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Transcriptomic Analysis of Coagulation Gene Expression in COVID-19 Patients

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***Abstract*—The immunopathogenesis of COVID-19 infection is reported to begin with the entry of the SARS-CoV-2 virus into the human body through droplets, entering the lungs, and binding to the ACE-2 receptor. Meanwhile, activated macrophages stimulate an immune and inflammatory response, activating the coagulation cascade. This activation in COVID-19 patients can exacerbate the condition and may result in acute respiratory distress syndrome (ARDS). The coagulation cascade is influenced by the expression of related genes, including F3, F5, F8, F12, F13A1, VWF, THBD, PROC, PROCR, PROS1, SERPINE1, A2M, and PLAUR. This cross-sectional study was conducted in 2022, with 22 mild COVID-19 cases, 35 moderate-to-severe cases, and 20 healthy control individuals from Dr. M. Djamil Padang General Hospital in West Sumatra, Indonesia. Coagulation gene expression data using the RNA-Seq method with transcriptomic analysis were collected and recorded in transcript per-million (TPM) units. The results showed a significant difference in the expression of coagulation genes in moderate-to-severe compared to mild COVID-19 patients and healthy controls. The analysis of $\log_2$ folds change has a statistically significant increase observed in the expression of coagulation genes and a significant difference in the expression of coagulation genes among moderate-to-severe and mild patients, as well as healthy controls. The results underscored the impact of COVID-19 infection on the activity of coagulation cascade genes, which could influence the condition of patients and serve as a reference for therapy.**

***Keywords*—Transcriptomic; coagulation; gene expression; inflammatory; COVID-19 patients.**

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I. INTRODUCTION

The WHO declared the SARS-CoV-2 infection as a global pandemic that spreads through the respiratory system with a high mortality rate, specifically among patients with comorbidities. Although many infected patients do not show severe symptoms, approximately 10% experience severe respiratory symptoms leading to acute respiratory distress syndrome (ARDS) [1]–[4]. COVID-19 infection caused by SARS-CoV-2 in the lungs leads to disruptions in epithelial and endothelial cells and the infiltration of inflammatory cells. These changes result in the formation of proinflammatory cytokines and the triggering of an exaggerated immune response. In this context, the excessive immune response is characterized partly by increased coagulation activity [5]–[7].

Angiotensin-converting enzyme 2 (ACE2), which acts as the SARS-CoV-2 receptor when binding with the virus, undergoes reduced expression and activates the renin-angiotensin system (RAS), leading to increased platelet adhesion and aggregation. Meanwhile, RAS regulates the decrease in fibrinolytic activity [8], [9]. Recognizing COVID-19 antigens by phagocytic cells triggers the activation and production of pro-inflammatory cytokines such as TNF-α, IL-6, and IFN-ɣ. These changes activate the coagulation cascade through the intrinsic pathway initiated by FXII activation (encoded by the F12 gene) and subsequently by FXI, FIX, and FVIII encoded by the F8 gene. On the other hand, the extrinsic pathway is initiated by tissue factors encoded by the F3 gene and continues with FVII. Both of these pathways activate FX with the assistance of the cofactor FV encoded by the F5 gene, which converts prothrombin into thrombin. In response,

thrombin converts fibrinogen into fibrin, transforming into a cross-linked clot with the help of FXIII encoded by the F13A1 gene. The cross-linked fibrin undergoes fibrinolysis through the action of plasmin, resulting in the degradation of products such as D-Dimer. Additionally, α2-macroglobulin (A2M) plays a role in maintaining hemostatic balance in the regulation of fibrinolysis [10], [11]. In addition, the ABO group has a substantial quantitative and qualitative effect on VWF. It also influences several aspects of platelet function, and It has a relationship between von Willebrand factor and primary hemostasis [12]–[14].

Endothelial injury leads to an increase in the levels of PAI-1 (plasminogen activator inhibitor-1) and VWF (von Willebrand factor) encoded by the SERPINE1 gene encoded by the VWF gene, heightened platelet activation, and a hypercoagulable state, resulting in venous, arterial, and microvascular thrombosis [15],[16]. Furthermore, in the lungs, there is a decrease in the regulation of endothelial cell genes thrombomodulin and protein C receptor encoded by the PROCR gene, compared to the non-COVID group [17]. Activated protein C encoded by the PROC gene inactivates coagulation factors Va and VIIIa with the help of the protein S cofactor encoded by the PROS1 gene [18]-[20].

Characterizing key molecular and cellular pathways involved in COVID-19 is essential for disease prognosis and management. The dysfunction of human host genes and proteins in COVID-19 caused by SARS-CoV-2 is a key factor impacting clinical symptoms and outcomes [21]. The rapid transcriptome analysis of nasopharyngeal swabs can be a powerful approach to quantifying host molecular response associated with platelet hyperreactivity and may provide valuable insights into COVID-19 pathophysiology [22], [23]. Utilizing transcriptome approaches, previous researchers have been able to ascertain various aspects of SARS-CoV-2 structure, entry, and replication [24]. Additionally, the use of tocilizumab treatment has been shown to accelerate the resolution of lymphopenia and myeloid dysregulation linked to severe COVID-19 [25].

There has been no comprehensive mRNA transcriptomic analysis study on coagulation factors using blood samples. Existing studies only describe the DNA expression of genes separately. Meanwhile, transcriptomic studies of several coagulation genes only use alveolar lavage samples [21]-[22], [24]-[25]. The limited number of genes studied resulted in transcriptomic analysis information only providing a small-scale analysis. This study attempted to describe the gene expression in the control, mild, and moderate-to-severe groups. This urgent research must be done immediately to understand the expression of certain genes in moderate-to-severe and mild patients for COVID-19, which could influence the condition of patients and serve as a reference for therapy.

Investigations using genetic approaches to describe gene expression play a crucial role in understanding the biochemical events in response to the COVID-19 virus within the body. Gene expression during transcription can be crucial in assessing the morphological changes of a disease and its therapy. Therefore, this study aimed to analyze changes in coagulation gene expression in the blood of COVID-19 patients using transcriptomic methods.

II. Materials and Method

This research was an analytical observational study with a cross-sectional design conducted from October 2021 to April 2022. The samples included 20 healthy control, 22 mild, and 35 moderate-to-severe COVID-19 patients at Dr. M. Djamil Central General Hospital in Padang, West Sumatra, Indonesia. In determining the minimum sample through a categorical test of unpaired data with a 5% significance level, a minimum of 20 patients were obtained in each group.

The study was conducted at the Biomedical Laboratory, Faculty of Medicine, Andalas University. The inclusion criteria for the samples included individuals willing to participate, providing informed consent, healthy controls with negative RT-PCR results, confirmed COVID-19 patients with RT-PCR-tested clinical symptoms ranging from mild asymptomatic to severe, and aged 18 years or older. On the other hand, exclusion criteria included patients with liver cirrhosis, HIV (+) positive, pregnant, and those with blood coagulation disorders such as hemophilia.

The laboratory conducted sample collection, RNA isolation, library preparation, and sequencing. Basic patient data, results of routine blood laboratory tests comprising hemoglobin, leukocytes, and platelets, as well as D-Dimer and comorbidities, were obtained from patient's medical records. Whole blood samples from healthy controls and patients with mild to severe COVID-19 symptoms underwent transcriptomic mRNA examination. This was achieved following the Illumina stranded Total RNA prep kit protocol, including ligation with Rebo-zero, until the final RNA sequencing stage.

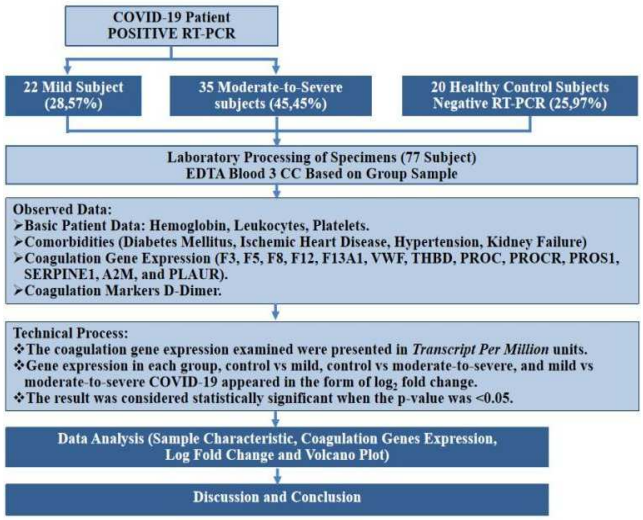

Fig. 1 The flowchart in the block diagram of the research.

Confidentiality was protected by adhering to the principles of medical ethics, and ethical clearance was obtained with permit number 454/KEPK/2021. The results of the coagulation gene expression examined were presented in Transcript Per Million (TPM) units. Gene expression in each group, control vs. mild, control vs. moderate-to-severe, and mild vs. moderate-to-severe COVID-19, appeared in $\log_2$ fold change. The result was considered statistically significant when the *p*-value was <0.05. The flowchart of this study is presented in a block diagram, as shown in Fig. 1. Each

step in this research method is explained according to the research flow.

III. Results and Discussion

A. Sample Characteristics

The samples included 20 healthy controls, 22 mild, and 35 moderate-to-severe COVID-19 patients, totaling 77 people. Table I shows more females than males; the majority in the moderate-to-severe group were aged >50-65 years (42.86%). Meanwhile, in the mild COVID-19 and healthy control groups, the majority were aged 18-50, 77.27% and 85%, respectively. The most common comorbidity among the patients was hypertension, particularly in moderate-to-severe (51.43%) and mild cases (22.73%).

The samples, based on laboratory examinations of hemoglobin, leukocytes, platelets, and D-Dimer levels, had different variations in the conditions of patient groups. The median hemoglobin (Hb) across all groups had nearly the same value. However, the distribution in the moderate-to-severe group showed a fairly wide range of Hb levels, ranging from 6.70 to 16.60 g/dL. The median leukocyte levels increased with the severity of the COVID-19 condition in patients, namely in mild cases 10.80 (7.50-23.80) ($x10^3/mm^3$) and in moderate-to-severe cases 11.03 (4.48-27.75) ($x10^3/mm^3$). The platelet levels in the moderate-to-severe group had a median of 220.00 (0.24-561.00) ($x10^3/mm^3$). D-Dimer values appeared to significantly increase in moderate-to-severe COVID-19 patients compared to mild cases and controls, with a value of 2203.00 (289.00-10000.00) ng/ml.

TABLE I
CHARACTERISTICS AND DISTRIBUTION OF MILD AND MODERATE-TO-SEVERE COVID-19 PATIENTS AND CONTROLS

Variable	Healthy Control	Mild COVID-19 Patients	Moderate-to-Severe COVID-19 Patients
Gender			
Male	4 (20%)	5 (22.72%)	17 (48.57%)
Female	16 (80%)	17 (77.27%)	18(51.42%)
Age			
18-50 years	17 (85%)	17 (77.27%)	10 (28.57%)
>50-65 years	3 (15%)	5 (22.72%)	15 (42.86%)
>65 years	0	0 (0)	10 (28.57%)
Comorbid			
Diabetes Mellitus	0	2 (9.09%)	9 (25.71%)
Ischemic Heart Disease	0	2 (9.09%)	3 (8.57%)
Hypertension	1 (5%)	5 (22.73%)	18 (51.43%)
Kidney Failure	0	0	3 (8.57%)
Laboratories			
Hemoglobin (g/dL), median (min-max)	13.00 (10.60-14.00)	12.00 (9.70-16.80)	11.60 (6.70-16.60)
Leukocyte ($x10^3/mm^3$), median (min-max)	8.30 (5.8-10.40)	10.80 (7.50- 23.80)	11.03 (4.48-27.75)
Thrombocyte ($x10^3mm^3$), median (min-max)	174.00 (145.00-280.00)	187.00 (134.00-561.00)	220.00 (0.24-561.00)
D-Dimer (ng/ml), median (min-max)	260.00 (190.00-450.00)	290.00 (110.00-1160.00)	2203.00 (289.00-10000.00)

B. Coagulation Gene Expression in Healthy Controls Along with Mild and Moderate-to-severe COVID-19 Patients

The results showed a difference in the expression of coagulation genes in patients with moderate-to-severe COVID-19 compared to mild and healthy controls. The coagulation gene expression, collected and recorded in TPM units, was compared between groups using log_2 fold change results using a volcano plot and bar diagram. Table II compares coagulation gene expression in healthy controls vs mild COVID-19 patients. The results showed mild F12 gene expression was 0.94x higher than control, while F3 gene expression was 4.21x control. The mild VWF gene expression was 1.21x control, F13A1, THBD, PROC, and PROS1 expression was 0.89x, 0.33x, 0.56x, and 0.92x control, respectively. Mild A2M gene was 0.74x greater than the control, PLAUR gene was 1.11x control, SERPINE1 was 1.55x control, F5 gene was -0.33x control, F8 was -0.78x control, and PROCR gene was -1.03x control.

The comparison between the mild and the control group is illustrated in Fig. 2. In the volcano plot, the genes with decreased and increased expression are depicted on the left and right sides respectively. The Closer to zero indicates less change for only few genes while moving away from zero in either direction happened for more genes expression to indicate more change between healthy controls versus mild COVID-19 patients, further illustrated with a bar diagram of log_2 fold change also available to indicates that differ significantly for healthy controls vs mild COVID-19 patients.

TABLE II
COMPARISON OF COAGULATION GENE EXPRESSION IN HEALTHY CONTROLS VS MILD COVID-19 PATIENTS

Gen	Log_2 fold change	p-value
F5	-1.85	2.93E-07
PROS1	0.92	0.01
F3	4.21	2.58E-07
VWF	1.21	1.38E-03
F8	-0.78	0.02
F13A1	0.89	1.59E-03
THBD	0.33	0.31
SERPINE1	1.55	8.13E-04
F12	0.94	2.13E-03
A2M	0.74	0.16
PROCR	-1.03	0.04
PROC	0.56	0.18
PLAUR	1.11	1.51E-04

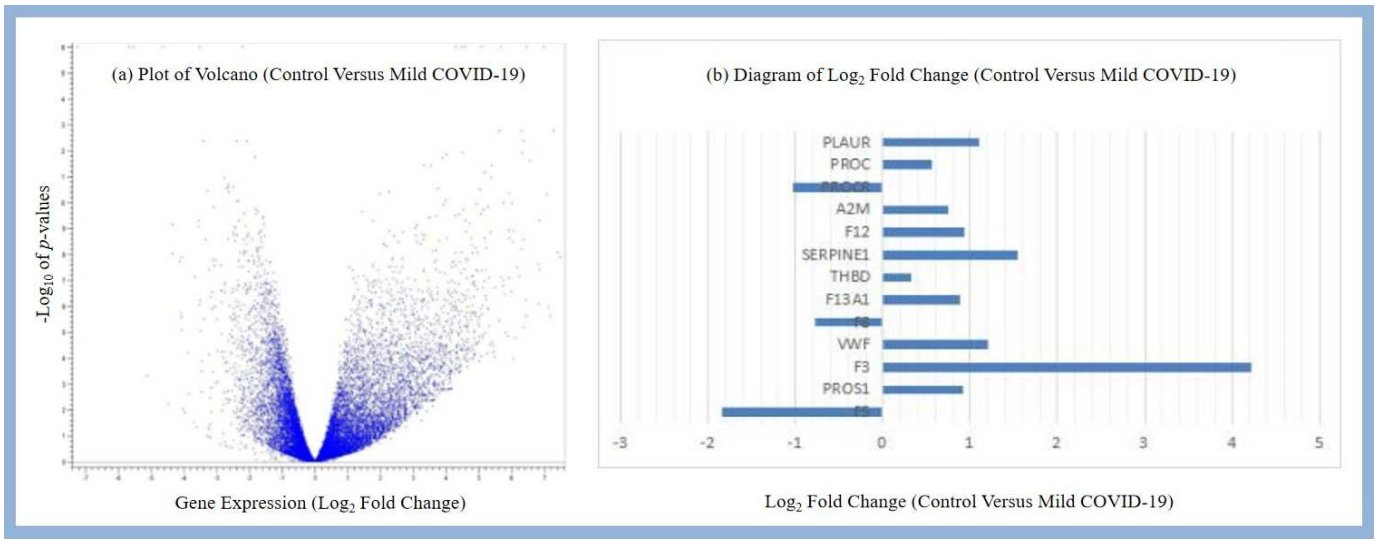

Fig. 2 Comparison of coagulation gene expression in healthy controls vs mild COVID-19 patients in volcano plot and log2 fold change.

TABLE III
COMPARISON OF COAGULATION GENE EXPRESSION IN HEALTHY CONTROLS VS MODERATE-TO-SEVERE COVID-19 PATIENTS

Gen	Log_2 fold change	p-value
F5	1.94	1.44E-05
PROS1	2.69	8.47E-07
F3	3.45	6.61E-06
VWF	1.66	7.21E-04
F8	1.09	2.14E-03
F13A1	1.63	1.80E-04
THBD	2.37	3.49E-09
SERPINE1	2.36	4.27E-05
F12	1.77	2.39E-05
A2M	0.34	0.5
PROCR	-1.61	4.46E-04
PROC	-0.24	0.64
PLAUR	1.56	5.86E-06

Table III compares the coagulation gene expression between healthy controls and moderate-to-severe COVID-19 patients. The expression of the moderate-to-severe F12 gene was 1.77x higher than the control, and there was also an increase in the expression of the moderate-to-severe F3 gene by 3.45x of the control. Furthermore, the moderate-to-severe F5 gene was 1.94x control, the VWF gene was 1.66x, the F8 gene was 1.09x, and the F13A1 gene was 1.63x control. Moderate-to-severe THBD gene was 2.37x, PROS1 was 2.69x, A2M gene was 0.34x, and PLAUR gene was 1.56x control. Moderate-to-severe SERPINE1 gene was 2.36x, PROC was -24x, and PROCR gene was -1.61x control.

The comparison between the moderate-to-severe and the control group is illustrated in Fig. 3. In the volcano plot, decreased and increased gene expressions were represented on the left and right sides. The Closer to zero indicates less change for only a few genes, while moving away from zero in either direction happened for more gene expression to indicate more change between healthy controls vs moderate-to-severe COVID-19 patients, further illustrated with a bar diagram of log_2 fold change also available to indicates that differ significantly for healthy controls vs moderate-to-severe COVID-19 patients.

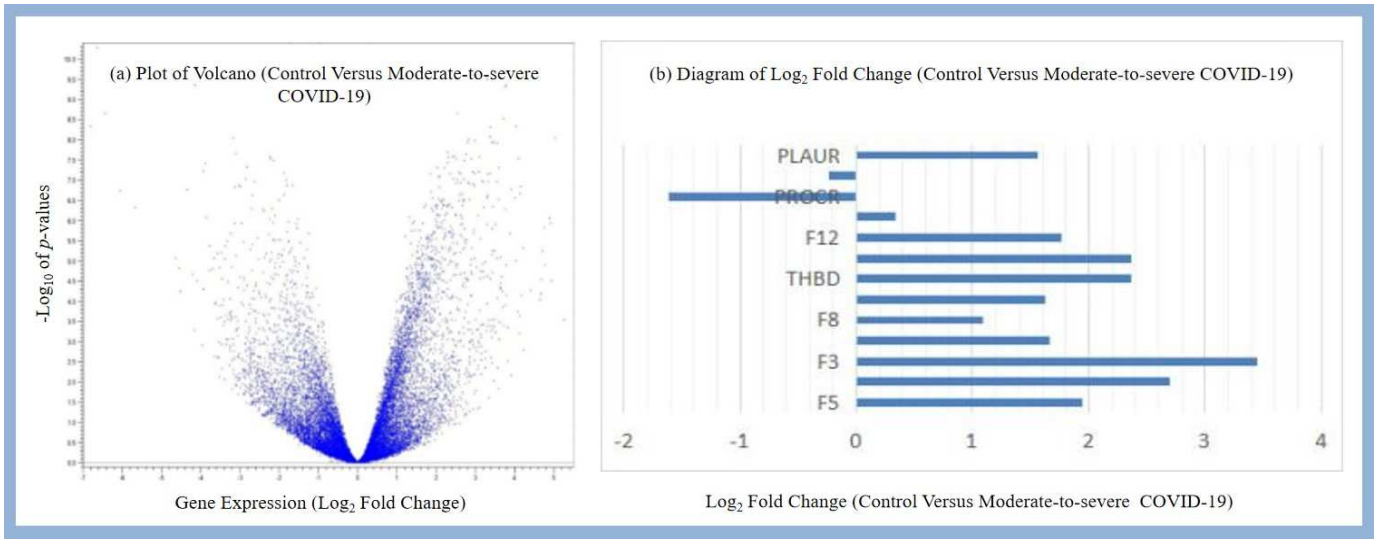

Fig. 3 Comparison of expression of coagulation genes in healthy controls vs moderate-to-severe COVID-19 patients in volcano plot and log_2 fold change.

TABLE IV
COMPARISON OF COAGULATION GENE EXPRESSION IN MILD VS MODERATE-TO-SEVERE COVID-19 PATIENTS

Gen	Log_2 fold change	p-value
F5	3.73	1.21E-12
PROS1	1.72	4.51E-04
F3	-0.74	0.26
VWF	0.49	0.26
F8	1.74	7.20E-06
F13A1	0.77	0.06
THBD	2.08	5.22E-07
SERPINE1	1.21	0.01
F12	0.89	0.03
A2M	-0.48	0.37
PROCR	-0.58	0.28
PROC	-0.92	0.1
PLAUR	1.87	5.51E-06

Table IV compares the coagulation gene expression between mild and moderate-to-severe COVID-19 patients. The expression of the F12 gene in the moderate-to-severe COVID-19 patients was 0.89x higher than in the mild group, while the F5 gene in the moderate-to-severe was 73x mild. The moderate-to-severe VWF gene was 0.49x mild, and the moderate-to-severe F8 gene was 1.74x mild. Furthermore, the moderate-to-severe F13A1 gene was 0.77x, the THBD gene was 2.08x, the PROS1 gene 1 was 72x, the PLAUR gene was 1.87x, and the SERPINE1 gene was 1.21x mild. The moderate-to-severe F3 gene was 0.74x greater than the mild, while the PROC gene was -0.92x, the PROCR gene was -0.58x, and the A2M gene was -0.48x lower than the mild.

The comparison between the mild and moderate-to-severe groups is illustrated in Fig. 4. In the volcano plot, decreased and increased gene expressions were illustrated on the left and right sides. The Closer to zero indicates less change for only a few genes, while moving away from zero in either direction happened for more gene expression to indicate more change between mild vs moderate-to-severe COVID-19 patients, further illustrated with a bar diagram of log2 fold change also available to indicate that differ significantly for mild vs moderate-to-severe COVID-19 patients.

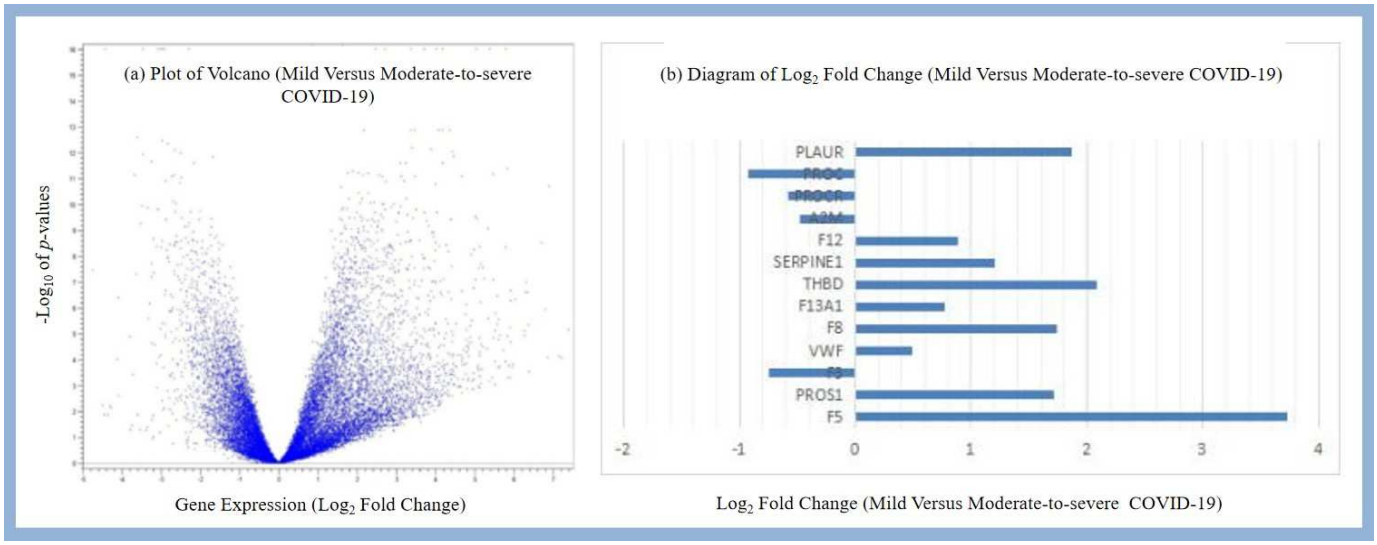

Fig. 4 Comparison of expression of coagulation genes in mild vs moderate-to-severe COVID-19 patients in volcano plot and log_2 fold change.

C. Discussion Based on the Results and Finding

In this study, the number of female COVID-19 patients was higher than that of males; a similar trend was found, reporting a higher percentage of female patients at 55.9% compared to males [26]. However, some other studies show that males have higher morbidity and mortality rates related to COVID-19 compared to females. In a study conducted in Denmark, the male gender was associated with higher rates of death, severity, and ICU care compared to females [27]. Most mild COVID-19 patients were between 18 and 50 years old, while in the moderate-to-severe group, most were aged >50-65 years. It also found similar results, where, out of a total of 49 moderate-to-severe cases, the median age of patients was 56 years, ranging from 39-73 years [28]. Furthermore, the most common comorbidity among the patients in this study was hypertension. Many other studies also describe similar results, and this is often associated with the high prevalence of hypertension in various regions worldwide, including Indonesia [29].

Based on the results, the expression of the F12 gene increased 0.94 times higher in mild COVID-19 patients compared to control. The expression of the F12 gene was 1.77 times higher in moderate-to-severe patients compared to control and 0.89 times higher in moderate-to-severe compared to mild patients. This study also found increased F12 gene expression in postmortem lung tissues of COVID-19-positive patients. The increase was attributed to the role of neutrophil extracellular traps (NETs) in activating contact activation of FXII [30]. In this context, an excessive accumulation of NETs occurs in the plasma and lung tissues of COVID-19 patients. NETs act as a pathway for contact activation of FXII in COVID-19 and potentially other NET-mediated disease states. FXIIa levels also increase in the plasma, coinciding with a rise in endothelial barrier permeability, leading to pulmonary edema in COVID-19 patients [30].

The results showed increased expression of the VWF gene in COVID-19 patients. The expression was 0.49 times higher in mild, 1.66 times higher in moderate-to-severe, and 1.21

times higher in mild patients compared to the control. The changes in VWF reflect activated/infected endothelium, leading to the loss of endothelial barrier function and the release of VWF. These changes enable platelet binding and TF expression, activating the subsequent coagulation system [31].

THBD gene expression in COVID-19 patients increased, namely moderate-to-severe patients 2.08x mild, moderate-to-severe patients 2.37x control, and mild 0.33x control. Furthermore, THBD values increased significantly in patients requiring ICU admission compared to non-ICU [32]. There was a significant increase in thrombomodulin TM (THBD) transcripts in CD14+ monocytes. Increased expression of CD14+ monocyte THBD transcripts also dampens excessive coagulation and inflammatory responses to SARS-CoV-2 infection [33].

PROC gene expression in the COVID-19 patient group varied, with the level in mild patients being 0.56x higher than in controls. In moderate-to-severe patients, the decrease was 0.92x for mild, and in moderate-to-severe patients, the decrease was 0.24x for controls. A study conducted at a Cairo hospital reported a statistically significant decrease in protein C encoded by the PROC gene associated with increased disease severity. Correspondingly, the PROC gene encoded by protein C in BALF samples experienced a 226.5-fold increase in expression compared to the control [34],[35].

The protein C receptor (PROCR) gene is a single-pass transmembrane protein expressed in endothelial cells, with crucial roles in anticoagulation and inflammation. PROCR gene expression in moderately severe COVID-19 patients was 0.58x higher than in mild cases. Moderately severe PROCR was -1.61x control, while in the mild patient group, it decreased to 1.03x control. PROCR-bound APC triggers protease-activated receptor-1 (PAR-1), producing anti-inflammatory and cytoprotective effects. This limits thrombus formation by binding FVII/FVIIa procoagulants, facilitating FVIIa clearance, and limiting the tissue factor (extrinsic) coagulation pathway [17].

There was an increase in F3 gene expression in COVID-19 patients, namely moderate-to-severe patients with 3.45x controls and mild 4.21x controls. Meanwhile, moderate-to-severe F3 gene expression decreased by 0.74x mild. Another study reported a 5.2-fold increase in the expression level of the tissue factor F3 gene transcript [33]. Generally, TF expression increases in various thrombo-inflammatory disorders, and its overexpression is important for promoting consumptive coagulation. The lack of increase in F3, coupled with the rise in TFPI (Tissue factor pathway inhibitor) transcripts, raises the possibility that other pathways unrelated to the FVIIa/TF complex may play an important role in COVID-19-associated thrombosis [35].

F5 gene expression in moderately severe COVID-19 patients increased by 3.73x in mild patients, 1.94x in controls in moderately severe, and 0.33x in mild patients. A previous study reported increased factor V activity in moderate-to-severe COVID-19 patients. This was attributed to an elevation in the number of megakaryocytes in the lungs, heart, and other organs of COVID-19 patients. Megakaryocytes produce platelets, which usually contain about 20%-25% of factor V in the blood, accounting for its increase in COVID-19 patients [36].

Factor VIII (FVIII) functions as a cofactor in the intrinsic pathway of blood coagulation during FX activation by FIXa. There was an increase in F8 gene expression in moderately severe COVID-19 patients by 1.74x compared to mild cases. The equivalent of moderate-to-severe patients was 1.09x controls, while mild patients decreased by 0.78x controls. The F8 gene encoding FVIII in BALF samples experienced a 4.6-fold decreased expression in moderate-to-severe COVID-19 patients compared to the control group [35].

Proteomic analysis using plasma showed decreased prothrombin and thrombin activation factors XIII (F13A1 and F13B) in COVID-19 compared to non-infected patients. This decline was more pronounced in moderate-to-severe patients [37]. The expression of the F13A1 gene in moderate-to-severe cases increased by 0.77 times compared to mild, 1.63 times compared to the control, and in mild cases, it was 0.89 times higher compared to the control. In contrast, it is found that the expression of the F13A1 gene in moderate-to-severe was 7.55 times lower than in mild cases [38]. The decrease in moderate-to-severe and critical COVID-19 cases was attributed to the rapid increase in the consumption of anticoagulant and procoagulant proteins in the early phase. This may lead to coagulopathy in the lungs or a poor prognosis [39].

An increase was observed in the expression of the PROS1 gene among COVID-19 patients, with a 1.72 times higher level in mild patients, 2.69 times higher in moderate-to-severe compared to the control, and 0.92 times greater in mild compared to the control. This was in contrast to other studies where a decrease in protein S was observed. The decrease may be caused by uncontrolled inflammation observed in COVID-19 patients and the resulting multi-organ failure. Protein S may be depleted due to its role in the coagulation process or production failure by endothelial cells attributed to SARS-CoV-2 infection [40].

PLAUR expression in moderately severe COVID-19 patients was 1.87x higher than in mild cases. The expression increased by 1.56x in the moderately severe group compared to the control and 1.11x in a mild group than the control. Local activity of PAI-2 (plasminogen activator inhibitor-2) can significantly inhibit the effects of PLAU/PLAUR and thereby contribute to the formation of pulmonary embolism and distal coagulopathy [33].

There was an increase in the expression of the SERPINE1 gene in moderate-to-severe COVID-19 patients, with 1.21 times higher levels than in mild patients. In moderate-to-severe patients, it increased by 2.36 times; in mild cases, the expression was 1.55 times higher than in the control. This increase was attributed to the renin-aldosterone-angiotensin system (RAAS) associated with the SARS-CoV-2 spike protein binding to the ACE2 receptor in host cells. In alveolar type II cells, this leads to a decrease in surfactant and induction of the p53 pathway, resulting in an increase in PAI-1 (SERPINE1 gene) and a decline in uPA and uPAR [41].

The expression of the A2M gene in moderately severe COVID-19 patients decreased by 0.48x compared to mild cases. Meanwhile, the expression in the moderate-to-severe group was 0.34x higher, and the mild group increased by 0.74x compared to the control. This increase was attributed to the endothelium in COVID-19. The α2-M gene protects the

vascular endothelium by modulating various protease-producing reactions adjacent to the endothelial surface [10].

In this study, diabetes, recognized as the second most common comorbidity after hypertension, was found to be associated with a worse prognosis in COVID-19. The A2M gene can bind to insulin in the serum and influence insulin activity in target cells. Elevated serum A2M reduces insulin bioavailability, disrupting blood sugar control [42].

In this study, all cofactors seemed to drop in moderate-to-severe patients compared to mild patients. Gene mutations that code for the production of proteins are just one of the several factors that might impact the rise or fall in gene expression. Non-coding RNA, another term for expressed genes, is RNA that does not code for the production of proteins. The expression of coagulation-related genes is influenced by numerous positive and negative pathways [39]. Genetic diversity is the primary factor separating each individual; therefore, it cannot be used as a point of comparison between individuals. This difference also relates to an individual's age or gender. This process may be interrupted if sampling for coagulation gene expression testing reveals variations in gene expression linked to sites associated with higher internal concentrations and activity in COVID-19 patients' areas of initial infection [39]–[42].

Furthermore, the procedure at the moment of sampling will also present a varied picture of the coagulation process. At each sample time point, the coagulation cascade, which starts with the intrinsic and extrinsic routes and ends with forming a fibrin clot, will present a distinct value image. Following the completion of one coagulation step, the next one will begin, increasing the levels and expressions under the still-in-progress reaction. The expression of the coagulation gene itself will be influenced by the patient's condition at the time of sampling, including any prior treatments.

In the end, nevertheless, each of these phases will result in a state of bodily homeostasis with coagulation factors balanced about one another, including procoagulant factors, anticoagulants, cofactors, and fibrinolytic inhibitors, and fibrinolytic factors. All stages that affect the expression of these coagulation genes are an effort by the body to balance all regulations related to COVID-19 infection so that therapy does not need to be given too aggressively in clinical practice. Symptomatic therapy is sufficient to accompany the healing process, except in certain emergencies, including in COVID-19 patients with comorbidities and requiring further comprehensive treatment.

IV. Conclusion

In conclusion, infection with the COVID-19 virus disrupts coagulation regulation and disturbs the balance of hemostasis, leading to a hypercoagulable state. These changes were observed in gene levels, showing alterations in expression and subsequently affecting the entire coagulation system. In the whole blood of COVID-19 patients, changes in coagulation regulation were found, as shown by differences in the expression of genes F12, VWF, THBD, PROC, PROCR, F3, F5, F8, F13A1, PROS1, PLAUR, SERPINE1, and A2M in mild and moderate-to-severe COVID-19 patients. Changes in coagulation regulation, whether through increased or decreased gene expression, constitute part of a process to maintain balance. However, when this balance shifts, it might result in mild to moderate-to-severe manifestations, such as disseminated intravascular coagulation (DIC), ARDS, or even death. Recognizing changes in gene expression can be a specific target in providing therapy to improve the condition of patients and prevent further deterioration. In clinical practice, medication does not need to be administered too aggressively because the body is attempting to balance the regulations linked to COVID-19 infection through all stages that affect the expression of these coagulation genes. Except in specific situations, such as COVID-19 individuals who require more extensive treatment due to comorbidities, symptomatic therapy is adequate to support the healing process.

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