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Heparin Binding Protein as a Predictive Marker for Sepsis and Septic Shock in Critically Ill Patients: A Cross Sectional Study

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ABSTRACT

Background: Sepsis is a serious inflammatory condition caused by infection, accompanied by high morbidity and mortality. An early effective diagnostic and predictive tool is needed to improve the management and prognosis of patients.
Objectives: This study was aimed to assess the role of serum levels of Heparin Binding Protein in early detection of sepsis and circulatory failure in critically ill patients, compared with the currently used biomarkers.
Subjects and methods: This is a cross-sectional study of 66 patients with sepsis, carried out in emergency ICU and surgical ICU in Zagazig University Hospitals. Patients' data were collected at admission, after 48 and 72 hours from medical records, bedside sheets, and radiographic reports. The concentration of heparin Binding Protein (HBP) is determined and compared to that of procalcitonin (PCT), lactate, and total leucocytic count. **Results:** Median range level of HBP in sepsis (11.8, 9.8 and 8.083 ng/ml), septic shock (20, 22.6 and 19.25 ng/ml), and in uncomplicated infections (1.57, 0.9 and 7.3 ng/ml) on admission, after 48 and 72 hours respectively. HBP had 90.3% sensitivity, 62.9% specificity and 75.8% accuracy at cut of value ≥13.35 ng/ml AUC= 0.822, in diagnosis of septic shock on admission.
Conclusion: serum heparin binding protein level is positively correlated with the severity of sepsis and septic shock and showed a good performance as an early diagnostic marker in patients with suspected sepsis and septic shock.
Keywords: Heparin Binding Protein, Sepsis, Septic Shock, Biomarker, Critically Ill.

INTRODUCTION

Sepsis is a life-threatening condition that develops as a result of a severe infection, and it is the leading cause of death in hospitalized patients[(1)]. Although the majority of hospitalized patients do not exhibit classic signs of sepsis, such as organ failure, about 20% – 30% of patients develop sepsis and septic shock within the first day after admission[(2)].

Early detection of septic shock -risk patients can decrease morbidity and mortality [(3,4)]. The traditional biomarkers such as procalcitonin (PCT), C-reactive protein (CRP), and neutrophil and lymphocyte counts are used mostly in the early diagnosis of bacterial cause in septic patients , but they have an ineffective role in prognostic evaluation of critically ill patients[(5)]. Procalcitonin was the biomarker most frequently assessed, but still demonstrates significant differences in the assessment of mortality rates, ranging between 7.5% to 10%. So , none of these biomarkers are adequate for routine clinical use in diagnosing sepsis[(6)]. Heparin-binding protein (HBP) is also called as azurocidin, a member of the serine proteinase family precomposed and mainly found in the azurophilic granules (almost 74 percent in content) and secretory vesicles (almost 18 percent in content) of neutrophils. Azurophilic granules show low affinity to be secreted from cells, and they are only released when neutrophils break into tissues. It is secreted in response to the stimulation of leukocytic membrane bound b2-integrins [(7)].

HBP is a multifunctional protein as it has wide range of antimicrobial activities, particularly against gram-negative bacteria[(8)]. Also, it is considered a powerful chemoattractant for many types of cells, mainly monocytes, and inducing vascular leakage and edema formation[(9,10)]. Previous studies documented HBP levels in the blood were found to be higher in patients with sepsis and septic shock with circulatory and organ failure[(11,12)].

These characteristics make HBP a hopeful candidate for early sepsis and septic shock diagnosis. This study was aimed to evaluate the role the serum level of HBP in diagnosis and prediction of sepsis in critically ill patients.

PATIENTS AND METHODS

This cross-sectional study included a total of 72 patients with sepsis, attending at emergency ICU and surgical ICU, Zagazig University Hospitals. This study was conducted between October 2017 to Jan 2020. Written informed consent of all the subjects was obtained.

Inclusion criteria included patients of both sexes between the ages of 18 and 70 with suspected infection after evaluation by the clinician. Clinical criteria for the systemic inflammatory response syndrome (SIRS) at least presence of 2 criteria as follows: Temp >38°C or < 36°C, Heart Rate > 90 bpm, Respiratory Rate > 20 or Pa CO_2 < 32 mm Hg, WBC > 12,000/mm^3, < 4,000/mm^3, or > 10% bands [(13)].

Exclusion criteria included patients under 18 or above 70 years old, antibiotic treatment for infection and Neutropenia due to primary abnormalities of coagulation, hematological malignancy, immunosuppressive therapy, immune compromised patient, and being on hemodialysis. Withdrawal criteria included; death within 24 hours from

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suspicion as the first sample at time of suspicion is the most important one to predict diagnosis.

The patient's final diagnosis was confirmed by attending physicians. All patients received treatment in the form of fluids, antibiotics, and vasopressors as needed.

Data collection:

Patients' data were collected at admission and after 48,72 hours from medical records, bedside sheets, radiographic reports (chest x-ray, abdominal ultrasound, echocardiography), and reports of laboratory studies of the patients including WBC and the serum levels of HBP, PCT, and lactate. Microbiological studies as cultures of sputum, blood, urine or biological fluid according to clinical suspicion before antibiotic administration.

Assessment of Vital signs: HR, mean arterial blood pressure (MAP), respiratory rate (RR), oxygen saturation (SpO2), and temperature were measured. Glasgow coma scale (GSC), sepsis-related organ failure assessment (SOFA Score) [(1)],quick sepsis-related organ failure assessment (QSOFA Score) [(1)]**.** The suspected source of infection was noted.

The patients were classified clinically into: ***Uncomplicated infection***: SIRS[(13)] with infection without organ failure[(1)]. ***Sepsis***: Organ dysfunction that is characterized as a 2 point or greater increase in total SOFA score as a result of infection. The existence of two QSOFA parameters is also a predictor of sepsis and increased mortality**.** [(1)]. ***Septic shock***: septic shock can be clinically described as a vasopressor need to keep a mean arterial pressure of 65mmHg or greater and serum lactate level greater than 2 mmol/L (>18mg/dL) in the absence of hypovolemia[(1)].

Sample collection and biomarker assays:

Blood samples were taken at 0, 48, 72 hours from suspected patient and distributed between EDTA containing tube for TLC and plain tube for serum which stored at -80ºC until analysis.

Laboratory analysis:

The concentration of HBP was determined by enzyme-linked immunosorbent assay (ELISA). Briefly the kit used (a double-antibody sandwich) to assay the level of Human Azurocidin/Heparin Binding Protein (AZU/HBP) in samples. Add Azurocidin/Heparin Binding Protein (AZU/HBP) to monoclonal antibody enzyme which was pre-coated with Human Azurocidin/Heparin Binding Protein (AZU/HBP) monoclonal antibody. Procalcitonin, WBCs, CRP, and lactate analysis were performed at the clinical chemistry laboratories, Zagazig University Hospitals.

Ethical considerations:

Approval from the Institutional Review Board (IRB) at Zagazig University Hospitals was performed. The study followed the recommendations of the Declaration of Helsinki, 2008.

Study outcome measures:

Primary outcome measures: evaluate the use of plasma HBP levels to predict the occurrence of sepsis and septic shock. *Secondary outcome measures* Comparing HBP performance relative to commonly used sepsis biomarkers (PCT, lactate, and TLC) in ICU patients.

Sample size:

Assuming that the total population of sepsis present at Zagazig university hospital found to be 100 during 12 months. Using Epi info version 6 with positive predictive value of HBP among people with sepsis is 57.3%. So the total sample will be 72 patients after exclusion of dropout value.

Statistical analysis

Analysis of data was done using Statistical Program for Social Science version 20 (SPSS Inc., Chicago, IL, USA). Quantitative data were described in the form of mean and standard deviation. Qualitative variables were described as number and percent. Qualitative variables were compared using chi-square ($\chi 2$). Friedman test (Fr) was used to compare three or more matched groups. The Kruskal-Wallis (KW) test is a nonparametric test was used to assess for significant differences on a continuous dependent variable by a categorical independent variable. Spearman´s non-parametric correlation coefficient (rho) was used for calculating correlations between HBP and other investigated parameters in the patient groups. ROC curve for cut off. Logistic regression for independent predictors. P value was set at <0.05 for significant results.

RESULTS

The current study included 72 patients, of them, 6 patients were excluded as they were died within 24 hours. The included 66 patients showed slight female predominance; 37 females (56.1%) and 29 males (43.9%). Their ages ranged from 18 to 70 years and the mean age was 49.12 years **(Table 1)**.

Table (1): Distribution of the studied patients according to demographic data:

	N=66	**%**
Gender		
Male	29	43.9
Female	37	56.1
Age (years):		
Mean ± SD	49.12 ± 15.45	
Range	18 – 70	

On admission 34/66 patients were diagnosed with sepsis and their number decline to 31 and 24 patients after 48 and 72 hours respectively while number of patients with septic shock were 31, 34, and 32 on admission, 48 and 72 hours respectively. Only one patient was diagnosed with uncomplicated infection on admission and after 48 hours while after 72 hours the number increased to 5/66 patients. There was no statistically significant difference in patient's distribution over the 72 hours of hospitalization p=0.678 **(Figure 1).**

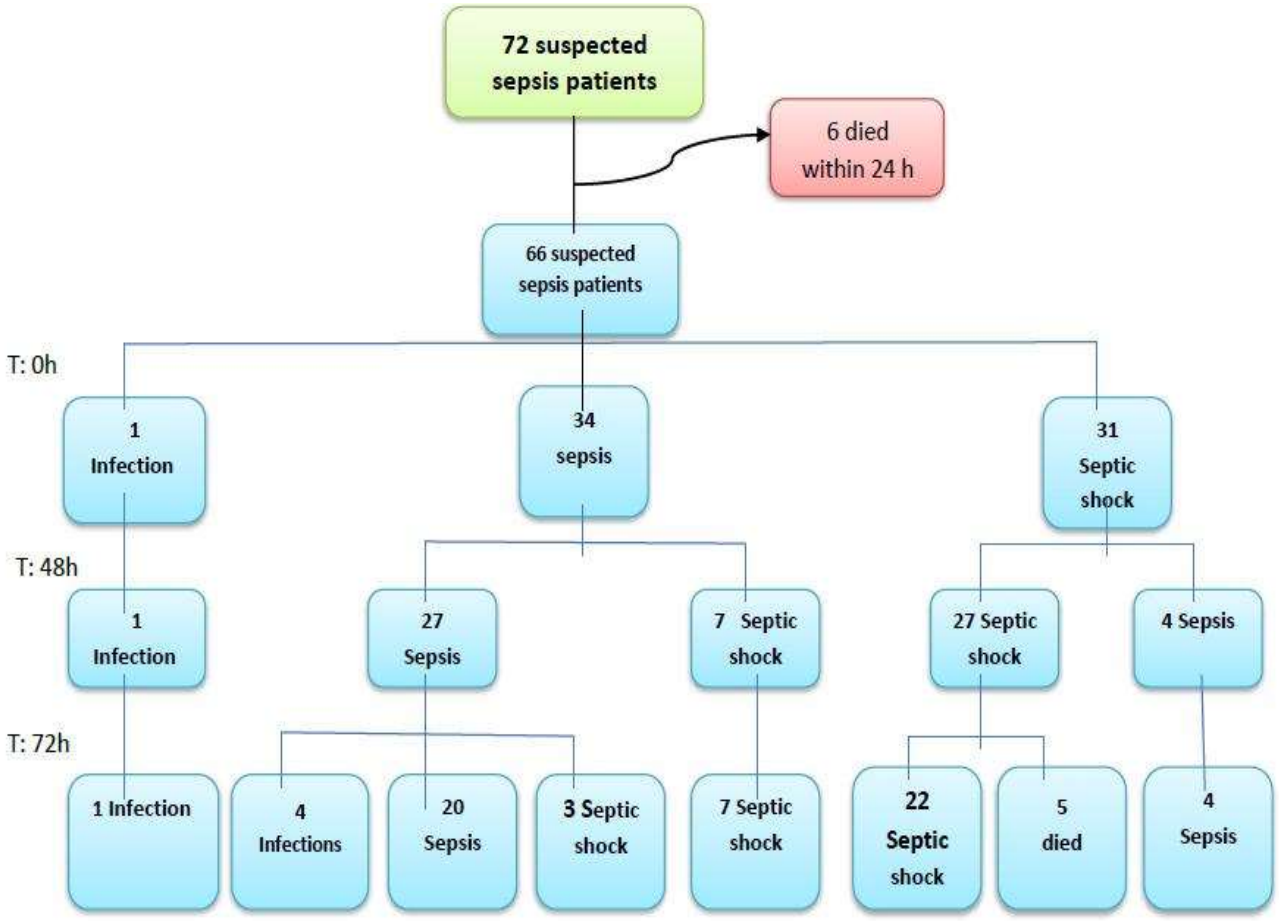

Figure (1): Flow chart of the patients in the cross-sectional study.

HBP median range showed significant decrease from 16 on admission to 13.813 ng/ml after 72 hours p<0.001 **(Table 2)**.

Table (2): Distribution of patients according to Heparin binding protein (HBP) level in the first 72 hours:

Time	HBP (ng/ml)		Test	
	Median	Range	Fr	p
On admission (n=66)	16	1.57 – 31	15.777	<0.001*
At 48 hours (n=66)	14.693	0.06 – 41		
At 72 years (n=61)	13.813	0.07 – 35		

Fr Friedman test

On a boxplot figure showing that level of HBP was highly significant correlation with severity of condition. HBP level was high in septic patients and more high in septic shock patients during the first 72 hours ,while level of HBP in infection was non-significant.

Spearman correlation coefficient between HBP in the first 72 hours and the other study parameters revealed there was statistically **significant** positive Correlation between heparin binding protein on admission, and SOFA, QSOFA, serum lactate, total leucocytic count on admission. There is statistically **non-significant** Correlation between heparin binding protein on admission and PCT on admission. There is statistically **significant** positive Correlation between heparin binding protein at 48 hours, and SOFA, PCT, QSOFA, serum lactate, total leucocytic count on admission at 48 hours. There is statistically **significant** positive Correlation between heparin binding protein at 72 hours, and SOFA, QSOFA, PCT, serum lactate, total leucocytic count on admission at 72 hours **(Table 3)**.

Table (3): Correlation between HBP in the first 72 hours and the studied parameters:

Parameters	Heparin binding protein (HBP)					
	at admission		At 48 hours		At 72 hours	
	r	p	r	p	r	p
Age (years)	-0.009	0.943	-0.008	0.947	-0.133	0.359
SOFA on admission	0.355	0.003*	0.359	0.003*	0.284	0.026*
SOFA at 48 hours	0.435	<0.001*	0.53	<0.001*	0.502	<0.001*
SOFA at 72 hours	0.42	0.001*	0.583	<0.001*	0.6	<0.001*
QSOFA on admission	0.343	0.005*	0.359	0.003*	0.307	0.016*
QSOFA at 48 hours	0.237	0.055	0.294	0.017*	0.362	0.004*
QSOFA at 72 hours	0.363	0.004*	0.448	<0.001*	0.521	<0.001*
Procalcitonin on admission	-0.06	0.63	0.142	0.255	0.077	0.558
Procalcitonin at 48 hours	0.264	0.032*	0.426	<0.001*	0.381	0.002*
Procalcitonin at 72 hours	0.417	0.001*	0.57	<0.001*	0.599	<0.001*
S. lactate on admission	0.446	<0.001*	0.274	0.026*	0.148	0.256
S. lactate at 48 hours	0.263	0.033*	0.344	0.005*	0.284	0.026*
S. lactate at 72 hours	0.384	0.002*	0.424	0.001*	0.496	<0.001*
TLC on admission	0.454	<0.001*	0.417	<0.001*	0.369	0.003*
TLC at 48 hours	0.465	<0.001*	0.514	<0.001*	0.44	<0.001*
TLC at 72 hours	0.394	0.002*	0.454	<0.001*	0.403	0.001*

r Spearman correlation coefficient *p<0.05 is statistically significant.

Measuring HBP on admission showed a good performance in the diagnosis of septic shock with 90.3% sensitivity, 62.9% specificity and 75.8% accuracy at cut of value ≥13.35 ng/ml. however its sensitivity declines after 48 and 72 hours to 79.4% and 87.5% respectively **(Table 4)**.

Table (4): Performance of HBP on admission, at 48 and 72 hours in diagnosis of septic shock:

Time	Cutoff (ng/ml)	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
On admission	**≥13.35**	0.822	90.3%	62.9%	68.3%	88%	75.8%
At 48 hours	**≥13.473**	0.692	79.4%	70.9%	75%	76.7%	75.8%
At 72 hours	**≥8.852**	0.737	87.5%	65.5%	73.7%	82.6%	77.1%

*P<0.05 is statistically significant

HBP was significantly more efficient in identifying patients who developed septic shock compared with the other investigated markers as the diagnostic accuracy for the identification of patients progressing to sepsis and septic shock was highest for HBP, the best cutoff was ≥13.35with area under curve 0.822,with sensitivity 90.3%specificity 62.9%positive predictive value (PPV) 68.3%negative predictive value (NPV) 88%,accuracy 75.8% compared with PCT with the best cutoff of value on admission was≥5 with area under curve 0.546 with sensitivity 58.1%, specificity 48.6%, accuracy 53%. The best cutoff value lactate on admission was≥1.95 with area under curve 0.774with sensitivity 83.9%specificity 40%, accuracy 60.6% .while for TLC on admission cutoff value was≥14.5 with area under curve 0.769 with sensitivity 87.1%specificity 74.3%, accuracy 80.3% **(Table 5).**

Table (5): Performance of HBP compared with other biomarkers (PCT, lactate, TLC) in prediction of septic shock on admission:

	Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
HBP	**≥13.35** ng/ml	0.822	90.3%	62.9%	68.3%	88%	75.8%
PCT	**≥5** ng/ml	0.546	58.1%	48.6%	50%	56.7%	53%
Lactate	**≥1.95** mmol/L	0.774	83.9%	40%	55.3%	73.7%	60.6%
TLC	**≥14.5**×10^3/ /mm^3	0.769	87.1%	74.3%	75%	86.7%	80.3%

*P<0.05 is statistically significant

DISCUSSION

Heparin-binding protein is a powerful inducer of endothelial degradation and an inflammatory mediator. HBP levels in the blood were found to be higher in patients with sepsis and septic shock in previous studies [14,15].

In addition, the value of HBP to localize patients with impending circulatory failure was justified previously[16]. Therefore, this study hypothesized that measurement of plasma level of HBP in patients with suspected sepsis is an effective marker for diagnosis of sepsis and prediction for septic shock.

A total of 66 patients with sepsis were enrolled in this study. Within the 72-h study period, of these 66 patients, 51.5%, 47% and 39.3% of patients at admission, 48-h and at 72-h had signs of sepsis respectively, 47%, 51.5% and 52.5% of patients developed septic shock at admission, 48-h and 72-h respectively, and 1.5% of patients at admission and 48-h and 8.2% of patients at 72-h had signs of infection. There were no significant differences between the three groups with respect to diagnosis (P>0.05).

In a study done by **Zanfaly *et al.*** [12] they found that a total of 90 patients with sepsis were prospectively enrolled in their study. Within the 12-h study period, of these 90 subjects, 41 patients (45.6%) had signs of infection, 29 patients (32.2%) developed sepsis, and 20 patients (22.2) complicated with septic shock. There were no significant differences between the study groups regarding patient criteria (P>0.05).

In the current study, there was a statistically significant increase in SOFA score in the first 48 hours with significant positive correlation with heparin binding protein on admission, at 48 and 72 hours. This came in agreement with **Tverring *et al.*** [17] who found that a high plasma HBP was associated with a higher maximum SOFA score within 5 days and an increased 28-day mortality, which is also in line with earlier research done by **Linder *et al.*** [14] that correlated a high plasma HBP with greater sepsis severity and death and concluded that patients diagnosed with severe sepsis and septic shock showed a significantly high levels of HBP at admission time and this elevation was associated with an increased mortality risk. In contrast, **Chew *et al.*** [18] have recorded elevated levels of HBP in 53 patients with shock in the ICU without infectious etiology and did not find an association with severity and outcome. The loss of a correlation between HBP level and infection or severity of illness was referred to the unselective nature of the patients.

Also, **Zhou *et al.*** [11] reported that HBP level was higher in patients with septic shock than in those with only sepsis without signs of circulatory or organ failure. In 2010 **Linder *et al.*** [19] also found that HBP is strongly involved in the pathophysiology of sepsis and septic shock, indicating a promising diagnostic marker and also a target for treatment.

Bentzer *et al.* [20] found that HBP also causes cytoskeletal rearrangement and cell contraction, resulting in endothelial gaps, vascular leakage, and neutrophil extravasation, which contributes to further HBP release from azurophilic granules

The presence of HBP within the secretory granules, which mobilized quickly after neutrophil activation, is responsible for the early increase in HBP. Following release, HBP leads to endothelium permeability changes, resulting in vascular leakage [21,22].

Linder *et al.* studies[15,23] stated the same findings and concluded that, as compared to the other biomarkers studied, HBP was the best predictor of circulatory failure. The previously investigated biomarkers such as PCT, CRP, and TLC count are mainly used in the early detection of bacterial infection in critically ill patients and they have limited roles in the detection of circulatory failure[24].

In the current study, there was statistically significant correlation between HBP and patient criteria on admission. On pairwise comparison, the difference is significant between groups of sepsis and septic shock (higher in patients with septic shock). This came in agreement with **Zanfaly *et al.*** [12] who found that on comparing the mean values of HBP at enrollment (baseline), there were no significant differences in the infection group but there was a significant increase in the sepsis and septic shock groups (P<0.001 for each).

In the present study, there was statistically significant positive correlation between heparin binding protein on admission, and serum lactate, total leucocytic count on admission while there was statistically non-significant correlation between heparin binding protein on admission and PCT on admission. On the other and **Zanfaly *et al.*** [12] found that there was a statistically significant positive correlation between HBP level and serum lactate (R=0.26, P=0.02, and R=0.49, P<0.001, respectively) in all septic patients. But there was no significant correlation between HBP and PCT or TLC count.

Linder *et al.* [15] found that there was a statistically significant positive correlation between HPB level and lactate, but there was no significant correlation between HBP and PCT or TLC count. Previous studies reported that the elevated levels of lactate, cortisol, and interleukin-6 in the blood of patients with different etiologies of shock had significant predictive values [25,26].

However, these biomarkers have some limitations: lactate levels are less influenced by arterial sampling and endogenous cortisol levels are down regulated by corticosteroids used in the treatment of

septic shock, or by relative adrenal insufficiency and interleukin-6 analysis is not generally available in regular hospital laboratories[27].

In the present study, the best cutoff of HBP in diagnosis of septic shock on admission was≥13.35 with sensitivity 90.3%, specificity 62.9%. These results were close to results of **Tydén *et al.*[28]** who found that the best cutoff of HBP on admission in diagnosis of septic shock on admission was≥15.0 with sensitivity 87.1%, specificity 95.1%.

Additionally, **Linder *et al.*[15]** reported that plasma HBP level >15 ng/ml indicated presence of severe sepsis and/or septic shock with better diagnostic performance than any other evaluated biomarker (total leucocytic count, IL-6, procalcitonin and CRP)**.**

Kahn *et al.*[29] also demonstrated that HBP has a good prognostic role in predicting critical infections with an AUC of 0.88 and outperformed all other investigated markers in the study. The cut-off value in critical infection was 22.85 ng/ml.

In the current study, ROC-curves looking at sensitivity and specificity of plasma levels of HBP at admission showed areas under the curve of 0.822. The risk model's ROC area is comparable to that of previous studies: 0.80 in **Kashani *et al.*[30]** and 0.86 in **Honore *et al.*[31].**

Recently **Zhou *et al.*[11]** found that AUC of HBP was 0.893, and the optimal cut-off value was HBP≥28.1ng/mL, showed a sensitivity of 84.9%, a specificity of 78.3%, a positive predictive value of 94.0% and a negative predictive value of 65.9% in diagnosing septic shock and these values were better than other investigated markers. The second-best probe was the PCT level.

In the present study, HBP was significantly more efficient in identifying patients who developed septic shock compared with the other investigated markers as the diagnostic accuracy for the identification of patients progressing to sepsis and septic shock was highest for HBP, the best cutoff was ≥13.35with area under curve 0.822, with sensitivity 90.3%specificity 62.9%

This is in accordance with the results of **Kahn *et al.*[29]** found that HBP had an AUC of 0.82, sensitivity 64% which was better performance than PCT with an AUC of 0.76, sensitivity 36%, serum lactate with an AUC of 0.53, sensitivity 53% and TLC with an AUC of 0.67, sensitivity 62%. **Linder *et al.*[23] ,**studied the role of HBP in prediction of septic shock compared with other sepsis biomarkers (PCT , lactate, TLC, CRP and IL6) he reported that HBP with cutoff value ≥15 had an AUC of 0.85 with Sensitivity 87.1%and Specificity 95.1% which was significantly higher than PCT, serum lactate and TLC.

CONCLUSION

It could be concluded that serum heparin binding protein level is positively correlated with the severity of sepsis and septic shock and showed a good performance as an early diagnostic marker in patients with suspected sepsis and septic shock.

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