

Correlation between VCAM-1 Level and Absolute Monocyte Count in Coronary Artery Disease

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ABSTRACT

To prove the correlation between VCAM-1 level and absolute monocyte count among Coronary Artery Disease (CAD) patients. The study was an observational analytic study with a cross-sectional approach. The research subjects were 74 CAD patients who had been proven by coronary angiography and were treated in the Cardiology Department of Central General Hospital by Dr. Kariadi Semarang. This study was conducted from March to July 2021. VCAM-1 levels were examined with the ELISA method, using BioTek ELX800 Microplate reader (USA), and absolute monocyte count was analyzed with flow cytometry method, using Hematology Analyzer SYSMEX XN-1000 (Japan). Statistical analysis was done by Pearson's correlation coefficient test, in which $p < 0.05$ was considered significant. The mean VCAM-1 level was (361.78 ± 128.01) ng/mL. The mean absolute monocyte count was $(0.48 \pm 0.17) \times 10^3/\mu\text{L}$. The result of the Pearson correlation test showed a moderate positive correlation between VCAM-1 levels and absolute monocyte count in CAD patients ($p = 0.00$; $r = 0.46$). There was a moderate positive correlation between VCAM-1 level and absolute monocyte count in CAD patients. A positive correlation exists between VCAM-1 level and absolute monocyte count among CAD patients.

Keywords: VCAM-1, absolute monocyte count, CAD

INTRODUCTION

Coronary Artery Disease (CAD) is a pathological condition where there is narrowing or blockage of the coronary arteries (arteries that supply blood to the heart muscle) due to plaque buildup in the artery walls, usually in adults aged 35-40 years. It is a significant cause of death and morbidity worldwide.^{1,2} There are 500.000-700.000 deaths due to CAD each year, and it is the cause of death for one-third of all deaths that occur in the male and female populations aged over 35 years in both developed and developing countries.³ The Indonesian Association of Cardiovascular Specialists/Perhimpunan Dokter Spesialis Kardiovaskuler Indonesia (PERKI) stated that cardiovascular disease is still a global threat in 2019, where CAD was the leading and first cause of all deaths in Indonesia.⁴ Basic Health Research/Riset Kesehatan Dasar (RISKESDAS) data in 2013 shows that CAD has the highest prevalence (0.5%) for cardiovascular disease in Indonesia. The incidence and severity of CAD in females triple after menopause.^{2,5}

An example of chronic inflammatory disease in the cardiovascular system is CAD, which is characterized by the narrowing of the coronary artery as an impact

of atherosclerosis.¹ Atherosclerosis begins with the release of Low-Density Lipoprotein (LDL) from the bloodstream into the subendothelial space, which is then oxidized by Reactive Oxygen Species (ROS) and becomes oxidized LDL. Oxidized LDL is an excellent chemotactic molecule in stimulating the expression of adhesion molecules, such as Vascular Cell Adhesion Molecule-1 (VCAM-1) and Intercellular Adhesion Molecule-1 (ICAM-1), and the secretion of proinflammatory cytokines, such as Tumor Necrosis Factor-alpha (TNF- α).¹ VCAM-1 on the endothelial surface will stimulate leukocyte migration to the subendothelial space and increase the adhesion between these inflammatory cells. Monocytes differentiate into macrophages in the tunica intima.⁶ Macrophage binds oxidized LDL and turns it into foam cells with proinflammatory function. Accumulation of lipids, necrotic nuclei, inflamed smooth muscle cells, endothelial cells, inflammatory cells, and foam cells will form an atherosclerotic plaque.⁷ Risk factors such as hypercholesterolemia contribute to the stimulation of VCAM-1 expression.

CAD often occurs in developing countries with limited facilities and resources; simple and inexpensive biochemical markers are needed, one of which is monocytes. Monocytes have an essential role

in atherosclerosis, namely stimulating the recruitment of leukocytes into atherosclerotic plaques and playing a role in the innate immune system. This critical role makes absolute monocytes an excellent and affordable parameter in detecting atherosclerosis in CAD.⁶ The study of Ji *et al.* showed that Monocyte-Lymphocyte Ratio (MLR) is an independent risk factor for CAD and a predictor of the severity of atherosclerosis.⁸ Although there have been several studies that examined the levels of VCAM-1 and absolute monocyte count in CAD, none have specifically examined the relationship between VCAM-1 levels and absolute monocyte counts in CAD patients. Hence, the researchers wanted to examine this as CAD cases in Indonesia continue to increase.^{8,9}

METHODS

This cross-sectional analytical observational study was conducted from March to July 2021 at the Cardiology Department of the Central General Hospital Dr. Kariadi Semarang. It received permission from the Health Research Ethics Committee (KEPK), Faculty of Medicine, University of Diponegoro, at 82/EC/KEPK/FK-UNDIP/III/2021.

This study selected 74 CAD patients who came to the Cardiology Department to do check-up routines; 61 were male, and 13 were female. The inclusion criteria for the study were patients aged 30 to 65 years diagnosed with CAD by coronary angiography, with average body temperature (36.5-37.5°C), and willing to participate in this study. The coronary angiography shows the location and severity of coronary atherosclerosis. The exclusion criteria included subjects with hematologic malignancy, leukopenia (leukocyte count <4000/ μ L), or infection. Data from questionnaires, physical examination, and laboratory examination of venous blood samples were obtained after written consent. The subjects do not need fasting before the venous blood sampling. The blood was taken from patients immediately after regular follow-up consultations. The blood sample for VCAM-1 was centrifuged to separate the serum

and red blood cells; then, the serum was stored in a freezer box (-20°C). The independent variable was the level of VCAM-1, and the dependent variable in this study was the absolute monocyte count. VCAM-1 level testing was performed at the Iodine Deficiency Disorders (IDD) Laboratory of the University of Diponegoro Medical School with the ELISA method, using BioTek ELX800 Microplate reader (USA); meanwhile, the absolute monocyte count testing was performed at the Clinical Pathology Laboratory of Central General Hospital Dr. Kariadi, Semarang with flow cytometry method, using Hematology Analyzer SYSMEX XN-1000 (Japan). The data obtained were then analyzed statistically using the SPSS program. A descriptive test was carried out on each data, then tested for data normality using the Kolmogorov-Smirnov test. Data of VCAM-1 levels and absolute monocyte counts were normally distributed, so the Pearson's correlation test was used. Independent sample T-test was also performed on VCAM-1 levels and absolute monocyte counts. The results were considered statistically significant if $p < 0.05$.

RESULTS AND DISCUSSIONS

The number of research samples that met the inclusion and exclusion criteria was 74 subjects. All subjects were CAD patients aged 30-65 years, which were dominated by male patients (82.4%). The primary data collected in this study was obtained from samples of research subjects. Of the total of all research subjects, the subjects consisted of 61 males (82.4%) and 13 females (17.6%). The mean age of the subjects was 58 years, with the minimum age of 42 and the maximum age of 65. The mean level of VCAM-1 was (361.78 \pm 128.01) ng/mL. The absolute monocyte count was obtained by multiplying the percentage of monocytes with the number of leukocytes. The absolute monocyte count was (0.48 \pm 0.17) $\times 10^3$ / μ L. The normality test was carried out using the Kolmogorov-Smirnov test because there were more than 50 subjects. The characteristics of the subjects are shown in Table 1.

Table 1. Characteristics of the research subjects

Variable (n=74)	Mean \pm SD	Median (Min-Max)	p
Gender			
Male n=61 (82.4%)			
Female n=13 (17.6%)			
Age (years)	57 \pm 5.44	58 (42 – 65)	0.00
VCAM-1 level (ng/mL)	361.78 \pm 128.01	363.5 (124 - 604)	0.08
Leukocyte count (10^3 / μ L)	7.88 \pm 1.63	7.80 (4.34 – 12.02)	0.20
Monocyte percentage (%)	6.22 \pm 2.14	5.95 (2.3 – 13.8)	0.00
Absolute monocyte count (10^3 / μ L)	0.48 \pm 0.17	0.47 (0.15 – 0.88)	0.20

p, normality test (Kolmogorov-Smirnov)

Table 2. Independent T-test of VCAM-1 level and absolute monocyte count

Variable	Mean±SD	p
VCAM-1 level (ng/mL)		0.001
Absolute monocyte count $\leq 0.5 \times 10^3/\mu\text{L}$, n=32 (43%)	319.88±133.55	
Absolute monocyte count $> 0.5 \times 10^3/\mu\text{L}$, n=42 (57%)	416.78±97.47	

p, independent T-test

Researchers divided the subjects into two groups based on their absolute monocyte count: subjects with normal and increased absolute monocyte counts. Based on the independent T-test on VCAM-1 level and the absolute monocyte count, which were normally distributed, it was found that there was a significant increase ($p < 0.05$) in the group of subjects with an increased absolute monocyte count (see Table 2).

The normality test results using the Kolmogorov-Smirnov test showed $p = 0.08$ for VCAM-1 level and $p = 0.20$ for absolute monocyte counts, indicating that both VCAM-1 level and absolute monocyte counts were normally distributed. Pearson correlation analysis showed $p < 0.05$, indicating a significant relationship between VCAM-1 levels and absolute monocyte counts in CAD patients (see Figure 1). The Pearson correlation value of 0.46 indicates a positive relationship with moderate correlation strength.

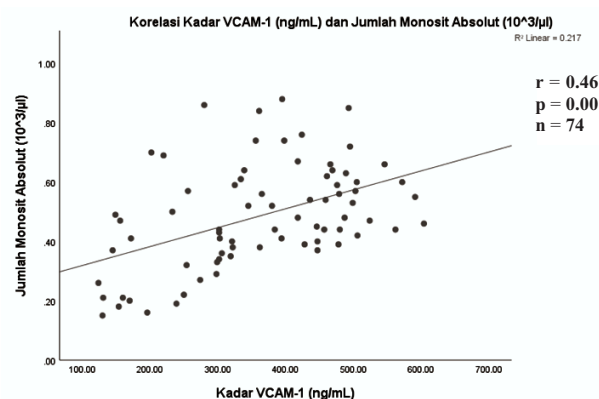


Figure 1. Correlation graph of VCAM-1 level and absolute monocyte count

This study involved 74 subjects consisting of 61 male CAD patients (82.4%) and 13 female CAD patients (17.6%). The mean age of the subjects was 58, with a minimum age of 42 years and a maximum age of 65 years, while the average age of the study subjects was (57 ± 5.44) years. These results were in accordance with the results of the Hajilooi *et al.* study, which stated that the incidence of CAD was most common in males with an average age of

(57.9 ± 9.6) years and increased three times in post-menopausal females compared to pre-menopausal females due to the cardioprotective nature of estrogen.²

The normal value for VCAM-1 level is 349–991 ng/mL. The subject's VCAM-1 level did not increase in this study, with an average of (361.78 ± 128.01) ng/mL. This result was supported by the study of Hajilooi *et al.*, who found that VCAM-1 and selectin-p levels neither increased nor decreased in stable atherosclerotic plaques.¹⁰ Ross *et al.* also stated that VCAM-1 level did not significantly correlate with endothelial dysfunction in CAD.¹¹ Increased expression of VCAM-1 in atherosclerotic conditions of CAD patients was not followed by an increase of soluble VCAM-1. It may occur because the release mechanism of endothelial adhesion molecules by proteases to the blood involves various intercellular communications that haven't yet been clear, so the measurement of the level of soluble adhesion molecules can't fully determine the condition and severity of endothelial dysfunction.¹² Some studies reported that matrix metalloproteinase (MMPs) is Zn^{2+} -endopeptidase, which has a vital role in the release of adhesion molecules from endothelium, such as VCAM-1.¹³ Release of VCAM-1 by MMPs can be stimulated by phorbol 12-myristate 13-acetate (PMA), a tumor promoter involved in gene transcription, cell growth and differentiation and immunity via Protein Kinase C (PKC) signaling pathway.¹⁴

The normal value for absolute monocyte count in adults is $0.285 \times 10^3 - 0.5 \times 10^3/\mu\text{L}$.¹⁵ This study showed a significant increase in absolute monocyte count ($p = 0.001$) with absolute monocyte count $> 0.5 \times 10^3/\mu\text{L}$ in 32 subjects (43%). The increase in absolute monocyte count was caused by the upregulation of VCAM-1 expression in atherosclerotic plaques.^{16,17} Meanwhile, the absolute monocyte count in the other 42 subjects was normal. Previous studies have found that medication consumption, such as statins and Angiotensin-Converting Enzyme (ACE) inhibitors, can exert a sufficiently large anti-inflammatory effect on endothelial dysfunction so that it is possible not to increase the absolute monocyte count.^{18,19}

This study showed a moderate positive relationship ($r=0.46$, $p=0.00$) between VCAM-1 level and absolute monocyte count in CAD patients. This finding follows Myron *et al.*, which stated that VCAM-1 is an endothelial molecule that plays a significant role in atherogenesis by promoting monocyte accumulation in the tunica intima.¹⁶ This finding also follows Cunningham and Gotlieb's study, which stated that disruption of shear stress in atherosclerotic conditions triggers modulation of VCAM-1 and Monocyte Chemoattractant Protein-1 (MCP-1) expression in response to TNF-alpha cytokine and LDL level escalation, thereby increasing the number of monocytes in the blood.²⁰ The mechanism of increasing absolute monocyte count in increasing VCAM-1 level can be associated with increasing proinflammatory cytokines, such as TNF-alpha, IL-4, IL-6, IFN-gamma, and lysophosphatidylcholine (LPC).^{10,21} Proinflammatory cytokines exposure on endothelium triggers VCAM-1 expression, which is major in initiating atherosclerosis by mediating firm adhesion and trans-endothelial migration of leukocytes. VCAM-1 then binds to integrin $\alpha_5\beta_1$, expressed on the monocyte surface, thereby increasing the number of monocytes in the blood.²² blood monocytes migrate to the tunica intima and bind to oxidized LDL. Monocytes then differentiate into macrophages due to Macrophage-Colony Stimulating Factor (M-CSF) stimulation to form early plaques called fatty streaks in the tunica intima.²³⁻²⁵

Several factors, such as the consumption of therapeutic drugs, such as statins and ACE inhibitors, and genetic traits whose effects on VCAM-1 levels and absolute monocyte counts were not analyzed, limited this study.

CONCLUSIONS AND SUGGESTIONS

This study concluded that there is a moderate positive relationship between VCAM-1 levels and absolute monocyte count in CAD patients. Further research is needed to investigate medication consumption (statins or ACE inhibitors) and genetic factors and their effect on soluble VCAM-1 levels and absolute monocyte counts in coronary artery disease.

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