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Endotheliopathy, Soluble Thrombomodulin and Its Role in Predicting Prognosis in Severe Coronavirus Disease-2019 Pneumonia

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ABSTRACT

Introduction: The main reason for acute respiratory distress syndrome (ARDS) and mortality during coronavirus disease-2019 (COVID-19) infection is thrombotic events due to the tendency to coagulopathy. Systemic inflammation, endothelial dysfunction caused by severe hypoxia and thrombocyte abnormalities lead to coagulopathy. Here, we aimed to search for the relationship of soluble thrombomodulin (TM) and von Willebrand factor (vWF) antigen levels as endothelial dysfunction biomarkers with an early stage severe COVID-19 infection.

Methods: Fifty-four patients admitted to our hospital with severe COVID-19 infection and 25 healthy asymptomatic patients were included in the study. Both the patient (at hospital admission date) and healthy control group gave venous blood samples for soluble TM and vWF antigen level measurements. The level of the searched parameters were compared between groups and hospital admission duration and mortality rate of the patient group. Results were evaluated using the SPSS program.

Results: vWF antigen levels did not show any difference between groups, but soluble TM was significantly higher in the patient group. Thus, soluble TM level did not show a statistically significant relationship with duration of hospitalization or mortality.

Conclusion: Early elevation of soluble thrombolic level can be considered as an early defense mechanism of endothelium against thrombosis in a severe COVID-19 infection.

Keywords: Solubl thrombomodulin, von Willebrand factor, COVID-19 infection

Introduction

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), also called as coronavirus disease-2019 (COVID-19) infection, raised from China, city of Wuhan in 2019 and spread globally in a short time to cause a pandemic that resulted in millions of people to get infected and so many to die. The most important feature of the virus was rapid contamination and the respiratory system was the most affected system. Its infection ranges from asymptomatic infection/mild respiratory infection to severe pneumonia and acute respiratory distress syndrome (ARDS) (1).

Severe hypoxia and inflammation set the ground for thrombotic diseases. Venous thromboembolic was seen in hospitalized patients between 16-49% (2-4). Thrombotic complications present in a wide range from pulmonary emboli to macrothrombosis in large arteries and veins and are accepted as a poor prognostic predictors (5-14). The virus is known to directly invade the alveolar epithelium to enter the body and cause a cytopathic effect on the alveolar epithelium, but it is not shown directly in the endothelium. Extensive endothelium dysfunction causes microthrombosis (15).

Thrombomodulin (TM) is a thrombin receptor with high affinity present in the endotelium membrane and presents as a natural anticoagulant. It acts as a cofactor of protein C thrombin - catalyzed activation and inhibits thrombin's procoagulant functions. Endothelial TM enzymatically splits in the presence of cytokines, active neutrophils, and macrophages. It releases soluble fragments that circulate in blood and are excreted urinally. These soluble parts are called soluble TMs. TM level is set as a molecular biomarker that reflects the damage of endothelial cells (16,17). vWF is a platelet - adhesive protein and the carrier of coagulation factor VIII synthesized by endothelial cells and megakaryocytes (18). The role of increased vWF in the prothrombotic picture in the course of COVID-19 has been demonstrated.

Here, we aimed to search for the level and role of soluble TM and vWF antigen levels as endothelial damage indicators and endothelial dysfunction biomarkers in early stage severe COVID-19 infection.

Methods

Ethical Statement

The protocol for this study was approved by the Ethical Committee of University of Health Sciences Turkey, İstanbul Training and Research Hospital (approval number: 2800, date: 02.04.2021). All included patients

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provided written informed consent for the publication of their medical details.

Study Design and Participants

The study was designed as a cross-sectional case-control study. 54 patients (29 F, 34 M) admitted to our hospital with severe COVID-19 infection and 25 (F: 11, M: 14) healthy asymptomatic patients were included in the study. Clinical and laboratory features of all patients were recorded.

Both the patient (at hospital admission date) and healthy control group gave venous blood samples for soluble TM and vWF antigen level measurements. The level of the searched parameters were compared between groups and hospital admission duration and mortality rate of the patient group.

Severe COVID-19 pneumonia was described according to clinical, radiological, and laboratory criteria for severe COVID-19 pneumonia according to the Living Guidance for Clinical Management of COVID-19 (19). COVID-19 infection was confirmed from oropharyngeal and nasal swap samples by real-time reverse transcriptase polymerase chain reaction testing. After admission to the hospital, venous blood samples were collected using standard blood sampling and laboratory methods and sent to the laboratory. Blood samples were centrifuged at 1500 rpm in tubes with EDTA and kept in -80 °C. Blood samples were tested for soluble TM and vWF antigen levels. Inflammatory parameters [C-reactive protein (CRP), ferritin, procalsitonin] were also tested. The effect of these values on the duration of hospitalization and mortality rates was evaluated. The same blood parameters were also measured in the healthy control group and compared with the patient group statistically.

Statistical Analysis

Statistical analysis was performed using SPSS 28.0 for the Windows program. $P<0.05$ was considered statistically significant. Descriptive statistics were reported as the mean, standard deviation, median, minimum, maximum, frequency, and percentage values. Quantitative independent data analysis was performed with independent sample t-test and Mann-Whitney U test. Qualitative independent data analysis was conducted with the chi-square test and the Fisher's test when this was not applicable. The effect size and cutoff values were evaluated with receiving operating characteristic analysis.

Results

Mean age was 53.2±9.1 years (F: 24/M: 35) in the patient group and 48.2±10.4 years (F: 11/M: 14) in the control group. In the patient group with accompanying diseases, 24 patients (40%) had hypertension 12 patients (20%) had diabetes mellitus, and 4 patients (6%) had NHYA 1-2 heart failure mean duration of hospitalization was 8 days (4-41). Demographic and clinical features of the patients are summarized in Table 1.

There was no statistically significant difference between the patient and control groups for orgender distribution ($p>0.05$). The mean vWF

Table 1. Laboratory, clinical and demographic characteristics of patients

		Min.-Max.	Median	Mean ± SD/(n, %)
Age		30.0-70.0	53.0	51.7±9.7
Sex	Female			24 (41.7%)
	Male			35 (58.3%)
Comorbidity	(-)			30 (50.8%)
	(+)			29 (49.2%)
Hypertension				24 (40.7%)
Diabetes mellitus				12 (20.3%)
Cardiovascular disease				4 (6.8%)
vWF, ng/mL		1.9-54.3	40.3	37.8±12.0
Trombomodulin, ng/mL		3.1-10.1	5.1	5.2±1.0
Haemoglobin, g/L		8.8-15.3	13.0	12.7±1.3
Urea		12.0-111.0	25.0	29.8±17.4
Creatinine, µmol/L		0.1-2.2	0.7	0.8±0.3
CRP (mg/L)		1.0-229.0	30.0	57.4±62.0
Procalcitonin, ng/mL		0.0-1.3	0.1	0.1±0.2
Ferritin, pg/dL		10.0-1500	147.5	284.0±334.8
Aspartate aminotransferase, U/L		12.0-195.0	33.0	37.6±28.1
Alanine aminotransferase, U/L		6.0-142.0	26.0	33.3±24.0
Lactate dehydrogenase, U/L		0.0-803.0	251.0	271.4±126.5
Creatine kinase, U/L		15.0-1049.0	69.5	135.0±175.7
Prognosis	Discharge			52 (88.1%)
	Death			7 (11.9%)
Hospitalization (day)		4.0-41.0	8.0	9.5±6.5

Min.: Minimum, Max.: Maximum, SD: Standard deviation, vWF: von Willebrand factor, CRP: C-reactive protein

antigen level was 37.8 ng/dL in the patient group and 44.3 ng/dL in the control group, and there was no significant difference between 2 groups (p>0.05). There was no difference in Hb and creatinine levels between the groups (p>0.05). In the patient group, TM, CRP, procalsitonin, ferritin, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and creatine kinase levels were significantly higher than those in the control group (p<0.05) Table 2.

In different patient and control groups, TM level was found to have a significant effect [area under the curve (AUC): 0.765 (0.650-0.880)]. The 5 cut-off value for TM was found to be effective to differentiate patient and control groups [AUC: 0.739 (0.623-0.855)]. Sensitivity 67.8%, positive prediction 88.9%, specificity 80.0%, negative prediction 51.3% Table 3.

Discussion

This study showed that in early stage severe COVID-19 solubl TM level was increased while vWF level did not show a significant change. These findings can be interpreted as in early stages of severe COVID-19 infection endothelial activation developed and this feature provided defense and protection against thrombosis.

Studies have shown that abnormal coagulation parameters are related to poor prognosis and fibrin degradation products, and D-dimer levels are predictors of mortality in COVID-19 infection (20). This opinion was supported when a decrease in mortality was seen after heparin had been used during the disease (21).

The endothelium has some physiological functions such as vascular tonus control, tissue homeostasis, barrier integrity, anti-inflammatory and antioxidant effects, vascular permeability regulation, structural and vascular integrity, and prevention of thrombosis (22). Endothelitis and endothelium dysfunction and accompanying platelet abnormalities that occur during COVID-19 infection cause a tendency to thrombosis and affect the prognosis of the disease.

Goshua et al. (23) showed in their study that in patients admitted to intensive care unit (ICU), vWF antigen levels were increased when compared to on-ICU patients. Solubl TM level was higher than 3.26 ng/mL and this level was negatively correlated with hospital discharge. Also, vWF vs solubl TM levels were related to mortality (23). It is known that in late stages of the disease, severe hypoxia and systemic inflammation cause endothelium dysfunction and a tendency to thrombosis (24). In

Table 2. General features and laboratory values of case and control groups

Mean ± SD/(n, %)		Control group		Case group		p	
		Median	Mean ± SD/(n, %)	Median			
Age (year)		48.2±10.4	51.0	53.2±9.1	54.0	0.052	t
Sex	Female	11 (44.0%)		24 (40.7%)		0.778	X^2
	Male	14 (56.0%)		35 (59.3%)			
vWF (ng/mL)		42.5±6.4	44.3	35.8±13.2	37.8	0.066	m
Trombomodulin (ng/mL)		4.6±0.8	4.5	5.4±1.0	5.5	**0.001**	t
Haemoglobin, g/L		12.9±1.1	13.0	12.7±1.4	12.9	0.425	t
Urea		23.8±15.9	20.0	31.5±17.5	26.5	**0.004**	m
Creatinine, µmol/L		0.72±0.20	0.70	0.79±0.33	0.80	0.544	m
CRP (mg/L)		3.8±2.4	4.0	80.1±61.1	61.0	**0.001**	m
Procalcitonin, ng/mL		0.01±0.01	0.02	0.11±0.18	0.08	**0.001**	m
Ferritin, pg/dL		60.9±37.7	54.0	378.5±359.6	269.0	**0.001**	m
Aspartate aminotransferase, U/L		22.9±9.9	22.0	44.0±30.9	37.5	**0.001**	m
Alanine aminotransferase, U/L		23.0±10.1	22.0	37.7±26.9	29.0	**0.005**	m
Lactate dehydrogenase, U/L		166.9±43.1	150.0	316.5±124.0	299.5	**0.001**	m
Creatine kinase, U/L		53.9±22.6	54.0	170.6±200.5	114.0	**0.001**	m

t: T-test, m: Mann-Whitney U test, X^2: Chi-square test, vWF: von Willebrand factor, CRP: C-reactive protein, SD: Standard deviation

Tablo 3. Predictive value of thrombomodulin with receiving operating characteristic analysis

		Area under the curve		CI 95%	p
Thrombomodulin, (ng/mL)		0.765		0.650-0.880	**0.001**
Thrombomodulin, cut-off 5		0.739		0.623-0.855	**0.001**
		Control	Case		
Thrombomodulin, (ng/mL)	≤5	20	19	Sensivity	67.8%
	>5	5	40	Positive prediction	88.9%
				Specificity	80.0%
				Negative prediction	51.3%

CI: Confidence interval

postmortem examination, in pulmonary endothelium cells of patients who died because of COVID-19 infection, TM expression was found to be decreased and vWF expression was increased. The increased number of immun cell infiltration was thought to decrease TM levels (25).

Decreased oxygenization, which means hypoxia is a direct risk factor for thrombosis (26,27). Hypoxia stimulates some cell signal pathways to initiate thrombosis and also suppresses TM, which is an anticoagulant (28-30). Here, it should be considered that TM is increased in the early stages of the disease while it is decreased in the advanced period and contributes to thrombosis. In our study, the increase in TM at early stages can be explained as the defense of endothelial cells against thrombosis. This finding can lead the way for treatment. Studies showed that replacement of recombinant soluble TM in septic patients with disseminated intravascular coagulation decreased mortality (24,31,32). TM is an endothelium function biomarker that has anticoagulant as well as anti-inflammatory effects immune cell adhesion and complement system activation (16,17).

Our study showed that vWF antigen levels did not have a significant difference in patients compared with healthy controls. This could be explained by the early stages of the disease. In different studies, vWF antigen levels were shown to be increased in the advanced stages (21st day) of the disease parallel to an increase in factor-8 levels. This was described as strong stimulation and damage of the endothelium and increase of vWF levels in Weibel-Palade bodies (33). vWF levels were found to be higher in ICU patients than in non-ICU patients and was related to mortality (23). This is also supported by another study (34-36).

This shows that vWF-level increases with duration and severity of the disease, contributes to thrombosis and mortality in critically ill patients.

In COVID-19 infection first target is alveolar target and there is no evidence showing that virus directly infects the endothelium and this was supported with postmortem studies (25,37-39). But there are limited number of studies contradicting this idea showing that decreased angiotensin-converting enzyme-2 expression caused decreased endothelium cell sensitivity to SARS-CoV-2 (40-46). Phenotypic changes of endothelium in COVID-19 infection are not enlightened yet but studies showed that endothelial cell activation and damage played an important role in ARDS, pulmonary edema and procoagulant stage related COVID-19 pathogenesis (22,47).

Study Limitations

The small number of patients in our study is a limitation of the study.

Conclusion

It can be considered that an increase in soluble TM in early stages of COVID-19 infection has a protective effect against thrombosis, but in advanced stages, a decrease in TM due to endothelial dysfunction plays an active role in thrombosis development and negatively affects prognosis of the disease.

Ethics Committee Approval: The protocol for this study was approved by the Ethical Committee of University of Health Sciences Turkey, İstanbul Training and Research Hospital (approval number: 2800, date: 02.04.2021).

Informed Consent: All included patients provided written informed consent for the publication of their medical details.

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