

Evaluation of Some Immunological Parameters in Patients with COVID-19 in Mosul City, Iraq

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Abstract

Background: Coronavirus disease 2019 (COVID-19), also known as severe acute respiratory syndrome coronavirus 2, is a viral disease caused by a coronavirus involved in severe acute respiratory syndrome that causes mild, moderate, and severe respiratory system infections. World Health Organization has documented that COVID-19 infections are rapidly spreading and have become a global pandemic. **Objectives:** This study aimed to investigate the association of specific immunological parameters in COVID-19-infected patients in Mosul, Iraq. **Materials and Methods:** A total of 268 confirmed COVID-19 patients were enrolled in this study (129 males and 139 females). One hundred seventy-nine healthy participants were chosen randomly (81 males and 98 females) as a control. All patients admitted to Al-Salam Teaching Hospital, Iraq from February to May 2021 were included in the study. Blood samples were collected from the participants for serum to estimate levels of C-reactive protein (CRP), interleukin-6 (IL-6), and interleukin-10 (IL-10) using an enzyme-linked immunosorbent assay technique. The total white blood cells (WBC), neutrophils, lymphocytes, and monocytes were also measured. **Results:** This study revealed that most COVID-19 patients showed high levels of serum CRP, IL-6, and IL-10. The patients with COVID-19 had a significantly higher total WBC count than healthy controls, with 12.21 and 6.93 cells per $10^3/L$, respectively. However, the results showed a reduction in lymphocyte counts in COVID-19 patients. The hematological analysis was statistically significant for all parameters that were estimated in all COVID-19 patients, and controls and was compatible with clinical significance ($P = 0.005$). **Conclusion:** Infections with COVID-19 may elevate the levels of serum CRP, IL-6, and IL-10. Therefore, monitoring these immunological markers is essential, particularly in individuals with severe COVID-19 infection.

Keywords: COVID-19, CRP, IL-10, IL-6, immunological parameters

INTRODUCTION

Currently, more than 200 countries and regions around the world have been infected by a novel coronavirus. More than four million people were infected with a novel coronavirus called coronavirus disease 2019 (COVID-19), and more than 300,000 were dead.^[1] The International Committee on Taxonomy of Viruses identified COVID-19 in 2019 and classified it as an enveloped ribonucleic acid beta-coronavirus belonging to the Coronaviridae family.^[2] It is involved in many different mild to severe respiratory symptoms.^[3] COVID-19 has developed rapidly to a pandemic level since it was first identified in Wuhan, China, by the Chinese Center for Disease Control and Prevention on January

7, 2020.^[4,5] Then, in February 2020, the new virus, the coronavirus was officially termed “COVID-19” by the World Health Organization (WHO) and represented as an emerging infectious agent, causing a worldwide pandemic and a public health emergency declaration.^[4] COVID-19 was first identified in Iraq on February 24, 2020, in Al-Najaf City. After that, more cases were diagnosed sequentially.^[6]

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COVID-19 is fatal to older people, immunocompromised patients, patients with chronic diseases, and others. However, younger COVID-19 patients may also develop potentially deadly complications, such as fulminant myocarditis and the dissemination of intravascular coagulopathy, as well as hematological changes. Immunological changes are represented by increasing interleukin (IL) levels, C-reactive protein (CRP), white blood cell (WBC) count, neutrophils, and lymphocytes.^[7-10]

The immune response to COVID-19 is similar to that in severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and other viral types of pneumonia.^[11] However, the complications, severity, and clinical picture of the immune changes in patients with COVID-19 are still not fully studied in Iraq. The inflammatory cascade of cytokines, including IL-1, IL-2, IL-6, IL-8, IL-10, and tumor necrosis factor (TNF), are indicated to predict case prognosis and survival.^[12] Cytokines are proteins formed by various cell types, including lymphocytes and monocytes, that perform their actions within a local environment or systemically regulate immunological and inflammatory reactions. Inflammatory cytokine-mediated pathways have been involved in acute and chronic infections with COVID-19.^[13] Differential leukocytes, such as neutrophils, lymphocytes, monocytes, and eosinophils, may also serve as essential biomarkers for many infectious diseases concerning COVID-19. For example, many recently published studies indicated that an elevation in total WBC and neutrophil count and a low lymphocyte count might be considered a risk biomarker for acute and chronic COVID-19 infection.^[14]

This study aimed to evaluate some immunological and hematological parameters in patients with COVID-19 disease and indicate the relationship of these findings with the COVID-19 infection.

MATERIALS AND METHODS

Study design and patients

This study enrolled 268 hospitalized COVID-19 patients diagnosed who were confirmed with infection by real-time reverse-transcription polymerase chain reaction test, and 179 healthy participant controls were admitted to Al-Salam Teaching Hospital in Mosul, Iraq, from February to May 2021. The ages of the patients ranged from 18 to 74 years old.

All patients had symptoms and signs of COVID-19, and all patients or their families obtained well-informed consent. Venous blood was collected from the patient and the control group. Blood samples were separated into two portions. The first one (2 mL) was collected in anticoagulant (ethylene diamine tetra acetic acid) tubes to evaluate hematological tests, such as total WBC, neutrophils, lymphocytes, and monocytes. The second portion, kept in plain tubes, was allowed to clot and centrifuged for

10 min at 3000 rpm for separation of the serum, and then, stored at -20°C to be used for immunological tests. The anticoagulant blood samples were transported to the hospital's laboratory to measure WBC count and differential leucocyte count as neutrophils, lymphocytes, and monocytes automatically by the Beckman Counter instrument (Ac•T 5diff CP, Brea, CA, USA) for each patient and control group.

Detection of serum CRP levels

A suspension of latex particles coated with anti-human CRP antibodies using a kit obtained from (Biosystems, Barcelona, Spain) was used to detect the serum level of CRP. The agglutination of the latex particles is proportional to the CRP concentration and was measured by turbidimetry. The CRP kit was obtained from Biosystems. The reference values for CRP are up to 6 mg/L.

Estimation of serum levels of IL-6 and IL-10

IL-6 and IL-10 concentrations were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits for humans (Elabscience®, Houston, TX, USA) according to the manufacturer's instructions. The optical density was measured at 450 nm using an ELISA reader (Bio-Tek, Winooski, VT, USA). The concentration of human cytokines (IL-10 and IL-6) in each sample is calculated by comparing the optical density of the samples to the standard curve.

Statistical analysis

The data were statistically analyzed using the Statistical Package for Social Sciences statistical package version 23 (IBM Corp., Armonk, NY, USA). Observed results were arranged and analyzed by groups of variables using an independent *t* test and a one-way analysis of variance. A *P* value of 0.05 or less was considered significant. To express the effects of continuous, the mean $\pm$ standard deviation was used.

Ethical approval

The study was conducted following the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol, the subject information, and the consent form were reviewed and approved by a local ethics committee according to document number 32 on January 2, 2021.

RESULTS

Participants of this study were classified into two groups of COVID-19 patients: 268 (60%) patients and 179 (40%) healthy controls, based on clinical symptoms and validated by COVID-19 tests to compare the immunological parameters. The mean age of all COVID-19 patients

and healthy controls was 38.55 years, ranging from 17 to 73 years old. The COVID-19-diagnosed patients had symptoms, such as difficulty in breathing, fever, cough, and dysfunctions of many organs, whereas the healthy control group had no defined symptoms and a negative virological test. In addition, the test results showed that the prevalence of COVID-19 patients according to gender was 129 (48.1%) males and 139 (51.9%) females. At the same time, healthy controls were 81 (45.3%) males and 98 (54.7%) females [Figure 1].

Figure 2 shows that the distribution of the participants (study group) through age groups in COVID-19 patients was inconsistent compared to healthy controls. For example, the age group 70–80 years old of COVID-19 patients was 2.24% of positive infections with COVID-19,

whereas the age group 40–50 years old was the highest percentage (24.2%) [Figure 2].

This study showed that patients with COVID-19 had a significantly higher total WBC count than healthy controls, with 12.21 and 6.93 cells per $10^3/L$, respectively. In addition, the results showed an increase in neutrophils and monocytes with lower lymphocytes in patients with COVID-19 compared with controls [Table 1]. However, according to the gender variable, the results showed no significant difference in total WBC, neutrophils, or monocytes between the genders of COVID-19 patients. In contrast, the results revealed that the lymphocyte mean was slightly higher in male patients with COVID-19 than in female patients compared with healthy controls at 1.27 and 1.12 cells/L, respectively [Table 1].

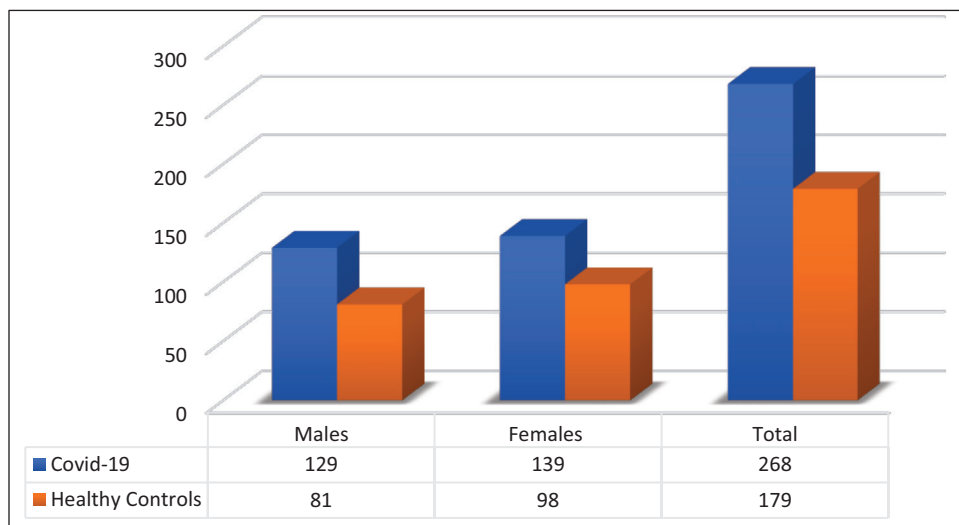


Figure 1: Distribution of the participants of the study groups according to gender

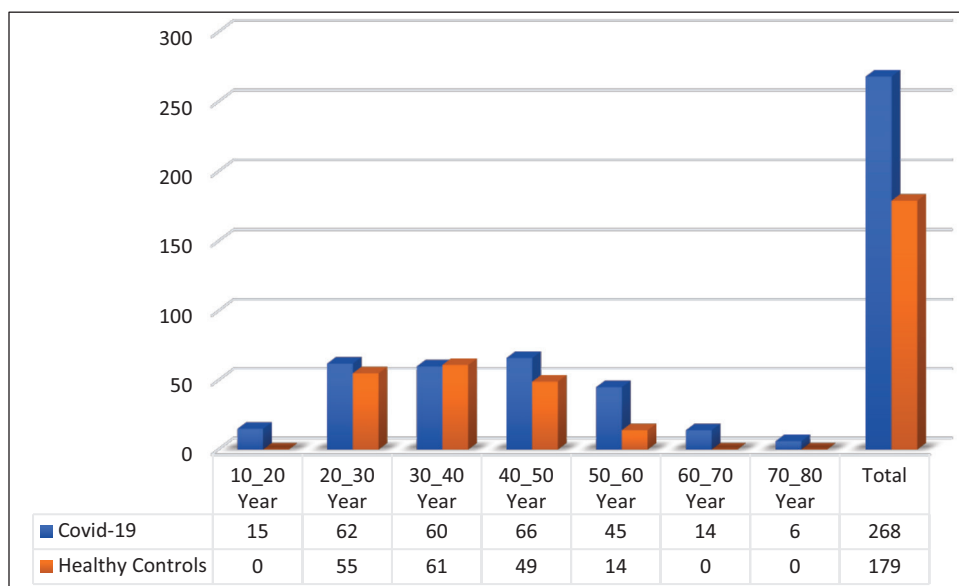


Figure 2: Distribution of the participants of the study groups according to age groups

A comparison of hematological parameters among COVID-19 patients and healthy controls according to age groups is presented in Table 2. The results showed a significantly higher mean of total WBC in the age group younger than 10 years, whereas the lower mean of total WBC was shown in the age group older than 70 years as 13.60 and 11.60 cells $10^3/L$, respectively.

With neutrophils and monocytes tests, the results showed the highest mean of neutrophils was 11.63 cells/L with the age group older than 70 years, and the highest mean of monocytes was 0.84 with the age group (50–60) years, whereas the lowest mean of neutrophils and monocytes

showed with the age groups 10–20 and 60–70 years, respectively.

In contrast, the result of lymphocytes reveals the lowest mean for the age group older than 70 years, followed by the age group 40–50 years old at 0.81 and 0.98, respectively. The hematological analysis was statistically significant for all parameters that were estimated in all COVID-19 patients and controls and was compatible with clinical significance ($P = 0.005$) [Table 3].

Table 4 shows that CRP levels in COVID-19 patients are significantly higher than in healthy controls, at 31.42 and

Table 1: Mean $\pm$ standard deviation of total WBC, neutrophils, lymphocytes, and monocytes among study groups according to gender

Parameters		Total WBC ($\times 10^3/L$)			Neutrophils (cells/L)			Lymphocytes (cells/L)			Monocytes (cells/L)		
Study groups		Mean	Standard deviation	N	Mean	Standard deviation	N	Mean	Standard deviation	N	Mean	Standard deviation	N
Patients	Males	12.22	3.72	129	9.27	1.83	129	1.27	1.50	129	0.82	0.08	129
	Females	12.21	3.14	139	9.79	1.91	139	1.12	1.21	139	0.82	0.09	139
	Total	12.21	3.43	268	9.54	1.89	268	1.19	1.36	268	0.82	0.08	268
Controls	Males	6.79	1.42	81	4.18	1.09	81	2.47	0.50	81	0.36	0.09	81
	Females	7.06	1.40	98	4.33	1.00	98	2.35	0.49	98	0.35	0.10	98
	Total	6.93	1.41	179	4.26	1.04	179	2.40	0.49	179	0.35	0.10	179

Table 2: Mean $\pm$ standard Deviation of total WBC, neutrophils, lymphocytes, and monocytes among study groups according to age groups

Parameters		Total WBC ($\times 10^3/L$)			Neutrophils (cells/L)			Lymphocytes (cells/L)			Monocytes (cells/L)		
Study groups	Age groups (years)	Mean	Standard deviation	N	Mean	Standard deviation	N	Mean	Standard deviation	N	Mean	Standard deviation	N
COVID-19 patients	10–20	13.60	3.41	15	9.13	2.18	15	1.42	1.83	15	0.81	0.08	15
	20–30	11.81	3.15	62	9.48	1.91	62	1.36	1.77	62	0.81	0.09	62
	30–40	12.29	3.79	60	9.34	1.80	60	1.27	1.57	60	0.82	0.08	60
	40–50	12.06	3.50	66	9.46	1.85	66	0.98	0.28	66	0.82	0.09	66
	50–60	12.37	3.46	45	9.82	1.83	45	1.07	1.06	45	0.84	0.08	45
	60–70	12.74	3.08	14	9.57	1.72	14	1.44	1.90	14	0.80	0.10	14
	70–80	11.60	2.29	6	11.63	2.34	6	0.81	0.16	6	0.83	0.14	6
Healthy controls	1–20	—	—	—	—	—	—	—	—	—	—	—	—
	20–30	7.00	1.37	55	4.53	1.08	55	2.36	0.47	55	0.36	0.10	55
	30–40	7.01	1.38	61	3.93	0.87	61	2.45	0.51	61	0.35	0.08	61
	40–50	6.81	1.45	49	4.33	1.14	49	2.38	0.49	49	0.35	0.11	49
	50–60	6.77	1.67	14	4.42	0.94	14	2.48	0.50	14	0.37	0.09	14
	60–70	—	—	—	—	—	—	—	—	—	—	—	—
	70–80	—	—	—	—	—	—	—	—	—	—	—	—

Table 3: Statistical significance of hematological tests

Statistics	Total WBC	Neutrophils	Lymphocytes	Monocytes
Mean difference	5.280	5.276	−1.209	0.466
Standard error difference	0.270	0.155	0.106	0.009
Sig. (two-tailed test)	0.00	0.00	0.00	0.00
P (t test)	19.497	33.976	−11.380	50.955
df	445	445	445	445

Table 4: Mean $\pm$ standard deviation of CRP, IL-6, and IL-10 among study groups according to gender

Parameters		CRP (mg/L)			IL-6 (pg/mL)			IL-10 (pg/mL)		
Statistics	study groups	Mean	Standard deviation	N	Mean	Standard deviation	N	Mean	Standard deviation	N
Patients	Males	30.28	17.97	129	30.99	10.25	129	26.65	9.01	129
	Females	32.48	15.87	139	29.35	12.16	139	27.57	8.04	139
	Total	31.42	16.92	268	30.14	11.29	268	27.13	8.52	268
Controls	Males	0.35	0.11	81	3.21	1.20	81	3.53	1.09	81
	Females	0.37	0.12	98	2.94	1.18	98	3.44	1.11	98
	Total	0.36	0.12	179	3.06	1.20	179	3.48	1.10	179

Table 5: Mean $\pm$ standard deviation of CRP, IL-6, and IL-10 among study groups according to age groups

Parameters		CRP (mg/L)			IL-6 (pg/mL)			IL-10 (pg/mL)		
Study groups	Statistics age groups (years)	Mean	Standard deviation	N	Mean	Standard deviation	N	Mean	Standard deviation	N
COVID-19 patients	10–20	20.92	17.08	15	28.43	9.42	15	28.88	9.75	15
	20–30	27.90	17.83	62	32.48	11.37	62	26.94	7.95	62
	30–40	31.05	17.47	60	29.48	11.15	60	28.94	10.37	60
	40–50	36.18	14.49	66	29.04	11.48	66	25.70	7.11	66
	50–60	32.31	16.85	45	30.68	12.22	45	26.82	8.05	45
	60–70	37.71	12.32	14	29.68	10.28	14	27.22	9.53	14
	70–80	24.07	19.97	6	25.87	9.54	6	24.50	4.17	6
Healthy controls	1–20	—	—	—	—	—	—	—	—	—
	20–30	0.36	0.12	55	2.78	1.07	55	3.24	1.03	55
	30–40	0.36	0.11	61	3.24	1.23	61	3.61	1.08	61
	40–50	0.35	0.12	49	3.10	1.23	49	3.51	1.22	49
	50–60	0.37	0.11	14	3.28	1.27	14	3.77	0.89	14
	60–70	—	—	—	—	—	—	—	—	—
	70–80	—	—	—	—	—	—	—	—	—

Table 6: Statistical significance of some immunological tests

Tests statistics	CRP	IL-6	IL-10
Mean difference	31.061	27.077	23.650
Standard error difference	1.265	0.847	0.640
Sig. (two-tailed test)	0.00	0.00	0.00
<i>P</i> (<i>t</i> test)	24.547	31.937	36.909
df	445	445	445

0.36mg/L, respectively. In addition, the results showed an increase in IL-6 and IL-10 in patients with COVID-19 compared with controls [Table 4]. According to gender, the results revealed a significantly higher level of CRP (32.48mg/L) and IL-10 (27.57 pg/mL) in female COVID-19 patients than in male patients (30.28mg/L and 26.65 pg/mg, respectively). In contrast, the results showed that IL-6 was slightly higher in male COVID-19 patients than in female patients compared with healthy controls (30.99 and 29.35 pg/mL, respectively) [Table 4].

The results revealed a significantly higher mean of CRP with the 60–70 years age group, whereas the lower mean of CRP was shown with the 10–20 years age group at 37.71 and 20.92mg/L, respectively. The IL-6 test results showed that the age group 20–30 years had the highest

mean of IL-6 at 32.48 pg/mL, followed by the age group 50–60 years at 30.68 pg/mL, and the age group older than 70 years had the lowest mean of IL-6 at 25.87 pg/mL. Furthermore, the result of IL-10 showed that the lowest mean with age groups older than 70 years was 24.50 pg/mL, followed by age groups 40–50 years at 25.70 pg/mL, and the highest mean with age groups 30–40 years was 28.94 pg/mL [Table 5]. However, the statistical significance of all parameters estimated in all COVID-19 patients and controls is compatible with clinical significance ($P = 0.005$) [Table 6].

DISCUSSION

COVID-19 has represented the second cause of cold infections, with a high incidence after influenza and rhinoviruses, which documented that it may be more readily transmitted horizontally from one person to another, leading to a worldwide epidemic.^[15] In the current study, the outcome disclosed that the counts of total WBC, neutrophils, and monocytes were elevated and showed the highest counts in both females and males in patients with COVID-19 compared with controls [Table 1]. The results also showed that the lymphocyte

count was decreased and showed low counts in both males and females of COVID-19 patients compared with healthy controls. These findings were in harmony with other substantial numbers of studies conducted in several regions in which observations have been documented for leukocytosis, neutrophilia, monocytosis, and lymphopenia in patients significantly confirmed as having COVID-19 illness.^[1,16-18]

Huang *et al.*^[2] reported that 1:4 of COVID-19 cases were suffering from leukopenia, with some way below 4×10^9 cells/L, and a decrease in lymphocytes below 1×10^9 cells/L in the majority (63%) of patients. They also revealed that 45% of infected patients with COVID-19 have WBC counts within the reference range ($4-10 \times 10^9$ cells/L), whereas about 30% of COVID-19 patients have a WBC count above 10×10^9 cells/L. Furthermore, blood from severely COVID-19-infected patients featured more neutrophils than blood from nonsevere COVID-19 patients.^[2] At this point, the reason COVID-19 causes lymphocytopenia in patients is not apparent.

When most viruses infect people, lymphocytosis might happen because lymphocytes act as effector cells that fight the infection.^[19] However, only a few members of the coronavirus family—including SARS-CoV, MERS-CoV, and COVID-19—inflict individuals with a drop in lymphocytic activity.^[20,21] This might be a result of their processes, which include immune-mediated apoptosis or the indirect coronavirus attack on lymphocytes.^[22] Some pilot investigations have looked at the role of lymphocyte subpopulations in the host response to COVID-19 infection, with several finding lower than normal levels of CD3+, CD4+, and CD8+ T cells on admission and 1 week later in patients with severe disease symptoms.^[16]

The most common cause of death in patients with COVID-19 is acute respiratory distress syndrome (ARDS). Cytokine storm, also termed cytokine release syndrome, is a lethal, uncontrolled systemic inflammatory response induced by the production of enormous amounts of pro-inflammatory cytokines, which causes ARDS.^[23]

The blood had increased levels of CRP, IL-6, and IL-10 in COVID-19 patients compared with healthy controls. This finding is in harmony with other studies that recorded an elevation in the levels of CRP and other inflammatory cytokines, such as IL-2R, IL-6, IL-8, IL-10, and TNF- α in the majority of COVID-19 patients with severe illness compared with nonsevere cases.^[16,24] An increase in serum cytokines such as IL-6 and IL-10 was one of the most important predictors of nonsurvivors.^[25] As a result, balanced immunosuppression (e.g., by using inhibitors of selected cytokines, IL-6, and IL-1), might be another effective strategy for reducing and modulating viral-induced hyperinflammation (storm) and thus preventing severe and irreversible organ damage, which contributes to the relatively high mortality of COVID-19 patients.^[26,27]

IL-6 and IL-10 may perform a pro-inflammatory function and have a role in immune activation in the COVID-19 development of pathogenesis. This hypothesis is supported by: First, the inflammatory and immune-stimulating cytokines [IL-4, IL-6, IL-7, IL-10, IL-18, interferon (IFN-), TNF-, and chemokines] are increased in the blood of severe illness patients with COVID-19.^[28,29]

Second, despite a reduction in the total peripheral CD8+ T-cell count, severe COVID-19 patients had circulating hyperactivated and expanding cytotoxic CD8+ T cells.^[30,31] In addition, in severe cases of COVID-19 patients with high serum IL-6 and IL-10, the percentages of IFN-producing effector CD4+ and CD8+ T cells might be increased in the blood.

Third, as COVID-19 disease progresses, the number of exhausted programmed cell death-1, T cell immunoglobulin and mucin domain 3, and CD8+ T cells in the patient's blood increases, and these levels correlate with the concentration of serum IL-10 in patients with COVID-19, implying that IL-10 plays a role in T cell exhaustion, presumably through proliferation and over-activation.^[27] Therefore, we postulate that IL-6 and IL-10 may have a pathogenic role in the development of COVID-19 illness because of these immunological characteristics in the severe illness of patients with COVID-19 who have significantly increased IL-6 and IL-10. This hypothesis, however, has to be thoroughly explored.^[29,32,33]

CONCLUSION

Patients with COVID-19 had elevated total WBC, neutrophil, lymphocyte, and monocyte counts, as well as elevated cytokine levels in their blood. Those with severe cases were more likely to exhibit these biomarkers. To enhance disease outcomes during COVID-19 infection, it is essential to keep an eye on these immunological and hematological assays, especially in patients with more severe illnesses.

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Conflicts of interest

There are no conflicts of interest.

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