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Relationship between Vascular Cell Adhesion Molecule-1 (Vcam-1) Level with Severity Degree of COVID-19 Patients at General Hospital H. Adam Malik Medan

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ABSTRACT

Background: The mechanism and pathogenesis of COVID-19 that distinguishes asymptomatic, mild, moderate, severe to critical symptoms is not yet known with certainty. Endothelial dysfunction and immune thrombosis serve as a profound pathogenic mechanism in COVID-19. Vascular Cell Adhesion Molecule-1 (VCAM-1) is a major regulator of leukocyte adhesion that contributes to the formation of thrombosis. The purpose of the study determine the relationship between VCAM-1 level with the severity degree in COVID-19 patients.

Methods: An analytical study with a cross-sectional design was conducted from September – March 2022. This study was followed by 50 patients aged over 18 years with moderate, severe, and critical degrees of COVID-19 admitted to Haji Adam Malik General Hospital Medan by excluding malignancy and pregnancy patients. Serum VCAM-1 levels were measured using Chemwell Analyzer.

Results: Among 50 patients, 33 patients (66%) were male and 17 patients (34%) were female. The mean age was 57 years with the youngest 19 years old and the oldest 81 years old. The median level of VCAM-1 was 23,02 ng/mL (10.96 ng/mL - 50.63 ng/mL). The median concentration of VCAM-1 was 37.85 ng/mL (15,59 – 50.63), 36.68 ng/mL (19.58 – 49.71), and 18.83 ng/mL (10.96 – 46.32) for critical, severe, and moderate degree respectively (p = 0.001). The cut-off value of VCAM-1 levels to predict the severity of COVID-19 is 32.01 ng/mL.

Conclusions: There is a significant relationship between VCAM-1 levels and the severity of COVID-19.

Keyword: VCAM-1; COVID-19; Endothelial Dysfunction, Thrombosis

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ABSTRAK

Latar belakang*: Mekanisme dan patogenesis COVID-19 yang membedakan seseorang tanpa gejala, gejala ringan, sedang, berat hingga kritis belum diketahui dengan pasti. Disfungsi endotel dan trombosis imun berperan sebagai mekanisme patogen utama dalam COVID-19. VCAM-1 merupakan regulator terbesar adhesi leukosit yang berkontribusi dalam terbentuknya trombosis. Tujuan penelitian adalah mengetahui hubungan kadar VCAM-1 dengan derajat keparahan COVID-19.*

Metode*: Penelitian analitik dengan desain cross-sectional dilakukan dari bulan September 2021 – Maret 2022 Penelitian ini diikuti 50 orang pasien COVID-19 rawat inap dewasa (>18 tahun) dengan derajat sedang, berat, dan kritis di RSUP Haji Adam Malik Medan. Kriteria eksklusi adalah pasien keganasan dan kehamilan. VCAM-1 diukur dari sampel serum menggunakan Chemwell Analyzer (metode ELISA).*

Hasil*: Dari 50 pasien COVID-19, terdapat 33 pasien laki-laki (66%) dan 17 pasien wanita (34%). Median usia 57 tahun dengan usia termuda 19 tahun dan tertua berusia 81 tahun. Median kadar VCAM-1 adalah 23,02 ng/mL (10.96 ng/mL - 50.63 ng/mL). Median kadar VCAM-1 tertinggi dijumpai pada kelompok derajat kritis, diikuti derajat berat dan derajat sedang secara berurutan 37.85 ng/mL (15,59 – 50,63), 36.68 ng/mL (19,58 – 49,71), and 18.83 ng/mL (10,96 – 46,32). Dengan uji Kruskall-Wallis didapati hubungan yang signifikan antara kadar VCAM-1 dengan derajat keparahan COVID-19 (p = 0,001). Nilai cut off kadar VCAM-1 untuk memprediksi derajat keparahan COVID-19 adalah 32,01 ng/mL.*

Simpulan: *Terdapat hubungan signifikan antara kadar VCAM-1 dengan derajat keparahan COVID-19.*

Kata Kunci: *VCAM-1,COVID-19, Disfungsi Endotel; Trombosis*

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1 Introduction

Corona Virus-19 Disease (COVID-19) is still a major problem in the world. WHO data shows an increase in cases that continue to occur up to 225 million cases have been reported and the number of deaths is around 4.6 million. Most cases are found in America, India, and Brazil. In Indonesia alone, confirmed cases ranged from 4,167,511 cases with 138,889 deaths as of September 12, 2021.[1] COVID-19 was caused by the novel beta coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which was first encountered in Wuhan, China in 2019. Based on the World Health Organization (2020), COVID-19 symptoms vary from mild (40%), moderate (40%), severe (15%), and critical (5%) with complications such as respiratory failure, sepsis and sepsis shock, thromboembolism, and the presence of multiple organ failure. In general, 3-10% of people infected with SARS-CoV-2 require hospitalization, but up to 20% have severe cases and require intensive care (ICU) with a high mortality rate.[2] The mechanism and pathogenesis of COVID-19 that distinguishes a person without symptoms, mild, moderate, severe to critical symptoms are not yet known with certainty.

Endothelial dysfunction and immune thrombosis play a major pathogenic mechanism in COVID-19. Strong evidence suggests that hyperthrombosis occurs, especially in the pulmonary alveoli due to immune thrombosis. Patients with COVID-19 also in post-mortem patients who died as a result of being infected with SARS-CoV-2 experienced a prothrombotic status with

manifestations of the presence of microthrombosis. The concept of immune thrombosis was first introduced in 2013, namely the symbiotic relationship of the coagulation system with the innate immune system, especially in the acute infectious phase.[3]

Leukocytes are the main regulator in the thrombi inflammatory process and an important component of the cause of thrombosis. The activation of platelets and leukocytes not only goes both ways to fight pathogens entering the body, but also activates each other's proinflammatory mediators as the body's defenses to form platelet aggregates- leukocytes where their expression depends on mediator classes such as cytokines (IL-1, IL-6 and IL-8), chemokines, and adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).[4]

Pathological signs of the presence of thromboembolism, viral infections of endothelial cells, and endothelium inflammation are also reported.[5] Endothelial activation indicates the severity of COVID-19. VCAM-1 is a glycoprotein compound induced by various inflammatory cytokines that form a strong bond between α4β1-integrin leukocytes and endothelium.[6] VCAM-1 levels are used to prove that there is endothelium involvement with the severity of COVID-19 disease, so endothelium marker examination is needed. The purpose of the study determine the relationship between VCAM-1 level with the severity degree in COVID-19 patients.

2 Method

This research is an analytical study with a cross-sectional data collection method. Among 50 samples of COVID-19, patients were collected from January 2022 – March 2022, who met the inclusion criteria, namely adult patients (≥ 18 years) with moderate, severe, and critical degrees yet patients with malignancy and pregnancy are excluded. This research was carried out at the Department of Clinical Pathology FK USU / RSUP Haji Adam Malik Medan. VCAM-1 levels were measured from serum samples using the Chemwell Analyzer (ELISA method).

This research was carried out after obtaining permission (ethical clearance) from the Ethics Committee for Research in the Health Sector, Faculty of Medicine, University of North Sumatra /RSUP. H. Adam Malik Medan. All patients who were willing to participate in this study provided informed consent in writing from the study subjects or were represented by their families. The patient will be explained in advance the benefits of research and the side effects that may occur.

3 Results

This study was attended by as many as 50 people who were COVID-19 patients who were hospitalized at RSUP Haji Adam Malik Medan. All patients included in this study met the inclusion and exclusion criteria. The characteristics of the subjects are presented in Table 1. The subjects were mostly male 33 people (66%) and 17 people (34%) were women. The median age

of the subjects was 57 years with the youngest age being 19 years and the oldest aged 81 years. A total of 23 subjects (46%) had a history of hypertension and 13 subjects (26%) had a history of DM. A history of CKD was found in as many as 11 people (22%) subjects. From the results of the COVID-19 severity examination, most of them were 36 people (72%) with moderate severity followed by 11 people (22%) with severe severity and 3 people with critical severity (6%). Of the 50 people with COVID-19 involved in this study, 14 patients (28%) died

Table 1 Characteristics of the Research Subjects

Subject Characteristics	**n = 50**
Gender, n (%)	
Man	33 (66)
Woman	17 (34)
Age, years	
Average (SD)	53,38 (15,71)
Median (Min-Max)	57 (19 – 81)
Hypertension, n (%)	
With Hypertension	23 (46)
Without Hypertension	27 (54)
Diabetes Mellitus, n (%)	
With Diabetes Mellitus	13 (26)
Without Diabetes Mellitus	37 (74)
CKD, n (%)	
With CKD	11 (22)
Without CKD	39 (78)
Severity of Covid 19, n (%)	
Moderate	36 (72)
Severe	11 (22)
Critical	3 (6)

Based on Table 2 displays the VCAM-1 levels of all subjects involved in this study. The median content of VCAM-1 is 23.02 ng/mL with the lowest content of 10.96 ng/mL and the highest level of 50.63 ng/mL.

Table 2 VCAM-1 Levels in COVID-19 Patients

VCAM-1, ng/mL	**n = 50**
Average (SD)	25.47 (11.37)
Median (Min-Max)	23.02 (10.96 – 50.63)

Table 3 shows the relationship between VCAM-1 levels and the severity of COVID-19. In subjects of moderate degree 36 people showed VCAM-1 levels with a median of 18.84 ng/mL (10.96 ng/mL – 46.32 ng/mL). In subjects with a severe degree of 11 people showed VCAM-1 levels with a median of 36.68 ng/mL (19.58 ng/mL – 49.71 ng/mL). In subjects with a critical degree of 3 people showed VCAM-1 levels with a median of 37.85 ng/mL (15.59 ng/mL – 50.63

ng/mL). Using the Kruskal Wallis test, it showed that there was a significant relationship between VCAM-1 levels and the severity of COVID-19 patients (p = 0.001).

Table 3 Relationship of VCAM-1 Levels with Severity of COVID-19 Patients

Degree of Severity COVID-19	n	VCAM-1, ng/mL		p*
		Average (SD)	Median (Min – Mak)	
Moderate	36	21.43 (8.96)	18.84 (10.96 – 46.32)	0.001
Severe	11	36.21 (8.82)	36.68 (19.58 – 49.71)	
Critical	3	34.69 (17.73)	37.85 (15.59 – 50.63)	

*Kruskal Wallis

The ability of VCAM-1 as a Predictor of COVID-19 Disease Severity

In this study, the division of COVID-19 severity degrees was divided into two categories, namely mild-moderate degrees with a total of 36 people and severe-critical degrees totaling 14 people. The results of the analysis using the ROC curve (Figure 1) obtained the AUC area of the VCAM-1 value in predicting the severity of the COVID-19 disease was 85.5% with a p-value < 0.001 and 95% IK 73.8% - 97.3%. This shows that the VCAM-1 value can be used to predict the severity of the COVID-19 disease with a good level of ability (AUC > 80% - 90%).

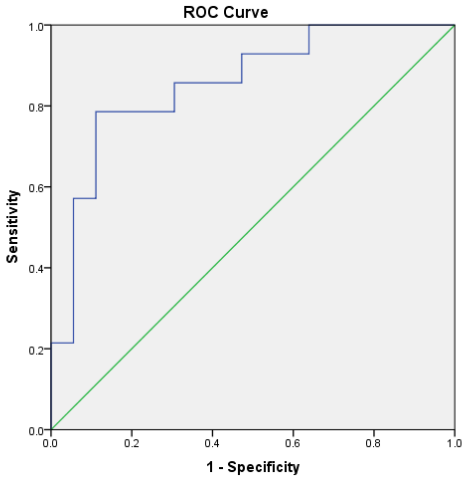

Figure 1 ROC Curve VCAM-1 Value as a Predictor of COVID-19 Disease Severity

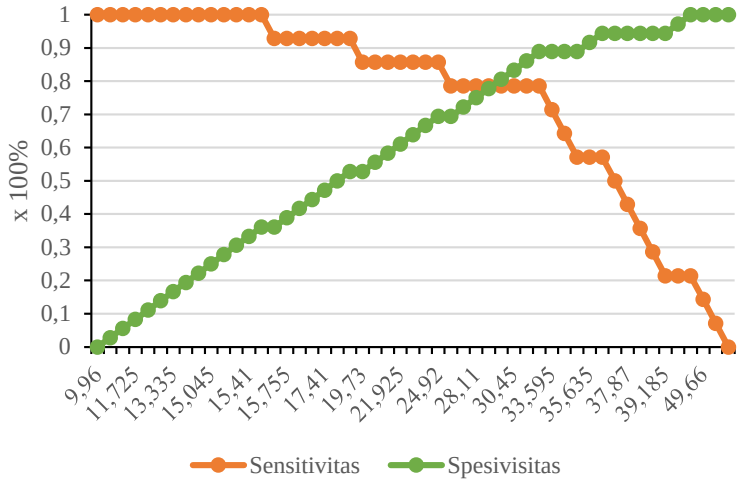

Figure 2 Graph of Sensitivity and Specificity of VCAM-1 Values to the Severity of COVID-19 Disease

Using the VCAM-1 cut-off value of 32.01 to predict the severity of COVID-19 disease, sensitivity, and specificity values were obtained at 78.6% and 88.9%, positive suspect values of 73.3% and negative suspect values of 91.4%, Accuracy of VCAM-1 values was 86% (Table 4).

Table 4 Accuracy of VCAM-1 Values in Predicting the Severity of COVID-19 Disease

VCAM-1	Degree of Severity		Sensiti Vitas	Spesivi sites	NDP	NDN
	Heavy Critical	Light Keep				
≥ 32.01 ng/mL	11	4	78.6%	88.9%	73.3%	91.4%
< 32.01 ng/mL	3	32				

4 Discussion

This research was conducted at the Department of Clinical Pathology FK USU / RSUP Haji Adam Malik Medan. Blood samples are taken at the beginning of treatment of patients who have been confirmed positive for COVID-19 with an RT-PCR test from January 2022 – March 2022. In this study, COVID-19 patients were 33 men (66%), and 17 women (34%). Previous research conducted by Fortunato where proportion of COVID-19 patients, 50.7% of women and men 49.3% who were hospitalized was 45.4% in men compared to 37.9% in women.[7] Gender differences are caused by various factors, namely the function of immunity related to the X chromosome, the effects of the hormone estrogen, and lifestyle. Localization of the ACE2 gene so that ACE2 levels are lower in women and the toll-like receptor 7 (TLR7) gene in the X chromosome may explain the increased risk of COVID-19 in men compared to women.[7] In the adaptive immune system, women have a higher CD4+ T cell count, stronger cytotoxicity activity of CD8+ T cells, and increased production of immunoglobulin B cells compared to men.[8] The protective effect of estrogen on viral infections by suppressing reticular stress endoplasma as caused by infection with the SARS-CoV 2 virus.[9] Smoking, alcohol consumption, and poor

eating habits, more commonly found in men than women, can lead to a higher incidence of comorbidities in men.[10]

In this study, the percentage of VCAM-1 levels in this study was 23.02 ng / mL (10.96 ng/ mL - 50.63 ng/ mL). The highest VCAM-1 was found in the critical degree group, followed by the severe degree and moderate degree respectively 37.85 ng/mL (15. 59 – 50. 63), 36.68 ng/mL (19. 58 – 49. 71), and 18.83 ng/mL (10. 96 – 46. 32). This is in line with the study by Tong that it was found significantly that there was a significant increase in VCAM-1 level in patients with severe severity compared to mild degree i.e. median level VCAM-1 4991.3 pg/mL and 3742.3 pg/mL with a p-value < 0.05.[11] Cohort research by Tong found that adhesion markers of FKN, VCAM-1, and ICAM-1 leukocytes increased in COVID-19 patients, the severity of COVID-19 was also related to serum levels of CRP, IL-18, TNF-α, IFN-γ, FKN, VCAM-1, ICAM-1, and VAP-1 levels which were then accompanied by a decrease in serum levels of CRP, IL-18, TNF-α, FKN, VCAM-1, ICAM-1, D-dimer in the recovery phase.[11] This is also in line with research by Yao namely that the median serum levels of sVCAM-1 are significantly higher in COVID-19 patients (1396.0 ng/mL, IQR: 1019.1– 1774.8 ng/mL) compared to healthy controls (612.4 ng/mL, IQR: 466.4 – 689.3 ng/mL).[12] Increased levels of VCAM-1 indicate that inflammation of the VCAM-1 is usually not found in endothelial cells at rest. Due to the presence of inflammatory mediators such as TNF- α and IL-1, it can significantly induce VCAM-1 expression so that elevated VCAM-1 levels reflect the presence of endothelial activation and infiltration of leukocytes that can cause immune thrombosis.[6]

Expression of vascular cell adhesion molecule (VCAM-1) has been demonstrated in the membranes of endothelial cells, smooth muscle cells, macrophages, and several others.[13] VCAM-1 mediates the attachment of circulating leukocytes to the endothelium, at sites of inflammation, and favors diapedesis, and accumulation in arterial walls.[14] Increased levels of adhesion molecules have been documented in chronic kidney disease (CKD).[15] VCAM-1 is involved in atherogenesis,[13] peripheral artery disease,[15] and in neointimal formation after arterial injury.[16] The latter is a crucial remodeling vascular process occurring in AVF. Their association with AVF patency has been inadequately investigated to date, and most studies have been focused on thrombosis of vascular access concerning VCAM-1 in dialysis patients.[17] Therefore, there is a relationship between increased VCAM-1 level and with severity of degree in COVID-19 patients that VCAM-1 can have a big impact in predicting the severity of COVID-19. This research is the first research in Indonesia. The limitation of this research is did not evaluate mild severity. A larger number of samples are needed in this study to prevent bias, the absence of follow-up in this study, and the need to determine the standard value of VCAM-1 level

The results of this study showed an accuracy of 86% in predicting the severity of COVID-19 and can significantly distinguish mild-moderate degree patients from severe-critical.

5 Conclusion

Based on the presentation of the results of the study, it can be concluded that there is a relationship between VCAM-1 levels and severity in COVID-19 patients. The median concentration of VCAM-1 was 37.85 ng/mL (15,59 – 50,63), 36.68 ng/mL (19,58 – 49,71), and 18.83 ng/mL (10,96 – 46,32) for critical, severe, and moderate degree respectively ($p = 0.001$). The cut-off value of VCAM-1 levels to predict the severity of COVID-19 is 32.01 ng/mL.

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