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EVALUATION OF C-REACTIVE PROTEIN LEVELS AS BIOMARKERS FOR IMMUNE RESPONSE AMONG SEPTIC PATIENTS IN YEMEN

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Abstract

Background: Sepsis is a severe and potentially fatal illness characterized by an uncontrolled immune response to infection, resulting in significant morbidity and mortality. This study aimed to evaluate C-reactive protein (CRP) levels as biomarkers for immune response among septic patients in Sana'a, Yemen and assess their correlation with other clinical factors. Methods: A cross-sectional study was conducted among 100 septic patients admitted to three major hospitals in Sana'a city between January and April 2024. Patients' demographic data, CRP levels, cortisol levels, complete blood count (CBC) results, and bacterial isolates were collected and analyzed using SPSS version 27. Spearman's correlation and logistic regression tests were employed to assess relationship between variables. Results: Among the 100 patients, 56.0% were male and 48.0% were over 40 years old. Elevated CRP levels were seen in 95.0% of patients, with 67.0% showing elevated cortisol levels. Klebsiella spp. was the most common bacterium isolated (24.1%). Significant correlations were found between CRP levels and age ($r = 0.343$, $p < 0.001$), cortisol levels ($r = 0.209$, $p = 0.037$), hemoglobin ($r = -0.401$, $p < 0.001$), platelet count ($r = -0.254$, $p = 0.011$), and red blood cell count ($r = -0.277$, $p = 0.005$). Multivariate logistic regression identified lower hemoglobin levels as a significant predictor of higher CRP levels (OR: 0.424, 95% CI: 0.203-0.887, $p = 0.023$). Conclusion: This study highlights the clinical relevance of CRP levels in assessing immune response and disease severity in septic patients. Lower hemoglobin levels were consistently associated with higher CRP levels, highlighting potential implications for patient management and treatment strategies in sepsis.

Keywords: C-Reactive Protein, Sepsis, Biomarkers, Immune Response, Yemen.

INTRODUCTİON

Sepsis is a medical condition manifested by an abnormal host response to infection[1], leading to tissue and organ damage [2]. The severity of sepsis ranges from mild to severe, including septic shock, with mortality rates estimated at ≥10% for sepsis and ≥40% when shock is present [1, 3]. Sepsis involves physiological, biological, and biochemical abnormalities that can lead to multiple organ failure and death [4].

Globally, sepsis remains a leading cause of death in the critical care units.[5]. It is linked to high morbidity and mortality rates and requires considerable medical resources. Therefore, understanding the pathogenesis and identifying prognostic predictors for sepsis is of great clinical significance [6].

During sepsis, the inflammatory response leads to increased cortisol secretion through activating the hypothalamic-pituitary-adrenal (HPA) axis. Elevated levels of circulating cortisol help counteract inflammation and low blood pressure associated with sepsis.

Cortisol's anti-inflammatory properties work to mitigate the cytokine storm triggered by sepsis. However, in case of severe sepsis and septic shock, cortisol levels may not rise adequately despite HPA axis activation, a condition known as critical illness-related corticosteroid insufficiency [7, 8]. These patients tend to have worse outcomes, including higher mortality rates. Conversely, excessive cortisol release can also be detrimental, emphasizing the need for a delicate balance to achieve optimal anti-inflammatory effects without further compromising the immune system [9-11]. This study aims to evaluate C-reactive protein (CRP) levels as biomarkers for the immune response among septic patients, and the association between CRP and other biomarkers.

METHODS

Study Design, Setting, and Sampling technique

This cross-sectional study was carried out among three main tertiary care hospitals in Sana'a: University of Science and Technology Hospital, Al-Jomhori Hospital, and Modern European Hospital. The study period spanned from January to April 2024. Using convenient sampling, 100 septic patients from the intensive care units of these hospitals were included.

A checklist was used to collect data, which included demographic characteristics (age, gender), complete blood count (CBC), and culture sensitivity. Blood samples were taken from patients under sterile conditions and sent to the laboratory to measure levels of cortisol and C-reactive protein (CRP) according to standardized protocols. To ensure consistency in testing methods, all cortisol and CRP level tests were performed by a single laboratory.

Data Analysis

Data analysis utilized IBM SPSS Statistics version 27.0 for Windows® (IBM Corp., Armonk, NY, USA). Frequencies and percentages described nominal and categorical variables, while means and standard deviations summarized continuous variables. Spearman's correlation test assessed relationships between CRP levels and other variables, with a scatter plot displaying the coefficient of determination (R^2).

The severity of CRP was classified based on the 75th percentile into two groups: CRP > 141 mg/L and CRP ≤ 141 mg/L. Independent variables with a p-value below 0.25 in univariate logistic regression were included in a multivariate binary logistic regression model [12].Odds ratios quantified the impact of each predictor on the severity of CRP elevation. A p-value of less than 0.05 indicated statistical significance.

RESULT

Participant Demographics of the septic patients and their Clinical Profile

Table 1 shows the demographic and clinical characteristics of the participating septic patients. The participants spanned various age groups, with 30.0% (n=30) aged ≤1 year, 22.0% (n=22) aged between 1 and 40 years, and 48.0% (n=48) aged over 40 years. Males constituted the majority, comprising 56.0% (n=56) of the participants, while females accounted for 44.0% (n=44).

Regarding clinical markers, 95.0% (n=95) of the participants had high C-reactive protein (CRP) levels, while only 5.0% (n=5) had normal CRP levels. For corticosteroid

levels, 67.0% (n=67) of participants had elevated levels, whereas 33.0% (n=33) had normal levels. In terms of gram stain results, 65.5% (n=19) were gram-negative, and 34.5% (n=10) were gram-positive.

The bacterial isolates identified in the study showed a diverse range of species. *Klebsiella Spp*. was the most frequently isolated, accounting for 24.1% (n=7) of cases, followed by E. coli and *Enterococcus Spp*. at 13.8% (n=4) each. Other isolated bacteria included *Klebsiella pneumoniae* at 10.3% (n=3), Staphylococci at 6.9% (n=2), and several others, such as *Enterococcus faecium, Enterococcus faecalis, Pseudomonas aeruginosa, Pseudomonas Spp., Staphylococcus aureus, Staphylococcus hemolytic, Acinetobacter baumanii complex, Acinetobacter spp., and Citrobacter Spp*., each representing 3.4% (n=1) of the cases.

Table 1: Participant Demographics and their Clinical Profile(n=100)

		Count	(%)
Age	≤ 1 year	30	(30.0)
	1-40 years	22	(22.0)
	> 40 years	48	(48.0)
Gender	Male	56	(56.0)
	Female	44	(44.0)
CRP	Normal	5	(5.0)
	High	95	(95.0)
Corticosteroid	Normal	33	(33.0)
	High	67	(67.0)
Gram stain	Gram positive	10	(34.5)
	Gram negative	19	(65.5)
Isolated bacteria	*Klebsiella pneumoniae*	3	(10.3)
	Klebsiella Spp.	7	(24.1)
	E. coli	4	(13.8)
	Enterococcus Spp.	4	(13.8)
	Enterococcus faecium	1	(3.4)
	Enterococcus faecalis	1	(3.4)
	Pseudomonas aeruginosa	1	(3.4)
	Pseudomonas Spp.	1	(3.4)
	Staphylococcus aureus	1	(3.4)
	Staphylococcus Spp.	2	(6.9)
	Staphylococcus hemolytic	1	(3.4)
	Acinetobacter baumanii complex	1	(3.4)
	Acinetobacter spp.	1	(3.4)
	Citrobacter Spp.	1	(3.4)

Correlation between CRP and other parameters in patients with sepsis

Table 2 presents correlations between CRP levels and various parameters among patients with sepsis. Age showed a moderate positive correlation with CRP levels ($r = 0.343$, $P < 0.001$). Gender did not show a significant correlation with CRP ($r = -0.019$, $P = 0.850$), suggesting that CRP levels were not influenced by gender in this study.

Corticosteroid levels exhibited a weak positive correlation with CRP ($r = 0.209$, $P = 0.037$). Hemoglobin ($r = -0.401$, $P < 0.001$), RBC count ($r = -0.277$, $P = 0.005$), PCV ($r = -0.338$, $P < 0.001$), MCV ($r = -0.241$, $P = 0.016$), MCH ($r = -0.303$, $P = 0.002$), and lymphocyte count ($r = -0.302$, $P = 0.002$) all showed significant negative correlations with CRP levels. These findings indicate that lower levels of hemoglobin, RBCs, PCV,

MCV, MCH, and lymphocytes were associated with higher CRP levels, suggesting a potential link between anemia and immune response reflected by CRP elevation in septic patients.

Among other parameters tested, neutrophil count showed a weak positive correlation with CRP (r = 0.254, P = 0.011), while no significant correlations were found for MCHC, RDW, WBCs, monocytes, eosinophils, basophils, or platelet counts.

Table 2: Correlation between CRP and other parameters in patients with sepsis

Parameter	Count	Pearson Correlation	P value
Age	100	.343**	**<0.001**
Gender	100	-.019	0.850
Cortisone	100	0.209*	**0.037**
Hemoglobin	100	-.401**	**<0.001**
RBC	100	-.277**	**0.005**
PCV	100	-.338**	**<0.001**
MCV	100	-.241*	**0.016**
MCH	100	-.303**	**0.002**
MCHC	100	-.157	0.119
RDW	100	.046	0.648
WBCs	100	.163	0.105
Neutrophils	100	.254*	**0.011**
Lymphocytes	100	-.302**	**0.002**
Monocytes	100	-.036	0.722
Eosinophils	100	.047	0.639
Basophils	100	-.007	0.943
Platelets	100	-.178	0.076

"*" and "**" denote significance at the 0.05 and 0.01 levels, respectively.

The study found that 25.0% (n=25) of patients had CRP levels > 141 mg/L, indicating a higher severity of systemic disease, while 75.0% (n=75) had CRP levels ≤ 141 mg/L, as shown in Figure 1. The scatter plot (Figure 2) illustrates that variations in hemoglobin (Hb) levels explain approximately 11.7% of the variance in CRP levels, as indicated by the coefficient of determination ($R^2 = 0.117$).

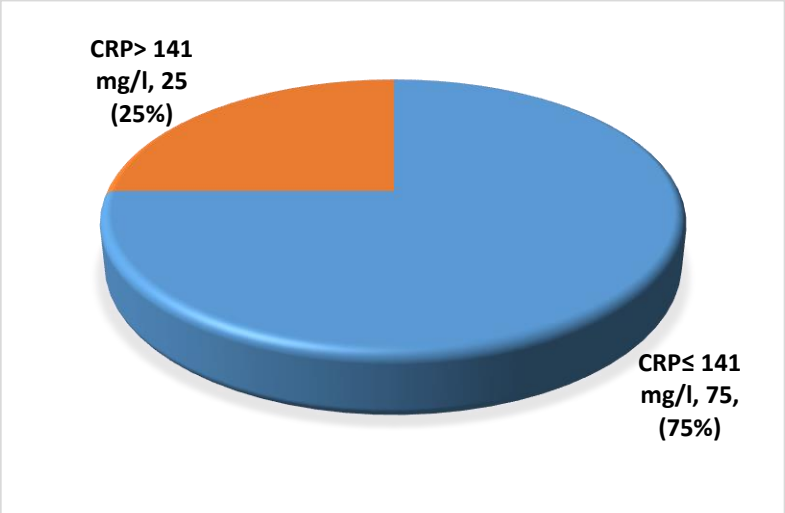

Figure 1: Severity of CRP elevation.

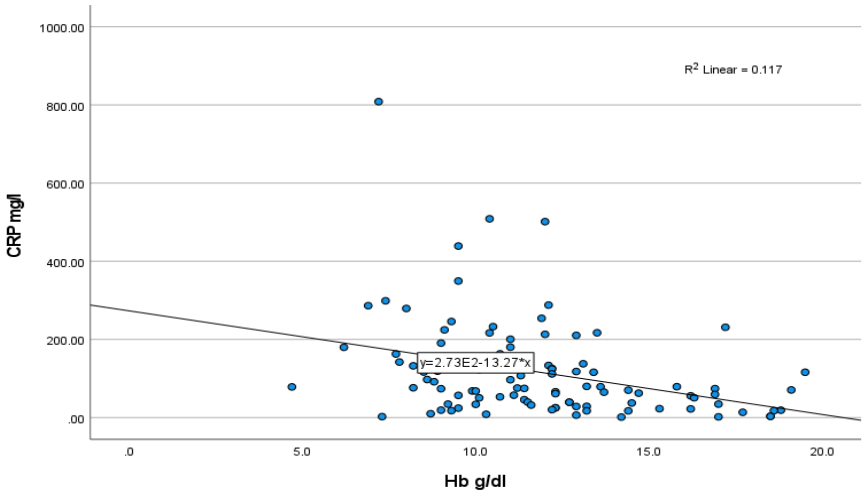

Figure 2: Scatter plot of the relationship between Hb and CRP. R2 = 11.7%

Factors associated with the severity level of CRP

The analysis of factors influencing high C-reactive protein (CRP) levels among sepsis patients revealed several significant associations in the univariate logistic regression. Age was positively associated with high CRP levels, with an odds ratio of 1.028 (95% CI: 1.009, 1.047, p=0.004). Hemoglobin levels exhibited a protective effect, with an odds ratio of 0.752 (95% CI: 0.622, 0.908, p=0.003). Similarly, packed cell volume (PCV) also exhibited a protective effect, with an odds ratio of 0.945 (95% CI: 0.896, 0.997, p=0.037). Conversely, a higher neutrophil percentage (N%) was linked to increased CRP levels (odd ratio: 1.029, 95% CI: 1.002-1.057, p=0.036), while higher lymphocyte percentage (L%) (odd ratio: 0.952, 95% CI: 0.913-0.992, p=0.018) and platelet count (odd ratio: 0.996, 95% CI: 0.992-0.999, p=0.021) were associated with lower CRP levels. In multivariate logistic regression, only hemoglobin levels remained significantly associated with elevated CRP levels (adjusted odd ratio: 0.424, 95% CI: 0.203-0.887, p=0.023), indicating that lower hemoglobin predicts higher CRP. Other variables, including age, PCV, neutrophil percentage, lymphocyte percentage, and platelet count, did not maintain significance. The model showed 79% accuracy in classification, and the Hosmer-Lemeshow Test confirmed its good fit (p=0.646) for predicting high CRP levels in sepsis patients.

Table 3: Factors influencing high CRP level among sepsis patients (n=100)

	Univariate logistic regression		Multivariate logistic regression	
	COR(95% C.I.)	P value	COR(95% C.I.)	P value [a]
Age	1.028 (1.009, 1.047)	**0.004***	1.012 (0.987, 1.038)	0.357
Gender	0.804 (0.320, 2.018)	0.642		
Hb g/dl	0.752 (0.622, 0.908)	**0.003***	0.424 (0.203, 0.887)	**0.023***
PCV %	0.945 (0.896, 0.997)	**0.037***	1.266 (0.993, 1.615)	0.057
WBCs	1.012 (0.963, 1.063)	0.638		
N%	1.029 (1.002, 1.057)	**0.036***	1.010 (0.977, 1.044)	0.542
L%	0.952 (0.913, 0.992)	**0.018***	0.994 (0.938, 1.052)	0.827
PLT	0.996 (0.992, 0.999)	**0.021***	0.997 (0.993, 1.001)	0.106
Cortisone	1.011 (0.990, 1.033)	0.318		

Note : a, variables with p value < 0.25 in univariate regression were included in multivariable regression. Model shows classification table of 79; Hosmer and Lemeshow Test =0.646.

75th quartile was used to classify CRP into two groups.

Abbreviation: White Blood Cells; PCV, Packed Cell Volume; Hb, Hemoglobin; WBCs, N%, Neutrophil Percentage; L%, Lymphocyte Percentage; PLT, Platelets

DISCUSSION

Our findings revealed that patients' age, cortisol level is positively correlated with the CRP. However, RBC indices (except neutrophils) negatively correlated with the CRP level. The abnormalities in hemoglobin and RBC indices among septic patients, indicating anemia and alterations in red blood cell production and function. These correlations indicate that lower levels of these hematological parameters are linked to higher CRP levels, reinforcing the link between anemia and inflammation in septic patients. This study's findings align partially with those of Agnello et al.[13].

Our findings revealed that patients' age, cortisol levels, Neutrophils, were positively correlated with CRP levels, indicating a more severe inflammatory response in older patients and those with higher cortisol. Conversely, most RBC indices, except for neutrophils, were negatively correlated with CRP levels. These correlations indicate that lower levels of these hematological parameters are linked to higher CRP levels, reinforcing the connection between anemia and inflammation in septic patients. This is partially compatible with the findings of Chauhan et al.[14].

The severity of CRP levels, reflecting the severity of the immune response, was associated with patients' age, cortisol levels, and certain RBC indices, including hemoglobin, neutrophils, and lymphocytes. These results are consistent with Chauhan et al. [14], who found that elevated CRP levels serve as a marker of systemic inflammation, aiding in the assessment of sepsis severity and treatment response monitoring.

Our study showed that the most frequently isolated bacteria in sepsis include Klebsiella species, E. coli, Pseudomonas aeruginosa, and Staphylococcus aureus, consistent with previous research [15]. Nearly two-thirds of sepsis cases in our study were attributed to gram-negative bacteria, aligning with prior studies [16]. However, other studies have shown that gram-positive bacteria are also frequent causes of sepsis [17]. A meta-analysis revealed higher incidence and CRP levels in sepsis caused by gram-negative bacteria compared to gram-positive bacteria [18].

Previous studies have shown that gram-positive bacteria leading to sepsis are most commonly due to Enterococcus and Staphylococcus species [19]. In our study, Enterococcus and Staphylococcus were the exclusive gram-positive bacteria leading to sepsis.

CRP concentrations exceeding 100 mg/l generally signify a notable inflammatory response. In our study, 66.5% of patients exhibited CRP levels above this threshold, with infections, rheumatologic diseases, inflammatory conditions, malignancies, and drug reactions commonly contributing to elevated CRP [20]. However, only 41% of our patients had severely elevated CRP levels, likely due to differences in comorbidities, patient age, blood sample timing, and other sociodemographic factors. Additionally, more than one-third of this study is pediatric age group.

Age was found to be associated with the severity of CRP elevation, consistent with other studies [21, 22]. Additionally, a negative association between hemoglobin levels and CRP elevation was observed, aligning with prior research conducted among

emergency unit patients [23]. Regarding lymphocyte count, a significant negative correlation with CRP was found, consistent with findings in individuals with family contact with COVID-19 [24].

Limitations

This study has several limitations. First, CRP levels can be influenced by the timing of blood collection, which was not accounted for in this study. Second, patients' comorbidities were not considered, may affect the results. Lastly, the reduced sample size limited the ability to explore in-depth statistical associations between CRP and other variables.

CONCLUSIONS

In conclusion, the study highlights significant correlations between CRP levels and various hematological parameters, as well as age in septic patients, reinforcing the complexity of the inflammatory response in sepsis. After adjusting for other factors, hemoglobin (Hb) remained statistically significant in its association with CRP levels. Further research with larger sample sizes and consideration of comorbidities is needed to deepen the understanding of these associations.

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