Study of Adhesion Molecules' (ICAM-1 and VCAM-1) Effects on Immunothrombosis in Severe and Critical COVID-19 Infections in Comparison with Mild Cases

Abdul Qader Wasfi Muhammed[1], Hadi Rasool Hassan[2], Jabbar Fadhail Mahdi[3]

[1, 2]Department of Clinical Laboratories/College of Applied Medical Sciences/University of Kerbala/Iraq

[3]Al-Hussein Medical City/ Kerbala/Iraq

Corresponding Author: Abdul Qader Wasfi Muhammed Email: (abdwasfi4877@gmail.com)

Abstract

Background: The study aimed to evaluate the association among the adhesion molecules (ICAM1 and VCAM-1) with immunothrombosis in severe and critical COVID-19 patients in comparison with mild cases. Materials and Methods: A cross sectional study was conducted. From 1 October 2021 to 30 May 2022, a total of 82 COVID-19 patients were recruited at the hospital in Imam AL-Hussein Medical City/Kerbala, consisting of 44 males and 38 females, and their ages ranged from 25 to 85 years old. All participants were hospitalized after testing positive for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). 27 of those were diagnosed with mild COVID-19 and 27 with severe cases, while 28 patients had critical diseases. Results: In mild cases, ICAM-1 adhesion molecule level increased approximately more than four folds over the normal range, and dramatically elevated with COVID-19 progression ($p = < 0.001$). VCAM-1 level also significantly elevated ($p = 0.002$), but his elevation was slower than ICAM-1 elevation. Conclusion: There is a more significant correlation among immunothrombosis, cellular adhesion molecules (ICAM-1, VCAM-1) elevation and COVID-19 severity.

Keywords: COVID-19, SARS-CoV-2, Immunothrombosis, ICAM-1, VCAM-1.

1. Introduction

The novel disease, termed coronavirus disease 2019 (COVID-19), that is the most significant pandemic in the past century remains a significant threat to public health. As of March 15, 2022, COVID-19 has taken nearly 6 million lives and infected more than 535 million people, and the number of cases continues to increase worldwide (WHO, 2022).

Even though lung infections are the most common symptom of coronavirus disease, the infection is frequently made worse by coagulopathy and thrombo-embolic events can be observed in a number of affected individuals (1). Dehydration, acute inflammatory conditions, diabetes, obesity, or hypertension, prior ischemic stroke, peripheral artery disease, and other conditions are frequently present in COVID-19 hospitalized subjects and may increase the risk of thrombo-embolic events. However, there are still other possible causes that can be found, such as increased synthesis of adhesion molecules that might cause endothelial activation and vascular inflammation (1).

COVID-19 causes a systemic inflammatory response in which many inflammatory cells are dysregulated and misexpressed. Some types of inflammatory mediators, such as adhesion molecules (intercellular adhesion molecule 1 [ICAM-1] and vascular cell adhesion molecule-1 [VCAM-1]), cytokines, and chemokines, are required for the activation and recruitment of inflammatory cell (2). Recent investigations have found pathological evidence of venous thromboembolism, direct viral infection of endothelial cells, and widespread endothelial inflammation (3). It was discovered that critical illness is related to markers of coagulation activation, specifically higher D-dimer and fibrinogen levels. On the other hand, relatively minimal alterations were identified in prothrombin time and platelet counts. In addition, a series of autopsies conducted on deceased COVID-19 patients described many instances of thrombosis. According to these data, vascular microthrombotic disease is likely the predominant contributor to mortality in critically ill COVID-19 patients (4).

The mechanisms underlying increased thrombotic events are not fully understood; however, mounting evidence suggests that endothelial activation and elevated expression of endothelial cell adhesion molecules such as (ICAM-1and VCAM-1) in COVID-19 patients plays a critical role, as of now becoming clear that endothelial cells actively and reactively participate in hemostasis of immune and inflammatory reactions, and thus, thrombosis (Immunothrombosis) occurs (5).

Inter-cellular adhesion molecule (ICAM-1) or cluster of differentiation 54 (CD54) is a protein that may be activated by IL-1 or TNF and is produced by vascular endothelial cells, macrophages, and lymphocytes. ICAM-1 is a ligand for the lymphocyte function-

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associated antigen-1 (LFA-1) integrin, which is present on lymphocytes and other leukocytes. Leukocytes attach to endothelial cells via ICAM-1/LFA-1 when activated and subsequently transmigrate into tissues (6).
also known as cluster of differentiation 106 (CD106), is a protein that facilitates lymphocyte, monocyte, eosinophil, and basophil adherence to vascular endothelium. It also participates in leukocyte-endothelial cell signaling and may be involved in the development of atherosclerosis and rheumatoid arthritis. Pathological evidence of venous thrombosis, direct viral infection of endothelial cells, and extensive endothelial inflammation has been discovered in recent research. As a result, studying the expression of endothelial cell adhesion molecules in COVID-19 is critical (3).

2. Materials and Methods

In accordance with the WHO, RT- PCR and CT scans were used to diagnose COVID-19 in all patients. For the purpose of this study, a total of 82 COVID-19 patients were admitted to the hospital in Imam AL-Hussein Medical City/Kerbala, consisting of 44 males and 38 females. Their ages ranged anywhere from 25 to 85 years old, on average.
These patients are divided into three groups: mild (27 patients: 14 males and 13 females); severe (27 patients: 14 males and 13 females); and critical (28 patients:17 males and 11 females). Patients were chosen at random from the local community, taking into consideration their ages and genders.
Blood samples were taken from all participants and each sample divided into three parts:
Part one- separated in EDTA tube to perform hematological tests (CBC).
Part two- transferred into sodium citrate tube to perform (D-dimer, PT, PTT).
Part three – in a gel tube for biochemical tests (C. reactive protein, Blood urea, and Serum creatinine) and immunological tests (ICAM-1 and VCAM-1).

3. Statistical Analysis

The data was entered into a Specific Software Statistical Package for the Social Sciences (SPSS) version 21 for Windows computer for statistical analysis. Results were presented as mean standard deviation (mean ± SD). When a *p*-value was less than 0.05, it was deemed statistically significant; a *p*-value less than 0.001 was considered highly significant. Additionally, ICAM-1 and VCAM-1 relationship with hematological, biochemical, and coagulation parameters is also explained by the Pearson correlation (*r*-value).

4. Results and Discussion

Demographic Data of the Studied Groups

The current study included 82 patients with COVID-19 identified by specific SARS-COV-2 real-time PCR on nasopharyngeal (NP) swab specimens.
According to SpO2 percentage and respiratory rate (RR), the precipitant patients divided into mild (SpO2 ≥ 94, RR ≤ 22), severe (SpO2 ≤ 93, RR ≥ 30) and critical which were the same as severe but required mechanical support such as ventilator and Continuous positive airway pressure (CPAP) therapy. Computerized topography (CT) percentage has been taken into consideration, and it is elevated with the severity of disease. As a result, the following patient groups were chosen (n=27 mild, n=27 severe, and n=28 critical). There were 44 (53.7%) males and 38 (46.3%) females among them. The average age was 59.9 years, with a range of 25 to 85 years.

Table 1: Demographic Data of the Studied Groups:

Total number			82		
Age			Mean (59.9) yr Average (25 – 85) yr		
Gender			Male 44 (53.7%) Female 38 (46.3%)		
Groups	Gender		SpO_2 % Mean ± SD	RespiratoryRate (RR) R per min	(CT%) Computerized topography%
	Male No. (%)	Female No. (%)			
Mild (N=27)	14 (51.9%)	13 (48.1%)	96% ± 2	22.1 ± 5.4	12% ± 4
Severe (N=27)	14 (51.9%)	13 (48.1%)	89% ± 4	36.3 ± 6.1	39% ± 14
Critical (N=28)	17 (60.7%)	11 (39.3%)	81% ± 3	40.8 ± 9.1	57% ± 11
			p = < 0.001	p = < 0.001	p = < 0.001

According to this table, there was no significant difference between male and female patients. This was in agreement with (2) who claimed that the percentage of men and women among ICU patients and non-ICU patients was the same. Despite the fact that there were no statistically significant differences between illness progression and gender in this specimen, male gender was discovered to be a risk factor for disease severity by (7,8) who found that the frequency of symptomatic COVID-19 was greater in males than in women.
Oxygen saturation (SpO2) and respiratory rate (RR) were significantly correlated with disease severity. Patients with severe and critical disease had significantly greater respiratory rates than those with moderate disease, whereas those with severe and critical disease had significantly lower SpO2 levels than those with mild disease. All patients with critical illness required mechanical ventilation. These findings were similar to those mentioned by (9), in which concluded that patients with severe and critical COVID-19 have significantly higher RR and lower SpO2 than mild patients.

Age Distribution for COVID-19 Studied Patients

In table 2, the ages of the subjected patients were distributed into three groups; the first young group

aged from 25–45 years, and they mostly occurred in mild cases and decreased in severe and critical cases. The second post-young group that aged from 46–65 years old showed a decrease in mild cases and an increase in both severe and critical cases. The third elderly group ranged in age from 66 to 85 years old, and they mostly presented in critical and severe cases, with a decrease in mild cases. This means that the severity of the disease significantly correlates with aging, and the elderly are more susceptible to severe and critical diseases. This means that there is a significant correlation between patients' age and disease severity.

Table 2: Age Distribution for COVID-19 Studied Patients:

Age group (yrs)	Mild (N=27) No. (%)	Severe (N=27) No. (%)	Critical (N=28) No. (%)
(25 – 45) Young	18 (66.6)	7 (25.9)	3 (10.7)
(46 – 65) Post young	5 (18.56)	8 (29.7)	5 (17.9)
(66 – 85) Elderly	4 (14.8)	12 (44.4)	20 (71.4)
Total	27 (100)	27 (100)	28 (100)

The explanation for this link is that older people frequently have low immunity and are suffering from one or more chronic conditions such as (hypertension, diabetes mellitus, smoking, etc.), resulting in increased complications and illness severity. A comparable set of data was recently published by (10), which in their investigation, they discovered that older people had the highest percentage of critical cases.

Mortality Distribution Among the Groups of COVID-19 Patients

There are no dead cases in mild group, while 3 patients are dead within severe group and they represented 3.6% from total patients' groups. The mortality number in critical group was higher more than three folds from severe group, there were 11 deaths within critical group and they represented 13.4% from total patients. The total mortality percentage was 17% and all patients who died were between (66-85) years old.

Table 3: Mortality Distribution Among the Groups of COVID-19 Patients:

Classified groups	No. Of Cases	Mortality distribution within age groups No. (%)			
		Young	Post young	Elderly	Total
Mild	27	0	0	0	0
Severe	27	0	1 (1.2%)	2 (2.4%)	3 (3.6%)
Critical	28	0	1 (1.2%)	10 (12.2%)	11(13.4%)
Total	82	0	2 (2.4%)	12 (14.6%)	14(17%)

As shown in above, mortality was significantly increased with disease severity and the patients aging in this study because no death recorded within mild patients, while 3 deaths in severe cases and 11 deaths in critical cases were recorded. These outcomes suggest that younger individuals are more prone to have mild symptoms, and they are less prone to die, but older individuals are more likely to die and have severe symptoms. This was agreed upon by a large number of other researchers, including (11), which stated that elderly COVID-19 patients had a significant death rate due to a high case fatality rate and symptomatic infection rate.

Hematological Parameters in COVID-19 Patients

white blood cell (WBC) counts were not significantly elevated but the percentage of neutrophils was significantly elevated in severe and critical compared with mild COVID-19 cases (p = <0.001). In contrast, the percentage of lymphocytes was significantly lower in severe and critical cases, as demonstrated by a significant correlation (p = 0.001). This might imply that the COVID-19 infection is preferentially targeting lymphocytes. Hemoglobin (Hb) levels were slightly lower in all three groups (mild anemia), and this slight reduction was also statistically significant (p = 0.001).

Table 4: Hematological Parameters in COVID-19 Patients Classified According to Degree of Disease Severity:

Variables	Normal range	Mild Mean ± S	Severe Mean± SD	Critical Mean ± SD	p- value
White blood cell counts x109/L	4.0 – 11.0	11.73 ± 4.77	13.09± 5.92	11.43± 5.12	p= 0.253
Neutrophil %	40-75 %	73% ± 12	80% ± 8↑	86% ± 5↑	p =< 0.001
Lymphocyte%	20 – 40 %	↓18% ± 7	↓14% ± 5	8% ± 3↓	p =< 0.001
Haemoglobin g/l	13.5 – 17.5	13.8 ± 1.8	↓11.5 ± 1.5	↓10.8 ± 1.8	p= 0.001

Total WBC counts showed slightly not significant increase with the disease severity. While neutrophil percentages were significantly increased in severe and critical COVID -19 patients, and this may be due to increase in inflammatory process and excessive cytokines release (cytokine storm). This explanation is similar to that reported by (12), who said that the elevation in neutrophils associated with increased secretion of some cytokines that include IL-8, IL-6, interferon 10 (IP-10), granulocyte- macrophage colony stimulating factor (GM-CSF), IL-1b, IL-10 and TNF.

In contrast, lymphocyte percentages decreased significantly with illness severity, which might be due to functional depletion of lymphocytes such as natural killer (NK) cells and cytotoxic T lymphocytes (CTLs), which are involved in viral infection management. Zhang C and colleagues have found that the overall number of CTLs and NK cells was significantly reduced in SARS-CoV-2 patients. These results suggest that SRAS-CoV-2 infection is associated with functional depletion of cytotoxic lymphocyte (13).

Hb significantly decreased in severe and critical cases in comparison with mild cases in both males

and females. This is explained by the possibility that COVID-19 patients' inflammation might result in an alteration of iron hemostasis and decreased intestinal iron absorption, which reduces the amount of the metal available for erythropoiesis and the formation of hemoglobin (anemia of inflammation) (14). Another cause of Hb decrease is impaired renal function that may occur in severe and critical COVID-19 cases, where the kidneys are the primary organs that regulate erythropoiesis. This agreed by (14), who reported that the altered iron homeostasis and retention within macrophages caused by the higher production of ferritin during COVID-19 infection resulted in reduced iron absorption by the gut and decreased metals for erythropoiesis. Also, patients with anemia were older, had a reduced renal function, and had significantly higher levels of inflammatory markers such as C- reactive protein or interleukine-6 (IL6). However, another study found that there was no significant correlation between hemoglobin levels and COVID-19 severity (15).

Some Biochemical Parameters in COVID -19 Patients

The most prominent elevation in the mild cases occurred by C. reactive protein (CRP), where it significantly elevated more than three folds compared to the normal range, and it increased in severe and critical more than mild cases (p = < 0.001). This makes it the most sensitive COVID-19 biomarker predictor, particularly for mild cases. Blood urea (BUN) was only slightly higher in mild cases, while it was significantly elevated in severe and critical cases. Blood urea levels is significantly elevated with disease progression (p=<0.001). Serum creatinine levels in mild cases were within the normal range. However, Serum creatinine levels were significantly elevated in the severe and critical cases in comparison with mild cases (p=<0.001).

Table 5: Some Biochemical Parameters in COVID -19 Patients:

Variable	Normal Range	Mild Mean ± SD	Severe Mean ± SD	Critical Mean ± SD	*p*- value
C-reactive protein (CRP) mg/l	0 – 5	20.0 ± 9.6↑	47.9 ± 20.4↑	63.4 ± 25.6↑	p= < 0.001
Blood urea (BUN)mg/l	15 – 45	54.5 ± 20.2↑	71.3 ± 34.8↑	121.1 ± 59.5↑	p= < 0.001
Serum creatinine (S.creatinine) mg/l	0.2 - 1.2	1.0 ± 0.4	1.3 ± 0.6↑	1.9 ± 0.8↑	p= < 0.001

CRP is an acute-phase protein of that increase following interleukin-6 secretion
by macrophages and T cells. Therefore, SAR-CoV-2 infection significantly caused CRP secretion from the beginning of the disease and it more elevated with COVID-19 severity. This was in agreement with (16) who stated that although CRP levels are often low in viral infections, adaptive immunity seems to be necessary for the clearance of the COVID-19 virus, and the macrophage activation syndrome may explain the higher serum CRP levels and lead to increase the severity of the disease. cytokines such as IL-6, TNF-α, stimulate hepatocyte to produce CRP during a cytokine storm that can be triggered by the process of COVID-19 pneumonia.

Blood urea (BUN) and serum creatinine levels elevation during COVID-19 may indicate early injury of the kidney. One possible reason for the high incidence of kidney involvement is that the systemic immune response to SARS-COV-2 can be harmful in certain individuals, resulting in what is known as a cytokine storm. As a result, the kidney may be a vulnerable target for this novel coronavirus. These were agreed with (17) who demonstrated that proximal tubule cells in the kidneys exhibit high levels of ACE2 implies that they may be targeted by SARS-CoV-2 at an early stage of illness. The spike glycoprotein of SARS-CoV-2 has an affinity for binding to ACE2 and this is considered a major determinant of disease severity.

Some Coagulation Parameters in COVID -19 Patients

In mild cases, no one of these parameters increased more than the normal range, but in severe and critical cases, only D-dimer levels increased more than normal. And this elevation was statistically more significant (p = < 0.001). There is no significant elevation in platelets count, prothrombin time and partial thromboplastin time (p=0.422; p=0.053, p=0. 0.256, respectively). Therefore, D-dimer considered the single routine marker that indicated the presence of thrombosis (immunothrombosis) in COVID-19 patients during this study. All mortalities in the current study were associated with high D-dimer levels. This indicate that D-dimer is a significantly correlates with COVID-19 severity, hypercoagulability that increased the risk of venous thromboembolism (VTE) events, leading to thrombo-inflammation and even death in severe and critical conditions.

Table 6: Some Coagulation Parameters in COVID-19 Patients:

Variable	Normal range	Mild Mean ± SD	Severe Mean ± SD	Critical Mean ± SD	p - value
D. dimer ng/ml	0-500	328.8 ± 114.9	1031.5 ± 305.2	2351.4 ± 1133.8	p= <0.001
Platelet count x109/L	150 - 450	239.23 ± 70.92	260.3 ± 109.3	231.4± 89.2	p= 0.422
Prothrombin time (PT)	10 - 14	13.1 ± 1.1	13.1 ± 1.2	13.9 ± 1.4	p= 0.053
Partial thromboplastin time (PTT)	30 – 38	32.3 ± 2.6	32.6 ± 4.1	33.5 ± 2.6	p= 0.256

In mild COVID-19 cases, D-dimer levels were slightly elevated in the patients but remain within high

normal rang. However, D-dimer levels in severe and critical cases were significantly elevated in comparison with mild cases. These results agreed by (15), they claimed that D-dimer, ferritin and LDH levels were significantly increased in patients with critical disease.

D-dimer is a fibrin breakdown product that has a mechanistic role in COVID-19 thrombo-inflammation. As a result, D-dimer can be employed as an essential coagulation biomarker that can assist establish patient screening, therapy options, and prognosis management (18).

Patients who have D-dimer levels >1000 ng/ml have a 20-fold increased mortality risk than those who have lower D-dimer levels. Therefore, D-dimer is a screening test for VTE in COVID-19 patients, and changing therapeutic anticoagulant dosages based on D-dimer elevation is more useful to patients than preventive doses (19).

Platelet counts showed no significant correlation with disease progression. This may be due the early and good prognosis of the COVID-19 cases in this study because the platelets (PLTs) at the time of admission were not affected and the number of PLTs was normal. This agreed with (15), They discovered that platelet count had no statistically significant link with COVID-19 severity. Other researchers agree that COVID-19-related thrombocytopenia is a delayed occurrence after infection, and that thrombocytopenia in COVID-19 patients develops in the intensive care unit (ICU) and threatens the life in severe cases (20).

Prothrombin time (PT) was normal in most COVID-19 patients with just 5% who have extended PT. Results like these also agreed with (15) who stated that prothrombin time did not show significant increase with COVID-19 progression.

Partial thromboplastin time (PTT) also did not show statistically significance with disease severity in the current study. PTT is frequently normal in patients with COVID-19 infection, with only 6% developing PTT prolongation, and the average length of PTT appears to be similar in COVID-19 critically ill and non-critically ill patients, with no significant link to disease severity or death. As a result, PTT does not seem to be a good predictor of COVID-19 development (21).

The Levels of ICAM-1 and VCAM-1 in COVID -19 Patient's

In mild cases, ICAM-1 adhesion molecule level increased approximately more than four folds over the normal range, and dramatically elevated with the severity of disease (p=<0.001). VCAM-1 level also significantly elevated (p=0.002), but his elevation was slower than ICAM-1 elevation.

Table 7: The levels of ICAM-1 and VCAM-1 in COVID -19 patient's:

Variable	Normal Range	Mild Mean± SD	Severe Mean± SD	Critical Mean± SD	p - value
ICAM-1 ng/ml	100 - 200	831.8 ± 307.8↑	1550.6 ± 673.8↑	1875.0 ± 626.7↑	p= <0.001
VCAM-1 ng/ml	0.14 – 9.0	13.9 ± 4.8↑	23.0 ± 4.4 ↑	30.1 ± 13.4 ↑	p = 0.002

ICAM-1 very significantly increased with disease progression. This might be related to the nature of ICAM-1, which is a main mediator of cell adhesion expressed throughout the inflammatory process and is frequently used to determine the severity of all inflammatory disorders. As a result, the inflammatory process that occurred by SARS-CoV-2 causes increasing in ICAM-1 expression. These results agreed by (22) who reported that in COVID-19 patients, viral infection of endothelial cells induced by SARS-CoV-2 can generate vascular alterations and enhance ICAM-1 expression, which might be employed as a biomarker to monitor disease severity or recovery. ICAM-1 also contributes in the recruitment and activation of inflammatory cells and facilitates leukocyte-endothelial binding and the migration of leukocytes across the endothelial barrier (23). The roles of VCAM-1 in COVID-19 are similar to that of ICAM-1 because they belong to the same endothelial adhesion molecules. Therefore, patients with SARS-CoV-2 infection also have elevated VCAM-1 levels. However, elevation both of ICAM-1 and VCAM-1 during COVID-19 indicates endothelial damage (often in alveolar epithelium) that caused by excessive inflammation or cytokine storm. Data similar to these were published by (24), who reported that during COVID-19 the endothelial damage biomarkers (ICAM-1 and VCAM-1) were significantly increased.

The Correlations of ICAM-1 and VCAM-1 with Hematological Parameters within Mild, Severe and Critical COVID-19 Patients

ICAM-1 level show no correlation with total WBC count or hemoglobin in any of the groups studied. However, ICAM-1 had a strong positive and significant correlation with low lymphocyte percentages (r =0.686, p=0.002) in mild cases, but in severe cases this correlation changed to medium negative significant correlation (r = -0.496, p=0.009), while in critical cases the correlation of ICAM-1 with diseases severity did not show statistical significance (r = -0.142, p = 0.461). In contrast, ICAM-1 had a strong negative and significant correlation with high neutrophils percentages (r =-0.508, p=0.037) in mild cases, but in severe cases this correlation changed to strong positive and significant correlation (r= 0.545, p=0.003) and then became weak positive not significant in critical cases (r =0.254, p=0.182). These results may indicate differential effect of ICAM-1 on lymphocytes and leukocytes in COVID-19.

VCAM-1 did not show significant correlation with lymphocyte and leukocyte percentages nor with any other hematological parameters within studied groups.

Table 8: The Correlations of Serum Levels of ICAM-1 and VCAM-1 with Hematological Parameters in Mild, Severe and Critical COVID-19 Patients					
Variables		ICAM-1 (831.8 ± 307.8) ng/ml	Correlation	VCAM-1 (13.9 ± 4.8) ng/ml	Correlation
Mild	White blood cell counts, x109/L	11.73 ± 4.77	r = 0.012 p = 0.592	11.73 ± 4.77	r = -0.285 p = 0.266
	Neutrophils%	73% ± 12	r =-0.508 p = 0.037	73% ± 12	r = -0.001 p = 0.996
	Lymphocyte%	18% ± 7	r = 0.686 p = 0.002	18% ± 7	r = 0.205 p = 0.429
	Hemoglobin g/l	13.3 ± 1.6	r = 0.129 p = 0.619	13.3 ± 1.6	r = 0.129 p = 0.619
Variables		ICAM-1 (1550.6 ±673.8) ng/ml	Correlation	VCAM-1 (23.0 ± 4.4) ng/ml	Correlation
Severe	White blood cell counts, x109/L	13.1 ± 5.92	r = 0.184 p = 0.365	13.1 ± 5.92	r = 0.349 p = 0.080
	Neutrophils%	80 % ± 8	r = 0.545 p =0.003	80 % ± 8	r = -0.092 p = 0.653
	Lymphocyte%	14 % ± 5	r = -0.496 p = 0.009	14 % ± 5	r = 0.166 p = 0.415
	Hemoglobin g/l	11.5 ± 1.5	r = 0.015 p = 0.941	11.5 ± 1.5	r = -0.024 p = 0.903
Variables		ICAM-1 (1875.0 ±626.7) ng/ml	Correlation	VCAM-1 (30.1 ± 13.4) ng/ml	Correlation
Critical	White blood cell counts, x109/L	11.4 ± 5.1	r = -0.070 p = 0.716	11.4 ± 5.1	r = 0.177 p = 0.358
	Neutrophils%	86 % ± 5	r = 0.254 p = 0.182	86 % ± 5	r = 0.106 p = 0.582
	Lymphocyte%	8 % ± 3	r = -0.142 p = 0.461	8 % ± 3	r = -0.154 p = 0.422
	Hemoglobin g/l	10.8 ± 1.8	r = -0.085 p = 0.658	10.8 ± 1.8	r = -0.065 p = 0.737

The explanation of ICAM-1 significant correlation with neutrophil and lymphocyte percentages is that SARS-CoV-2 infection causes inflammatory process which leads to activation of many inflammatory cell including leukocytes that should bind to endothelium at inflammation site, ICAM-1 is necessary to leukocyte/endothelium interaction. Therefore, ICAM-1 significantly increased along with COVID-19 severity. These agreed by (25) who mentioned that ICAM-1 during SARS-CoV-2 infection binds and interacts with leukocyte markers (lymphocyte function-associated antigen or LFA-1 or αLβ2) and Macrophage-1 antigen (integrin αMβ2 or macrophage integrin or Mac-1) and this interaction is necessary to leukocyte adhesion to endothelial cells. Additionally, SARS-CoV-2 has a protein known as open reading frame7a (ORF7a) which has a structural homology with ICAM-1. According to one research, the ORF7a protein of SARS-CoV2 contains a conserved Ig immunoglobulin-like structure with an integrin binding site, which gives a mechanistic explanation for SARS-CoV2's interaction with the human immune system. This implies that more research into ORF7a-mediated effects on immune cells such as T lymphocytes and macrophages (leukocytes) might aid in understanding the illness and developing effective therapies (25).

Ethical approval

The approval of the ethics was obtained from the Kerbala Health Directorate. In addition, prior to taking the sample, verbal consent from the patients and/or their parents was obtained before the procedure was carried out. When conducting the sampling, we followed all appropriate safety and health protocols.

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References

1Allegra, A., Innao, V., Allegra, A.G. and Musolino, C. (2020). Coagulopathy and thromboembolic events in patients with SARS-CoV-2 infection: pathogenesis and management strategies. Annals of Hematology, 99(9), pp.1953-1965.
2. Wang, Z., Yang, B., Li, Q., Wen, L., & Zhang, R. (2020). Clinical features of 69 cases with coronavirus disease 2019 in Wuhan, China. Clinical infectious diseases, 71(15), 769-777.
3. Varga, Z., Flammer, A. J., Steiger, P. K., Haberecker, M., Anne Y., Andermatt, R., Zinkernagel, A. S., ... & Moch, H. (2020). Endothelial cell infection and endotheliitis in COVID-19. The Lancet, 395(10234), 1417-1418.
4. Wichmann, D., & Sperhake, J. (2020). P. Z., Lütgehetmann Jan M. Z., Autopsy findings and venous thromboembolism in patients with COVID-

19. Ann Intern Med, 173, 268-277.
5. Galley, H. F. M (2004). Webster NR. Physiology of the endothelium. Br J Anaesth, 93(1), 105-113.
6. Yang L, Froio RM, Sciuto TE, Dvorak AM, Alon R, Luscinskas FW (2005). "ICAM-1 regulates neutrophil adhesion and transcellular migration of TNF–alpha-activated vascular endothelium under flow". Blood. 106 (2):584–92. doi:10.1182/blood-2004124942.
7. Abate, B. B., Kassie, A. M., Kassaw, M. W., Aragie, T. G., & Masresha, S. A. (2020). Sex difference in coronavirus disease (COVID-19): a systematic review and meta-analysis. BMJ open, 10(10), e040129.
8. Gebhard, C., Regitz-Zagrosek, V., Neuhauser, H. K., Morgan, R., & Klein, S. L. (2020). Impact of sex and gender on COVID-19 outcomes in Europe. Biology of sex differences, 11(1), 1-13.
9. Suresh, S., Tiwari, A., Mathew, R., Bhaskararayuni, J., Sahu, A. K., Aggarwal, P., ... & Sinha, T. P. (2021). Predictors of mortality and the need of mechanical ventilation in confirmed COVID-19 patients presenting to the emergency department in North India. Journal of Family Medicine and Primary Care, 10(1), 542.
10. Statsenko, Y., Habuza, T., Talako, T., Pazniak, M., Likhorad, E., Pazniak, A., ... & Das, K. M. (2022). Deep Learning-Based Automatic Assessment of Lung Impairment in COVID-19 Pneumonia: Predicting Markers of Hypoxia with Computer Vision. Frontiers in medicine.
11. Kang, S. J., & Jung, S.J. F. I. (2020). Age-related morbidity and mortality among patients with COVID-19. Infection & chemotherapy, 52(2), 154.
12. Masso-Silva, J. A., Moshensky, A., Lam, M. T., Odish, M. F., Patel, A., Xu, L., ... & Crotty Alexander, L. E. (2022). Increased peripheral blood neutrophil activation phenotypes and neutrophil extracellular trap formation in critically Ill coronavirus disease 2019 (COVID-19) patients: A case series and review of the literature. Clinical Infectious Diseases, 74(3), 479-489.
13. Zhang, C., Wang, X. M., Li, S. R., Twelkmeyer, T., Wang, W. H., Zhang, S. Y., ... & Tang, H. (2019). NKG2A is a NK cell exhaustion checkpoint for HCV persistence. Nature communications, 10(1), 1-11.
14. Bellmann-Weiler, R., Lanser, L., Barket, R., Rangger, L., Schapfl, A., Schaber, M., ... & Weiss, G. (2020). Prevalence and predictive value of anemia and dysregulated iron homeostasis in patients with COVID-19 infection. Journal of clinical medicine, 9(8), 2429.
15. Taj S, Kashif A, Arzinda Q, Fatima S, Imran S, Lone A, Ahmed QA. (2021). Role of hematological parameters in the stratification of COVID-19 disease severity. 62:68-72.
16. Azar, M. M., Shin, J. J., Kang, I., & Landry, M. (2020). Diagnosis of SARS-CoV-2 infection in the setting of the cytokine release syndrome. Expert review of molecular diagnostics, 20(11), 1087-1097.
17. Werion, A., Belkhir, L., Perrot, M., Schmit, G., Aydin, S., Chen, Z., ... &Vancraeynest, D. (2020). SARS-CoV-2 causes a specific dysfunction of the kidney proximal tubule. Kidney international, 98(5), 1296-1307.
18. Bikdeli, B., Madhavan, M. V., Jimenez, D., Chuich, T., Dreyfus, I., Driggin, E., ... & Lip, G. Y. (2020). COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. Journal of the American college of cardiology, 75(23), 2950-2973.
19. Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., ... & Cao, B. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The lancet, 395(10229), 1054-1062.
20. Delshad, M., Safaroghli-Azar, A., Pourbagheri-Sigaroodi, A., Poopak, B., Shokouhi, S., & Bashash, D. (2021). Platelets in the perspective of COVID-19; pathophysiology of thrombocytopenia and its implication as prognostic and therapeutic opportunity. International immunopharmacology, 99, 107995.
21. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. 395:497–506.
22. Smith-Norowitz, T. A., Loeffler, J., Norowitz, Y. M., & Kohlhoff,. S. (2021). Intracellular Adhesion Molecule-1 (ICAM-1) Levels in Convalescent COVID-19 Serum: A Case Report. Annals of Clinical & Laboratory Science, 51(5), 730-734.
23. Tong, M., Jiang, Y., Xia, D., Xiong, Y., Zheng, Q., Chen, F., ... & Zhu, Y. (2020). Elevated expression of serum endothelial cell adhesion molecules in COVID-19 patients. The Journal of infectious diseases, 222(6), 894-898.
24. Spadaro, S., Fogagnolo, A., Campo, G., Zucchetti, O., Verri, M., Ottaviani, I., ... & Contoli, M. (2021). Markers of endothelial and epithelial pulmonary injury in mechanically ventilated COVID-19 ICU patients. Critical Care, 25(1), 1-9.
25. Nizamudeen, Z. A., Xu, E. R., Karthik, V., Halawa, M., Arkill, K. P., Jackson, A. M., ... & Emsley, J. (2021). Structural assessment of SARS-CoV2 accessory protein ORF7a predicts LFA-1 and Mac-1 binding potential. Bioscience Reports, 41(1).