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ORIGINAL ARTICLE CARDIOLOGY

Plasminogen activator inhibitor-1 levels as an indicator of severity and mortality for COVID-19

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ABSTRACT

OBJECTIVE: Coronavirus disease-19 (COVID-19) is a multisystemic disease that can cause severe illness and mortality by exacerbating symptoms such as thrombosis, fibrinolysis, and inflammation. Plasminogen activator inhibitor-1 (PAI-1) plays an important role in regulating fibrinolysis and may cause thrombotic events to develop. The goal of this study is to examine the relationship between PAI-1 levels and disease severity and mortality in relation to COVID-19.

METHODS: A total of 71 hospitalized patients were diagnosed with COVID-19 using real time-polymerase chain reaction tests. Each patient underwent chest computerized tomography (CT). Data from an additional 20 volunteers without COVID-19 were included in this single-center study. Each patient's PAI-1 data were collected at admission, and the CT severity score (CT-SS) was then calculated for each patient.

RESULTS: The patients were categorized into the control group (n=20), the survivor group (n=47), and the non-survivor group (n=24). In the non-survivor group, the mean age was 75.3±13.8, which is higher than in the survivor group (61.7±16.9) and in the control group (59.5±11.2), (p=0.001). When the PAI-1 levels were compared between each group, the non-survivor group showed the highest levels, followed by the survivor group and then the control group (p<0.001). Logistic regression analysis revealed that age, PAI-1, and disease severity independently predicted COVID-19 mortality rates. In this study, it was observed that PAI-1 levels with >10.2 ng/mL had 83% sensitivity and an 83% specificity rate when used to predict mortality after COVID-19. Then, patients were divided into severe (n=33) and non-severe (n=38) groups according to disease severity levels. The PAI-1 levels found were higher in the severe group (p<0.001) than in the non-severe group. In the regression analysis that followed, high sensitive troponin I and PAI-1 were found to indicate disease severity levels. The CT-SS was estimated as significantly higher in the non-survivor group compared to the survivor group (p<0.001). When comparing CT-SS between the severe group and the non-severe group, this was significantly higher in the severe group (p<0.001). In addition, a strong statistically significant positive correlation was found between CT-SS and PAI-1 levels (r: 0.838, p<0.001).

CONCLUSION: Anticipating poor clinical outcomes in relation to COVID-19 is crucial. This study showed that PAI-1 levels could independently predict disease severity and mortality rates for patients with COVID-19.

Keywords: Coronavirus disease-19; disease severity; mortality; plasminogen activator inhibitor-1; thromboembolism.

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a beta-coronavirus that originated in China and causes the acute respiratory syndrome known as coronavirus disease-19 (COVID-19). In 2020, the spread of COVID-19 resulted in a global pandemic [1]. Clinical findings and mortality rates in relation to COVID-19 have generally been associated with the respiratory system and cardiovascular system [2]. Infections associated with COVID-19 can cause numerous cardiovascular complications, such as myocardial injury, ischemia, myocarditis, arrhythmic events, and thromboembolism [3]. In patients with COVID-19, venous and arterial thrombi formation causes serious diseases, including myocardial damage, heart failure, stroke, and respiratory distress syndromes, as well as common pulmonary and cardiovascular complications [4]. These findings show that endothelial dysfunction plays a crucial role in the pathophysiology of COVID-19 disease [5]. Many biomarkers such as troponin, brain natriuretic peptide, and D-dimer are important to diagnose cardiac involvement and coagulopathy and to demonstrate the severity of COVID-19 [6, 7].

In many infections, endothelial cells secrete IL-6, TNF-α, and IL-1 cytokines, both of which increase the proliferation of tissue factor (TF) in the endothelial liner while increasing plasminogen activator inhibitor-1 (PAI-1) levels [8, 9]. Endotoxin-induced proliferation of TF and PAI-1 in the endothelial cells may, therefore, constitute a stimulus that can support thrombotic events [10]. As PAI-1 is a glycoprotein and serine proteinase inhibitor, it plays a critical role within the fibrinolytic system and is synthesized by hepatocytes, megakaryocytes, and endothelium [11]. Low levels of tissue plasminogen activator (tPA) or high PAI-1 levels provide tPA inhibition and can have an adverse effect on the fibrinolytic system [12]. The overexpression of PAI-1, which provides arrangement of fibrinolysis, causes thrombotic events to improve [13]. In patients with COVID-19, the risk of thrombotic events is increased due to plaque ruptures and increased coagulability in conjunction with intense systemic inflammation and direct vascular inflammation [14]. The presence of abnormal coagulation parameters and the correlation between coagulation parameters, including D-dimer and international normalized ratio (INR), and disease severity have been observed in hospitalized patients with severe COVID-19 [15]. However, no existing studies evaluate the relationship between PAI-1, disease severity, and mortality.

The goal of our study is to demonstrate the relationship between PAI-1 levels and disease severity and mortality in hospitalized patients with COVID-19.

Highlight key points

- Patient PAI-1 levels were evaluated separately in patients that died and patients that showed severe levels of the disease. These levels were observed to be high in both groups and showed a general increase in all patients with COVID-19 compared to healthy individuals.
- Radiological evaluation with CT scans was also performed, and a strong correlation was found between radiological disease severity and high PAI-1 levels.
- Patient PAI-1 levels were found to predict mortality and disease severity separately with a high sensitivity and specificity percentage.

MATERIALS AND METHODS

Study Population

This study examined 71 hospitalized patients that were diagnosed with COVID-19 using real time-polymerase chain reaction (RT-PCR) tests and chest computerized tomography (CT) between June 2020 and August 2020 within a single center. Twenty healthy individuals, matched by age and gender, with no known cardiac or pulmonary diseases, were recruited into the control group for biomarkers. Patients whose cardiac biomarkers were not measured at the time of admission, who were under the age of 18, who were diagnosed with acute coronary syndrome or advanced renal failure (GFR <30 mL/min), or who were undergoing dialysis were excluded from this study. Chest CT scans were performed during hospital admission, and patients who did not receive these were excluded from this study.

The RT-PCR test was performed with SARS-CoV-2 ribonucleic acid using the established RT-PCR method approved by the Ministry of Health's General Directorate of Public Health (HSGM) Microbiology Laboratory. Treatment guidelines prepared by the HSGM were adhered to when treating patients. Using hospital medical records, relevant data, including demographics, laboratory and clinical information, medical treatment, and outcome data were obtained for patients. The PAI-1 levels measured during hospital admission were collected, and patients were categorized according to the severity of the disease and whether they survived.

In Turkiye, the vaccination process began in early 2021 using the CoronaVac vaccine. The Pfizer-BioNTech vaccine became available later in 2021. No patients had been vaccinated at the time of this study or displayed symptoms of any recurrent disease. No patients were fully vaccinated against COVID-19 at the time of the follow-up session for this study.

When conducting this study, the 1975 Helsinki report and subsequent revisions were taken into account. Informed consent was obtained from patients, and the study was approved by the Goztepe Training and Research Hospital Ethics Committee and the Turkish Ministry of Health. The ethical approval submission was numbered 2020/0311 and dated May 18, 2020.

Definitions and Clinical Outcomes

Patient medical information was investigated in terms of the severity and mortality of COVID-19. Patients with any of the following criteria were defined as severe patients: (1) oxygen saturation in resting ≤93%; (2) respiratory deficiency (respiratory rate [RR] ≥30 breaths per min); (3) ratio of partial oxygen arterial pressure to fractional inspired oxygen (PaO_2/FiO_2, ≤300 mmHg); and (4) critical complications (respiratory collapse, mechanical ventilation, multiple organ failure, or intensive care unit [ICU] admission) [16]. Acute cardiac injury (ACI) was described as high-sensitive cardiac troponin I (hs-TnI) serum levels above the 99th percentile upper reference limit, independent of abnormalities in the electrocardiogram [17]. Acute respiratory distress syndrome (ARDS) was described while taking into account the Berlin definition [18].

Acute kidney injury (AKI) was defined according to the definition provided by kidney disease improving global outcomes. The estimated glomerular filtration rate for the diagnosis of chronic kidney disease was defined as 30–59/1.73 m^2 [19]. Acute hepatic injury (AHI) was defined >200 U/L for AST and >300 U/L for ALT [20]. The clinical outcomes were determined as the length of stay, disease severity, discharges, and death. These clinical outcomes were then followed up on June 20, 2020.

CT as Evidence of Disease

A CT severity score (CT-SS), as put forward by Yang et al. [21], was used to evaluate the severity of COVID-19 pneumonia. Establishing CT-SS required evaluating ground glass views, crazy pavement patterns, and consolidations. Each of the 18 segments for both lungs was redefined as 20 zones within the CT scans. All three lung regions were evaluated for the chest CT scan using a system that attributed scores of zero, one, or two depending on a parenchymal opacification of 0%, <50%, or >50%, respectively. Using this method, the CT-SS was calculated within a range of 0–40 [21]. A combination of clinical assessments, chest CTs, and RT-PCRs was used to diagnose COVID-19 in this study [22].

PAI-1 Analysis

During admission, blood samples from all patients were taken into serum tubes using the peripheral venous approach. All samples were centrifuged at 1.000–1500 g for 15–20 min and stored at -80°C until analysis. Serum PAI-1 levels were determined using the Enzyme-linked Immunosorbent Assay Kit (Bioassay Technology Laboratory, Shanghai, China), according to the producer's protocol. To obtain certain measurements, all samples were analyzed in duplicate by investigators blinded to clinical data.

Statistical Analyses

Statistical analysis was performed using the Statistical Package for the Social Sciences 21.0 ([SPSS] Inc., Chicago, IL, United States of America). The Kolmogorov–Smirnov test was also utilized to analyze data normality. Continuous data were indicated as mean±SD and categorical data as a percentage (%). The Chi-square test was utilized to determine differences in categorical variables. In addition, the analysis of variance test was used to compare parametric variables in the primary analysis. The Kruskall-Wallis test was then utilized to make comparisons among non-parametric variables. Relationships between parameters were evaluated using Pearson's and Spearman's analyses. Logistic regression analysis was used to determine independent variables in relation to mortality and disease severity. The results of logistic regression analyses were introduced with 95% CI. Receiver operating characteristic (ROC) curves were obtained to determine optimal values with the highest overall specificity and sensitivity for mortality estimation. The significance level was accepted as $p<0.05$.

RESULTS

The clinical and demographic characteristics of the 71 patients hospitalized with COVID-19 and the 20 volunteers used in this study are shown in Table 1. The subjects were grouped accordingly as the control group (n=20), the survivor group (n=47), and the non-survivor group (n=24). The mean age was 75.3±13.8 in the non-survivor group, which was higher than in the survivor group (61.7±16.9) and in the control group (59.5±11.2), (p=0.001). Of the 24 non-survivors, 13 (54%) were male, while 17 of 47 survivors (36%) were male. There was no statistical differentiation between the groups in terms of gender, systolic arterial pressure (SAP), diastolic arterial pressure (DAP), RR, heart rate

Table 1. Demographic and clinical characteristics of patients surviver and non-surviver group

Variables	Total (n=91)	Control (n=20)	Survivor (n=47)	Non-survivor (n=24)	p
Clinical characteristics					
Age (years)	64.8±16.2	59.5±11.2[a]	61.7±16.9[e]	75.3±13.8[ae]	0.001
Male, n (%)	43 (47)	13 (65)	17 (36)	13 (54)	0.071
BMI (kg/m^2)	23.2±3.5	23.5±3.3	23.0±2.9	23.4±4.4	0.642
HR, beats/min	92.6±16.6	–	93.8±15.6	90.5±18.5	0.476
RR, times/min	24.2±5.0	–	24.3±5.6	23.9±3.0	0.793
SAP, mmHg	124.0±21.6	–	122.4±19.1	127.1±25.9	0.429
DAP, mmHg	69.2±11.6	–	70.5±8.0	66.8±16.2	0.236
CT-SS	11 (6–17)	–	8 (4–13)*[e]	17 (11–24)[ae]	<0.001
Chronic medical illness					
HT (%)	46	30	46	58	0.170
DM (%)	17	10	29	29	0.205
HL (%)	3	5	2	4	0.805
CAD (%)	20	20	19	25	0.843
CVE (%)	6	5	2	16	0.062
COPD (%)	5	0	6	8	0.448
Malignite (%)	8	0	10	12	0.281
Laboratory findings					
Hgb (g/dL)	14.0±1.4	14.8±1.2	12.2±1.9	11.7±2.2	0.627
Platelet (10^3/µL)	219.3±68.3	238.2±64.1*[a]	208.0±83.0*[e]	167.1±58.3[ae]	0.006
WBC (10^3/µL)	8 (5–11)	8 (6–10)a	6 (5–9)e	10 (5–16)[ae]	0.043
Creatinine (mg/dL)	0.9 (0.8–1.5)	0.8 (0.7–0.9)*[a]	0.8 (0.7–1.4)*[e]	1.5 (0.8–2.1)[ae]	0.005
Sodium (mmol/L)	136.0±10.3	138.4±2.3	133.8±10.9	138.2±7.7	0.335
Potassium (mmol/L)	4.2±0.6	4.5±0.3	4.1±0.6	4.0±0.7	0.056
Glucose (mg/dL)	126.1±44.8	103.4±32.9[a]	127.2±32.9	141.9±63.2[a]	0.018
CRP (NR <5 mg/dL)	5 (2–13)	3 (1–4)*[a]	5 (3–11)*[e]	14 (6–29)[ae]	<0.001
hs-TnI (NR<14 pg/mL)	30 (12–77)	5 (4–7)*[a]	36 (18–80)*[e]	48 (27–107)[ae]	<0.001
d-dimer (NR<500 ng/mL)	859 (390–1798)	250 (132–467)*[a]	970 (631–1826)*[e]	1525 (659–3305)[ae]	<0.001
PAI-1 (ng/mL)	6 (1–12)	1(0.8–2)*[a]	5(2–11)*[e]	14(11–20)[ae]	<0.001
sO_2 (%)	91.0±12.1	–	93.2±5.7	87.0±18.4	0.041
Fibrinogen (mg/dL)	484.7±172.3	–	522.1±155.4	414.8±185.7	0.056
PT (sn)	16.4±5.4	13.3±0.6[a]	15.6±4.9[e]	19.0±6.4[ae]	0.008
APTT (sn)	37.3±20.6	28.2±1.2	40.2±5.5	35.1±10.1	0.225
INR	1.2±0.4	0.9±0.0[a]	1.1±0.3[e]	1.4±0.5[ae]	0.008
AST (IU/L)	27 (18–44)	19 (7–22)*[a]	27 (17–43)*[e]	38 (26–77)[ae]	<0.001
ALT (IU/L)	24 (15–44)	21 (14–30)	28 (15–49)	27 (15–46)	0.358
Total protein (g/dL)	6.5±0.9	–	6.7±0.9	6.0±0.7	0.007
Albumin (g/dL)	3.6±0.6	–	3.8±0.5	3.2±0.5	<0.001
Treatments					
Antiviral therapy (%)	49	–	44	58	0.276
Antibiotic therapy (%)	100	–	66	34	–
Anticoagulant (%)	81	–	76	91	0.120
Oxygen therapy (%)	57	–	40	91	<0.001
High-flow oxygen (%)	21	–	15	33	0.090
IMV (%)	22	–	2	62	<0.001
ICU admission (%)	38	–	17	79	<0.001
Hospital stay (days)	8 (4–13)	–	7 (3–11)	14 (4–26)	<0.001
Complications					
Acute cardiac injury (%)	64	–	53	87	0.004
Acute kidney injury (%)	35	–	21	62	0.001
Acute hepatic injury (%)	24	–	14	41	0.012
Severe disease (%)	53	–	36	87	<0.001

*: P<0.05 Between control group and survivor group; a: P<0.05 between control group and non-survivor; e: P<0.05 between survivor group and non-survivor group; ALT: Alanin aminotransferaz; AST: Aspartat aminotransferaz; APTT: Activated partial thromboplastin time; BMI: Body mass index; CAD: Coronary artery disease; COPD: Chronic obstructive pulmonary disease; CRP: C-reactive protein; CT: Computed tomography; CVE: Cerebro vascular event; DAP: Diastolic arterial pressure; DM: Diabetes mellitus; Hgb: Hemoglobin; HL: Hyperlipidemia; HR: Heart rate; hs-TnI: High sensitive-Troponin I; HT: Hypertension; ICU: Intensive care unit; IMV: Invasive mechanical ventilation; INR: International normalized ratio; NR: Normal range; PAI-1: Plasminogen activator inhibitor-1; PT: Prothrombin time; RR: Respiratory rate; SAP: Systolic arterial pressure; sO_2: Oxygen saturation; WBC: White blood cell.

(HR), body mass index (BMI), hypertension (HT), diabetes mellitus (DM), hyperlipidemia (HL), coronary artery disease, cerebrovascular event, chronic obstructive pulmonary disease, or malignancy. In the non-survivor group, CT-SS was measured as higher than in the survivor group ($p<0.001$).

White blood cells, creatinine, glucose, hs-TnI, C-reactive protein (CRP), D-dimer, PAI-1, SO_2, INR, PT, and AST values showed statistically significant differences and were higher where platelet (PLT) levels were lower in the non-survivor group. When in-hospital complications were examined, ACI, AKI, AHI, and severe disease were observed significantly more frequently in the non-survivor group than in the survivor group (Table 1). In the non-survivor group, a greater amount of oxygen therapy, more admissions to the ICU, and an increased use of invasive mechanical ventilation (IMV) were observed in addition to longer hospital admissions compared to the survivor group, ($p<0.001$). Patients were, then, divided into severe (n=33) and non-severe (n=38) according to the severity of the disease (Table 2). There was no significant difference between the groups in terms of age, gender, BMI, HR, SAP, DAP, and chronic diseases. The CT-SS was measured as higher in the severe group than in the non-severe group ($p<0.001$). The laboratory tests indicated statistically significant differences for hs-TnI, CRP, D-dimer, PAI-1, creatinine, and albumin values for both groups. In addition, more oxygen therapy and high flow, an increased need for IMV, more ICU admissions, and longer hospital admission durations were observed in the severe group compared to the non-severe group ($p<0.001$). Among in-hospital complications, ACI, AKI, and death were seen significantly more often in the severe group than in the non-severe group (Table 2).

The relationship between CT-SS and PAI-1 was evaluated using the Spearman correlation analysis, and a strong positive correlation was found (r: 0.838, $p<0.001$) (Fig. 1).

The parameters used to predict the development of mortality were evaluated using logistic regression analyses. First, the parameters of age, CRP, hs-TnI, D-dimer, PAI-1, AKI, and disease severity were evaluated using a univariate analysis. The parameters of age, PAI-1, and disease severity were found to independently predict mortality (Table 3).

The specificity and sensitivity of PAI-1 values were evaluated using ROC analysis to predict patient mortality. In the figure, the blue mark shows the PAI-1 value,

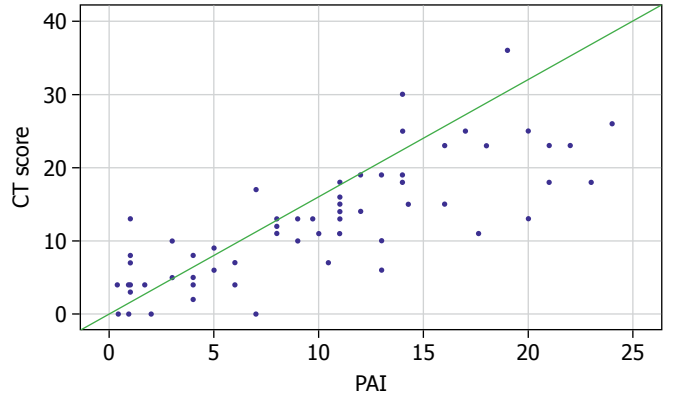

FIGURE 1. Spearman correlation analysis of CT-SS and PAI-1 levels.

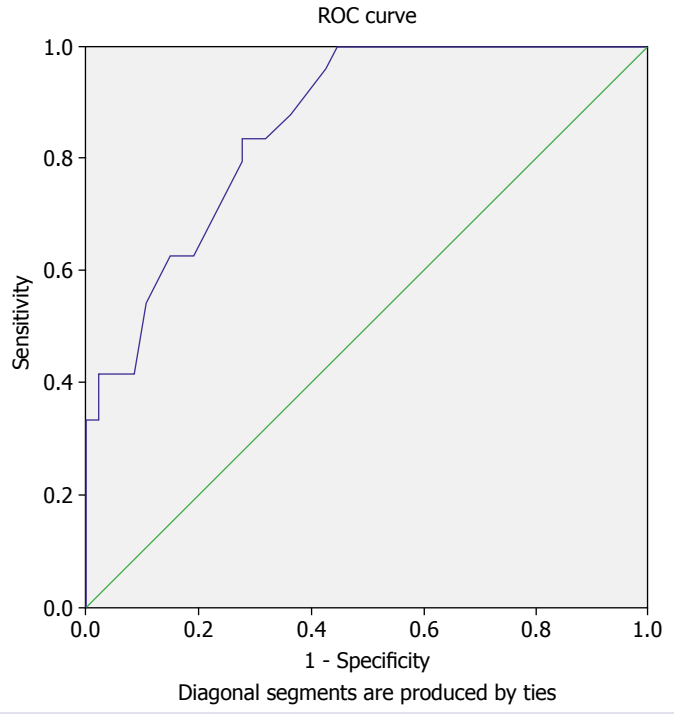

FIGURE 2. Predictive ability of serum PAI-1 levels for mortality.

and the area under the curve was measured at 0.860. In addition, the PAI-1 value predicted the development of mortality with a sensitivity of 83%, a specificity of 83%, and a cutoff value of 10.2 (Fig. 2).

The parameters used to predict COVID-19 severity were also evaluated using regression analysis. The parameters of age, gender, HT, DM, CRP, hs-TnI, D-dimer, and PAI-1 were evaluated using a univariate analysis. The parameters hs-TnI and PAI-1 were found to independently predict disease severity (Table 4).

Table 2. Demographic and clinical characteristics of patients non-severe and severe group

Variables	Non-severe (n=33)	Severe (n=38)	p
Clinical characteristics			
Age (years)	63.4±18.1	68.7±16.0	0.195
Male, n (%)	12 (36)	18 (47)	0.349
BMI (kg/m^2)	23.2±2.5	23.3±4.1	0.722
HR, beats/min	93.7±18.7	91.8±14.9	0.661
RR, times/min	23.5±4.5	25.0±5.4	0.270
SAP, mmHg	121.0±19.7	126.3±23.0	0.351
DAP, mmHg	70.1±8.4	68.5±13.6	0.618
CT-SS	7 (4–12)	13 (10–18)	<0.001
Chronic medical illness			
HT (%)	42	58	0.193
DM (%)	24	34	0.359
HL (%)	3	2	0.919
CAD (%)	27	15	0.237
CVE (%)	6	8	0.763
COPD (%)	6	8	0.763
Malignite (%)	15	8	0.335
Laboratory findings			
Hgb (g/dL)	12.2±1.8	11.9±2.2	0.507
Platelet (10^3/µL)	202.5±90.1	186.8±65.5	0.403
WBC (10^3/µL)	7 (5–10)	9 (4–13)	0.604
Creatinine (mg/dL)	0.8 (0.7–1.3)	1.2 (0.8–2.0)	0.047
Sodium (mmol/L)	133.2±22.6	137.1±5.1	0.309
Potassium (mmol/L)	4.0±0.6	4.1±0.6	0.527
Glucose (mg/dL)	125.9±35.8	137.9±52.9	0.283
CRP (NR <5 mg/dL)	4 (2–13)	11 (4–27)	0.005
hs-TnI (NR<14 pg/mL)	28 (17–43)	81 (34–112)	<0.001
d-dimer (NR<500 ng/mL)	826 (450–1650)	1555 (874–2167)	0.029
PAI-1 (ng/mL)	5 (1–10)	11 (8–16)	<0.001
sO_2 (%)	92.4±6.2	89.4±15.3	0.246
Fibrinogen (mg/dL)	510 (357–686)	480 (386–617)	0.572
PT (sn)	15.9±5.4	17.5±5.8	0.227
APTT(sn)	30 (28–34)	32 (28–40)	0.416
INR	1.2±0.4	1.3±0.4	0.325
AST (IU/L)	28 (17–49)	35 (24–47)	0.201
ALT (IU/L)	26 (15–49)	28 (15–48)	0.899
Total protein (g/dL)	6.5 (4.7–7.2)	6.0 (4.5–6.8)	0.083
Albumin (g/dL)	3.8 (2.6–4.1)	2.9 (3.2–3.7)	0.008
Treatments			
Antiviral therapy (%)	33	63	0.012
Antibiotic therapy (%)	46	54	–
Anticoagulant (%)	72	89	0.069
Oxygen therapy (%)	36	76	0.001
High-flow oxygen (%)	0	40	<0.001
IMV (%)	0	42	<0.001
ICU admission (%)	6	65	<0.001
Hospital stay (days)	4 (2–8)	12 (6–23)	<0.001
Complications			
Acute cardiac injury (%)	36	89	<0.001
Acute kidney injury (%)	15	52	0.001
Acute hepatic injury (%)	18	29	0.289
Death (%)	9	55	<0.001

*: P<0.05 Between control group and non-severe group; a: P<0.05 between control group and severe; e: P<0.05 between non-severe group and severe group; IQR (25–75%); ALT: Alanin aminotransferaz; AST: Aspartat aminotransferaz; APTT: Activated partial thromboplastin time; BMI: Body mass index; CAD: Coronary artery disease; COPD: Chronic obstructive pulmonary disease; CRP: C-reactive protein; CT: Computed tomography; CVE: Cerebro vascular event; DAP: Diastolic arterial pressure; DM: Diabetes mellitus; Hgb: Haemoglobin; HL: Hyperlipidemia; HR: Heart rate; hs-TnI: High sensitive-troponin I; HT: Hypertension; ICU: Intensive care unit; IMV: Invasive mechanical ventilation; INR: International normalized ratio; NR: Normal range; PAI-1: Plasminogen activator inhibitor-1; PT: Prothrombin time; RR: Respiratory rate; SAP: Systolic arterial pressure; sO_2: Oxygen saturation; WBC: White blood cell.

Table 3. Univariate and multivariate logistics regression analysis on predicting mortality in patients with COVID-19

Variable	Univariate			Multivariate		
	OR	95% CI	p	OR	95% CI	p
Age	1.058	1.020–1.098	0.003	1.130	1.045–1.221	0.002
CRP	1.310	1.105–1.712	0.005	1.120	0.910–1.321	0.316
Hs-TnI	1.002	1.001–1.003	0.028	0.999	0.997–1.001	0.126
d-dimer	1.215	1.100–1.341	<0.001	1.089	0.902–1441	0.431
PAI-1	1.340	1.165–1.541	<0.001	1.481	1.162–1.887	0.001
AKI	2.154	1.456–5.012	<0.001	1.231	0.872–2.013	0.531
Severe disease	7.353	3.208–11.561	<0.001	4.111	1.348–7.862	0.014

OR: Odd ratios; CI: Confidence interval; AKI: Acute kidney injury; Hs-TnI: High sensitive troponin I; CRP: C-reactive protein; PAI-1: Plasminogen activator inhibitor-1; AKI: Acute kidney injury.

Table 4. Univariate and multivariate logistics regression analysis on predicting disease severity of COVID-19

Variable	Univariate			Multivariate		
	OR	95% CI	p	OR	95% CI	p
Age	1.019	0.991–1.048	0.194			
Gender	1.575	0.607–4.085	0.350			
HT	0.536	0.208–1.377	0.195			
DM	1.625	0.574–4.601	0.361			
CRP	1.078	1.007–1.154	0.003	1.028	0.966–1.094	0.386
Hs-TnI	1.036	1.014–1.059	0.001	1.040	1.015–1.066	0.002
d-dimer	1.315	1.121–1.691	<0.001	1.003	0.962–1046	0.884
PAI-1	1.186	1.077–1.306	0.001	1.217	1.082–1.369	0.001

OR: Odd ratios; CI: Confidence interval; CRP: C-reactive protein; DM: Diabetes mellitus; hs-TnI: High sensitive troponin I; HT: Hypertension, PAI-1: Plasminogen activator inhibitor-1.

DISCUSSION

This study examined the relationship between PAI-1 levels, disease severity, and mortality in patients hospitalized with COVID-19. This study found that the PAI-1 levels of patients hospitalized with COVID-19 were higher than in the control group. In addition, the PAI-1 levels were higher in the non-survivor group than in the survivor group and higher in the severe group compared to the non-severe group. A strong positive correlation between CT-SS and PAI-1 was also observed. Multivariate analysis revealed that PAI-1 levels predicted both mortality and disease severity for COVID-19. In addition, PAI-1 levels predicted the development of mortality with a sensitivity of 83%, a specificity of 83%, and a cut-off value of 10.2.

A homeostatic balance exists between coagulation and fibrinolysis. Excessive fibrinolysis can cause abnormal bleeding, whereas a deficiency in fibrinolysis can cause diffuse intravascular coagulopathy and thrombosis [23]. The fibrinolytic system is complicated by COVID-19 due to various determinantal effects. The pathophysiology of coagulopathy in COVID-19 is thought to occur due to platelet aggregation, inflammation, and microthrombosis, as well as fibrinolysis impairment [24]. Serpin PAI-1 plays a crucial role across multiple disease processes within the lungs [25]. Impaired fibrinolysis

during ARDS causes fibrin deposition [26]. Suppressed fibrinolysis in ARDS is thought to be caused by the increased circulation of PAI-1 levels [27].

As reported in similar studies, high D-dimer concentrations indicate disease severity and mortality in COVID-19 [7]. Similarly, increased troponin levels during hospital admission have been shown to detect poor clinical outcomes and high mortality rates for COVID-19 patients [6]. The findings of this study suggest that troponin levels also predict disease severity independently.

In a study that compared 118 hospitalized COVID-19 patients and 30 healthy subjects, the PAI-1 levels were higher in patients with COVID-19 and associated with worse outcomes in terms of respiratory endpoints [28]. In another study that compared 63 hospitalized COVID-19 patients and 43 healthy individuals, the PAI-1 levels were higher in patients that had been admitted to the ICU. In addition, patients diagnosed with AKI have been shown to have higher PAI-1 levels at admission [29]. A different study compared 113 patients with COVID-19 and 24 patients with non-COVID-19 respiratory tract infections. In this study, PAI-1 levels were reported to be higher in COVID-19 patients [9]. One further study observed elevated PAI-1 levels in 55 patients with severe COVID-19. These increased API-1 levels were associated with severe hypoxemia, respiratory deficiency, and thromboembolism [30]. The findings of this present study suggest that PAI-1 levels can independently predict mortality and disease severity.

The biomarkers identified for endothelial damage due to COVID-19 are clearly related to the disease severity and exhibit a different pattern from other septic and severe inflammatory syndromes [31]. The SARS-CoV-2 virus connects to ACE-2 to gain entry into the cell. The subsequent activation of the renin-anjiotensin-aldosterone system pathway stimulates endothelial cell dysfunction by stimulating the production of reactive oxygen species (ROS) and excreting proinflammatory cytokines, including TNF-a, IL-1, and IL-6. This then activates a coagulation cascade which results in an increase in disease severity and the escalation of thrombotic events [32]. As a known fibrinolysis inhibitor, PAI-1 levels have been shown to increase the risk of atherothrombotic events [11, 33]. The main source of elevated PAI-1 levels in COVID-19 patients is thought to be endothelium. However, this could also be due to the release of PAI-1 from activated platelets. Various proinflammatory mediators, including IL-6 and TNF alpha, which are known components of cytokine storm syndrome in COVID-19, also cause PAI-1 to be produced by extravascular tissues [33, 34].

Limitations

This study contained several key limitations. First, the study was single-centered, and the sample number was small. In addition, the PAI-1 levels were evaluated during patient hospitalization, and repeated PAI-1 disease progression measurements were not performed. Furthermore, imaging methods such as echocardiography and ultrasonography could not be applied due to the risk of transmission, and thrombosis was not detected due to difficulties in diagnosing thrombotic events such as microthrombosis.

Conclusion

The data from this study show that increased PAI-1 levels may be a biomarker related to poor clinical outcomes for COVID-19. As the prothrombotic risk of COVID-19 is known, early detection for patients with high prothrombotic risk, which can then lead to early initiations of prophylactic anticoagulation therapy, is extremely important. Further studies on PAI-1 levels are required as the spread of COVID-19 progresses.

Ethics Committee Approval: The Istanbul Medeniyet University Goztepe Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 18.05.2020, number: 2020/0311).

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