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Correlation between Increased Serum Vascular Endothelial Growth Factor Levels and 30-Day-Outcome After Acute Myocardial Infarction

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ABSTRACT

Vascular Endothelial Growth Factor (VEGF) plays an important role in the process of regeneration and vascular repair. Atherogenesis and angiogenesis contribute to the formation of coronary collateral circulation as an alternative source of blood supply during the repairing process of Acute Myocardial Infarction (AMI). This study aimed to investigate the elevation of VEGF and its correlation to Peripheral Blood Mononuclear Cells (PBMC) and Major Adverse Cardiovascular Events (MACE). Serum VEGF measurements were carried out on 1st, 2nd, and the 5th-day onset of angina on 20 patients using Chemwell Analyzer based on ELISA method. PBMC was counted on days 1st and 5th according to CBC results from Automatic Cell Counter Sysmex XN-1000i. The major adverse cardiovascular event was recorded 30 days after AMI onset. It was found that serum VEGF level in this study was 169.3±34.5 pg/mL on the 1st day, 217.0±49.7 pg/mL on the 2nd day, and 249.2±48.5 pg/mL on the 5th day. Serum VEGF levels increased gradually and the highest value was found on the 5th day (p=0.000). There was no correlation between elevated serum VEGF levels with PBMC on the 1st day (p=0.429, r=-0.035) and the 5th day of AMI (p=0.225, r=+0.081). There was no correlation between elevated serum VEGF levels with incidence of MACE on 30 days after onset of AMI (OR=0.959, 95% CI, p=0.302). Serum VEGF concentrations are increased in Acute Myocardial Infarction and can be used as a marker of myocardial injury. However, this study was unable to prove its role in the outcome of AMI.

Keywords: Vascular endothelial growth factor, acute myocardial infarction, peripheral blood mononuclear cells, major adverse cardiovascular event

INTRODUCTION

Acute Myocardial Infarction (AMI) is a condition in which evidence of cardiomyocyte necrosis in a clinical setting consistent with acute myocardial ischemia.[1,2] AMI is a fatal disease and still has become a health issue in developed as well as developing countries. According to World Health Organization (WHO), cardiovascular disease is the most common cause of death worldwide, including Indonesia.[3] Acute myocardial infarction is the second leading cause of death after stroke and its prevalence has reached 1.7% in Indonesia.[4,5]

In the repair process, the formation of coronary collateral circulation is very important as an alternative source of blood supply that can suppress myocardial ischemia thereby preventing necrosis and triggering the left ventricular remodeling.[6] Angiogenesis is a vital compensatory mechanism in myocardial ischemia, which supplies the ischaemic tissue with blood.[7] Angiogenesis is the process of formation of new blood vessels from preexisting vessels, involving cell proliferation, migration, differentiation, tube formation, and regulation of angiogenic factors. It is responsible for a great variety of physiological and pathological processes, including myocardial infarction.[8] Vascular endothelial growth factor (VEGF) is the key and strongest angiogenic factor to induce angiogenesis.[9,10] VEGF is a homodimeric glycoprotein, which is the main regulator of the angiogenesis process, which can increase vascular permeability, accelerate the formation of collateral pathways in the myocardium, and trigger tissue repair.[11]

VEGF is produced by endothelial cells, smooth muscle cells, macrophages, cardiac fibroblast, lymphocytes, polymorphonuclear cells, megakaryocytes, monocytes, and platelets.[12] It is already known that monocytes and lymphocytes can release high amounts of VEGF in a variety of clinical conditions.[13] Peripheral Blood mononuclear cells

(PBMCs) consists of lymphocytes (T, B, and natural killer cells), monocytes, and dendritic cells. It is noted that PBMC count increases in the myocardium with necrosis after the onset of AMI.[14] PBMC is probably one of the candidates, which play a role in improving VEGF circulation in patients with AMI.

The vascular endothelial growth factor is crucially involved in the repair process of vascular endothelial function and vascular regeneration.[6] Vascular endothelial growth factor is a signal protein, which can increase vascular permeability, stimulate the expression of vascular endothelial cells and induce angiogenesis.[15,16] Vascular endothelial growth factor secretion is stimulated by hypoxia and ischemia condition in patient with AMI.[6,15] Study conducted in China found high levels of VEGF on the 7th day after the onset of AMI. It is also found that patients with high VEGF levels have lower MACE incidence than those with low VEGF levels, suggesting a low VEGF level as an independent risk factor against MACE.[12]

The role of VEGF in the progress of AMI disease remains to be proven. No laboratory parameter can assess the occurrence of hypoxia in AMI. The purpose of this research was to determine the increase in serum VEGF levels in AMI patients in Adam Malik Hospital and its correlation with disease outcomes.

METHODS

The research was conducted from January–March 2019 with a cohort research design. Subjects in this study consisted of AMI patients who attended the IGD Department of Cardiology and Vascular medicine, Adam Malik Hospital, Medan. Complete blood count and serum VEGF levels were tested in all subjects. Acute myocardial infarction was diagnosed based on the criteria of PERKI 2018 as follows: a typical angina complaint increased cardiac marker (Troponin I and CKMB) and EKG showing an ischemia sign.

Laboratory tests were carried out in the Clinical Pathology laboratory, Adam Malik Hospital. Tests on serum VEGF levels were carried out using the Chemwell Analyzer ELISA method and the reagent of Human Vascular Endothelial Growth Factor (VEGF) ELISA Kit-Qayeebio. Peripheral blood mononuclear cells were measured based on the CBC test results using the Automatic Cell Counter Sysmex XN-1000i instrument. The outcome was assessed based on the detection of MACE 30 days after the onset of AMI.

Statistical analysis was conducted using SPSS ver. 25.0. The difference between categorical variables was analyzed using the Wilcoxon test and Friedman test whereas the strength of the correlation between variables was determined using linear regression tests. The logistic regression test was conducted to analyze the increased relationship of VEGF serum by the occurrence of MACE. Analysis was conducted at a 95% confidence interval and a p-value < 0.05 was considered statistically significant.

This study has received approval from the Health Research Ethics Committee of the Faculty of Medicine, Sumatera Utara University/Adam Malik Hospital, Medan with number No: 105/TGL/KEPK FK USU-RSUP HAM/2019.

RESULTS AND DISCUSSIONS

Table 1 describes the clinical characteristics of 20 subjects diagnosed with AMI. The mean age of research subjects was 41–78 years (58.8±9.8).

Table 1. General characteristics of the study population

Characteristic	Value (%) or Mean±SD (n=20)
Gender	
Male	16 (80)
Female	4 (20)
Age (years)	59.2±9.7
Risk factors	
Obesity (BMI ≥25)	14 (70)
Smoking	13 (65)
Hypertension (BP ≥160 and/or 95 mmHg)	6 (30)
Diabetes (Random Blood Glucose ≥200 mg/dL)	8 (40)
Dyslipidemia	15 (75)
cTnI (ng/mL)	9.5±10.5
CKMB (U/L)	125.6±109.5

cTnI (cardiac Troponin I); CKMB (Creatinine Kinase Myocardial Band); BMI (Body Mass Index); BP (Blood Pressure)

Subjects consisted of male (80%) and female (20%). Of 20 subjects, 14 people had a risk factor for obesity (70%), 13 smokers (65%), 15 people with dyslipidemia (75%), 6 people with hypertension (30%), and 8 people with diabetes mellitus (40%). The mean value of cardiac markers on the first day of the attack was Troponin I with 9.5±10.5 and CKMB with 125.6±109.5.

Serum VEGF levels gradually increased and the highest value was found on the 5th day (Fig. 1). Serum VEGF levels in AMI patients were 169.3±34.5 on the 1st day, 217.0±49.7 on the 2nd day, and 249.2±48.5 on the 5th day. Serum VEGF levels were significantly higher on the 2nd day than on the 1st day (p=0.000). Serum VEGF levels on the 5th day were significantly higher than on the 2nd day (P=0.000) and compared to the 1st day (p=0.000) as described in Table 2.

Table 2. Serum VEGF levels in AMI patients

Parameter	Mean±SD	p-value
VEGF 1st day (pg/mL)	169.3±34.5	0.000[a]
VEGF 2nd day (pg/mL)	217.0±49.7	0.000[b]
VEGF 5th day (pg/mL)	249.2±48.5	0.000[c]

Note: a test between VEGF the 1st and 2nd day, b test between VEGF the 2nd and 5th day, c test between VEGF the 1st and 5th day

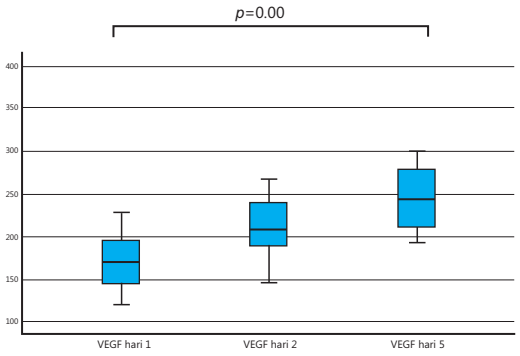

Figure 1. Boxplot Analysis of Serum VEGF Levels on 1st, 2nd, and 5th day

The vascular endothelial growth factor is expressed due to the stimulation of hypoxia during acute myocardial infarction. It has a pro-angiogenetic effect and pro-inflammatory properties to induce vascular permeability.[17] A study conducted by Harada *et al.* shown that levels of total VEGF-A increased after PCI and reached their peak at day 7 (470.1(309.1-743.7)) pg/mL.[18] Other studies found that the concentration of serum VEGF was high at 7 days after onset of AMI.[19] After AMI in mice, new microvessels form in the infarct border zone, the subendocardial space, and the epicardium. Between 2 and 4 days after coronary artery ligation, the capillary network in the border zone starts expanding, with extensive branching and vessel sprouting into the infarct core.[16] After 7 days, most capillary epithelial cells in the border zone cease to proliferate and some newly formed capillaries enlarge and acquire smooth muscle cell support.[16] In a classic autopsy series in patients from the pre-reperfusion era, newly formed capillaries penetrated the infarct core from the periphery starting on day 4; ingrowth of blood vessels was most prominent during the second week after AMI, indicating that the process takes longer in patients than in themuch smaller murine heart.[16,20]

Total leukocytes count and differential count in this study showed a higher number of leukocytes, absolute neutrophils, absolute lymphocytes, and PBMC on the 1st day than the 5th day, while the monocytes were higher on the 5th day than the 1st day. According to the results of compare mean analysis, there was no difference of absolute lymphocytes (p=0.764), absolute monocytes (p=0.489), and PBMC (p=0.852) between the first and fifth days. Contrastingly, there was a difference in leukocyte count (p=0.010) and absolute neutrophils (p=0.009) between the first and fifth days (Table 3).

Table 3. Difference number of leukocytes and differential count of leukocytes between the first and fifth day of onset in patients with AMI

Parameter	Value on 1st Day	Value on 5th Day	p-value
Leukocyte (/µL)	14,829±5,265	11,277±4,053	0.010
Absolute neutrophil (/µL)	11,979±5,340	8,294±4,367	0.009
Absolute lymphocyte (/µL)	1,788±937	1,711±630	0.764
Absolute monocyte (/µL)	915±464	991±356	0.489
PBMC (/µL)	2,753±1,202	2,702±646	0.852

PBMC (Peripheral Blood Mononuclear Cell) obtained from the summation of absolute lymphocytes with absolute monocytes

To assess the correlation and strength of the correlation between the number of PBMC with increased levels of serum VEGF on the first and fifth day, linear regression analysis was used (Fig.2; Fig.3). This study found no correlation between the number of PBMC with increased serum VEGF levels on both the first day (p=0.429, r=-0035) and the fifth day (p=0.225, r=+0.081).

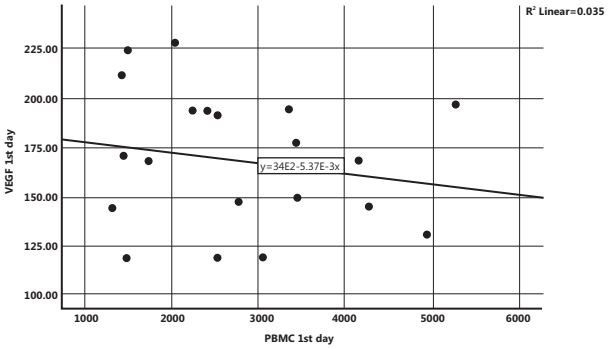

Figure 2. Correlation between number of PBMC and increased Serum VEGF levels on the first day of AMI

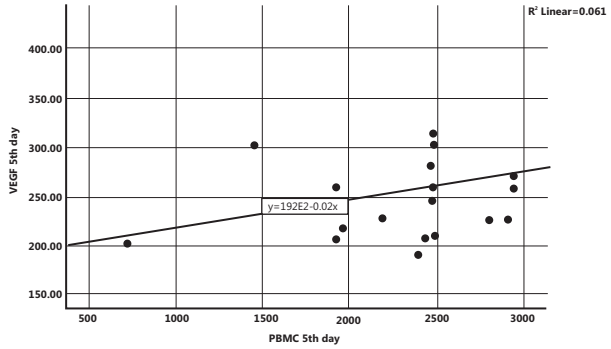

Figure 3. Correlation between number of PBMC and increased Serum VEGF levels on the fifth day of AMI

After MI in mice or rats, ECs in the infarct region express VEGFR2 for at least 7 days.[20,21] At the same time, monocytes and macrophages, fibroblasts, cardiomyocytes, and epicardium-derived cells produce VEGFA in the infarct region.[22] Inflammatory cell-derived VEGFA plays a crucial role specifically after injury.[23] In patients with acute MI, ECs in the infarct region have been shown to express VEGFA and VEGFA plasma levels are elevated.[6] VEGF can be released by various cell types, and its signals are mediated through the tyrosine kinase receptors, VEGFR-1 (or Flt-1) and VEGFR-2 (or KDR), which are expressed by endothelial cells, monocytes, and many malignant tumors.[24] Analysis of VEGFRs on PBMCs showed that monocytes expressed detectable but low amounts of VEGFR-1 and VEGFR-3 and no VEGFR-2.[13] Serum VEGF levels are linked to thrombocyte counts, probably because platelets release VEGF during blood coagulation. Without consideration of these factors, measurement of VEGF might not reflect the actual situation.[13]

A linear regression test was also used to determine the correlation and strength of the correlation between increased CKMB at the beginning of the disease with the serum VEGF levels. Figure 4 shows that there was no correlation between increased CKMB level and increased serum VEGF levels (p=0.424, r=-0036).

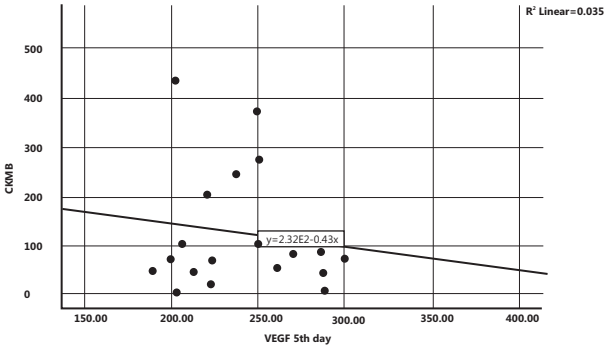

Figure 4. Correlation between increased CKMB and serum VEGF levels in patients with AMI

The same test was also used to assess the correlation and strength of the correlation between increased levels of Troponin I at the beginning of the disease and the serum VEGF levels. It was shown that there was no correlation between both parameters (p=0.916, r=+0.001) as described in Figure 5.

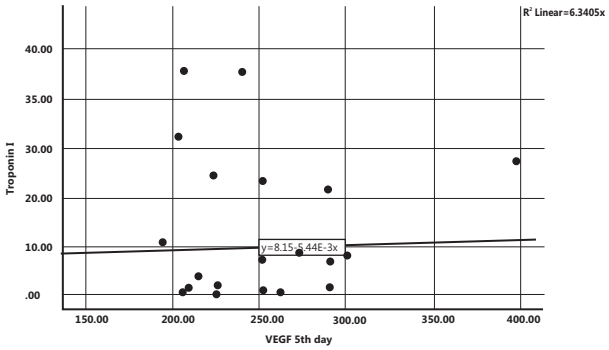

Figure 5. Correlation between increased Troponin I and serum VEGF levels in AMI

Infarct size is major prognostic relevance in patients suffering from AMI.[25] Study conducted by Tiller et al. reported that Troponin and Creatine Kinase (CK) is myocardial injury biomarkers that reflect infarct size (p < 0.001).[26] Previous study,

compare the diagnostic performance of these markers with VEGF in estimating infarct size using ROC curve. This study revealed that the area under the ROC curve (AUC) of VEGF used to predict CAD was 0.667 (sensitivity: 68.5%; specificity: 60.1%), which was remarkably powerful compared to CK-MB with an AUC of 0.622 (sensitivity: 60.7% and specificity: 54.4%).[27]

All patients who participated in this study followed by 30 days after the onset of angina attack to assess the outcome. The outcome was assessed by observing MACE in each patient. The major adverse cardiovascular event, which occurs 30 days after the onset of the AMI attack was experienced by 12 people (60%) and 8 people (40%) who were still alive and had not shown demonstrated complications. The emerging MACE was 6 people (30%) as a result of death and 6 people (30%) due to heart failure (Table 4).

Table 4. Frequency of MACE 30 days after AMI

MACE	Frequency, n(%)
Live with no outcome	8 (40)
Death	6 (30)
Heart failure	6 (30)
Total	20 (100)

To determine the role of increased serum VEGF levels in the AMI outcome, the logistics regression analysis was conducted at the peak serum VEGF with MACE 30 days after the onset of the AMI. In this research, it was found that increased serum VEGF levels did not affect the occurrence of MACE 30 days after AMI attack. Logistics regression analysis results can be seen in Table 5.

Table 5. Logistic regression analysis of peak serum VEGF levels against the occurrence of MACE 30 days after AMI attack

Parameter	Multivariate Analysis		
	OR	95% CI (Lower-Upper)	p-value
Peak serum VEGF (pg/mL)	0.959	0.885 – 1.039	0.302

The study by Niu *et al.* with multinomial logistic regression revealed that reduced VEGF levels (β=1.243; 95% CI, 1.018-1.326; p=0.026) were independent risk factors for MACE. They concluded that high plasma VEGF levels on the 7th day after the onset of the AMI facilitated long-term prognosis in patients with AMI, while low plasma VEGF levels were an independent risk factor for MACE incidence.[19] Other studies assessed the comparisons of concentration VEGF-A at days 1, 3, 7, and 30 after PCI in patients with and without MACE. The levels of total VEGF-A tended to be lower in patients with MACE than in those without.[18]

CONCLUSIONS AND SUGGESTIONS

Serum VEGF levels were increased in patients with AMI. Peripheral blood mononuclear cells do not become a major source of increased levels of VEGF circulating in serum. In addition, the study was unable to prove the increase in VEGF as a good prognosis marker in AMI patients. Further researches are required to determine the role of VEGF with long term outcome and source of serum VEGF in AMI patients.

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