepsis group). The samples were collected from both sexes (20 males and 20 females ranging in age from (17 to 72) years' old who were at the ICU at Al-Hussein teaching hospital in Karbala and the Obstetrics hospital in Karbala city/Iraq between (November 2021 and April 2022). For each patient, case information sheets comprising age, gender, and other factors were completed.

Patients were ruled out if they had a different diagnosis, such as pulmonary thromboembolism, burns, severe pancreatitis, anaphylaxis, adrenal insufficiency, thyrotoxicosis, or alcohol with drawal.

To assess organ dysfunction in the sepsis group, the Sequential Organ Failure Assessment (SOFA) score was generated daily for four days. The presence of pathogenic bacteria in blood cultures was used to make the etiologic diagnosis.

Blood collection

A 10 ml blood sample was taken from each patient after cleaning the area above the vein and using a new sterile needle for each vein puncture Within an hour of blood draining, the 10 ml blood samples were transported to the laboratory 5 mL of blood sample used for blood culture and cultured in a bottle for bacterial identification using the BACT/ALERT PLUS Culture bottle (BIOMERIEUX, France)culture system and incubated until the BacT/Alert instrument (BACTEC, Becton Dickinson) which will signal it as positive or negative.

The other 5 mL of blood was set in a gel tube to get the serum from patients, then centrifuged 1000 x g (or 3000 rpm) for 15 minutes to obtain serum, which was then frozen at -20 °C for subsequent immunological and blood biochemistry tests.

3. Result

Table 3.3 showed that calprotectin and presepsin levels in patient group were elevated compared to control group which is significant for calprotectin (P=0.019) but did not reach statistical significance for presepsin.

Table 3.3 Calprotectin & Presepsin levels in study group:

Parameter	Mean +SD Patient group)(	Mean+ SD (Control group)	P-value
Calprotectin (ng)	69.33±45.59	49.03±28.45	0.019*
Presepsin (ng)	255.71±357.3	137.53±180.2	0.066

T-student test, Results Are Presented as Mean ± Sd, P<0.05 Considered -Significantly Different, [S]= Significant, [Ns]= Non Significant

3.4. Diagnostic utility for Calprotectin & Presepsin Significant, [Ns]= non-Significant

ROC curve analysis revealed that both biomarkers had good specificity percent for diagnosis of sepsis 77.5%, 80% for presepsin and calprotectin respectively. Areas under curve were 75.8%, 65% for presepsin and calprotectin respectively Presepsin had highly significant diagnostic utility with p-value<0.001, while calprotectin had p-value of 0.02 which is also significant as shown in table 3.4 & figures 3.2.,3.3.

Table 3. 4: rea Under the Curve, optimal threshold, Sensitivity and specificity of Presepsin1 & Calprotectin levels obtained by the ROC curves Patient

Test Variable	AUC	P Value	Sensitivity %	Specificity %	Cut-Off Points	Youden Index	CI (95%)
Presepsin	75.8%	<0.001*	67.5%	77.5%	116.70	0.45	0.651 - 0.866
Calprotectin	65%	0.021*	55%	80%	64.98	0.35	0.528 -0.772

- significant

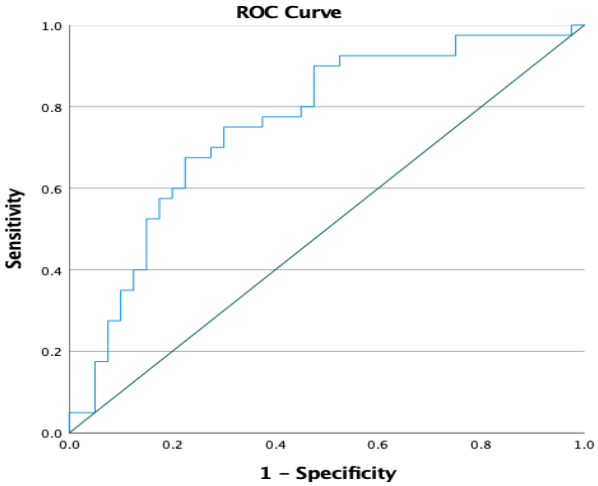

Figure 3.2: ROC curves for Presepsin1 in patients' group to analyse the optimal diagnostic points for predicting of sepsis cases compared to control group.

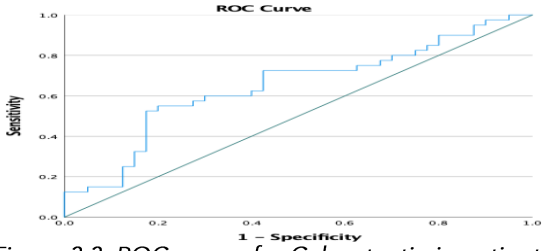

Figure 3.3: ROC curves for Calprotectin in patients' group to analyse the optimal diagnostic points for predicting of sepsis cases compared to control group.

3.5 Calprotectin & Presepsin serum levels in subgroups of sepsis:

Table 3.5 showed no significant differences in Calprotectin & Presepsin levels between subgroups of sepsis. Although presepsin increases in sever sepsis, (511.88±161.87) whereas calprotectin levels were more in septic shock group (59.53±18.82).

Table 3.5 Calprotectin & Presepsin serum levels in subgroups of sepsis:				
Biochemical Parameters	(N=40)			P Value
	Sepsis N= 20	Septic Shock N= 10	Sever Sepsis N= 10	
Presepsin	137.96±30.85	476.62±150.72	511.88±161.87	0.44[Ns]
Calprotectin	36.40±8.14	59.53±18.82	49.75±15.73	0.67[Ns]
ANOVA test, Results Are Presented as Mean ± Sd, P<0.05 Considered Significantly Different, [S]= Significant, [Ns]= non-Significant				

3.6 Calprotectin & Presepsin serum levels among sofa score in patients' groups:

Table 3.6 clarified association between Calprotectin & Presepsin serum levels and sofa score in patient's groups, which revealed that calprotectin serum levels mean were (67.99±47.9, 69.23±41.1, 77.69±55.6) from the lowest to highest score (no significant, P =0.929). Whereas mean Presepsin levels were (312.8±448.8, 153.3±89.9, 220.02±125.7) respectively and again non statically significance is obtained (P=0.452).

Table 3.6 Calprotectin & Presepsin serum levels among sofa score in patients' groups:					
Sofa score	N (%)	Calprotectin mean± SD	P value	Presepsin Mean± SD	P value
0-6	24(60)	67.99±47.9	0.929*	312.8±448.8	0.452*
7-9	12(30)	69.23±41.1		153.3±89.9	
10-12	4(10)	77.69±55.6		220.02±125.7	
ANOVA test, No significant difference at P<0.05					

4. Discussion

The current study founded those levels of calprotectin in patient group were significant (P=0.019) compared to control group while non-significant result obtained for presepsin (P=0.066). this could point the role of calprotectin in inflammatory process of sepsis. Calprotectin is one of the earliest biomarkers for detection of the inflammatory response to infections especially in sepsis [11],[12].

This result is consistent with past studies for measurement of serum levels of calprotectin in neonates and adult respectively (Decembrino et al, 2015, [13].

Current finding also declares that both markers were found to have good diagnostic utilities for differentiating sepsis patients from control group. this result is similar to study of Larsson et al, 2019 which find that the AUC for calprotectin was 0.79 (Larsson et al, 2019). Other study showed that calprotectin had a sensitivity of 62.5% and a specificity of 69.7% in neonate with sepsis which is near results of current study taking in consideration differences in age groups of sepsis [14],[15].

Past studies also showed that presepsin levels were significantly elevated in sepsis with good diagnostic utility[16], while our study fail to achieve the statistically significant level although levels were also elevated in patients' group. this could be due to small sample size.

Our study also showed that diagnostic utility of presepsin was significant for differentiating patients from control group. this result goes with past studies which conclude the importance of Presepsin as a new biomarker and play a crucial role as a supplemental method in the early diagnosis of sepsis[17], [18],[19].

Past study of Zhang et al. revealed that the overall diagnostic sensitivity of presepsin for sepsis was 0.83 (95% CI: 0.77–0.88), and specificity was 0.78 (95% CI: 0.72–0.83). The AUC was 0.88 (95% CI: 0.84–0.90). These results when compared to current data which are specificity of 77.5%, sensitivity of 67.5% and AUC= 75.8 showed a clear similar finding [20].

When comparing levels of Calprotectin & Presepsin

in subgroups of sepsis, no significant differences were found. Although presepsin increases in sever sepsis, (511.88±161.87) whereas calprotectin levels were more in septic shock group (59.53±18.82). The study of Wirtz et al., 20202 revealed that high concentration of calprotectin at admission to ICU is associated with more severity of sepsis which is in agreement current study [21].

Regarding past studies for presepsin Lee et al. study conclude higher levels in patients with septic shock than in those with sepsis ($p = 0.002$)[70]. Also similar findings found by Aliu-Bejta et al. study with a conclusion that Presepsin had a good diagnostic ability to differentiate septic shock from sepsis in the study groups [23].

Regarding Calprotectin & Presepsin serum levels among sofa score subgroups of patient's groups, anon-significant result obtained. Although higher levels of presepsin were within the lowest score i,e the worst sepsis state. Calprotectin levels in contrast is higher in the highest score this could be explained as mentioned above depending on admission date when it is high at admission this lead to bad prognosis and low sofa score and vice versa [24]. Past study showed that Higher values of presepsin were observed in septic patients at presentation (time 0)[24]. This is one of the drawbacks on current study, it doesn't correlate serum markers levels with duration of admission. Aliu-Bejta et al study found a strong correlation of presepsin with SOFA score ($p < 0.0001$) [25] .

5. Conclusions

1. calprotectin and presepsin levels elevated in adult sepsis.
2. calprotectin & presepsin could be regarded as good biomarkers for diagnosis of sepsis. In addition, both study markers have relevant specificity for diagnosis of sepsis.
3. no association between severity of sepsis and levels of study markers.
4. direct positive correlation between calprotectin and CRP.
5. gram negative bacteria Salmonella typhi is the most bacteria resulted from blood culture of sepsis and it is associated with more severe form of sepsis which is septic shock.
6. no association between positive bacterial growth and severity of sepsis.

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