Clinical and Experimental Health Sciences

Platelet Activating Factor Acetylhydrolase (PAF-AH) Activity: Could It Have a Role on Coagulation in Covid-19 Patients?

Tülin Bayrak[1], Gülsen Şener[2], Emel Sağlam Gökmen[3], Ahmet Bayrak[1]

[1] Ordu University, Faculty of Medicine, Department of Biochemistry, Ordu, Turkey.
[2] Başaksehir Çam and Sakura City Hospital, Department of Biochemistry, İstanbul, Turkey.
[3] Bağcılar Training and Research Hospital, Department of Internal Medicine, İstanbul, Turkey.

Correspondence Author: Tülin Bayrak
E-mail: bayrakt09@gmail.com

Received: 07.12.2021 **Accepted:** 22.03.2023

ABSTRACT

Objective: Abnormal immune inflammatory response and cytokine storm play an important role on the aspect of increasing mortality in Covid-19. We aimed to investigate whether the Platelet Activating Factor Acetylhydrolase activity (PAF-AH) and hematological parameters have prognostic and predictive value in determining the disease severity.

Methods: A total of 84 Covid-19 patients, 52 of whom were hospitalized in the ward and 32 in the intensive care unit (ICU), and 38 control patients were included in this study.

Results: Lymphocyte and serum albumin levels were significantly lower ($p < .001$) and age, neutrophils, CRP, procalcitonin, LDH, INR, D-dimer levels were significantly higher ($p < .001$) in Covid-19 patients compared to the control group. ICU patients had significantly lower ($p < .001$) lymphocyte, albumin values and significantly higher ($p < .001$) age, leukocyte, neutrophils, CRP, INR, aPTT, D-dimer levels compared to ward patients. PAF-AH activity was significantly increased in ICU patients compared to the control group ($p < .05$). A positive correlation was found between PAF-AH and D-Dimer in the ICU group.

Conclusion: We found increased PAF-AH activity in patients with Covid-19. It's important to spot the PAF-AH activity in cardiovascular events that develop due to coagulation problems, which are likely to be seen on these patients in the future.

Keywords: Hematological parameters, D-Dimer, INR, aPTT.

1.INTRODUCTION

An outbreak of pneumonia, the cause of that is unknown, which began in Wuhan, China in December 2019, has led to the identification of a new beta coronavirus, called Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). SARS-CoV – 2 is the seventh member of the identified RNA coronavirus family that can infect humans (1). According to WHO data, as of May 9, 2021, 156.496.592 confirmed cases of Covid-19 have been reported in 223 countries and regions all over the world, causing deaths in 3.264.143 patients (2). It has been observed that the clinical findings of the infection caused by Covid-19 are in a broad spectrum, starting from mild manifestations such as asymptomatic disease and mild upper respiratory tract infection to severe viral pneumonia that can be associated with respiratory failure and can result in acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC) and MOF (3) and finally, death. It has been revealed that advanced age, underlying diseases, abnormal immune inflammatory responses, and cytokine storms play an important role in the progression and the increasing rates of mortality of this infection, which does not yet have specific antiviral treatment other than supportive therapy (4).

It is thought that two different mechanisms run together during the process of this disease: Firstly, the pathogenicity caused by the virus itself; and the second is the inflammatory response of the body to this condition (5). Excessive inflammation leads to a predisposition to the formation of venous and arterial thromboembolic diseases as a result of endothelial dysfunction, platelet activation, hypoxia, immobilization (6). Therefore, the detection of circulating biomarkers that can represent immune status and inflammation is of critical clinical importance for early recognition of the systemic hyperinflammatory process in Covid-19 and predicting the progression of the disease. It is known that the systemic inflammatory process causes changes in leukocyte, lymphocyte, neutrophil, monocyte and platelet levels (2). Some studies have shown that peripheral blood parameters are markers of systemic inflammatory response and elevated values of these markers may lead to poor prognosis (7,8).

DOI: 10.33808/clinexphealthsci.1033882

PAF is one of the most powerful lipid mediators associated with inflammatory events, synthesized by various cells, including macrophages, neutrophils, lymphocytes, basophils, eosinophils, platelets, fibroblasts and vascular endothelial cells, which are at the center of most of the inflammatory systems (9). It plays a role in smooth muscle contraction, platelet activation, chemotaxis and degranulation of polymorphonuclear leukocytes and monocytes, and increases vascular permeability by altering vascular tension (10). Platelet Activating Factor Acetylhydrolase (PAF-AH) is a highly specific antioxidant enzyme released from mast cells that neutralizes both the acetyl group of PAF, which is a powerful pro-inflammatory lipid mediator, and the short-chained fatty acids of oxidized phospholipids by hydrolyzing and it also regulates inflammatory responses. Many studies show that PAF-AH plays an important role in reducing PAF-induced damage and terminating the signals of phospholipids such as PAF and oxidized PAF, which yield products unable to be recognized by the PAF receptor (11,12).

Due to its highly contagious nature, Covid-19 has placed a huge burden on hospitals. A group of laboratory tests will be useful for the efficient use of limited isolation facilities in outbreaks and for early classification, follow-up and therapeutic follow-up of patients. It has been reported that hematological parameters are significantly abnormal in most patients with Covid-19. Studies have shown that the development of coagulopathy in these patients is one of the poor prognostic features and most of the expired patients have DIC (13). Decreased PAF-AH activity is known to be accompanied by sepsis, inflammation, and coagulation (14). Determining the levels of PAF-AH activity, which has both antithrombotic and antioxidant properties, may be important for shedding light on the coagulation mechanism in these patients. There is no study in the literature yet in which hematological parameters and PAF-AH activity were evaluated together in Covid-19. We aimed to determine whether there is a relationship between them by evaluating PAF-AH enzyme activity and coagulation parameters in Covid-19 patients in this study. In addition, we wanted to determine the effectiveness of these parameters in predicting patients who should be admitted to the ICU and to investigate the usability of PAF-AH as a predictive and prognostic indicator in Covid-19 patients.

2.METHODS

2.1. Study Design and Participants

For this study, approval from the Ordu University Faculty of Medicine Ethics Committee with the date of 28.05.2020 and number 112 was obtained, as well as informed consent from all participants or their relatives.

This retrospective study was conducted with patients who visited the Emergency Room of İstanbul Bağcılar Training and Research Hospital between the dates of 15.03.2020-01.04.2020. In the study, a total of 84 adult patients that are aged 18 years and older, 52 of whom were treated in the ward and 32 in the ICU were included. The diagnosis of Covid-19 was confirmed by clinical examination, CT and rRT-PCR test positivity in nasal and/or pharyngeal swab samples. Patients with hematological disease or those who underwent blood transfusions while hospitalized were excluded. Again, 38 adult patients who were found to be negative with clinical examination, CT, and rRT-PCR tests and still admitted to the hospital on the same dates were treated as control patients. Inclusion criteria for the control group were determined as rRT-PCR test negativity, and those with the comorbid disease were once again excluded.

The routine parameters examined were analyzed retrospectively and the PAF-AH activities of these patients were studied from blood samples taken for routine tests and stored at-80ºC.

2.1.1. Clinical Classification

Covid-19 cases were evaluated according to the T.C. Ministry of Health's COVID-19 (SARS-CoV-2 infection) guidelines (15) and case classification was made. 52 patients who meet at least one of the following criteria were admitted to the ward; a. Fever, cough, nasal congestion, sore throat, muscle/joint pain, and tachypnea (≥30/min), SpO2 level below 90% in room air and b. Bilateral diffuse pneumonia findings detected on chest radiography or tomography 32 patients who meet one of the following criteria were transferred to the ICU;

Dyspnea and respiratory distress, respiratory rate>30/min, PaO2/FiO2 < 300, increased oxygen need during follow-up, PaO2 < 70 mmHg or SpO2 < 90% despite 5 L/min oxygen therapy, hypotension (systolic blood pressure < 90 mmHg and a decrease from usual SBP more than 40 mmHg) and tachycardia > 100/ min, mean arterial pressure < 65 mmHg, confusion, acute liver function tests disorder, acute kidney injury, arrhythmia and increased troponin, development of acute organ dysfunction and immune suppression, lactate > 2 mmol, capillary return disorder and cutis marmoratus.

The demographic, epidemiological, clinical, radiological data, and laboratory results of the patients were obtained by using data collection forms from electronic records. The parameters of the Covid-19 positive patients treated in the ward and ICU were compared to Covid-19 negative healthy control group.

2.2. Biochemical Study

Routine parameters taken when patients received their current diagnosis were studied in the Laboratory of Bağcılar Training and Research Hospital. For the complete blood count of the patients, blood samples were taken into a tube with EDTA and hematology analysis of the samples was performed by absorption photometry and fluorescence flow cytometry method on XN-900 (Sysmex, Japan) automatic analyzer. For coagulation parameters, with the plasma separated from the

blood samples taken into the citrate tube by centrifugation at 3500 rpm for 10 min, by immune turbidimetric and chromogenic substrate methods, on the SF-8200 (Succeder, China) automatic coagulation analyzer; biochemistry parameters, with serum obtained by centrifuging blood samples taken into the gel tube at 3500 rpm for 10 minutes were performed with the photometric assays in the AU 480 (Beckman coulter, USA) automatic biochemistry analyzer by utilizing. PAF-AH activity was analyzed from the blood samples taken for routine tests, by the enzymatic method using a spectrophotometry device, and stored at-80ºC in the Research Laboratory of Ordu University Faculty of Medicine Biochemistry.

PAF-AH activity levels have been determined spectrophotometrically by the method of Stafforini et al. (16). 2-thio-PAF (Cayman Chemical) was used as a substrate in the study. It is based on the spectrophotometric determination of 5-thio2-nitrobenzoic acid formed as a result of reaction with DTNB of free thiol groups, formed by hydrolysis of acetyl thioester bond in position sn-2 at of by at 412 nm. The molar absorbency coefficient for 5-Thio-2-nitrobenzoite acid was taken as (Ɛ= 13600 m-1cm-1). The unit of PAF-AH activity is defined as the amount of enzyme that hydrolyzes one micromole of 2-Thio PAF per minute. The detection range of the test is 30-300 nmol/min/mL.

2.3. Statistical Analysis

Data with normal distribution were expressed as the mean ± standard deviation (SD) while data with abnormal distribution were expressed as median ± IQR. Kolmogorov-Smirnov test was used to control the normal distribution of our data. Levene test was used to control the homogeneity of group variations. The group averages were compared using a one-way analysis of variance (ANOVA) when the normality assumption was provided. In addition, the Kruskal-Wallis test was also performed for assessing abnormality. Following these analyses, different groups were identified using Dunn tests or Duncan's multi-range. In the correlation analysis of parameters, Pearson or Spearman correlation test was used for parametric or nonparametric data. The statistical significance level was accepted as $p < .05$. SPSS.20 software (version 20.0) statistical package program was made using the for calculations.

3.RESULTS

3.1. Demographic Features

A total of 84 COVID-19 patients consisted of 52 in the ward and 32 in the ICU and 38 healthy people participated in the study. There was a significant difference between the groups in terms of age distribution ($p<0.001$). In the ward group, 25 of the patients were female, 27 were male, the average age was 53.3 ± 18.6; in the ICU hospitalized patients, 13 females, 19 males, mean age 72.5 ± 12.5 and Covid-19 negative control group, 24 females, 14 males, mean age 38.3 ± 11.2 was determined as ($p < .001$).

56% of the patients (n = 84) had comorbidity and the most common comorbid diseases were spotted as diabetes (34.91%, n=28), hypertension (26.19%, n=22) and coronary artery disease (17.86% n=15). (Table 1).

Table 1. *Demographic characteristics of the patients and control group.*

Groups	Control group (n=38)	Ward group (n=52)	ICU group (n=32)	p value
Age (mean ± SD)	38.3±11.2	53.3±18.6	72.5±12.5	<0.001
Gender				
Male (n) (%)	14 (36.84%)	27 (51.92%)	19 (59.38%)	
Female (n) (%)	24 (63.16%)	25 (48.08%)	13 (40.62%)	
DM		9 (17.31%)	19 (59.38%)	
HT		6 (11.54%)	16 (50%)	
CAD		3 (5.77%)	12 (37.5%)	
COPD		5 (9.62%)	5 (15.63%)	
Chronic renal failure		1 (1.92%)	6 (18.75%)	
Cerebrovascular disease		0	1 (3.13%)	

For age; data is presented as mean ± standard deviation (SD) and n (%).

3.2. Routine Parameters of the Covid 19 Ward and ICU Groups

Between the ward group and ICU group, there were various differences found in the routine hematological blood parameters and routine biochemistry parameters that show anti-inflammatory capacity. ICU patients had significantly lower lymphocytes (0.9±0.6; 1.5±0.7 $p < .01$) and lower albumin (2.8±0.5; 3.5±0.6 $p < .001$) levels compared to ward patients.

Compared to the levels of admitted ward patients', ICU patients had significantly higher Leukocyte 11.6 (0.6-36.4); 7.2(3.3-20.9) $p < .001$), higher neutrophils 10.0 (0.5-33.3); 4.84 (2.0-18.0) $p < .001$), higher CRP (132.5±104.8; 54±67.9 $p < .001$), higher LDH (384±168.5; 293.6±118.4 $p < .001$), higher INR 1.2(0.8-2.1); 1.1(0.9-2.1) $p < .001$), higher aPTT (37.7±12.2; 20.9±17.6, $p < .001$), higher D-dimer (2.41±2.5; 1.05±1.6 $p < .001$) (Table 2). PAF-AH levels were also statistically significantly increased in ICU patients (111.9 ± 26.6; 129.2 ± 27.6) ($p < .05$) compared to the control group. There was no significant difference in terms of PAF-AH levels between the patient groups. PAF-AH levels were statistically significantly increased in ICU patients, compared to the control group patients (111,9±26,6; 129,2±27,6) ($p < .05$) (Figure 1).

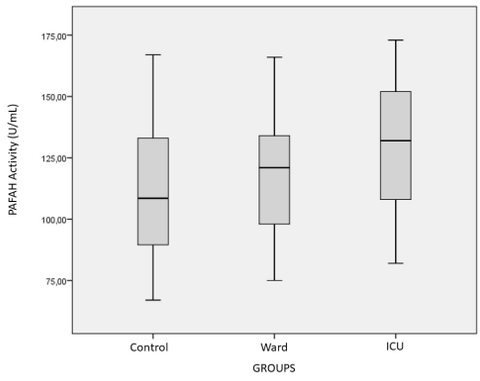

Figure 1. *Distribution of PAFAH Activity averages by groups*

Table 2. *Comparison of biochemical parameters according to Covid-19 patients and control groups.*

	Control group (n= 38)	Ward group (n= 52)	ICU group (n= 32)
Age (years)	38.3±11.2	53.3±18.6 [e]	72.5±12.5 [e, f]
Glucose (mg/dl)	90.7±26.2	111±64.6	164.6±101.8
Urea (mg/dl)	32.5±10.7	38.2±26.8	86.8±86.8 [a]
Albumin (mg/dl)	4.3±0.2	3.5±0.6 [e]	2.8±0.5 [e, f]
AST (U/L)	22.8±10.2	34.3±25 [a]	76.7±177.8
ALT (U/L)	22±19.2	30±33.9	44.2±87.5
GGT (U/L)	7.1±13.4	25,8±31	66.6±46.3
ALP (U/L)	75.3±20.8	91.2±40.1	117.5±66.8 [c]
Creatine Kinase (U/L)	92.5±16.2	88.4±93.1	127.1±165
Hemoglobin(g/L) (mg/dl)	12.2±3.4	12.1±3	10±2.4
Leucocytes (×10^9 per L)	7.5(4.1-12.4)	7.2(3.3-20.9)	11.6 (0.6-36.4) [e]
Neutrophils (×10^9 per L)	4.01 (2.1-6.8)	4.84 (2.0-18.0)[c]	10.0 (0.5-33.3) [c,f]
Lymphocytes (×10^9 per L)	2.4±0.9	1.5±0.7 [e]	0.9±0.6 [e,d]
Monocyte (×10^9 per L)	0.5±0.1	0,6±0.4	0.6±0.4
Basophil	0.05(0.02-0.1)	0.02(0.01-0.08) [c]	0.04(0.01-0.15)
Eosinophil (×10^9 per L)	0.1±0.2	0.1±0.5	0.1±0.5
Platelet (×10^9 per L)	269((158-422)	250(1.02-459)	314(102-683) [b]
CRP (mg/dl)	2.3±1.8	54±67.9 [e]	132.5±104.8 [e,f]
Procalcitonin (ng/ml)	0.02(0.01-0.09)	0.07(0.02-47.8)	0.7(0.05-100.4)
Ferritin (ng/ml)	37.7±48	169.5±320.6 [a]	542.8±749.9 [a,b]
Pt (sn)	13.3(12.3-16.6)	14.1(11.1-28.1) [a]	15.7(11.4-27.5) [c,f]
Inr	1.03(0.9-1.3)	1.1(0.9-2.1)[c]	1.2(0.8-2.1) [e, f]
aptt (sn)	32.8±8.2	20.9±17.6 [e]	37.7±12.2 [f]
D-dimer (µg/ml)	0.24±0.3	1.05±1.6 [c]	2.41±2.5 [e,b]
Fibrinogen (mg/dl)	349.3±111.3	287.2±254	391.7±255.8
Pafah (U/ml)	111.9±26.6	118±25.4	129.2±27.6 [a]
LDH (U/L)	192.4±38.1	293.6±118.4 [e]	384±168.5 [e,b]

Data is presented as mean ± standard deviation (SD) or median (interquartile range (IQR))
ap < 0.05 compared with control group
bp < 0.05 compared with Ward group
cp < 0.01 compared with control group
dp < 0.01 compared with Ward group
ep < 0.001 compared with control group
fp < 0.001 compared with Ward group

3.3. Comparison of the Relationship Between PAF-AH and Routine Parameters of the Control and Covid19 Group at Admission Ime

There was a significant positive correlation between age and ferritin (r = 0.370, p = .022), between PAF-AH-INR (r = 0.318, p = .045), between PAF-AH-ALT (r = 0.341, p = .031), between CRP-Fibrinogen (r = 10.701, p = 0.000), between CRP-D-Dimer (r = 0.814, p = .000), between ALT-INR (r = 0.580, p = .000), between ALT-Albumin (r = 0.417, p = .031), between AST-INR (r = 0.416, p = .009) in the control group. A significant negative correlation between age and aPTT (r =-0.356, p = .028) was present in the control group.

A significant positive correlation between CRP and INR level (r= 0.284, p= .042) was found in the ward group. There was a positive correlation between PAF-AH-D-Dimer (r= 0.376, p= .044), between ALT-D-Dimer (r= 0.376 p= .044), between Ferritin-D-Dimer (r=0.535, p= .03), between Ferritin-ALT (r=0.462, p= .008), between AST-Leukocyte (r=0.497, p= .004), between AST-Neutrophil (r=0.504, p= .003), between AST-Monocyte (r=0.572, p= .001), between ALT-Monocyte (r=0.381, p= .031), between Monocyte-INR (r=0.566, p= .001), between Monocyte-Ferritin (r= 0.421, p= .017) in the ICU group. A significant negative correlation between ALT-aPTT (r= – 0.579, p= .001) was present in the ICU group.

4.DISCUSSION

In this study, hematological parameters and PAF-AH enzyme activity levels were examined and compared in patients of SARS-CoV-2 in the ward and ICU. Abnormal routine results of hematological, biochemical parameters, and PAF-AH levels were available in patients with Covid-19. We found that examining PAF-AH and peripheral blood count, especially lymphocytes and neutrophils, would help to predict severe Covid-19 cases. Increased age, leukocyte, neutrophil, CRP, albümin, and D-Dimer levels as well as increased PAF-AH levels can be considered as independent biomarkers to show a deteriorating clinical picture. There was a significant positive correlation between D-Dimer and PAF-AH levels. Our findings may be useful as an earlier indicator for determining the disease severity and to help classify patients for ICU transfer. We ponder the reason for the changes in the measured parameters as the developing inflammatory process. It can be thought that PAF-AH is expressed and affected differently from post-transcriptional and post-translational mechanisms at different stages of systemic inflammation.

In three COVID-19 patients who underwent minimally invasive autopsies, marked irregularity of the lymphohematopoietic system, a decrease in lymphocytes in the spleen, necrosis, and cell degeneration was observed in addition to severe lung lesions (17). SARS-CoV-2 is a RNA coronavirus that enters human cells by binding to angiotensin-converting enzyme 2 (ACE2), which is highly expressed in lung alveolar cells, vascular endotelium, cardiac myocytes, and various cells (18). The binding of SARS-CoV-2 to the ACE2 receptor causes a high expression of ACE2, which can lead to damage

in alveolar cells. Damage to Alveolar cells can cause a range of systemic inflammatory responses, often involving varying degrees of hematopoietic system abnormalities and hemostasis activation. Especially in some COVID-19 patients, excessive inflammatory cytokine increase, which can lead to a "cytokine storm" such as increasing levels of IL-6, has the potential to cause severe diseases such as ARDS, DIC, MOF, shock as a result of uncontrolled inflammation (19).

In our study, in parallel with other studies, most of the patients were male. Also, the cases were older in comparison to other studies' patients and more likely to have underlying diseases as a result of poorer immune functions (3). Similar to other studies, more than half of the patients had comorbidity. Diabetes was the most common comorbidity, followed by hypertension, CAD, and COPD (20). Qin et al. stated that hypertension and cardiovascular disease are found at a higher rate in severe cases compared to mild cases (21). It has been stated that hypertension may play a role in the progression of COVID-19 by causing abnormal immune function as a result of immune activation (22).

In addition to the various parameters used to detect systemic inflammation, simple, low-cost and easily accessible hematological markers, which are widely used, are important (19). Lymphocytes play a decisive role in sustaining the inflammatory response and maintaining immune homeostasis. Potential mechanisms that could lead to lymphocyte deficiency were regarded as; lymphocytes being infected with the virus as a result of expressing ACE2, lymphocytic dysfunction, inflammatory cytokines leading to lymphocyte apoptosis and translocation of lymphocytes from peripheral blood to lungs (19). It has been stated that survival in COVID-19 may depend on the ability of lymphocytes killed by the virus to regenerate (23). Therefore, lymphocyte count in Covid-19 is extremely important as a clinical predictor of disease severity and prognosis. In the study of Zhou et al. it was shown that lymphocytopenia was seen in 40% of patients and the initial lymphocyte level was significantly higher in survivors than in non-survivors. This study confirmed that elevated IL-6 levels and lymphopenia were more common in severe patients, and increased age in patients with Covid-19 was related associated with increased mortality (3). Age-related defects in B and T cell function and excessive type 2 cytokine production have been shown to cause viral replication control difficulties and prolonged proinflammatory responses (24).

Regardless of whether leukocytosis represents neutrophilia, lymphocytosis, or both, it has been reported to be a harbinger of bacterial infection or superinfection for patients with COVID-19 (25). Our study showed that, unlike some previous studies, Covid-19 is associated with leukocytosis rather than leukopenia (26,27). We think that leukocytosis reflects severely increased inflammation. In one study, while an increase in leukocyte count was slightly seen in patients with severe disease, a significant increase was shown in patients who passed away (23). Consistent with the results we found in our study, it was suggested that the significant increase in leukocytes in patients with severe disease may indicate clinical worsening (23). In a study conducted on 75 patients, the leukocyte levels of the severe group were found to be higher than the moderate group (28). Leukocytosis is not correlated with lymphopenia, it is caused by the high amount of neutrophils, and lymphopenia may emerge in both inclining-declining cases of leukocyte count (23). In our study, leukocytosis was accompanied by lymphopenia and an increase in neutrophil count. In a study of Huang et al. involving 140 COVID-19 patients, he stated that leukocytosis, neutrophilia, and lymphopenia are important determinants of ICU admission (26). In our study, similar to other studies, ICU patients generally had deeper lymphopenia and neutrophilia (29,30).

Decreased lymphocyte count indicates immune system damage, while an increased neutrophil count indicates the intensity of the inflammatory response (20). Neutrophils are immunity cells that are increased in certain lung diseases, including viral respiratory infections. (31). Neutrophils may exit into the airways from the circulation via postcapillary venules in the systemic circulation or via the capillaries in the pulmonary circulation. Neutrophil production may be triggered by virus-related inflammatory factors such as interleukin-6, interleukin-8, interferon-gamma factors, tumor necrosis factor-alpha, and granulocyte colony stimulating factor (19). In one study, peripheral routine blood parameters of the severe and critical groups were analyzed, the inflammatory neutrophil count in the critical group was significantly higher than the severe group, lymphocyte count was found to be significantly lower than the severe group (29). Our results show that lymphocyte and neutrophil levels at the moment of admission are related to the prognosis of the ailment. These data propose that the degree of infection and inflammation triggered by the virus, becomes more intense, augmenting the storm of inflammation and leading to increased tissue and cell damage. Our results are consistent with previous studies that found a relationship between prognosis and disease severity (25,28).

In COVID-19 infection, the virus damages bone marrow cells directly or with the effect of overproduction of proinflammatory cytokines, and this leads to the suppression of hematopoiesis, which explains low hemoglobin, leukopenia, lymphopenia, eosinophilic cytopenia, thrombocytopenia as a result of a series of immune responses in patients. It has been stated that this critical condition may be related to the ICU patients' tendency towards low immune response and sepsis (32). There was no significant difference in hemoglobin levels between patient groups in our study. Although the number of thrombocytes decreased in ward patients, it was not statistically significant. There was significant thrombocytosis in the ICU group compared to the control group. Thrombocytopenia is common in critical patients and indicates severe organ damage, and it has been noted that there is a greater decline in platelet count, especially in those mortal cases (33). The mechanism of thrombocytopenia in COVID-19 patients is multifactorial. Chen et al. reported that some of the patients had thrombocytosis (27). One study found that patients with significantly higher platelets and PLRs had longer average hospitalization days. It

has been stated that this turn of events may be caused by the release of excessive cytokines that stimulate the formation of megakaryocytes (34). Platelets trigger the degranulation of mast cells, leading to inflammatory responses and tissue damage.

SARS-CoV-2 directly attacks vascular endothelial cells, initiating localized inflammation, endothelial activation, irregular cytokine release, and tissue damage. Vascular endothelial damage causes hypercoagulability in infected patients by COVID-19, as a result of excessive thrombin production and inhibition of fibrinolysis (35). Tang et al. found higher levels of longer PT, APTT, and D-dimer values in those who could not survive the disease compared to survivors in their study. These results suggested that patients have a tendency for hypercoagulability and supported microthrombosis (36). Wang et al. showed that D-Dimer levels were significantly higher in the ICU group in line with our results (20).

Mast cell degranulation has been reported in the alveolar septa of those who died due to COVID-19 (37). Mast cells are immunity cells that can be activated by many factors, including viruses, and have the attribute of PAF secretion (38). Recently held responsible as a factor for COVID-19 pneumonia severity, mast cells have been shown as one of the rich sources of pro-inflammatory cytokines in the lungs (39). It is also known that PAF activates platelets and promotes clot formation. PAF is known to be one of the most potent lipid mediators involved in various physiological occasions. Activation of cells responsive to pathogens, cytokine response, free radicals, and the formation of oxidized phospholipids recognized by the PAF receptor cause uncontrolled systemic inflammation and DIC by activating the coagulation system. Increased vascular permeability, plus increased leukocyte count and platelet aggregation lead to hypoperfusion and tissue hypoxia, which are the main cause of multiple organ dysfunction (11). There is evidence that the PAF signaling system is irregular in traumatic injuries, sepsis, and shock, and that interruption or termination of effector responses has beneficial consequences and thereby regulates the inflammatory response (14). PAF-AH is an enzyme that inactivates PAF and oxidized phospholipids with proinflammatory and prothrombotic features and controls systemic inflammation. PAF-AH levels increase with the stimulation of inflammatory agonists and lipopolysaccharides, however, decrease with anti-inflammatory drugs and cytokines. Circulating PAF-AH levels are found to be decreased in systemic infection and multiple organ failure. In oxidative stress-based diseases such as asthma, sepsis, stroke, acute CVH, and MOF; PAF-AH levels have been found to be low and it has been reported to contribute to organ dysfunction (40).

In one study, low PAF-AH levels in severe patients admitted to the ICU, and showed that the enzyme level increased over time. But, in this study, high PAF-AH activities were reported in the nonsurvivors group compared to survivors, and no significant predictive value was found (41). It has been reported in previous studies that PAF-AH plays an important role in controlling systemic inflammation as part of the compensatory anti-inflammatory response (42). It has been recommended that it is possible that there are a number of variations in the enzyme activity of patients (41). An earlier study had shown that plasma PAF-AH activity did not decrease in the ARDS risk group and ARDS patients, on the contrary, in bronchoalveolar lavage (BAL) fluid, increased PAF-AH levels were spotted in the acute phase of ARDS, compared to patients with ARDS risk and control patients (42). It is thought that PAFAH is increased by the leakage into the alveoli as a result of increased pulmonary capillary endothelial and alveolar epithelial permeability, release from injured cells, or by active synthesis of local PAFAH in the lungs of patients with ARDS by alveolar macrophages, alveolar type 2 pneumocytes. Previous studies also suggest that an extracellular form of PAF-AH may be vital in the local regulation of inflammation in the lung by inactivating PAF and related phospholipids released in the airways (43).

In their study, Benli et al. detected that PAF-AH level is increased in patients with high PSA levels and in the group diagnosed with prostate cancer compared to the control group. They expressed their belief that this variation may be caused by the increased inflammatory process (44). Similarly, in our study, although PAF-AH levels were increased in patients in the ward compared to the control group, it was not statistically significant. However, there was a statistically significant increase in the ICU group compared to the control group. A statistically significant positive correlation was found between D-Dimer and PAF-AH in our ICU patients. Macrophages, megakaryocytes, and dendritic cells each respond to inflammatory and hemostatic signaling factors (45). Moreover, conditions such as inflammation and sepsis play a role in controlling acute inflammation caused by PAF and oxidized phospholipids by increasing plasma PAF-AH expression and synthesis. This suggests that endogenous PAF-AH is a systemic circulating marker of inflammation that is regulated temporarily and expressed differently. In our study, the increase in PAF-AH activity in the severe (ICU group) course of the disease compared to the moderate severe (inpatient group) course of the disease is an important discovery due to the antioxidant and antithrombotic effect of the enzyme. In cardiovascular events that develop due to clotting problems that are likely to occur in patients in the future, it becomes important to determine how PAF-AH levels and PAF levels are affected. PAF has many potent biological effects on almost all tissues and organs (46). Therefore, considering these findings, it was stated that it would be reasonable to try to prevent the effect of PAF with various treatments (47).

There were some limitations in this study. Our control patients were younger than the COVID-19 patients. However, this study was the first to determine PAF-AH activity in Covid-19 patients as far as we know. In new studies to be designed, we believe that determining PAF levels as well as PAF-AH activity will provide better knowledge of the literature. More large-scale and well-designed and qualified clinical studies are needed to elucidate coagulation mechanisms in COVID-19 patients.

5.CONCLUSION

In summary, COVID-19 causes abnormalities in hematological parameters and PAF-AH activity. Therefore, measurement of PAF-AH and routine peripheral blood parameters shows that it can have an important reference value in determining the inflammatory process, predicting the patients who should be admitted to the ICU at the initial stage, classifying the patients, and evaluating the progression and prognosis of COVID-19.

Acknowledgements: *The researchers would like to thank the patients who participated in the study.*

Funding: *The author(s) received no financial support for the research.*

Conflicts of interest: *The authors declare that they have no conflict of interest.*

Ethics Committee Approval: *This study was approved by Ethics Committee of Ordu University Faculty of Medicine, (approval date 28.05.2020 and number 112)*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: TB, GS
Design of the study: AB, GS, TB
Acquisition of data for the study: ESG
Analysis of data for the study: TB, AB
Interpretation of data for the study: TB, AB, ESG
Drafting the manuscript: TB, GS, ESG
Revising it critically for important intellectual content: AB, ESG, GS
Final approval of the version to be published: TB, GS, ESG, AB

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How to cite this article: Bayrak T, Şener G, Sağlam Gökmen E, Bayrak A. Platelet Activating Factor Acetylhydrolase (PAF-AH) Activity: Could It Have a Role on Coagulation in Covid-19 Patients? Clin Exp Health Sci 2023; 13: 503-510. DOI: 10.33808/clinexphealthsci.1033882