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The Association between Protein C and Antithrombin III Levels with the Severity of Coronavirus Disease-2019 Symptoms

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Abstract

BACKGROUND: Coronavirus disease-2019 (COVID-19) has various symptoms ranging from mild to critical. Hypercoagulation state is often observed in severe and critical COVID-19. Both coagulation and inflammation are interrelated and amplifying each other, with protein C and antithrombin (AT) III as two important mediators.

AIM: The aim of the study was to determine the association between protein C and AT III levels with the severity of COVID-19 symptoms.

METHODS: This analytical study was conducted at Haji Adam Malik Hospital from April to July 2021. Subjects were obtained by consecutive sampling method. Inclusion criteria were patients with confirmed COVID-19 using reverse transcription polymerase chain reaction and willing to participate. Subjects were divided into two groups: Mild-moderate and severe-critical symptom groups. Demographic and blood sample was obtained from each subject. Blood samples underwent examination for leukocyte, thrombocyte, PT, aPTT, protein C, and AT III.

RESULTS: A total of 50 patients were obtained with female domination (58%) and mean age of 41.44 (standard deviation 20.90) years. Most subjects (86%) were in mild-to-moderate symptom group. There were significant differences in the level of protein C and AT III in both group ($p = 0.029$ and 0.034, respectively). Using the cutoff value for AT III of 45.6%, subjects who had mediator level below the value tend to develop severe and critical symptoms compared to their counterparts (odds ratio = 6.458).

CONCLUSION: AT III is associated with severity of COVID-19 symptoms. Lower AT III level increases the risk for developing severe and critical symptoms.

Introduction

The most common symptoms of coronavirus disease-2019 (COVID-19) are fever, tiredness, and dry cough. Some patients may experience aches or pains, nasal congestion, runny nose, sore throat, or diarrhea. The symptoms experienced are usually mild and appear gradually. Some infected people do not show any symptoms and still feel well. Infected patients who managed to recover without the need for special treatment are quite high, about 80% of the number of sufferers. It also said about one in six people who contracted COVID-19 developed severe pain and had difficulty breathing. Elderly people and people with pre-existing medical conditions such as high blood pressure, heart problems, or diabetes are more likely to develop more serious complications. The severity of the disease in patients is not only determined by viral virulence but also the host response [1], [2], [3], [4], [5].

Although the majority of COVID-19 patients have respiratory tract infections, approximately 60–70% of hospitalized patients have coagulation disorders such as thrombocytopenia, hypercoagulation, disseminated intravascular coagulation (DIC), and venous thrombosis [6], [7]. Activation of coagulation as well as activation of extensive vascular inflammation-associated disease has been shown to be present in severe COVID-19 patients, and autopsies revealed that nearly 58% of patients died from venous thrombosis and pulmonary embolism [8], [9].

Coagulation and inflammation are interrelated. It is well known that an exaggerated innate immune response activates coagulation, whereas activation of coagulation and subsequent formation of thrombin leads to increased inflammation [10]. In addition to promoting clot formation by activating platelets and by cleaving fibrinogen into fibrin, thrombin also exerts several cellular effects through proteases activated receptor [11], [12]. Thrombin formation and its activity are tightly regulated by anticoagulant molecules such as antithrombin (AT), tissue factor pathway inhibitors, and activated protein C (APC) [13], [14]. During inflammation, most of these anticoagulant protective mechanisms are disrupted, as noted in COVID-19 patients. As a result of impaired regulation of procoagulant–anticoagulant molecules, natural homeostasis is further shifted toward procoagulant and pro-inflammatory phenotypes that predispose to the development of intravascular thrombosis, DIC, and multiorgan failure. Overproduction

of pro-inflammatory cytokines such as tumor necrosis factor α, interleukin (IL)-6, and IL-1β as found in COVID-19 patients causes a cytokine storm, which causes hypercoagulation, platelet activation, leukocyte infiltration, and vascular hyperpermeability [15]. Acute increases in pro-inflammatory molecules in the lungs, leading to the simultaneous formation of pulmonary edema and pulmonary embolism as observed in COVID-19 patients [16]. Treatment with natural anticoagulant molecules, APC, and AT which are known to have anticoagulant and anti-inflammatory cytoprotective effects can save COVID-19 patients with hyperinflammation and coagulopathy [17]. This study aims to investigate the association between protein C and AT III levels on the severity of COVID-19 symptoms.

Methods

This analytical study was conducted at Haji Adam Malik General Hospital in Medan, North Sumatera, Indonesia, from April to July 2021. This research has been approved by the Research Ethics Committee of the Universitas Sumatera Utara. Subjects were selected using consecutive sampling method. Inclusion criteria were confirmed COVID-19 patients using reverse transcription polymerase chain reaction, admitted at Haji Adam Malik General Hospital, and willing to participate in the study. This study had been approved by The Health Research Ethical Committee, Medical School, Universitas Sumatera Utara with registry number 728/KEP/USU/2021.

COVID-19 patients who were willing to participate in this study were then grouped into two groups based on severity of symptoms: Mild-to-moderate and severe-to-critical symptom groups. The criteria for the severity of symptoms were determined by the COVID-19 control and prevention guideline from the Ministry of Health of the Republic of Indonesia (Table 1). Demographical data were also collected from the subject's medical record.

Table 1: Severity criteria of coronavirus disease-2019 symptoms

Severity criteria	Symptoms
Mild	Fever >38°C
	Cough
	Sore throat
	Nasal congestion
	Malaise
Moderate	Fever >38°C
	Shortness of breath
	Persistent cough and sore throat
	Mild pneumonia (peripheral oxygen saturation>93%)
Severe	Fever >38°C
	Severe pneumonia (peripheral oxygen saturation<93%)
Critical	Septic shock
	ARDS

ARDS: Acute respiratory distress syndrome, COVID-19: Coronavirus disease-2019.

Blood sample from each subject was gathered. Samples were examined in the laboratory for leukocyte and thrombocyte count, PT, and APTT, and for determining AT III and protein C levels. To determine AT III levels, we used Coatron A6 (TECO Medical Instruments, Niederbayern, Germany) device with chromogenic method while protein C level evaluation was carried out using ChemWell (Megazyme Ltd, Bray, Ireland) device with enzyme-linked immunosorbent assay method. Samples were treated accordingly based on standard device instructions.

Qualitative data were presented in frequency and percentage while qualitative data were presented in mean and standard deviation (SD). The association between qualitative variables was analyzed using Fisher's exact test. Qualitative data undergo normality test before further statistical analysis. Independent T test was utilized for normally distributed data and Mann–Whitney test was utilized otherwise. The receiver operating characteristic (ROC) curve was used to assess the predictive accuracy of the protein C and AT III regarding COVID-19 symptom severity. After obtaining the optimal cutoff for the two parameters, the odds ratio (OR) values for predicting severe-critical symptom were calculated. $p < 0.05$ was considered statistically significant. All statistical analyses were carried out using Statistical Package for the Social Sciences software.

Results

A total of 50 subjects were involved in this study with 43 subjects in mild-to-moderate group and seven in severe-to-critical group. Of all subjects, 21 were males and 29 were females. The mean age of the patients in this study was 41.44 (SD 20.90) years. There was no significant difference in gender and age between both groups. Other laboratory parameters were also similar except protein C ($p = 0.029$) and AT III ($p = 0.034$). Demographic and laboratory characteristics of subjects in this study were presented in Table 2.

Table 2: Demographic and laboratory characteristics of subjects

Characteristics	Total, *n* (%)	COVID-19 symptom severity		*p*
		Mild-to-moderate	Severe-to-critical	
Sex (*n*)				
Men	21 (42)	17 (39)	4 (57)	0.434[a]
Women	29 (58)	26 (61)	3 (43)	
Mean age, year (SD)	41.44 (20.90)	39.19 (21.28)	53.14 (15.84)	0.074[b]
Mean hemoglobin, g % (SD)	13.50 (2.16)	13.42 (2.16)	14.24 (2.23)	0.603[c]
Mean leukocytes, mm^{-3} (SD)	8.03 (6.29)	7.02 (4.20)	13.96 (12.44)	0.106[c]
Mean thrombocyte, mm^{-3} (SD)	233.81 (78.14)	228.82 (77.73)	235.11 (41.44)	0.941[b]
Mean PT, second (SD)	17.29 (13.61)	16.24 (9.41)	24.30 (28.98)	0.784[c]
Mean aPTT, second (SD)	36.49 (15.63)	36.37 (15.61)	35.41 (17.42)	0.565[c]
Mean protein C, ng/mL (SD)	75.31 (25.38)	82.23 (19.84)	32.84 (7.57)	0.029*[c]
Mean AT III, % (SD)	62.80 (30.66)	66.78 (29.06)	38.37 (30.96)	0.034*[c]

[a]IFisher's exact test, [b]Independent *t*-test, [c]Mann–Whitney test, *$p < 0.05$. SD: Standard deviation, COVID-19: Coronavirus disease-2019, AT: Antithrombin, PT: Partial thromboplastin time (PTT); aPTT: Activated partial thromboplastin time.

According to the results of the Mann–Whitney test, there were significant differences in the levels of

protein C and AT III between mild-to-moderate symptom group compared to severe-to-critical symptom group. Higher levels of protein C and AT III were observed in mild-to-moderate symptom group as shown in Figure 1.

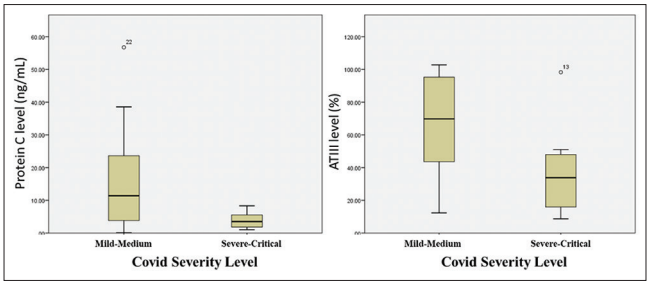

Figure 1: Comparison of protein C and antithrombin III levels between both study groups

Statistical analysis was continued to ROC curve analysis. The area under the curve (AUC) for protein C and AT III in predicting severe-to-critical symptoms was 0.759 and 0.751, respectively (Figures 2 and 3). The highest accurate cutoff point for protein C was 5.36 ng/mL, giving a sensitivity of 69% and a sensitivity of 71.4%. For AT III, the cutoff point was 45.6% with a sensitivity of 72.1% and a specificity of 71.4%.

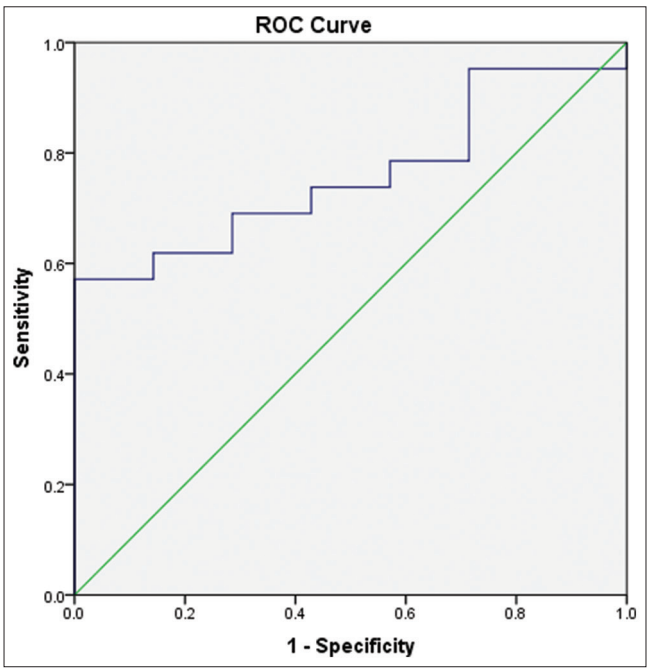

Figure 2: Receiver operating characteristic curve of protein C in predicting severe-to-critical COVID-19 symptoms

We further analyzed the cutoff value to determine the risk of severe-to-critical symptoms in patients with COVID-19. Patients with protein C level lower than 5.36 ng/mL had 5.769 times higher risk for developing severe-to-critical COVID-19 symptoms compared to those with protein C level higher than the cutoff. However, this result was not significant since the 95% CI interval crosses the value of 1. In the other hand, AT III level below 45.6% in patients with COVID-19 would increase the tendency for developing severe-to-critical symptoms as high as 6.458 times compared to their counterparts (Table 3).

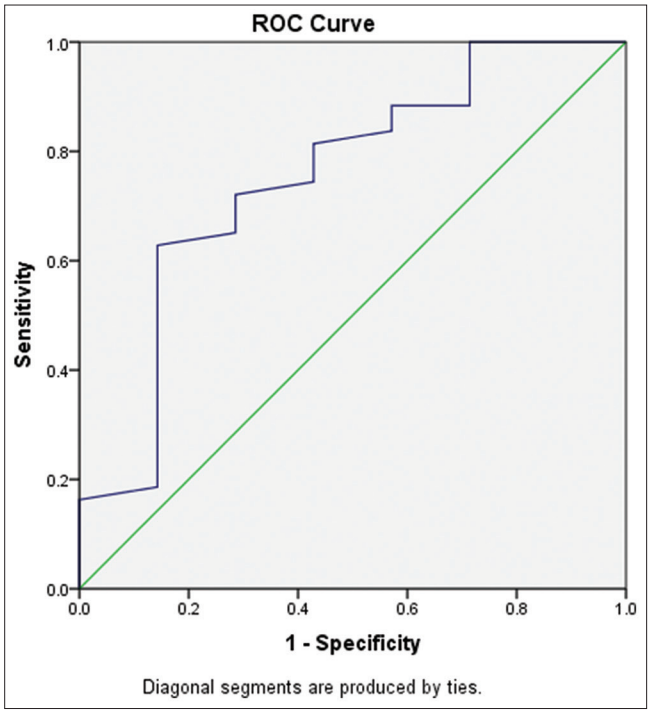

Figure 3: Receiver operating characteristic curve of antithrombin III in predicting severe-to-critical COVID-19 symptoms

Discussion

This study explored the relationship between protein C and AT III levels and severity of COVID-19 symptoms. Significantly higher protein C levels were found in COVID-19 patients with mild-to-moderate symptoms compared to subjects with severe-to-critical symptoms. Our analysis also found that COVID-19 patients with protein C levels below 5.36 ng/mL were at higher risk for severe or critical symptoms (OR 5.769). Unfortunately, the finding was not significant. The previous study has also shown that low protein C levels on admission indicate the severity of disease in patients with COVID-19 [18].

Table 3: Risk analysis of severe-to-critical coronavirus disease-2019 symptoms based on protein C and antithrombin III cutoff values

Variable	OR	95% CI	
		Lower	Upper
Protein C	5.769	0.988	33.676
AT III	6.458	1.1	37.198

AT: Antithrombin, OR: Odds ratio, CI: Confidence interval.

This happens because protein C has anticoagulant and anti-inflammatory properties. When thrombin binds to thrombomodulin on the surface of endothelial cells, protein C is converted to its active form, APC. APC cleaves activated factor V and activated factor VIII and neutralizes plasminogen-activator inhibitor-1. With a 10,000-fold reduction in prothrombinase activity [19], cleavage of activated factor V results in a significant reduction in thrombin formation. Protein S further enhances APC-activated cleavage of factor V and factor VIII. The anti-inflammatory effect of APC is mainly

through protease-activated receptor (PAR)1. PAR1 is present on the surface of endothelial cells and is unique in that it propagates both pro- and anti-inflammatory responses. APC interacts with the endothelin C receptor (EPCR), a complex that cleaves PAR1. Anti-inflammatory signaling is initiated through multiple intracellular pathways, including sphingosine kinase and inhibin 2 [20], [21], [22]. This pathway leads to anti-apoptotic, anti-inflammatory, and barrier-promoting changes in endothelial cells [20] that influence the severity of COVID-19.

In this study, we also found that patients with COVID-19 with severe and critical symptoms had significantly lower AT III levels than patients with mild-to-moderate symptoms. Likewise, the risk of COVID-19 patients with AT III levels above the cutoff showed a higher risk of exacerbation. The previous studies have also shown that AT III levels also decrease in COVID-19 cases [23], [24], [25], [26]. We suspect that this may be due to consumptive coagulopathy. Consumptive coagulopathy of varying severity can exacerbate the course of severe COVID-19 patients [24], [26]. AT III is the major endogenous thrombin inhibitor, and in addition to affecting the intrinsic, extrinsic, and general pathways of the coagulation cascade, it is a potent anti-inflammatory agent. AT III levels are reduced in coagulation disorders due to rapid uptake following formation of the AT complex and due to decreased synthesis and increased neutrophil clearance [25], [27]. Heparin treatment also results in a reduction in AT III levels [28].

Our study is the first study evaluating the association between protein C, AT III, and severity of COVID-19 symptoms in our center. This information may aid to the previous studies regarding the important role of protein C and AT III in predicting disease course of COVID-19 thus improving management and outcome of patients. However, both markers are not widely available particularly in remote area. This prevents a wide extrapolation of our study results. The main limitation of this study is that we did not adjust or match the factors that may influence protein C and AT III levels. In addition, there was an imbalance in total subjects in both groups which may affect the results.

Conclusion

Protein C and AT III are associated with severity of COVID-19 symptoms. Both are promising markers in predicting and managing severe-to-critical COVID-19 cases. Routine evaluation of protein C and AT III in COVID-19 patients is advised to improve disease outcome.

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