

RESEARCH ARTICLE

Role of some serological markers in evaluation COVID-19 infection severity

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Received: June 7, 2023; accepted: July 13, 2023.

Covid-19 is considered an epidemic disease that causes increasing the levels of some inflammatory proteins and biomarkers such as interleukins and acute phase proteins. Therefore, these biomarkers could be useful in knowing and monitoring the severity of disease. This study investigated some biomarkers that related to the immunological response including D-dimer, C-reactive protein (CRP), and interleukin-6 (IL-6), and their effects on the severity of infection and early diagnosis of Covid-19 patients. This retrospective study employed two groups of patients' information to investigate the effects of some immune factors associated to Covid -19 with the first group of 30 confirmed COVID-19 patients aged from 15 to 92 years old and the second control group of 30 patients aged from 18 to 73 years old. All patients' blood serum levels of CRP, IL-6, and D-dimer were measured by using Afias-6 luminescence immunoassay and compared between the two groups. The results showed that the highest level of the biomarker was D-dimer at a mean $2,029.65 \pm 143.31$ ng/mL, followed by CRP at 88.46 ± 10.41 ng/mL, and then, IL-6 at 26.03 ± 4.61 ng/mL. The highest percentages of these biomarkers were detected at the ages between 15 and 30 years old for D-dimer at $3,642.73 \pm 126.44$ ng/mL, 47 to 62 years old for CRP at 116.89 ± 16.61 ng/mL, and 79 to 94 years old for IL-6 at 60.57 ± 10.23 ng/mL. In addition, the rates of CRP, D-dimer, and IL-6 were the highest in females compared to males with CRP of 92.42 ± 10.44 ng/mL and 83.71 ± 7.84 ng/mL, D-dimer of $2,200.34 \pm 158.77$ ng/mL and $1,834.55 \pm 251.27$ ng/mL, IL-6 of 29.51 ± 6.27 ng/mL and 22.06 ± 6.88 ng/mL for females and males, respectively. Moreover, the results of the correlation coefficient (R) showed high correlation between those biomarkers. The R values of CRP/D-dimer, CRP/IL-6, and D-dimer/IL-6 were 0.343, 0.151, and 0.121, respectively. The results of this retrospective study concluded that COVID-19 infection significantly affected D-dimer, CRP, and IL-6 percentage in the patients, and those biomarkers could be used to monitor the severity of disease progression.

Keywords: C-reactive protein; D-dimer; interleukin- 6; COVID 19.

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Introduction

Coronavirus (SARS-CoV-2) is a single-stranded RNA virus with a positive genome sense. SARS-coronavirus and MERS-coronavirus are

genetically associated with other coronavirus species. In general, bats are the common hosts for both SARS-coronavirus and MERS-coronavirus. Some reports indicated that the human SARS-CoV-2 virus might be transmitted

through intermediate hosts such as pangolins. Since SARS-CoV-2 is an RNA virus, it can quickly develop through homologous, non-homologous recombination, and mutation, allowing it to propagate through a wider variety of hosts [1]. Coronavirus has a club-like appendix with spherical shapes on the top called "spikes." [2]. The coronavirus consists of four structural proteins including spike (S), envelope (E), membrane (M), and nucleocapsid (N) [3]. COVID-19's pathogenic and physiological processes as well as diagnostic techniques are still being investigated. Clinical surveillance and suitable treatment techniques are required to reduce morbidity cases. For example, pneumonia could be detected in the early stages of infection by the change in the levels of C-reactive protein (CRP), while the severe pneumonia patients had a high level of CRP [4]. About 20% of SARS-CoV-2 infected individuals possibly suffered from fatal diseases such as septic shock, cytokine storm, acute inflammation, coagulation, complications, dysfunction, hypoxia, and multiple organ failure [5]. This fatal disease has shown the relation to hemostatic abnormalities while COVID 19 infection is increasingly recognized [6].

There are many factors that increase the risk venous thromboembolism (VTE) including peripheral and coronary artery disease and heterogeneity in metabolism including obesity, diabetes, and high blood pressure in addition to family history of infections with VTE [7, 8]. Therefore, the severe cases of some types of viral infection such as coronavirus may be interfered by venous thrombosis, which leads to damage to the lining of blood vessels, neutrophil activation, and blood clot formation or disseminated intravascular coagulation (DIC) [9]. Decrease in white blood cell counts and increase of serum interleukin-6 (IL-6) levels were noticed in COVID-19 patients in addition to high production and release of CRP from the liver cells and other immune proteins [10, 11]. The D-dimer is released after lysis of fibrin by fibrinolysis which causes coagulation. Previous studies indicated the importance of measuring blood serum D-dimer level in COVID-19 patients because of its

essential role in monitoring the progression of the disease and the severity of the infection [12]. COVID-19 infection might be associated with hemostatic disorder, and non-survivors showed significantly increased D-dimer levels. Therefore, the D-dimer cutoff value was used for prognosis at the patient admission point and for monitoring the disease severity [13]. One of the risk variables for possible adult COVID-19 death was the patient's blood serum D-dimer level > 1 g/mL, which made the role of detection the D-dimer level in COVID-19 patients essential [14]. The increase in inflammatory cytokines levels such as IL-6 is linked to this hyperinflammatory response. It was concluded that IL-6 played an essential role in a cytokine storm (CRS) in patients with COVID-19 [15]. It is possible to use alternative therapies such as IL-6 inhibitors to reduce the risk of CRS, especially for patients with severe diseases in critical cases. According to international recommendations, some reports indicated that IL-6 inhibitors could also be used for people with COVID-19 as well [16]. COVID 19 progression is linked to serological biomarkers including IL-6, CRP, ferritin, fibrinogen, and D-dimer [17]. Some reports also indicated that IL-6, CRP, and D-dimer in COVID-19 patients could be used to predict respiratory failure [18]. This study was to verify the correlations between biomarkers of D-dimer, IL-6, CRP and the severity of COVID-19 patients, and to use them as the important factors to monitor progression and the severity of the disease.

Materials and Methods

Patient information and data collection

A total of 30 COVID-19 virus infected patients, aged from 15 to 92 years old, who were diagnosed by using polymerase chain reaction (PCR) technology and chest x-rays were involved in this study, while another 30 healthy volunteers were included as the normal control group. All COVID-19 infected patients were recruited from February 2021 to May 2021 in Marjan Medical City, Al-Hilla Teaching Hospitals, and private clinics (Al-Hilla city, Babylon, Iraq), and were non-

smokers, free of chronic diseases, and not diagnosed with the Corona virus before. All procedures of this retrospective study were approved by the Ethics Committee of the University of Babylon, Babylon, Iraq (Approval No. DSM/HO-26745). The patients' data including biomarkers of CRP, D-dimer, and IL-6 were retrieved from the Statistics and Information Center of the Marjan Teaching Hospital (Hillah, Babylon, Iraq) based on the agreement for scientific research collaboration between the Ministry of Higher Education and Scientific Research and the Ministry of Health in Iraq.

Data processing and biomarker measurement

All patients' medical information including gender, age, and address were included in this study. According to patients' medical records, peripheral venous blood samples were collected. The serums were then isolated by centrifugation at 5,000 rpm for 10 minutes at room temperature and were immediately stored at -20°C for biochemical analysis. The fluorescence immunoassays were employed for the measurements of serum CRP, D-dimer, and IL-6 by using the reagent kits and AFIAS-6 Automated Immunoassay Analyzer (Boditech Med Inc, Gangwon-do, Korea).

Statistical analysis

The data were analyzed by using SPSS version 23.0 (IBM, Armonk, New York, USA). One-way ANOVA and Duncan's test were employed to compare the data difference. The significance difference was set at $P < 0.05$ as significant difference and $P < 0.01$ as very significant difference.

Results and discussion

Diagnosis of coronavirus according to serological markers (CRP, D-Dimer, and IL-6)

In this retrospective study, the serological markers of CRP, D-Dimer, and IL-6 demonstrated a diagnostic behavior for coronavirus infection. The most predictor associated with symptoms of

coronavirus was D-dimer with the average of $2,029.65 \pm 143.31$ ng/mL, followed by CRP at 88.46 ± 10.41 ng/mL, and then IL-6 at 26.03 ± 4.61 ng/mL. On the other hand, in the control group, D-dimer was at 248.65 ± 26.27 ng/mL, while CRP at 1.85 ± 0.35 ng/mL and IL-6 at 2.87 ± 0.24 ng/mL. The statistical analysis showed very significant differences between the patient and control groups ($P < 0.01$) (Table 1). Globally, the incidence of COVID-19 rises quickly. Moreover, patients without symptoms are also a source of infection [19]. COVID-19 as a new public health issue, the increase of mortality rate has been observed in severe cases [20]. The results of this study showed that increased serum levels of IL-6 and CRP had higher correlations to respiratory failure, which indicated the importance of measuring these markers in COVID-19 patients to predict the development of the infection that led to pulmonary failure. Excessive clotting and hypoxemia cause an increase in D-dimer level in patients with COVID-19. D-dimer elevation is common in COVID-19 patients with severe conditions which increased the risk of blood clots and led to death [21]. This study showed an increase in the D-dimer as a serological marker for coronavirus detection, which was consistent with the results reported Righini, *et al.* [22]. In COVID-19 patients, the most sensitive change in coagulation parameters is an increase in D-dimer level that leads to an increase in the risk of clot formation. However, D-dimer has a limited specificity for venous thromboembolism and is more of a signal of fibrinolysis than of current thrombosis [23]. On the other hand, elevated D-dimer levels were substantially linked with severe types of COVID-19 and the existence of a CEP according to a study by Han, *et al.* [24]. Bilgir, *et al.* found that the mean of D-dimer levels in COVID-19 patients were considerably higher than that in healthy people ($P < 0.001$) and increased significantly with disease severity [25]. The concentration of CRP is influenced by many factors such as gender, age, and physical condition and also related to the inflammation severity. The results showed that CRP levels rose as the illness advanced and were shown to be linked to coronavirus infection. The increase of

Table 1. Serological markers (D-dimer, CRP, and IL-6) with coronavirus.

Parameters	Control	Patient	P value
CRP	1.85 ± 0.35	88.46 ± 10.41	0.0001**
D-dimer	248.65 ± 26.27	2,029.65 ± 143.31	0.002**
IL-6	2.87 ± 0.24	26.03 ± 4.61	0.0001**

Note: The data were expressed as mean ± SE. ** very significant difference ($P < 0.01$).

Table 2. The association between COVID-19 patients and control group according to age.

Parameters	Age (years)	Control	Patient	P value
CRP	15-30	0.50 ± 0.04	79.86 ± 17.22	0.038*
	31-46	2.61 ± 0.02	46.22 ± 8.21	0.0001**
	47-62	1.93 ± 0.01	116.89 ± 16.61	0.0002**
	63-78	2.63 ± 0.03	97.34 ± 10.10	0.006**
	79-94	-	89.25 ± 9.33	-
D-dimer	15-30	159.61 ± 12.55	3,642.73 ± 126.44	0.0001**
	31-46	215.98 ± 18.71	905.25 ± 111.27	0.0001**
	47-62	266.89 ± 15.32	2,114.81 ± 115.28	0.0001**
	63-78	360.73 ± 22.51	1,584.15 ± 244.12	0.0001**
	79-94	-	2,855.22 ± 150.23	-
IL-6	15-30	2.30 ± 0.30	4.32 ± 0.72	0.033*
	31-46	2.40 ± 0.40	7.18 ± 1.36	0.020*
	47-62	3.51 ± 0.42	26.82 ± 11.82	0.039*
	63-78	2.93 ± 0.68	33.97 ± 7.19	0.029*
	79-94	-	60.57 ± 10.23	-

Note: The data were expressed as mean ± SE. *significant difference ($P < 0.05$). **very significant difference ($P < 0.01$).

CRP might represent lung lesions and disease severity in the early stages of COVID-19 [26]. In the current study, the normal range of CRP level was compared with COVID-19 patients and control group, which looked at the probable variables linked to illness development and severity. In addition, the CRP's diagnostic significance in the progress of COVID-19 was noticed [27]. The correlation between CRP level and COVID-19 infection progress was examined in this study and the results found that patients with a CRP level > 88.46 mg/L were more likely to acquire the disease's severe form. Similar to recent studies, the results of this study showed that IL-6, CRP, and D-dimer levels were associated with the severity of COVID-19 infection, which were significantly elevated in D-dimer compared with the other markers, which was consistent with the results of Terpos, *et al.* [28].

Correlation between biomarkers and COVID-19 patients according to the age

The age is considered a strong predictor of coronavirus infection. The highest percentage of D-dimers was among those infected in the age group of 15 to 30 years old at $3,642.73 \pm 126.44$ ng/mL, while the highest percentage of CRP was among the age group of 47 to 62 years old at 116.89 ± 16.61 ng/mL, and the percentage of IL-6 in the age group of 79 to 94 years old at 60.57 ± 10.23 ng/mL. On the other hand, the control group showed CRP and D-dimer at 360.73 ± 22.51 ng/mL and 2.63 ± 0.03 ng/mL for the age group of 63 to 78 years old, while IL-6 was at 3.51 ± 0.42 ng/mL for ages between 47 to 62 years old. The statistical analysis demonstrated very significant difference ($P < 0.01$) (Table 2). IL-6 has an essential role in inflammation, and in severe cases, it is a good indicator of COVID-19 [29]. It is also an indicator for another elevated biomarkers

Table 3. The association between COVID-19 patients and control group according to gender.

Parameters	Gender	Control	Patient	P value
CRP	Male	2.08 ± 0.20	83.71 ± 7.84	0.00014**
	Female	1.55 ± 0.12	92.42 ± 10.44	0.0002**
D-dimer	Male	296.10 ± 52.11	1,834.55 ± 251.27	0.002**
	Female	190.66 ± 20.41	2,200.34 ± 158.77	0.014*
IL-6	Male	2.82 ± 0.24	22.06 ± 6.88	0.022*
	Female	2.93 ± 0.46	29.51 ± 6.27	0.005**

Note: The data were expressed as mean ± SE. *significant difference ($P < 0.05$). **very significant difference ($P < 0.01$).

such as fibrinogen, albumin synthesis inhibitor, hepcidin, serum amyloid A, and CRP [30]. A study by Luo, *et al.* demonstrated that the use of IL-6 inhibitors was essential to reduce the cytokine storm severity at COVID-19 patients, especially in severe cases [31]. The results of this study demonstrated that the sex and age of patients influenced the concentration of physiological parameters and the severity of COVID-19, as well as the morbidity rate. On the other hand, hypertension, diabetes, and stroke were found to affect the severity of the disease as well [32]. The current results showed increased infection with coronavirus in age between 15 to 30 years old for D-dimer and 47 to 62 years old for CRP, while the infection occurred in age 79 to 94 years old for IL-6, which were consistent with the results of M. Ozen, *et al.* [33] who found increased infection in old age for immunological protein compared to the young age. Furthermore, patients' age was linked to illness progression and a greater fatality rate [34]. According to our results with CRP and IL-6, an infection rate was significantly in older age cases due to reduced immunity and presented comorbidities. The lack of population screening, which undercounted patients, did not reflect the valid population infection rate [35]. The increases of those biomarkers might be the cause of increased inflammation by those immunological proteins (CRP and IL-6) in COVID-19 patients, notably with age older than 45 years old [36]. However, the low levels of the D-dimer in this study, especially in males, might be due to the missing information regarding patients such as the selection and exclusion criteria, existence of comorbidities, and treatment statistics.

Correlation of coronavirus to the gender

The results of this study demonstrated that the females showed significantly higher values of D-dimer, CRP, and IL-6 at the averages of 2,200.34 ± 158.77, 92.42 ± 10.44, and 29.51 ± 6.27 ng/mL compared to males at 1,834.55 ± 251.27, 83.71 ± 7.84, and 22.06 ± 6.88 ng/mL, respectively. On the other hand, the control group showed that males were higher in D-dimer and CRP at 296.10 ± 52.11 and 2.08 ± 0.20 ng/mL compared to females at 190.66 ± 20.41 and 1.55 ± 0.12 ng/mL, respectively, while males IL-6 at 2.82 ± 0.24 ng/mL was lower than that of females at 2.93 ± 0.46 ng/mL ($P < 0.01$) (Table 3). The percentages of patients and control according to gender were shown in Figure 1, where the highest percentage was in female at 53% (16 out of 30 patients), while the male was at 47% (14 out of 30 patients). The control group showed that the highest percentage was in male at 57% (17 out of 30 people), while the female was at 43% (13 out of 30 people). This study found that the mean of infection in females was more than it in males, which was inconsistent with Cai's result that found men were more infected with COVID-19 due to the increased expression of angiotensin converting enzyme (ACE) receptors for SARS-CoV-2 in pneumocytes than that in women [37]. The possible reason behind this difference might be that females were more than males in this study. The D-dimer test adopted in this study was because that this marker had essential role in monitoring the severity of disease. The results showed that both D-dimer and CRP demonstrated a higher percentage in males more than females, which was consistent with the

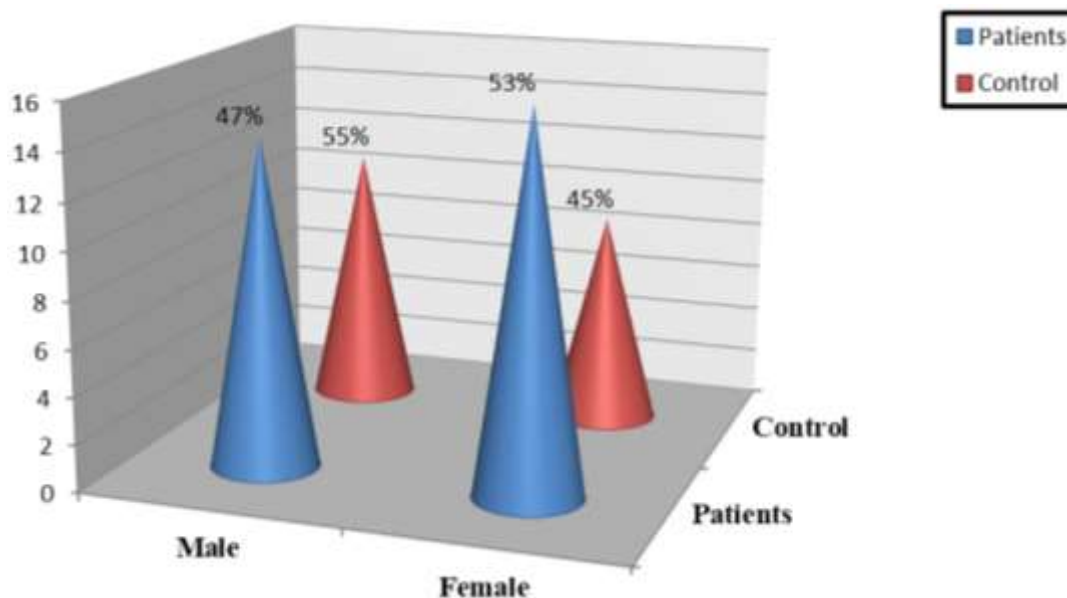


Figure 1. The number of patients and control according to gender.

previous report that increased age, male gender, and dyspnea symptoms all affected D-dimer value [38]. Furthermore, females in some cases were more likely to develop severe illness and symptoms due to the presence of ACE2 receptor in higher prevalence of unhealthy lifestyles women such as drinking too much alcohol and sleeplessness, which was also partially consistent with our results [39]. The baseline comorbidities across all groups were comparable except that the higher IL-6 group (29.51 ± 6.27 ng/mL) had female predominance ($P < 0.05$), which was also shown by the results of Hadi, *et al.* [40]. This study showed that D-dimer was higher in females than that in males and higher CRP and IL-6 serum levels were found in comorbidities patients. The gender and the biomarker distribution were partially comparable to the results of Borobia, *et al.* [41].

Correlation between D-dimer, CRP, and IL-6

The significant differences between the biomarkers in COVID-19 patients were observed in this study. The results showed a relatively significant correlation between CRP and D-dimer with the R value of 0.343 and CRP and IL-6 with the R value of 0.151. There was also a significant

difference between D-dimer and IL-6 with the R value of 0.121 (Table 4).

Table 4. Correlation between biomarkers of D-dimer, CRP, and IL-6 in COVID-19 patients.

Parameters	Correlation coefficients (R)
CRP and D-dimer	0.343*
CRP and IL-6	0.151*
D-dimer and IL-6	0.121*

Note: *significant difference when $R < 1$.

IL-6 was focused on this study because of its documented unique role in the cytokine storm, association with severity of disease, requiring mechanical breathing, and high mortality in COVID-19 patients, while it might also be exploited for treatments [42, 43]. The results showed that increased IL-6 levels correlated with disease severity, thus determining severe cases, which was similar as D-dimer and CRP. The serological markers investigated in this study were quantitative detected in clinical cases for COVID-19 patients [44]. Table 4 showed the relations between the biomarkers. When

evaluating a patient with a COVID-19 infection, these clinical markers could help doctors to initiate therapy and monitor the progress as well as aid in improving diagnosis and treatments. The current findings had revealed differences in biomarker levels that might be beneficial in the progress of a therapeutic intervention. For example, one research looked at the usage of several drugs in patients who had elevated D-dimers due to COVID-19 [45]. The results of this study demonstrated the relationships between elevated serum levels of CRP, D-dimer, and IL-6 in COVID-19 with the disease severity [46]. The high levels of these markers were significantly relevant to the cause of systemic hyperinflammation phase in COVID-19 patients. Moreover, the findings of this study showed that there was a highly correlation between these biological markers in COVID-19 patients, which confirmed their importance in early diagnosis and monitoring the development of the pathological condition of COVID-19 patients. The results were consistent with several previous studies on the importance of choosing these markers as a means of diagnosing the severity of infection in COVID-19 patients [47, 48].

Conclusion

The results of this study suggested that the COVID-19 infection was highly associated with CRP, IL-6, and D-dimer. In addition to their importance in diagnosis, they were essential in monitoring the response to treatment and the development of the infection.

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