Available online at https://www.sjomr.org

SCIENTIFIC JOURNAL
OF MEDICAL RESEARCH

Vol. 6, Issue 24, pp 10-16 , 2022

ISSN: 2520-5234

ORIGINAL ARTICLE

Evaluation The Effects of Platelet Activating Factor (PAF) on Immunothrombosis in Severe and Critical COVID-19 Infections Compared to Mild Cases

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ARTICLE INFORMATIONS

Article history:

Received : 29 September 2022

Revised: 8 November 2022

Accepted: 20 November 2022

Published: 24 December 2022

Keywords:

COVID-19, SARS-CoV-2, Immunothrombosis, PAF.

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ABSTRACT

Back ground: The study aimed to evaluate the correlation between the platelet activating factor (PAF) and immunothrombosis in severe and critical COVID-19 patients in comparison with mild cases.

Materials and Methods: A cross sectional study was conducted. From October 1 2021 to May 30 2022, a total of 82 COVID-19 patients were recruited at AL-Hussein Medical City in 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 were diagnosed with mild COVID-19 and 27 with severe cases, while 28 patients had acute diseases.

Results: In mild COVID-19 cases, PAF levels increased very quickly, but in severe and critical cases PAF levels stabilized within a limited range ($p = 0.447$).

Conclusion: Platelet-activating factor PAF increased in mild cases, but then it stabilized within a limited range in severe and critical COVID-19 cases.

INTRODUCTION

The novel disease, coronavirus disease 2019 (COVID-19), 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 hospitalized COVID-19 subjects and may increase the risk of thromboembolic events. However, other possible causes can still 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 inflammatory mediators, such as adhesion molecules, chemokines, and cytokines (such as platelet-activating factor [PAF]), must be expressed to activate and attract inflammatory cells.[2] Recent investigations have found

CITATION: Muhammed AQW, Hassan HR. "Evaluation The Effects of Platelet Activating Factor (PAF) on Immunothrombosis in Severe and Critical COVID-19 Infections Compared to Mild Cases". Sci. J. Med. Res. 2022;6(24):10-16. DOI: 10.37623/sjomr.v06i24.03

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 parameters that indicate an activated coagulation process, 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. Accordingly, vascular microthrombotic disease likely contributes 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 and platelet activation that leads to thrombosis (Immunothrombosis) plays a critical role. In light of the fact that viral inclusions have been identified in endothelial cells, it has been proposed that damage and activation of endothelial cells could be the driving force behind platelet activation and the resultant coagulopathy. Hence, understanding the involvement of platelets in critical COVID-19 sickness is essential for both comprehending the biology of SARS-CoV-2 infection and locating potential therapy methods.[5]

Recent studies have found significant endothelial damage, extensive pulmonary micro thrombosis, and enhanced angiogenesis in the lungs of dead coronavirus-infected individuals. These findings confirm previous recent publications from other centers that show the presence of elevated coagulation markers and microthromboses in the lungs and other organs of COVID-19 patients.[6] Platelet activation and aggregation have recently been documented in severe COVID-19 patients. Platelet-activating factor, or PAF for short, is a cytokine that is the most effective inducer of platelet aggregation. PAF is also a powerful phospholipid activator and a regulator of several leukocyte processes, including platelet aggregation and degranulation, inflammation, and anaphylaxis. In addition to this, it plays a role in the alterations of vascular permeability, the oxidative burst, the chemotaxis of leukocytes, as well as the enhancement of arachidonic acid metabolism in phagocytes.[7]

Many different types of cells produce platelet-activating factor, but it is most prevalent in cells that play an important role in the host's immune response. Platelets, neutrophils, monocytes, endothelial cells, and macrophages are examples of these cells. These cells continuously produce PAF, usually in small amounts, and the activity of PAF acetyl hydrolases regulates production. PAF is produced in larger amounts by inflammatory cells in response to specific stimuli such as COVID-19, and its biological effects resemble COVID-19 symptoms.[8] Recent research has shown that platelets, by releasing PAF into the bloodstream, are responsible for activating perivascular mast cells, which ultimately results in inflammation. In addition, mast cell degranulation accompanied by Interstitial edema and immuno-thrombosis were found recently in the alveolar septa of dead COVID-19 patients. Mast cells are a major generator of PAF and are plentiful in the lungs, where they may contribute to COVID-19 development.[9]

MATERIALS AND METHODS

In accordance with the WHO, RT- PCR and CT scans were used to diagnose COVID-19 in all patients. For 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 was divided into three parts:

Part one- placed in EDTA tube for hematological tests (Complete blood count (CBC)).

Part two- transferred into sodium citrate tube to perform (D-dimer, PT, PTT).

Part three – putted in a gel tube for biochemical tests (C. reactive protein, Blood urea, and Serum creatinine) and immunological test (PAF).

Statistical Analysis

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

RESULTS AND DISCUSSION

Demographic Data of the Studied Groups

According to Table 1 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 were 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 the 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, ranging from 25 to 85 years.

There was no significant difference between female and male patients, according to this table, as agreed by,[2] who

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	Respiratory Rate (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
			$p = < 0.001$	$p = < 0.001$	$p = < 0.001$

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 gender and illness severity in this specimen, male gender was discovered to be a risk factor for disease severity by,[10,11] who found that the frequency of symptomatic COVID-19 was greater in males than in women.

Oxygen saturation (SpO_2) and respiratory rate (RR) were significantly correlated with disease severity. The patients with severe and critical disease had a significantly higher respiratory rate as compared to mild patients, while the SpO2 of those with severe and critical disease was significantly lower as compared to those with mild disease. All patients with critical illness required mechanical ventilation. These findings were similar to those mentioned by,[12] which concluded that patients with severe and critical COVID-19 have significantly higher RR and lower SpO_2 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, 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 a significant correlation exists between the patient's age and disease severity.

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,[13] which discovered that older people had the highest percentage of critical COVID-19 cases.

Mortality Distribution Among the Groups of COVID-19 Patients

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

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)

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 Table 3, mortality was significantly increased with disease severity and the patients aging in this study because no death was recorded within mild patients. In contrast, three 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 many other researchers, including,[14] 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 in comparison with mild COVID-19 cases

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

Variables	*Normal range*	*Mild Mean ± SD*	*Severe Mean± SD*	*Critical Mean ± SD*	*p-value*
White blood cell counts x10^9/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*
Hemoglobin g/L	13.5 – 17.5	13.8 ± 1.8	↓11.5 ± 1.5	↓10.8 ± 1.8	*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*

(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) (Table 4). 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).

Total WBC counts showed a slightly not significantly increase with the disease severity. While neutrophil percentages were significantly increased in severe and critical COVID -19 patients, this may be due to increased inflammatory process and excessive cytokine release (cytokine storm). This explanation is similar to that reported by,[15] who said that the elevation in neutrophils is 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 the 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 the functional depletion of cytotoxic lymphocytes.[16]

Hb significantly decreased in severe and critical cases compared to 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). 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 with,[17] 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 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.[18]

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 are significantly elevated with disease progression (p = <0.001). Serum creatinine levels in mild cases were within the normal range (Table 5). However, Serum creatinine levels were significantly elevated in the severe and critical cases in comparison with mild cases (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 was more elevated with COVID-19 severity. This was in agreement with,[19] 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. The macrophage activation syndrome may explain the higher serum CRP levels and lead to an increase in the severity of the disease. Cytokines, such as IL-6, and TNF-α, stimulate hepatocytes to produce CRP during a cytokine storm that the process of COVID-19 pneumonia can trigger.

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

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 x10^9/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

a cytokine storm. As a result, the kidney may be a vulnerable target for this novel coronavirus. These were agreed with,[20] who demonstrated that proximal tubule cells in the kidneys exhibit high levels of ACE2, which implies that SARS-CoV-2 may target them at an early stage of illness. The spike glycoprotein of SARS-CoV-2 has an affinity for binding to ACE2, which is considered a significant determinant of disease severity.

Some Coagulation Parameters in COVID -19 Patients

According to Table 6 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 to 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, during this study, the D-dimer was considered the single routine marker that indicated the presence of thrombosis (immuno-thrombosis) in COVID-19 patients. All mortalities in the current study were associated with high D-dimer levels. This indicates that D-dimer significantly correlates with COVID-19 severity and hypercoagulability, that increases the risk of venous thromboembolism (VTE) events, leading to thrombo-inflammation and even death in severe and critical conditions.

In mild COVID-19 cases, D-dimer levels were slightly elevated in the patients but remained within the high normal range. However, D-dimer levels in severe and critical cases were significantly elevated compared to mild cases. These results agreed by,[18] they claimed that D-dimer, ferritin, and LDH levels were significantly increased in patients with the 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 in establishing patient screening, therapy options, and prognosis management.[21]

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.[22]

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 by[18] who reported that platelets count did not show statistically significant association with severity of disease. 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.[23]

Table 7: The levels of PAF in COVID-19 patients:

Variable	*Normal Range*	*Mild Mean ± SD*	*Severe Mean ± SD*	*Critical Mean ± SD*	*p-value*
PAF ng/mL	0 - 5	126.5 ± 49.5↑	130.5 ± 56.1	143.3 ± 65.3	p= 0.447

Prothrombin time (PT) is normal or near-normal in most COVID-19 patients with just 5% who have extended PT. Results like these also agreed by[18] who said 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.[24]

The Levels of PAF in COVID -19 Patients

PAF levels in mild cases elevated very quickly, but in severe and critical cases (Table 7), PAF levels stabilized within a limited range (p = 0.0447).

After elevation of PAF level in mild cases, PAF secretion is reduced by PAF inhibitors which are obtained by taking some nutritional supplements such as vitamins, minerals, and some medicines. This was in agreement with,[25] who reported that PAF synthesis is modulated by PAF inhibitors which inactivate PAF receptors and affect the metabolism of PAF. These inhibitors have been found to reduce the activity of PAF regulatory enzymes and/or increase the activity of the PAF degrading enzyme. Several medicines (such as statins and antiviral drugs) and micronutrients (such as vitamin C, vitamin A, vitamin D, vitamin E, fatty acids, omega-3, selenium, and minerals) act as inhibitors for PAF synthesis.

Correlation of Serum Level PAF with Hematological Parameters in Mild, Severe, and Critical COVID-19 Patients

PAF levels in mild cases also showed no statistically significant correlation with hematological parameters (Table 8). Only a

Table 8: Correlation of Serum PAF Levels with Hematological Parameters in Mild, Severe and Critical COVID-19 Patients

Variables		*PAF (126.5 ± 49.5) ng/mL*	*Correlation*
Mild	White blood cell counts, x10^9/L	11.73 ± 4.77	r = -0.212 p = 0.412
	Neutrophils%	73% ± 12	r = -0.271 p = 0.291
	Lymphocyte%	18% ± 7	r = 0.398 P = 0.113
	Hemoglobin g/L	13.3 ± 1.6	r = 0.210 P = 0.416
Variables		PAF (130.5 ± 56.1) ng/ml	Correlation
Severe	White blood cell counts, x10^9/L	13.1 ± 5.92	r = 0.184 p = 0.367
	Neutrophils%	80 % ± 8	r = -0.195 p = 0.338
	Lymphocyte%	14 % ± 5	r = 0.051 p = 0.804
	Hemoglobin g/l	11.5 ± 1.5	r = 0.513 p = 0.007
Variables		PAF (143.3 ± 65.3) ng/ml	Correlation
Critical	White blood cell counts, x109/L	11.4 ± 5.1	r = 0.154 p = 0.422
	Neutrophils%	86 % ± 5	r = -0.256 p = 0.179
	Lymphocyte%	8 % ± 3	r = 0.219 p = 0.252
	Hemoglobin g/L	10.8 ± 1.8	r = -0.236 p = 0.217

strong positive statistically significant correlation occurred between PAF and Hb in severe cases (r = 0.513, p= 0.007). However, no statistical significance remains between PAF and Hb in critical cases (r = 0.236, p = 0.217).

In mild cases, hemoglobin (Hb) levels began to decrease but remained within the normal range. While in severe and critical cases, Hb is down below the normal range because the inflammation in COVID-19 individuals can result in a disruption of iron hemostasis and decreased iron absorption in the intestine, resulting in intravenous hemolysis and this, in turn, triggers platelet activation.[26] PAF that is elevated in mild cases triggers platelet activation necessary to control hemolysis. This is the explanation of the significant correlation between PAF and hemoglobin levels in severe cases.[27] However, PAF elevation begins to be stable within a limited range during severe and critical cases, so no significant correlation remains between PAF and Hb in critical cases.

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.

ACKNOWLEDGMENTS

Thanks and appreciation go to the medical staff of Al-Hussein Medical city/ Kerbala for their help in the sampling.

All thanks and gratitude go to COVID-19 patients and the parents of their contribution to the study.

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