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Original Article

Transcriptomic analysis of profibrinolytic and fibrinolytic inhibitor genes in COVID-19 patients

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Abstract

The immunopathogenesis of COVID-19 infection is initiated by the entry of the SARS-CoV-2 virus into the human body through droplets, entering the lungs and binding to the ACE-2 receptor. Activated macrophages stimulate an immune and inflammatory response, leading to the activation of the coagulation cascade, including profibrinolytic and fibrinolytic inhibitor processes. One of the proteins involved in profibrinolytic is encoded by the *PLAUR* gene, while fibrinolytic inhibitor proteins are encoded by the *A2M* and *SERPINE1* genes. This research aims to assess the transcriptomic analysis of genetic expression data of profibrinolytic genes, fibrinolytic inhibitor genes and their correlation with serum D-dimer levels, which describe the clinical condition of coagulation in COVID-19 patients. This cross-sectional study included 25 patients each for mild and moderate-to-severe COVID-19 at Dr. M. Djamil Padang General Hospital, Padang, Indonesia. Inter-group gene expression comparisons will be analyzed using $\log_2$ folds change, and bivariate tests will be analyzed using correlation. The results show that the *PLAUR* gene has higher expression in moderate-to-severe compared to mild cases. Similarly, the *SERPINE1* and *A2M* genes expressions are higher in moderate-to-severe compared to mild cases. Furthermore, there is a significant correlation between serum D-dimer levels and profibrinolytic factor (*PLAUR* gene) expression in COVID-19 patients. The correlation between serum D-dimer levels with fibrinolytic inhibitor factor (*SERPINE*1 and *A2M* genes) expression was found. These conclude that there is a significant difference in the expression of the profibrinolytic and fibrinolytic inhibitor genes between mild and moderate-to-severe cases in COVID-19, demonstrating COVID-19 infection affects coagulation activities.

Keywords: Transcriptomic, profibrinolytic, fibrinolytic inhibitor, D-dimer, COVID-19

Introduction

Coronavirus disease 2019 (COVID-19) is caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that has rapidly spread worldwide in 2020 with a high mortality rate. The rapid and widespread distribution of SARS-CoV-2 in Indonesia was exacerbated by the lack of compliance with territorial restrictions and a prevalence of dishonesty to medical personnel. Data revealed that mutations can occur during transmission, caused by travel history and increased patient immunity [1]. The SARS-CoV-2 can damage an infected person's body defenses. The damage inflicted by SARS-CoV-2 to the endothelial wall can be classified into

Received: May 6, 2024 - Accepted: June 25, 2024 - Published online: July 5, 2024

indirect damage caused by hyperinflammation and elevated cytokine levels in circulation [2] and direct damage caused by SARS-CoV-2 on endothelial cells [3,4].

In the condition when endothelial damage is apparent, the body's physiological response is in the form of direct hemostasis. The hemostasis process triggers the initiation of the coagulation cascade through both intrinsic and extrinsic pathways, including the profibrinolytic and fibrinolytic inhibition processes. When a blood clot forms and consolidates, the fibrinolysis process has already commenced in a separate and interconnected system [4]. Urokinase plasminogen activator receptor (uPAR) acts as a receptor for urokinase plasminogen activator (uPA) in localizing and promoting plasmin formation on the cell surface [5,6]. This protein functions as a profibrinolytic factor in the process of activation of plasminogen. The uPAR is encoded by the *PLAUR* gene, which has a role in localizing and promoting plasmin formation [5,6].

Simultaneously, fibrinolytic inhibitors can impede fibrinolysis through the activation of plasminogen. Plasminogen is activated by tissue plasminogen activator (tPA) and uPA, which are serine proteases (serpins) bond to facilitate the conversion of plasminogen to plasmin. These serpin proteins are inhibited from becoming activated plasmin by plasminogen activator inhibitor type 1 (PAI-1), PAI-2, C1-esterase inhibitor, and protease nexin [7]. The PAI-1 acts as a major fibrinolysis inhibitor, especially as a plasmin inhibitor coded by the *SERPINE1* gene. The α2-macroglobulin coded by the *A2M* gene is a protease inhibitor and cytokine transporter that uses a bait-and-trap mechanism to inhibit a broad spectrum of proteases. The α2-macroglobulin binds free plasmin to inhibit fibrin breakdown. The *SERPINE1* and *A2M* genes are crucial as they code for many proteins involved in the regulatory role of fibrin components in the hemostasis process [8]. Various inhibitors regulate the coagulation process, and disruptions or mutations in the genes encoding these proteins can fail to achieve hemostasis [9]. The *SERPINE1* gene is expressed in endothelial cells and can also be found in plasma, platelets, hepatoma, and fibrosarcoma cells [6]. The *PLAUR* gene is expressed in various tissues, including adipose tissue, lung, bone marrow, monocyte, and lipid [10]. The *A2M* gene is expressed in the respiratory tract, particularly the bronchi, liver, fat tissue, and endometrial tissue [11]. The expression of these genes varies among individuals and between tissues within an individual.

Acute inflammation such as COVID-19 tends to shift the hemostatic balance towards a prothrombotic and antifibrinolytic state, characterized by an increased procoagulants and antifibrinolytics in circulation. Balancing the deposition and degradation of fibrin is crucial to preventing bleeding, controlling inflammation, and facilitating tissue repair [15]. Excessive coagulation activation, as observed in conditions such as disseminated intravascular coagulation (DIC), can impede oxygen supply, leading to multiorgan dysfunction [16]. Microscopic examination of patients with DIC often reveals intravascular fibrin deposition and multiorgan dysfunction [17].

D-dimer is the final product of fibrin degradation, measured in the blood and produced by various organs damaged throughout the body by COVID-19 infection [18]. The formation of D-dimer is maintained by the coagulation cascades, which involve feedback on uPA, tPA, plasmin, PAI1, and α2-macroglobulin that influence each other. Dynamic changes in D-dimer levels could indicate the progression and prognosis of COVID-19 [18]; fibrinogen and D-dimer levels at admission could serve as biomarkers for predicting COVID-19 prognosis. Routine monitoring and evaluation of laboratory testing, particularly D-dimer and fibrinogen, could be implemented to reduce the morbidity and mortality rate of COVID-19 [19]. In sepsis patients, assessing fibrinogen alongside D-dimer levels is essential for a more accurate prognostic evaluation [20]. A gradual decrease in fibrinogen, along with elevated D-dimer levels, could be used to diagnose the DIC status [21]. The aim of this study was to determine the expression of profibrinolytic (*PLAUR)*, fibrinolytic inhibitor genes (*SERPINE1* and *A2M*), and their correlation with serum D-dimer levels as a marker of coagulation state in COVID-19.

Methods

Study design and patient criteria

A cross-sectional study was conducted among mild and moderate-to-severe COVID-19 patients who received treatment at Dr. M. Djamil General Hospital, Padang, Indonesia, between October 2021 and February 2022. To be eligible for the research's involvement, the patients should be ≥18 years old with confirmed COVID-19 through an RT-PCR test, have clinical symptoms ranging from mild asymptomatic to moderate-to-severe, and be willing to participate in this research by signing an informed consent form. All patients in this study had never received COVID-19 vaccination before. All patients with HIV-positive, hemophilia, immunocompromised conditions, liver cirrhosis, and pregnant individuals were excluded. The criteria used to define mild and moderate-to-severe COVID-19 patients were based on the Indonesian COVID-19 Management Guidelines [22], which also referred to WHO guidelines [15].

Sample and sampling method

The research sample was the population of COVID-19 patients who received treatment at RSUP Dr. M. Djamil from October 2021 to February 2022. The research sample was calculated using the minimum sample calculation formula for unpaired categorical analytical tests, which resulted in a total of 25 samples for each group. Patients with comorbid HIV liver cirrhosis and pregnant women were excluded.

Study variables

There are two kinds of variables used in this research: dependent and independent variables. The dependent variables are expression of profibrinolytic factor (*PLAUR* gene) and expression of fibrinolytic inhibitor factor (*SERPINE1* and *A2M* genes), while the independent variable is D-dimer serum level.

Gene expression analyses

Blood samples from COVID-19 patients in both groups were collected. These samples were isolated using the QIAamp Kits, a product produced by Qiagen company in Germany. QIAamp Kits facilitate RNA purification from cell-free body fluids through fast spin-column, vacuum, and plate centrifugation. RNA in the whole blood specifically binds to the QIAamp silica membrane, with pure viral being eluted in either water or a buffer provided with the kit. The RNA isolation stages include lysis, binding, washing, and delution. The isolation results were immediately tested using the Qubit HS assay following its assay protocol. Samples with a value more than 100 ng will be diluted, while samples with a value below 20 ng were re-isolated by increasing the concentration or can be replaced with another sample meeting the criteria. Suitable isolates were stored in a refrigerator at -80°C to maintain good RNA quality until library preparation. Library preparation was carried out according to the Illumina stranded Total RNA prep, ligation with Ribo-Zero kit protocol.

There are eight stages in the process, including rRNA depletion, RNA fragmentation and denaturation, cDNA synthesis, A-tailing, ligation, amplification, quantification and normalization, and the final stage of RNA sequencing. At each stage, researchers were assisted by expert technicians from the provider Illumina. In the final stage, after completing the initial six steps of library preparation, the samples were analyzed for the quality of the results and their respective concentrations and then prepared for the sequencing stage. Samples with a uniform concentration were diluted to the value determined by the protocol. During the sequencing process, the transcriptomic system guide was followed under the guidance of Illumina-trained technicians. Subsequently, patients' data, including comorbidities, routine blood laboratory results, and D-dimer assessment, were collected from the patient's medical records. The D-dimer assessment was carried out by taking serum blood samples using the VIDAS® D-dimer Exclusion II tool. Furthermore, RNA sequencing (RNA-seq) is a state-of-the-art method for quantifying gene expression (mRNA abundance) and performing differential gene expression analysis with high resolution using next-generation sequencing (NGS). The RNA expression in this study is presented in unit transcripts per kilobase million (TPM).

Data analysis

The transcriptomic results of the profibrinolytic gene (*PLAUR)* and fibrinolytic inhibitor genes (*SERPINE1* and *A2M*) were analyzed using bioinformatics analysis, including $\log_2$ folds change, upregulated, and downregulated gene identification. The data were statistically analyzed using the non-parametric Mann-Whitney U test with 95% confidence interval and a significance level of p=0.05. Statistical tests were conducted to investigate if there were differences in the expression of the profibrinolytic gene (*PLAUR)* and fibrinolytic inhibitor genes (*SERPINE1* and *A2M*) between mild and moderate-to-severe COVID-19 patients. Furthermore, Spearman correlation tests were performed to examine the correlation between the expression of each gene and serum D-dimer levels.

Results

Sample characteristics

A total of 50 patients were included in the study, with 25 patients in each group, and their characteristics are presented in **Table 1**. The percentage of females was higher in both groups (68% and 56% for mild and moderate-to-severe groups, respectively). Mild cases were predominant among those aged 18–50 years (76%), while moderate-to-severe cases were more prevalent in the >50–65 years age group (44%), followed by those aged more than 65 years. The most common comorbidities in both groups were hypertension (56%), diabetes mellitus (36%), kidney failure (12%), and ischemic heart disease (4%). Laboratory test results revealed higher hemoglobin and leukocyte levels in mild cases, while platelet and D-dimer levels were elevated in moderate-to-severe cases. The findings of these COVID-19 patients showed differences in blood laboratory results.

Table 1. Distribution of characteristics of mild and moderate-to-severe COVID-19 patients

Variable	Severity of COVID-19	
	Mild, n (%)	Moderate-to-severe, n (%)
Gender		
Male	8 (32)	11 (44)
Female	17 (68)	14 (56)
Age		
18–50 years	19 (76)	7 (28)
>50–65 years	6 (24)	11 (44)
>65 years	0 (0)	7 (28)
Comorbid		
Diabetes mellitus	1 (4)	9 (36)
Ischemic heart disease	1 (4)	1 (4)
Hypertension	3 (12)	14 (56)
Kidney failure	0 (0)	3 (12)
Laboratory test		
Hemoglobin (g/dL), median (min-max)	13.0 (9.7–17.8)	11.6 (6.7–16.6)
Leukocyte ($\times 10^3$/mm^3), median (min-max)	40.8 (31.5–56.8)	11.0 (4.4–27.7)
Thrombocyte ($\times 10^3$ mm^3), median (min-max)	187.0 (134.0–561.0)	220.0 (0.2–561.0)
D-dimer (ng/mL) median (min-max)	290.0 (110.0–1160.0)	2203.0 (289.0–10,000.0)

Comparison of profibrinolytic and fibrinolytic inhibitor gene expressions between mild and moderate-to-severe COVID-19

The comparison of the profibrinolytic gene *(PLAUR)* and fibrinolytic inhibitor genes (*SERPINE1* and *A2M*) in mild and moderate-to-severe COVID-19 patients is presented in **Table 2**. The gene expression significantly increased in the moderate-severe group compared to the mild group for both gene groups. The number of $\log_2$ fold changes illustrates the increase in gene expression between moderate-severe and mild groups. A normalization factor was applied to these values to obtain the total number of expressed genes in each cell. A positive $\log_2$ fold change number indicated growth, and vice versa.

Table 2 revealed that *PLAUR* gene expression was 5.87 times higher (p<0.0001), *SERPINE1* gene expression was 10.9 times higher (p<0.05), and *A2M* gene expression was 8.89 times higher (p<0.0001) in moderate-to-severe COVID-19 patients compared to the mild group.

Moreover, the relative increase in gene expression can be measured using a basic function, as there are two powers of the value of the $\log_2$ fold change.

Table 2. Comparison of mild vs moderate-to-severe of profibrinolytic (*PLAUR)* and fibrinolytic inhibitor (*SERPINE1* and *A2M*) gene expression in COVID-19 patients

Gene		Log_2 fold change	Relative increase of gene expression	*p*-value
Profibrinolytic genes	*PLAUR*	5.87	58.4852	<0.0001
Fibrinolytic inhibitor gene	*SERPINE1*	10.9	1910.8516	<0.0001
	A2M	8.89	474.4131	<0.0001

The severity of COVID-19 cases correlates significantly with an increased *SERPINE1* gene. The *A2M* gene shows an increase in $\log_2$ fold change by 8.89 times, showing that the relative expression of the gene increased by 474.4131. The severity of COVID-19 aligns with an increase in the *A2M* gene, which is statistically significant. *PLAUR* also demonstrated higher $\log_2$ fold change values in patients in the moderate-to-severe group compared to the mild group (*PLAUR*) with a $\log_2$ fold change of 5.87 times, and the relative expression of the gene increased by 58.4852. The severity of COVID-19 infection is in line with an increase in the *PLAUR* gene, which is statistically significant.

Expression of profibrinolytic (*PLAUR)* and fibrinolytic inhibitor genes (*SERPINE1* and *A2M*) in mild and moderate-to-severe COVID-19 patients

In this research, the expression of the *PLAUR*, *SERPINE1*, and *A2M* genes, based on TPM in COVID-19 patients, is presented in **Table 3**. In both groups, the distribution of *PLAUR* gene expression data is non-normal and has a wide range. In the mild group, the minimum expression value is 0.0819 TPM, the maximum is 14.1108 TPM, and the median is 0.835100 TPM. In the moderate-to-severe group, the minimum expression value is 0.2585 TPM, the maximum is 10.7157 TPM, and the median is 2.699915 TPM.

Table 3. Expression value of profibrinolytic gene (*PLAUR)* and fibrinolytic inhibitor genes (*SERPINE1* and *A2M*) with unit transcripts per kilobase million (TPM) in mild vs moderate-to-severe COVID-19 patients

Gene		Variable	n	Median	Minimum	Maximum
Profibrinolytic gene	*PLAUR*	Mild	25	0.835100	0.0819	14.1108
		Moderate-to-severe	25	2.699915	0.2585	10.7157
Fibrinolytic inhibitor gene	*SERPINE1*	Mild	25	0.121494	<0.0001	1.6291
		Moderate-to-severe	25	0.462664	<0.0001	14.0299
	A2M	Mild	25	0.008475	<0.0001	0.0607
		Moderate-to-severe	25	0.024954	<0.0001	0.2132

The expression of the *SERPINE1* gene in both groups showed an abnormal distribution of data over a wide range. In the mild group, the minimum expression value was <0.0001 TPM, the maximum was 1.6291 TPM, and the median was <0.0001 TPM. In the moderate-to-severe group, the minimum expression value was <0.0001 TPM, the maximum was 14.0299 TPM, and the median was <0.0001 TPM. Similarly, the expression of the *A2M* gene in both groups showed that the data distribution was abnormal over a wide range. The minimum expression value was 0.000 TPM, the maximum was 0.0607 TPM, and the median was 0.008475 TPM in the mild group. The minimum expression value was <0.0001 TPM, the maximum was 0.2132 TPM, and the median was 0.024954 TPM in the moderate-to-severe group.

Correlation of serum D-dimer levels with expression of profibrinolytic gene *(PLAUR)* and fibrinolytic inhibitor genes (*SERPINE1* and *A2M*) in COVID-19 patients

The correlations between D-dimer levels with the profibrinolytic gene (*PLAUR)* and fibrinolytic inhibitor genes (*SERPINE1* and *A2M*) are quantified by correlation coefficient values ranging from -1 to 1. Values closer to -1 or 1 indicate a stronger correlation, whereas values approaching 0 indicate a weaker correlation. The results of these correlations are presented in **Table 4**. There

is a significant positive correlation between serum D-dimer levels and the *PLAUR* gene expression in COVID-19 patients, with a correlation coefficient of 0.370. This finding indicates that an increase in *PLAUR* gene expression correlates with increased serum D-dimer levels in COVID-19 patients.

Table 4. Correlation of serum D-dimer levels with expression of profibrinolytic gene (*PLAUR)* and fibrinolytic inhibitor genes (*SERPINE1* and *A2M*) in COVID-19 patients

Gene		Correlation between D-dimer level and *PLAUR*, *SERPINE1*, and *A2M* in COVID-19 patients			
				D-dimer	*PLAUR*
Profibrinolytic gene	*PLAUR*	D-dimer	Correlation coefficient	1.000	0.370**
			p-value	.	0.008
		PLAUR	Correlation coefficient	0.370**	1.000
			p-value	0.008	.
				D-dimer	*SERPINE1*
Fibrinolytic inhibitor gene	*SERPINE1*	D-dimer	Correlation coefficient	1.000	0.180
			p-value	.	0.210
		SERPINE1	Correlation coefficient	0.180	1.000
			p-value	0.210	.
				D-dimer	*A2M*
	A2M	D-dimer	Correlation coefficient	1.000	0.339*
			p-value	.	0.016
		A2M	Correlation coefficient	0.339*	1.000
			p-value	0.016	.

There is a positive relationship between serum D-dimer levels and the *SERPINE1* gene expression in COVID-19 patients, with a correlation coefficient of 0.180. However, this correlation is not statistically significant. Furthermore, a significant positive correlation exists between serum D-dimer levels and *A2M* gene expression, with a correlation coefficient of 0.339. This significant correlation indicates a strong relationship, wherein an increase in *A2M* gene expression corresponds with an increase in serum D-dimer levels in COVID-19 patients.

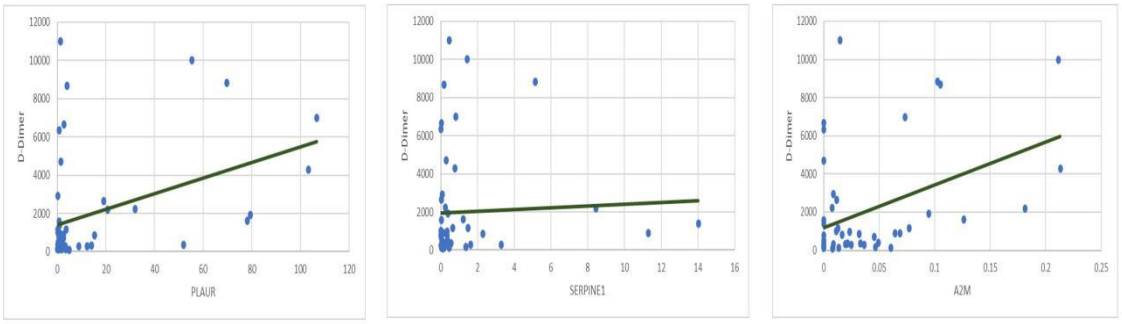

Figure 1. Correlation curve of D-dimer levels with expression of the *PLAUR*, *SERPINE1* and *A2M* genes in COVID-19 patients.

Figure 1 illustrates that the expression curves of the profibrinolytic gene *(PLAUR)* and fibrinolytic inhibitor genes (*SERPINE1* and *A2M*) in COVID-19 patients increase concomitantly with elevated D-dimer levels. Notably, the expression of the *PLAUR* and *A2M* genes exhibits a higher increase in correlation with D-dimer levels compared to the expression of the *SERPINE1* gene. This finding suggests that the elevated expression of *PLAUR* and fibrinolytic inhibitor genes contributes to the increase in D-dimer levels observed in COVID-19 patients.

Discussion

Our study found that *PLAUR* gene expression was higher in severe COVID-19 patients compared to those with mild cases. This finding is consistent with a study showing a significant increase in *PLAUR* expression in SARS-CoV-2-infected bronchial compared to the control group [24]. Subsequently, local plasminogen activator inhibitor-2 (PAI-2) activity could inhibit the effects of plasminogen activator urokinase/plasminogen activator urokinase receptor (uPA/UPAR),

contributing to pulmonary emboli and distal coagulopathy [24]. To accelerate protease catalytic activation, uPA (encoded by the *PLAUR* gene) is a serine protease that cleaves inactive plasminogen and converts it into plasmin by interacting with uPAR (encoded by *PLAUR*) on the cell surface. uPAR plays a crucial role in producing the serine protease plasmin [12]. uPAR is associated with diverse physiological processes such as cell differentiation, proliferation, migration, and fibrinolysis, and it contributes to the pathogenesis of airway remodeling, lung injury, and pulmonary fibrosis [12].

This study found a significant positive correlation was found between high *PLAUR* gene expression and the elevation of serum D-dimer levels in COVID-19 patients, with a correlation coefficient of 0.370. In contrast, a previous study found no significant relationship between the soluble uPAR and D-dimer levels in COVID-19 patients, with a correlation coefficient of only -0.114 [24]. uPAR and its soluble form mediate the conversion of plasminogen into plasmin. Subsequently, humoral immunity plays a crucial role in controlling infection after developing viremia. One of the chemotactic agents that plays a significant role in migrating these cells is uPAR, which is encoded by *PLAUR* gene [25].

The research findings from single-cell RNA sequencing (scRNA-seq) in 24 samples of peripheral blood mononuclear cells (PBMCs) revealed that the expression of the *SERPINE1* gene in severe cases was higher compared to mild cases, showing an increase of more than 1.75 times statistically non-significant ($p<0.05$) [24]. During transmission, the renin-aldosterone-angiotensin system (RAAS) interacts with the SARS-CoV-2 spike protein, attaching to the natural receptor for angiotensin-converting enzyme 2 (ACE2) on host cells. Both tPA and PAI-1 are closely related to the RAAS. ACE2 downregulation leads to decreased degradation of angiotensin II, resulting in a buildup of angiotensin II. Angiotensin II binds to the cellular receptor angiotensin II type 1a receptor in the lungs, which causes acute lung injury. Injury occurs to type II alveolar cells, which are the source of surfactant. With a decrease in surfactant, the p53 pathway was induced, which will cause an increase in PAI-1 (*SERPINE1* gene) and a decrease in both uPA and uPAR [26]. This process ultimately shifts the fibrinolytic balance to a hypofibrinolytic state [27].

The D-dimer produced in the lungs results from fibrinolysis acting on intra-alveolar fibrin membranes or local microthrombi. Patients with severe COVID-19 cases exhibit a local imbalance in the lungs, characterized by increased fibrin formation and inadequate fibrinolytic activity relative to the substantial fibrin burden. This imbalance suggests impaired fibrin clearance, contributing to clinical respiratory manifestations [28]. In this study, a weak correlation was found between increased expression of the *SERPINE1* gene in COVID-19 patients and the elevation of serum D-dimer levels, with a correlation coefficient of 0.180.

This research showed a significant correlation between the increased expression of the *A2M* gene in COVID-19 patients and the elevation of serum D-dimer levels, with a correlation coefficient of 0.339. This increase is associated with the condition of the endothelium in COVID-19, as the activity of α2-M was shown by immunofluorescence staining techniques that form a thin and continuous layer on the luminal surface of endothelial cells. The positioning of α2-M between the vessel wall and circulating blood underscores its crucial role in safeguarding the vascular endothelium. This protein modulates diverse protease-generating reactions near the endothelial surface, contributing to endothelial protection [29]. In addition to its role as a relevant plasma proteolytic enzyme inhibitor, *A2M* can modulate enzyme-substrate interactions, providing a mechanism to maintain and protect enzymatic activity in the presence of other circulating inhibitors. In the fibrinolytic system, two principal inhibitors are alpha 2 plasmin inhibitor (α-2 antiplasmin) and plasminogen activator inhibitor 1 (PAI-1) [30]. The function of α-2-antiplasmin is threefold: inhibition of plasminogen binding to fibrin, cross-linking fibrin, and plasmin proteolysis. α-2 antiplasmin binds to plasminogen, competitively inhibiting the binding of plasminogen to fibrin [31].

Alpha-2-macroglobulin is considered an acute-phase protein because its expression is regulated by inflammatory cytokines such as interleukin-6 (IL-6). The binding of IL-6 to its receptor activates the Janus kinase (JAK) pathway and its signal transducer and activator of transcription 3 (Stat3). Upon this activation, Stat3 binds to the *A2M* gene promoter and enhances its transcription. In hemostasis, *A2M* is involved in coagulation and fibrinolysis. Its anticoagulant

properties result from its ability to inhibit thrombin. However, the effects of plasma A2M concentrations on platelet activation have not been extensively studied. In fibrinolysis, it exhibits antifibrinolytic properties by inhibiting the activation of plasminogen into plasmin, which degrades fibrin fibers and removes thrombus. Plasminogen activation is facilitated by tissue plasminogen activator (tPA) and urokinase but also indirectly via kallikrein [32].

There is a positive correlation between the increased expression of the *A2M* gene in mild cases and the elevation of serum D-dimer levels, with a correlation coefficient of 0.162, and in severe cases, with a correlation coefficient of 0.136. Phylogenetically, an A2-M inhibitor binds a broad spectrum of proteases, capturing the protease separately from its substrate and physiological molecular receptors [30]. In another study, the average level of APC-α2-M complexes was lower in (venous thromboembolism) VTE patients (0.7 ng/mL; 0.0–1.3) compared to controls (1.3 ng/mL; 0.6–2.0). Low binding of APC-α2-M complexes is also a risk factor for VTE [33].

Conclusion

The variations in gene expression involved in coagulation cascade processes in COVID-19 patients were observed and showed the potential for increases or decreases. Transcriptomic analysis revealed increased expression of profibrinolytic gene (*PLAUR*) and fibrinolytic inhibitor genes (*SERPINE1* and *A2M*) in moderate-to-severe COVID-19 patients compared to those with mild cases. The final product in the coagulation cascade, chains of stable fibrin clots, must remain a balance between the formation and breakdown processes, which can be assessed through markers of increased serum D-dimer in COVID-19 patients. There is a relationship between increased D-dimer levels and increased expression of profibrinolytic (*PLAUR*) and fibrinolytic inhibitor (*SERPINE1* and *A2M*) genes in COVID-19 patients.

Ethics approval

This research protects and maintains the confidentiality of patient data and obtained ethical clearance with research approval number 454/KEPK/2021.

Acknowledgments

We thank everyone who helped with this research: the research participants, the reviewers for valuable comments, and the infectious diseases integrated diagnostic and research laboratory of Universitas Andalas.

Competing interests

All the authors declare that there are no conflicts of interest.

Funding

This research grant is supported by part of the doctoral dissertation research scheme of the Indonesian Ministry of Education, Culture, Research, and Technology with contract number 115/E5/PG.02.00.PL/2023.

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

Febrianti IK, Putra AE, Raveinal R, Elliyanti A. Transcriptomic analysis of profibrinolytic and fibrinolytic inhibitor genes in COVID-19 patients. Narra J 2024; 4 (2): e843 - http://doi.org/10.52225/narra.v4i2.843.

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