The Role of Procalcitonin and Serum Amyloid a As a Laboratory Biomarker in COVID-19

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ABSTRACT

Background: Recent research demonstrates the critical impact that cytokine storm and intensive immune response against the virus play in worsening the severity and death of illness. Biomarkers such as Procalcitonin (PCT), Serum Amyloid A (SAA) play a part in the etiology of severe COVID-19 and may serve as an early indicator for severity.
Objective: The current study aimed to determine the serum concentrations of these laboratory biomarkers (PCT and SAA) in asymptomatic, moderate, severe and critical COVID-19 patients and to compare them to healthy controls.
Patients and methods: A total of 100 patients by positive RT-PCR COVID-19 results besides a control group of 46 healthy participants with negative RT-PCR results. Patients were classified as 4 groups; critical, severe, moderate, or asymptomatic, according to WHO standards. Enzyme-Linked Immune Sorbent Assay (ELISA) was used by (CV Human Reader HS REF 16670) to measure PCT and SAA.
Results: Mean level of PCT is 404.59 pg/ml and SAA 19.1 pg/ml among patients more than controls (127.65 and 10.64, respectively). Mean levels were higher among both PCT and SAA in critical (680.84 and 28.46, respectively) and severe (565.86 and 23.27, respectively) of COVID-19 patients, other than moderate (226.98 and 13.71, respectively) and asymptomatic (144.67 and 10.75, respectively). **Conclusion**: There was a major alteration in the level of PCT and SAA among patients groups (critical, severe, moderate, and asymptomatic) and control group. A strong correlation was found between biomarkers high concentrations, old age, and chronic diseases with the disease severity.
Keywords: Serum Amyloid A, Procalcitonin, COVID-19, Prognosis, Case control study, University of Anbar.

INTRODUCTION

This respiratory infection has spread globally after the coronavirus disease 19 (COVID-19) outbreak in China in December 2019, becoming a pandemic that has infected millions of people and spread to numerous countries [1].

Furthermost patients infected by SARS-CoV-2 have mild illness and existing common symptoms for example fever, cough, besides fatigue [2].

A minor number of infected patients would development to severe cases with acute respiratory distress syndrome. Some patients with severe and critical illness would degenerate in a short period of time and die rapidly due to multiple organ failure, especially in elderly patients [3].

In severe and critical COVID-19, the number of inflammatory and non-inflammatory markers increased. Some of these indicators were utilized to assess the severity and prognosis of pneumonia. It is well recognized that the generation of pro-inflammatory cytokines typically characterizes dysregulated innate and adaptive immune responses [4].

Pro-inflammatory cytokines, such as COVID-19, IL-6, and IL-10, which have been used to support diagnosis, monitor treatment, and assess illness severity, can be utilized as predictors for quick detection of individuals at higher risk of disease and assist doctors [5].

Except for these strictures, many other parameters too can be easy to get after routine blood and biochemical investigation [6]. In order to identify whether other indices from common laboratory testing have prophetic value in patients with COVID-19, and

offer more evidence for clinical finding and management, this task will examine selected indices of peripheral blood analysis and biochemical analysis with [Procalcitonin (PCT), Serum amyloid A (SAA) and lymphocyte count] in COVID-19 patients, PCT has used as a diagnostic marker in clinical practice [7]. PCT is released by all parenchymal tissue under the influence of endotoxins and pro-inflammatory cytokines. Increased levels of PCT, the 116-amino acid precursor of the hormone calcitonin, are positively correlated with COVID-19 severity, a marker of secondary bacterial infection [8].

Various viral and bacterial infections have been proven to cause a rise in SAA, an acute-phase reactant that release by the liver [9]. The level of SAA has newly been measured in biological fluids from patients with different lung diseases [10].

In this study, we sought to determine whether, in addition to the primary parameters, it is possible to use these inflammatory markers to assess the severity of the disease in COVID-19 patients and to support the diagnosis of COVID-19 patients. We also sought to determine whether these indices from routine laboratory testing are useful in predicting prognosis in COVID-19 patients.

PATIENTS AND METHODS

Study was carried out on 146 persons, divided into two groups; 100 patients with positive RT-PCR COVID-19 test, and a control group of 46 healthy persons with negative RT-PCR test.

Patients with positive RT-PCR COVID-19 test also had divided into four subgroups; Critical, severe, moderate and asymptomatic with 25 patients for each group.

Patients ranged in age from 15 to 84 years. Measurement of SAA and PCT was done by using Enzyme-Linked Immune Sorbent Assay (ELISA) done by CV Human Reader HS REF 16670.

Peripheral blood samples of 5-10 ml were collected from 100 COVID-19 patients after real- time PCR test for nasopharyngeal swab. The samples were obtained from patients while they were suffering from an active illness, with the majority of their symptoms appearing between days 5 and 14 after contracting the infection.

Complete blood counts (CBC) including the count of lymphocytes were measured for all participants.

Ethical Approval:
This study was ethically approved by the Institutional Review Board of the College of Medicine, University of Anbar, and an informed written consent was taken from each participant in the study. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) for studies on humans.

Statistical Analysis
The collected data were introduced and statistically analyzed by utilizing the Statistical Package for Social Sciences (SPSS) version 20 for windows. Qualitative data were defined as numbers and percentages. Chi-Square test and Fisher's exact test were used for comparison between categorical variables as appropriate. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as mean and SD, and independent sample t-test and ANOVA test were used for comparison between groups. P value ≤ 0.05 was considered to be statistically significant.

RESULTS
A total 100 adult patients of COVID-19 with positive RT- PCR COVID-19 test with age group ranging from 25 to 84 years were included in this study. The patients were selected from different Hospitals in Iraq.
The mean ages of critical and severe COVID-19 patients were at the sixth decade, while the moderate and asymptomatic COVID-19 patients were at the fifth

to third decade of ages. In comparison with mean ages of control which recorded at the fourth decade of ages. These differences statistically were highly significant (**Table 1**).

Table (1): Comparison of the mean ages of the studied groups.

Study Groups	Mean	SD	P-value
Critical (n=25)	64.12	2.49	
Sever (n=25)	61.04	2.45	≤ 0.001 (H.S)
Moderate (n=25)	57.4	2.23	
Asymtomatic (n=25)	38.56	1.97	
Control (n=46)	43.97	2.84	
Total	51.71	1.43	

Most cases of COVID-19 were males, with no statistical significant difference regarding gender (**Table 2**).

Table 2: Distribution of studied groups according to genders.

Study Groups		Male	Female	Total	P Value
Critical	N	14	11	25	Chi-square 7.27 P-value 0.1
	%	56%	44%	100%	
Sever	N	13	12	25	
	%	52%	48%	100%	
Moderate	N	20	5	25	
	%	80%	20%	100%	
Asypmtomatic	N	19	6	25	
	%	76%	24%	100%	
Control	N	33	13	46	
	%	71.7%	28.3%	100%	
Total	N	99	47	146	
	%	67.8%	32.2%	100%	

The mean levels of PCT in patients were (404.59) more than control (127.65) were highly significant, while the mean levels of PCT were significantly higher among critical (608.84) and severe (565.86) of COVID-19 patients more than moderate (226.98), asymptomatic (144.67) and control (127.65) groups. These differences have P$\leq$0.001 (**Figure 1**).
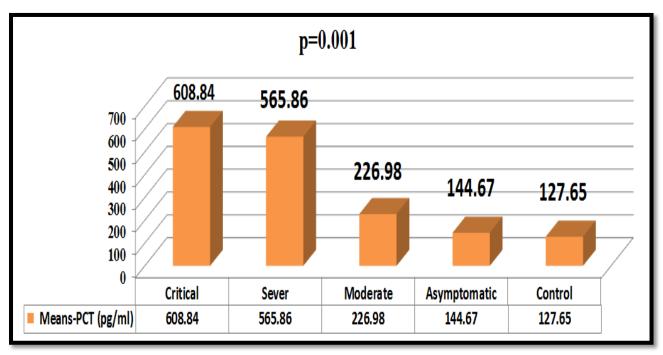
Figure 1: Levels of PCT among COVID-19 patients and control group.

The mean levels of SAA (19.1) in patients more than control (10.64) were highly significant, the levels of SAA in critical (28.46) and severe (23.27) of COVID-19 patients, while the levels of SAA were within normal levels among the moderate (13.71).

Figure 2 displays the changes in the levels between the control (10.64) and asymptomatic (10.95) COVID-19 patient groups that were highly significant (P<0.001).
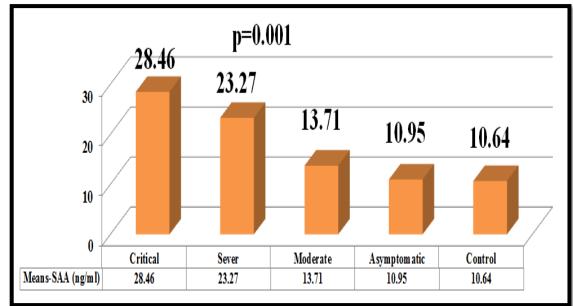

Figure 2: Levels SAA among COVID-19 patients and control group.

Table 2 shows the mean count of lymphocyte in the blood of patients was 1.42 which was lower than in control (2.51), with statistically significant difference.

Table 2: The count of lymphocyte cells in the patients and controls.

Test	Study Groups	N	Mean	SD	T-test	P-value
Lymphocyte	Case	100	1.42	0.09	6.82	<0.001 (H.S)
	Control	46	2.51	0.13		

DISCUSSION

Investigation of the COVID-19 patients in this study revealed that men made up the majority of cases. This may be due to the fact that men are more likely to engage in risky behaviors, such as working in low-skilled jobs that require them to be active, leave the house, and interact with others even during the containment phase. Men are also more likely to leave their homes, engage in social activities, sit with others, and remove their masks when drinking or smoking [11]. The types and quantities of sex hormones produced by men and women can differ, which may influence a person's susceptibility to COVID-19 infection [12].

According to the study, patients are in their sixth decade. People between the ages of 56 and 80 had the highest infection prevalence (43%), which may be explained by the fact that patients in this age group are more likely to have other predisposing illnesses, such as diabetes mellitus, hypertension, liver dysfunction, or renal failure. Contrary to what was discovered in an Italian study, this one revealed that patients over 70 were more likely to develop infections and pass away generally [13].

PCT levels appeared to depend on the degree of the disease and may be related to bacterial co-infection. According to this study, patients with severe COVID-19 had considerably greater PCT levels in their serum [14]. Additionally, it was discovered that patients in the critical and severe group had considerably higher elevated PCT levels than those in the moderate and asymptomatic group. This increase may be due to severe COVID-19 or bacterial co-infection, which likely develops later in the course of the illness. The immunological response to SARS-CoV-2 infection is brought on by mixed pneumonia caused by viruses or bacteria. Consequently, it is likely that any co-infection will increase the course and severity of COVID-19 [15]. A meta-analysis of four Chinese studies revealed that a five-fold increased incidence of severe COVID-19 is associated with raised PCT levels [16]. Elevated PCT levels have been linked in several studies to greater COVID-19 severity [17].

Circulating levels of SAA were found to be higher in severe cases and both were strongly correlated with the severity of COVID-19. SAA is directly chemotactic for neutrophils, monocytes, mast cells, and T cells, and can enhance inflammatory response through chemokine activation and chemotkaxis induction [18].

The critical and severe group had significantly lower lymphocyte numbers. These findings demonstrated that coronavirus consumed a large number of immune cells and impaired the body's cellular immunological activity because there was a significant decrease in the overall number of lymphocytes. A meta-analysis revealed that lymphopenia and severe COVID-19 were related [19]. In acute SARS-CoV infection, peripheral T cells, both CD4+ and CD8+, are rapidly decreased. Lymphocytes that express the ACE2 receptor and are sequestered in particular target organs may be a direct target of SARS-CoV-2 infection [20].

CONCLUSION

Serious and critical COVID-19 patients had significantly greater levels of procalcitonin, SAA, and indicators than moderate and asymptomatic patients, whereas critical and severe Covid-19 patients had significantly lower lymphocyte counts.

Conflict of interest: The authors declare no conflict of interest.
Sources of funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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