HYPOFIBRINOLYSIS IS ASSOCIATED WITH THE SEVERITY OF COVID-19 INFECTION: THE ROLE OF OBESITY

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ABSTRACT

Introduction*: Obesity, by causing hypofibrinolysis and thrombotic complications, ought to be a risk factor in terms of disease severity and mortality in COVID-19 infection.We aim to investigate the effects of obesity on fibrinolytic system in coronavirus patients while studying the changes of major fibrinolytic inhibitors plasminogen activator inhibitor (PAI-1) and thrombin activatable fibrinolysis inhibitor (TAFI).*

Material and methods*: Sixty-six patients and 21 healthy donors were investigated and observed until either their recovery or death. Body mass index (BMI) was calculated for each person and peripheral blood used for PAI-1 and TAFI measurements. PAI-1 antigen and TAFI activated/inactivated (TAFIa/i) were measured using ELISA kits.*

Results*: PAI-1 antigen and TAFIa/i plasma levels are higher in patients than in control group (P<00001 for both).Patients needing ICU had higher TAFIa/i values than non-ICU patients. The 15 patients who died had higher TAFIa/i levels than those staying alive.Obese patients (BMI ≥ 30) had higher PAI-1 levels than non-obese patients. TAFIa/i is associated with D-dimer, C-reactive protein (CRP), ferritin, creatinine and neutrophile count, whereas PAI-1 is associated only with the serum creatinine level.*

Conclusion*: Our study shows that the levels of the major fibrinolytic inhibitors PAI 1 and TAFI increase in patients with COVID-19 infection, and proves for the first time that PAI 1 levels increase more in obese patients than in non-obese ones. It is also revealed that the plasma TAFI level is associated with the severity of COVID-19 infection, which suggests that the inhibitory treatments against TAFI should be effective in preventing thrombotic complications in the course of COVID-19 infection.*

Keywords: *COVID-19, Plasminogen activator inhibitor-1, Thrombin activatable fibrinolysis inhibitor, obesity.*

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible and pathogenic virus belonging to the coronavirus family. It emerged at the end of 2019 in Wuhan, China and since then it has been responsible for an acute respiratory disease pandemic called the coronavirus disease 2019 (COVID-19). This disease has caused great risk for human health and still threatens public safety[(1)]. Advanced age, diabetes mellitus, cardiovascular diseases, cerebrovascular diseases, respiratory diseases, and male gender have been reported as risk factors in COVID-19 infections for mortality and morbidity[(2-4)]. In addition to these risk factors, obesity seems to be a prevalent risk factor for requiring an intensive care unit (ICU) and mortality in patients with COVID-19.Worldwide, reports have shown that 70-90% of SARS CoV-2 infected patients who were admitted to the ICU were overweight[(5-7)]. The fact that obesity is a risk factor in COVID-19 infections for increased mortality and morbidity beyond what is expected needs further explanation.

Although pulmonary damage and associated acute respiratory distress syndrome (ARDS) seem to be the main cause of high mortality in COVID-19 infections, life-threatening thromboses, especially pulmonary embolisms, appear in many patients and contribute to mortality[8,9]. The hyperinflammation/cytokine storm that occurs in COVID-19 infections is blamed for hypercoagulability and the thrombotic process; however, higher than expected thrombotic events are observed in those infected with COVID-19 and these complications cannot always be prevented, despite anticoagulant prophylaxis[8,10-12]. Recently, it has been suggested that hypofibrinolysis associated with abundant thrombin generation might contribute to the thrombosis observed in COVID-19 infections[9].

Plasminogen activator inhibitor-1 (PAI-1) is the primary physiological inhibitor of plasminogen activators tPA and uPA. Moreover, PAI-1, with other acute-phase proteins, plays an important role in inflammatory and immune responses following infectious and non infectious injuries[13]). Two characteristic features of ARDS, namely the formation of intravascular micro-thrombi and fibrin deposits in the alveolar space, are often the combined result of tissue factors generated by inflammatory cells (procoagulant) and PAI-1 produced by endothelial cells (antifibrinolytic)[14]. Recently, it has been shown that patients with severe COVID-19 have higher plasma PAI-1 levels compared to patients with bacterial sepsis or ARDS[15]. It has been demonstrated that adipocytes are an important source of PAI-1, underscoring the contribution of abundant adipose tissue deposits to circulating PAI-1 levels[16,17].

Thrombin activatable fibrinolysis inhibitor (TAFI) is a glycoprotein belonging to the metalloprotease family. TAFI is synthesized in the liver and circulates in blood as a proenzyme without any biological activity[18]. Thrombin converts TAFI to an active from (TAFIa) and, therefore, contributes to the stabilization of the fibrin clot against lysis (19). Increased TAFI activation due to an increased rate of thrombin generation might lead to thrombotic disorders. It has been reported that patients with COVID-19 who need ICU support have significantly higher levels of TAFIactivated/inactivated (TAFIa/i) compared to non-ICU patients[9]. The role of obesity on TAFI activation is not clear.

In our study, we aim to identify the changes in fibrinolytic inhibitors according to the course of the COVID-19 infection. We also aim to investigate the effect of obesity on the fibrinolytic system in patients with COVID-19 infections.

Material and methods

Patients and data collection

Included in this study were adult patients with a positive SARS-CoV-2 polymerase chain reaction test who were hospitalized in Çorlu State Hospital between 1 September, 2021, and 1 December, 2021. The patients were observed until their recovery or upon death. Twenty-one healthy donors, who were age- and sex-matched to the patients, were also included as control group. The study has been approved by the ethics committee of Namık Kemal University Medical Faculty. All patients were treated according to the national guidelines for COVID-19 infections. Favipiravir was given to all patients for 5 or 10 days according to their disease severity. Appropriate thromboprophylaxis was applied with low-molecular-weight heparin (LMWH; enoxaparin 40mg OD or 40mg BD if D-dimer>1,000 ng/mL) or subcutaneous unfractionated heparin (5000 UI BD) if there was significant renal failure. Dexamethasone (6mg OD) was administered for seven days to all patients who had lung involvement. All other supportive treatments and antibiotics were given as needed.

Clinical and laboratory data were collected from the electronic patient medical files. Body mass index (BMI) was calculated for each patient. Laboratory parameters were recorded at the time the patient was included in the study. Peripheral blood was drawn at inclusion for PAI-1 and TAFIa/i measurements.

Immunoassays

Platelet poor plasma was obtained by centrifugation of citrated blood at 2500 g for 20 min at room temperature. Aliquots of citrated plasma were stored, shortly after collection, at -80°C until the analysis. PAI-1 antigen and TAFIa/TAFIi were measured using ELISA kits (Asserachrome, Stago).

Statistical analysis

All statistical analyses were performed using the IBM SPSS Statistics (Version 25.0; IBM Corp., USA). Student's t test and Mann–Whitney U test depending to distribution of variables were used to compare the two independent groups. Chi-square test was used for comparison of categorical variables. Correlations between continuous variables were determined by Pearson method. All p values were

two-sided, and p <0.05 was considered statistically significant.

Results

Included in this study were 66 patients (34 females) with a mean age of 64.19±15.99 years, and 21 healthy participants (10 females) with a mean age of 59.85±7.10 years. Table 1 displays clinical characteristics and laboratory data of the COVID-19 patients and healthy controls. PAI-1 antigen and TAFIa/i plasma levels were higher in the patients than in controls (Table 1, Figure 1A and B).

Variables	n	Patients	n	Healthy donors	P Value
Age, year	66	64.19 ± 15.99	21	59.85 ± 7.10	0.23
Gender, female n(%)	66	34 (51.5)	21	10 (47.6)	0.80
BMI (kg/m^2)	59	28.97 ± 4.99	21	29.26±5.04	0.82
PAI-1, ng/ml	66	63.29 ± 33.15	21	31.00 ± 10.99	<0.0001
TAFIa/i, ng/ml	66	47.46 ± 28.90	20	19.97 ± 6,10	<0.0001
D dimer, ng/ml	63	2001.8 ± 5643.9	21	338 ± 345	0.023
Ferritin, ng/ml	50	1239.3 ± 3523.7	21	132.5 ± 221.0	<0.0001
CRP, mg/dl	65	51.27 ± 38.80	21	2.70 ± 2.35	<0.0001
PT, sn	41	15.70 ± 9.58	21	11.54 ± .53	<0.0001
PTT, sn	42	26.90 ± 4.75	21	23.38 ± 2.25	0.001
Fibrinojen, mg/dl	30	488.91 ± 158.38	21	329.7 ± 73.93	<0.0001
AST, U/L	63	112.7 ± 499.5	21	19.63 ± 6.36	<0.0001
ALT, U/L	64	83.18 ± 224.3	21	24.52 ± 17.90	0.017
Creatinine, mg/dl	65	1.22 ± 1.11	21	0.80 ± 0.14	0.042
LDH, U/L	36	690.7 ± 1191.7	21	175.4 ± 33.35	<0.0001
Leucocyte count(x10^9/L)	65	8.74 ± 5.20	21	7.30 ± 3.53	0.32
Neutrophil count(x10^9/L)	65	7.05 ± 4.84	21	4.25 ± 2.92	0.003
Lymphocyte count(x10^9/L)	64	1.00 ± 0.63	21	2.35 ± 0.79	<0.0001

Table 1: Demographic characteristics and laboratory results of the patients and the healthy donors.
Data are shown as mean ± SD if not specified, BMI: Body mass, index, PAI-1: Plasminogen activator inhibitor-1, TAFIa/i: Thrombin activatable fibrinolysis inhibitor-activated/inactivated, CRP:C-reactive protein, PT: Prothrombin time, aPTT: Activated partial thromboplastin time, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase

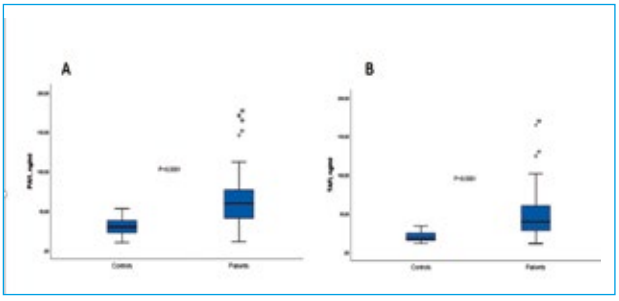

Fig. 1: Comparison between patients and healthy controls with regard to PAI-1(A) and TAFIa/i (B).

The female patients were older than the male patients (p = 0.036), and the male patients had higher D-dimer values than the female patients (p = 0.028), but there was no difference between male and female patients for PAI-1 and TAFIa/i. There were 21 patients (31.8%) admitted to the ICU; among them, 15 (71.4%) received invasive mechanical ventilation. The patients needing ICU had higher TAFIa/i values than non-ICU patients (Figure 2B), but there was no difference in PAI between ICU and non-ICU patients (Figure 2A). Table 2 shows clinical and laboratory findings of ICU patients and non-ICU patients. The patients who required mechanical ventilation had also higher TAFIa/i levels, but not PAI-1, than those who did not require ventilation (Figure 2C and D). The 15 patients who died, they had higher TAFIa/i levels than the patients who did not die (Figure 2F), but there was no significant difference between the two groups for PAI-1(Figure 2E).

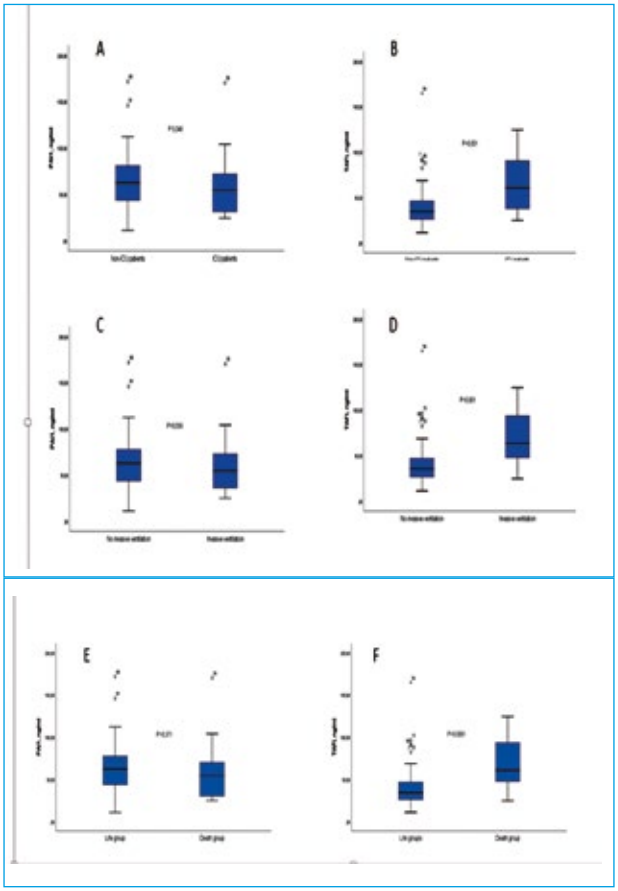

Fig. 2: Comparison between patients needing ICU and those not needing ICU with regard to PAI-1 (A) and TAFIa/i (B). Comparison between patients requiring mechanical ventilation and those not requiring it with regard to PAI-1 (C) and TAFIa/i (D).Comparison between patients who died and those staying alive with regard to PAI-1 (E) and TAFIa/i (F)

Plasma PAI-1 levels were positively correlated with BMI (r = 0.288, p = 0.027) but there was no correlation between TAFIa/i and BMI (r = -0.226, p = 0.341). Correlations between PAI-1, TAFIa/i and other parameters are displayed in Table 3. In the analysis made by classifying patients into obese (BMI≥30) and non-obese groups, obese patients had higher PAI-1 levels than non-obese patients but

there was no difference between the two groups for TAFIa/i (Figure 3A and B). Table 4 shows laboratory differences between obese and non-obese patients. TAFIa/i was associated D-dimer, C-reactive protein (CRP), ferritin, creatinine and neutrophil count; however, PAI-1 was associated only with the serum creatinine level.

Variables	n	ICU Patients	n	Non-ICU Patients	P Value
Age, year	21	67.47 ± 13.69	45	62.66 ± 16.88	0.328
Gender, female n(%)	21	7 (33.3)	45	27 (60.0)	0.043
BMI (kg/m²)	15	27.96 ± 4.72	44	29.41 ± 5.06	0.207
PAI-1, ng/ml	21	58.41 ± 33.63	45	65.57 ± 33.05	0.346
TAFIa/i, ng/ml	21	62.71 ± 29.02	45	40.34 ± 26.23	0.001
D dimer, ng/ml	18	4850.2 ± 8756.9	45	862.4 ± 3257.4	<0.0001
Ferritin, ng/ml	19	1941.8 ± 4610.8	31	808.7 ± 2649.5	0.164
CRP, mg/dl	20	60.86 ± 36.42	45	47.02 ± 39.45	0.136
Creatinine, mg/dl	21	1.40 ± 1.41	44	1.14 ± 0.94	0.259
Neutrophil count(x10⁹/L)	21	1.06 ± 5.68	44	5.33 ± 3.25	<0.0001
Lymphocyte count(x10⁹/L)	21	0.730 ± .572	43	1.145 ± 0.631	<0.0001

Table 2: Demographic characteristics and laboratory results of ICU patients and non-ICU patients.
Data are shown as mean ± SD if not specified, BMI: Body mass, index, PAI-1: Plasminogen activator inhibitor-1, TAFIa/i: Thrombin activatable fibrinolysis inhibitor-activated/inactivated, CRP:C-reactive protein

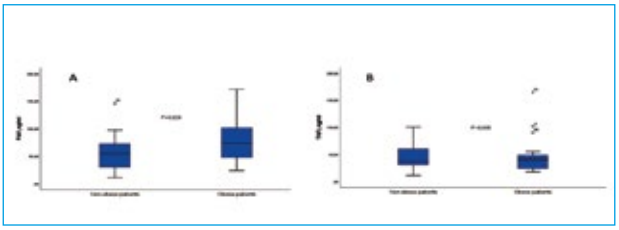

Fig. 3: Comparison between obese patients and those non-obese with regard to PAI-1 (A) and TAFIa/i (B).

Variables	n	Obese Patients	n	Non-obese Patients	P Value
Age, year	20	62.7 ± 18.3	39	65.8 ± 14.2	0.69
Gender, female n(%)	66	34 (51.5)	21	10 (47.6)	0.80
PAI1, ng/ml	20	74.93 ± 36.29	39	56.11 ± 27.63	0.029*
TAFIa/i, ng/ml	20	47.64 ± 35.23	39	45.10 ± 23.66	0.83
D dimer, ng/ml	20	1954 ± 4914	36	1901 ± 6296	0.17
Ferritin, ng/ml	16	1334 ± 3660	28	290.6 ± 238.7	0.27
CRP, mg/dl	20	60.28 ± 45.30	38	47.99 ± 36.08	0.32
Creatinine, mg/dl	20	1.26 ± 0.91	38	1.07 ± 0.88	0.51
Neutrophil count(x10⁹/L)	19	6.85 ± 3.96	39	5.98 ± 3.60	0.43
Lymphocyte count(x10⁹/L)	19	1.12 ± 0.76	39	0.94 ± 0.53	0.39

Table 4: Demographic characteristics and laboratory results of obese patients and non-obese patients.
Data are shown as mean ± SD if not specified, PAI-1: Plasminogen activator inhibitor-1, TAFIa/ai: Thrombin activatable fibrinolysis inhibitor-activated/inactivated, CRP:C-reactive protein

Discussion

Our study shows that the levels of the major fibrinolysis inhibitors PAI 1 and TAFI are increased in COVID 19 patients. PAI 1 is positively correlated with BMI and has higher levels in obese patients than it does in non-obese patients; TAFIa/i is positively correlated with disease severity (such as the need for ICU treatment) and mortality.

In the blood, PAI 1 circulates in two distinct pools, some free in the plasma and some retained in platelets[20]. Platelets hold the major circulating pool, which can contribute to a high local concentration of PAI 1 at the site of a growing fibrin clot (20), so that an increased PAI 1 level is responsible for hypofibrinolysis and fibrin persistence. The expression and release of PAI 1 are strongly regulated by growth factors, inflammatory cytokines, hormones, glucose, and endotoxins[21,22]. As adipocytes are important sources of PAI 1, enlarged adipose tissue contributes to circulating PAI 1 levels[16,17]). In addition, obesity, type 2 diabetes, and metabolic syndrome are often associated with a chronic state of inflammation characterized by overexpression of inflammatory cytokines, such as IL 6 and TNF alfa, which induce PAI 1 expression in adipose tissue[23-25]. It has been shown that obesity is associated with disease severity and mortality in COVID 19 infections[5-7]), so we hypothesized that PAI 1 levels would be more elevated in obese COVID 19 patients than in non-obese ones and could contribute to the disease's severity and mortality. Recently, it has been shown that, patients with severe COVID-19 have higher plasma PAI-1 levels compared to patients with bacterial sepsis or ARDS[15], but no relationship with obesity has been investigated. Nougier et al. found that PAI 1,tPA, and TAFI levels were more elevated in COVID 19 patients in need of ICU support than in those who did not[9]. The authors did not show any correlation between PAI-1and BMI.We are the first to demonstrate, in the context of Covid 19, that PAI-1levels are higher in obese patients than in non-obese patients.

TAFI is a glycoprotein in the metalloprotease family; it is synthesized in the liver and circulates in the blood as a proenzyme without biological activity[18]. Following coagulation activation, TAFI is cleaved by the thrombin-thrombomodulin complex or by the plasmin; this liberates an unstable fragment, activated TAFI (TAFIa), with carboxypeptidase activity[19,26,27]. The TAFIa removes the carboxy-terminal lysine and arginine residues from partially degraded fibrin, thus decreasing the binding rate of plasminogen to fibrin and its conversion into plasmin[28]. TAFIa is a heat-labile protein which is inactivated into TAFIi by conformational change[29].

Data from animal models and clinical studies indicate that despite its short half-life the amount of TAFIa might play a more crucial role in retarding fibrinolysis than the total amount of TAFI protein[30,31]. We measured TAFIa and TAFIi, finding that COVID 19 patients had higher TAFIa/i plasma levels than healthy donors did. The hypercoagulable state existing in COVID 19 infections can result

in the generation of high concentrations of the thrombin required to activate TAFI. Nougier et al. found that TAFIa was higher in COVID-19 patients who needed ICU support than in those who did not; the authors concluded that very high thrombin generation, despite heparin treatment, can result in hypofibrinolysis because of the increased PAI-1and TAFI levels.

We found that TAFI, unlike PAI-1, is associated with disease severity in COVID 19 patients; the TAFIa/i level is higher in patients requiring ICU support and is associated with high mortality. In addition, laboratory parameters related to disease severity, such as d dimer, ferritin, and CRP were positively correlated with TAFIa/i. Although increased levels of TAFI and other fibrinolytic system proteins (tPA and PAI 1) are reported in patients needing ICU support[9,32], no other data about the relationship of TAFI with disease severity have been reported.

When our study's findings and the medical literature on the subject are evaluated together, it can be inferred that PAI 1 levels increase in the early period of COVID 19 infection, with the increase being more pronounced in obese patients, and that PAI 1 plasma levels maintain a high level throughout the course of the disease while TAFI plasma levels become higher as the disease progresses, possibly due to the increasing thrombin generation. This elevated TAFI level perhaps prevents fibrinolysis and gives rise to thrombotic complications. It is noteworthy that TAFI has been reported as being more important than PAI 1 in dissolving the fibrin (33). In recent years, inhibitor molecules against TAFI have been developed and are being tested in some thrombotic diseases[34,35]. We believe that there is an urgent need for these molecules to be tested in COVID 19 patients.

Our study has two limitations. First, we measured the total PAI 1 antigen rather than PAI 1 activity and cannot claim that PAI 1 activity does not change with the severity of the infection. Second, missing data and the small size of the groups meant that the association of PAI 1 and TAFI with co-morbidities, such as diabetes, vascular diseases, and pulmonary diseases, all reported as risk factors in COVID 19 infections, could not be evaluated.

Conclusion

Our study has shown that levels of the major fibrinolytic inhibitors PAI 1 and TAFI increase in patients with COVID 19 infection. It has also revealed for the first time that PAI 1 levels increase more in obese patients than they do in non-obese patients. We have shown that the plasma TAFI level is associated with the severity of COVID 19 infection, and infer from this that the inhibitory treatments against TAFI might be effective in preventing thrombotic complications in the course of COVID 19 infections.

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