ORIGINAL ARTICLE

Association of Endothelial Nitric Oxyde Synthase (eNOS) Levels and Modifiable Risk Factors for Acute Myocardial Infarction with ST-Segment Elevation (AMI-STE)

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ABSTRACT

Background: The pathological process underlying myocardial infarction is atherosclerotic thrombosis that causes endothelial dysfunction. In the AMI-STE process, eNOS has functions as the body's defense mechanism to prevent more severe damage to the endothelium. Decreased eNOS levels were found in modifiable AMI risk factors. This study aimed to determine the association of eNOS levels based on modifiable risk factors for AMI-STE patients.
Methods: This study was an analytical observational study with a historical cohort design. The samples were patients with AMI-STE diagnosis who had undergone PPCI at M. Djamil Hospital Padang from March to June 2022. The eNOS levels were taken from the blood samples. Modifiable AMI risk factors were taken from patient's medical record. The T test was carried out to determine statistical analysis.
Results: The average age of the 38 subjects was 62.53 ± 8.2 years, and 92.1% among them were male, and 52.6% subjects were diagnosed with anterior AMI-STE. The average eNOS level in subjects was 47.47 ± 23.88 (normal level is 48.16 pg/mL). More than half (73.5%) of the subjects in low eNOS group had diabetes mellitus as risk factor (P<0.0001) and most subjects (78.9%) in group with low eNOS had hypertension (p=0.022). Other risk factors, namely dyslipidemia and smoking, were also more common in group with low eNOS. However, statistical tests showed that there was no statistically association between eNOS levels with dyslipidemia and smoking.
Conclusion: There was statistically significant association between eNOS levels with diabetes mellitus and hypertension as modifiable risk factors for AMI-STE.

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Introduction

Coronary heart disease (CHD) is one of main problems among all heart diseases. CHD that occurs acutely is known as Acute Myocardial Infarction (AMI) and is one of the main causes of death. Based on the World Health Organization (WHO) report in 2017, around 17.8 million people died due to cardiovascular disease. In the United States, every 40 seconds, a citizen suffers from AMI, of which there were 785,000 new cases and 470,000 cases of recurrent AMI in 2017.[1–2] In Indonesia, based on Basic Health Research (RISKESDAS) in 2018, the prevalence of heart disease was 1.5% of the total Indonesian population, in West Sumatra alone the prevalence of heart disease is higher than the national prevalence figure, namely 1.6%, in 2018 based on Riskesdas.[3] Exact data on the incidence, morbidity and mortality rates of myocardial infarction in Indonesia are still very limited. Acute Myocardial Infarction with ST-Segment Elevation (AMI-STE), a form of AMI, is a disease that occurs when there is total blockage of the coronary arteries.[4] Based on the Jakarta Acute Coronary Syndrome Registry from October 2014 – July 2015, there were 1024 patients with AMI-STE of which 81% underwent primary percutaneous coronary intervention (PPCI).[5] Based on the COMPLETE Trial study conducted on AMI-STE patients who underwent PCI in 2019, it was found that the death rate from cardiovascular disease, recurrent AMI, ischemia requiring revascularization, heart failure and unstable angina pectoris accounted for 34.5% of the total 4041 patients.[6]

The pathological process underlying myocardial infarction is atherosclerotic thrombosis, which is an interaction between susceptible plaque and thrombosis. In myocardial infarction, the inflammatory response plays an important role in the initiation of atherosclerotic plaque and the development of the plaque into a vulnerable plaque, characterized by thin fibrous vesicles, an extensive lipid core, and a large accumulation of inflammatory cells, especially macrophages that are highly susceptible to rupture and form thrombus.[7]

The cause of all this is a problem with the endothelium, known as endothelial dysfunction. Endothelial dysfunction is a decrease in the ability of the endothelium to carry out the functions of blood vessel homeostasis. This decrease in function causes disturbances in blood vessel tone, as well as a condition known as endothelial activation, namely pro-inflammatory, proliferative and pro-coagulation.[8] Decreased endothelial function is reflected in reduced levels of Nitric Oxyde (NO), where NO itself is produced by NO synthase. Currently, 3 isoforms of NO synthase are known and one of them is the largest contributor to NO formation, namely endothelial Nitric Oxide Synthase (eNOS). In the AMI-STE process, eNOS function decreases, where eNOS has functions as the body's defense mechanism to prevent more severe damage to the endothelium.[9] In 2016, Abdel Hamid et al conducted research on AMI-STE patients showing that there were circulating endothelial cells and endothelial cell dysfunction which could predict major cardiovascular events such as death, recurrent myocardial infarction and heart failure in patients with AMI-STE.[10] So that the occurrence Endothelial dysfunction correlates with reduced eNOS levels.

Decreased eNOS levels were also found in various CHD risk factors. eNOS expression was found to be decreased in metabolic syndrome[11]. Normal insulin signaling will increase eENOS enzymatic activity. On the other hand, conditions

of insulin resistance will disrupt the P13K-Akt pathway which will cause reduced eNOS activity, reduced NO formation and consequently vasoconstriction[12]. Likewise with other CHD risk factors, such as hypertension and dyslipidemia, eNOS levels have also been found to be reduced in various studies. In dyslipidemia, oxidative and non-oxidative LDL will interfere with immunity and regeneration of cells experiencing inflammation. In addition, oxidative LDL inactivates the eNOS/NO pathway[13]. Mean endothelial eNOS levels were significantly lower in the group of smokers compared to non-smokers.[14] Genetics may also influence eNOS expression because clinical studies in humans have shown that polymorphisms in the eNOS gene predispose to hypertension, insulin resistance, and type 2 diabetes, the three main clinical features of metabolic syndrome. which is a risk factor for CHD events. The literature shows that among people with CHD risk factors, the likelihood of developing severe CHD such as AMI-STE increases if they have low eNOS levels.[13]

Based on the background that has been explained, the aim of this study was to determine the association of Endothelial Nitric Oxide Synthase (eNOS) Levels based on Modifiable Risk Factors for Coronary Heart Disease in Acute Myocardial Infarction Patients with ST Segment Elevation.

Methods

This research was an analytical observational study with a historical cohort research design conducted during patient care. The study population was AMI-STE patients who had undergone PPCI at the Integrated Heart Care Installation at Dr. M. Djamil Hospital Padang in March 2022 to June 2022. The minimum sample size in this study obtained based on the sample formula was 38 people.

The inclusion criteria for this study were patients with a diagnosis of AMI-STE and who had undergone PPCI with TIMI Flow 3 MBG 3 and received standard medical therapy for the treatment of acute myocardial infarction at the Integrated Heart Services Installation of Dr. M. Djamil Hospital Padang. The exclusion criteria in this study were patients who were diagnosed with AMI-STE but had sepsis, had previous heart surgery, had a history of previous AMI, had burns, had a history of multiple trauma and had stage III as well as stage IV of chronic kidney disease.

The sampling technique for this research was simple random sampling, taking samples from the population randomly without paying attention to the strata in the population and each member of the population has the same opportunity to be sampled and meets the research inclusion criteria and selection to be included in the research until the required subjects are met[15]. The independent variable in this study was eNOS levels, while the dependent variable was modifiable CHD risk factors. The subjects who met the inclusion criteria were then divided into two groups, namely group I with low eNOS level and group 2 with normal eNOS level. Between both groups, the eNOS levels were then compared according to risk factor variables, such as diabetes mellitus, hypertension, dyslipidemia and smoking

The research procedures began from subjects who met the research criteria had their eNOS levels checked. The steps taken in this research were blood samples taken during treatment at Dr. M. Djamil Hospital Padang within

24 hours from the onset of acute myocardial infarction symptoms. A 3 ml blood sample was taken from a peripheral vein, placed in an EDTA tube. Each specimen that had been centrifuged was then placed into a 1.5 ml micro cup which had been given a name, research number and medical record number then stored at -80oC in the Biomedical Laboratory of the Faculty of Medicine, Andalas University. Examination of eNOS levels was carried out by the Biomedical Laboratory of the Faculty of Medicine, Andalas University. CHD risk factors that can be changed include hypertension, hyperlipidemia, diabetes mellitus, obesity, smoking informations were obtained from patients medical records.

Statistical analysis was carried out by univariate and bivariate. Univariate analysis was carried out by analyzing each research variable. For categorical research variables which included major cardiovascular events, gender, hypertension, dyslipidemia, smoking and family history of disease, analysis was carried out by looking at the frequency distribution which was presented in the form of frequencies and percentages. Meanwhile, continuous data, namely age and eNOS levels, are expressed in mean values and standard deviations. At the beginning of the bivariate analysis, a data normality test was carried out using the Shapiro Wilk test. Because the data were normally distributed then the T test was used to determine the association of eNOS levels with CHD risk factors in AMI-STE patients treated at Dr M Djamil Hospital. Data analysis was carried out with a confidence level of 95% and $\alpha = 0.05$. If the p value <0.05 means there is an association between the research variables studied. All analyzes were carried out with the SPSS for Windows computer program. This research has passed ethical review from the Research Ethics Committee, Medical faculty of Andalas University, with letter numbers 602/UN.16.2/KEP-FK/2023.

Results

Baseline Characteristics of AMI–STE Patients Undergoing Primary Percutaneous Coronary Intervention

Table 1 explains the basic characteristics of research subjects. Based on table 1, the average age of the research subjects was 62.53 ± 8.2 years. Almost all patients (92.1%) were male and only a small number (7.9%) of patients were female. Cardiovascular risk factors in research subjects were smoking, diabetes mellitus, hypertension and dyslipidemia, respectively. Only 1 patient with family history of CHD as one of unmodifiable risk factors was found in this study. More than half (52.6%) of the study subjects were diagnosed with anterior AMI-STE. Most of the research subjects (65%) were patients with Killip class I.

Table 1. Characteristics of Research Subjects

Variables	**n = 38, f (%)**	**mean ± SD**
Age (years), mean ± SD		62.53 ± 8.2
Gender, n (%)		
Male	35 (92.1)	
Female	3 (7.9)	
Blood Pressure (mmHg)		
Systolic		124 ± 22.032
Diastolic		70 (50 – 97)
MAP		89 ± 13

AMI-STE Diagnosis, n(%)		
Anterior	20 (52.6)	
Inferior	18 (47.40)	
Risk Factors, n (%)		
Diabetes Melitus	17 (44.7)	
Smoking	30 (78.9)	
Dyslipidemia	10 (26.3)	
Hypertension	23 (60.5)	
Family History of CHD	1 (2.6)	
Onset		6.82 ± 2.903
Killip Class (n=20)		
I	13 (65)	
II	7 (35)	

Average eNOS levels of AMI–STE patients undergoing primary percutaneous coronary intervention

For 38 research subjects, eNOS levels were measured at admission. Based on table 2, the average eNOS level in research subjects was 47.47 ± 23.88. Median eNOS level was 37,050. The eNOS levels of the subjects in this study ranged from 17,000 – 121,956. Based on the literature, eNOS levels in healthy subjects are 48.16 pg/mL[13]. This means that the eNOS levels of AMI-STE patients in this study were lower than the normal eNOS level.

Table 2. eNOS levels in AMI-STE patients

Measurement Results	**Value**
Mean ± SD (pg/mL)	47,47 ± 23,88
Median (Minimum – Maksimum)	37,050 (17,000 – 121,956)

Association of eNOS Levels with Modifiable Risk Factors in AMI-STE Patients

As seen on table 3, the research subjects were divided into two groups, 19 people on group I with low eNOS levels and 19 subjects were group 2 with normal eNOS levels. Group I had average eNOS level of 34,69 ± 7,007 pg/mL, while the group II with Normal eNOS level of 54.74 ± 26,13 pg/mL.

Table 3. Groups of subjects based on eNOS levels

Groups	**eNOS Level (Mean ± SD in pg/mL)**
Group I (Low eNOS)	34,69 ± 7,007
Group II (Normal eNOS)	54.74 ± 26,13

Based on table 4, it can be seen that overall risk factors were found to have low eNOS levels compared to normal ones. More than half (73.5%) of the subjects in group I (low eNOS) had diabetes mellitus as the risk factor and only a small portion (15.8%) of subjects in group II (normal eNOS) had risk factors in the form of diabetes mellitus. eNOS levels had a statistically significant association with diabetes mellitus as a risk factor for AMI-STE ($p<0.001$). Subjects with risk factors for hypertension also had similar findings. More than half of the subjects (78.9%) in group I had hypertension as the risk factor. However, in group

II almost half (42.1%) had hypertension. eNOS levels had a statistically significant association with hypertension as a risk factor for AMI-STE (p=0.022). Other risk factors, namely dyslipidemia and smoking, were also more common in group I. However, statistical tests showed that there was no statistically significant association between eNOS levels and dyslipidemia (p=0.135) and smoking (p=0.346) as risk factors for AMI-STE.

Table 4. Association of eNOS Levels with Modifiable CHD Risk Factors in Research Subjects

Variables	Group I (Low eNOS levels), N (%)	Group II (Normal eNOS) N (%)	P value
Diabetes Melitus	14 (73.5)	3 (15.8)	<0.001
Hypertension	15 (78.9)	8 (42.1)	0.022
Dyslipidemia	7 (36.8)	3 (15.8)	0.135
Smoking	16 (84.2)	14 (73.7)	0.346

Discussion

This research was carried out at the Integrated Heart Center Installation of Dr. M. Djamil Hospital Padang from March 2023 to December 2023. A total of 38 patients with AMI-STE had met the inclusion criteria. The subjects were then divided into groups with low eNOS levels and normal eNOS levels. This study showed that the average age of the research subjects was 62.53 ± 8.2 years. This is in line with Bloos et al's research on 576 AMI-STE patients, it was found that the majority (83%) of AMI-STE patients were over 50 years old and only a small portion (17%) of patients were under 50 years old**.**[16] However, based on various previous studies, there are also slight differences in terms of age. The average age at first onset of ACS in the United States is 65 years for men and 72 years for women. About two-thirds of myocardial infarctions occur in patients over 65 years of age and one-third in patients over 75 years of age. In addition, 60% of hospitalizations due to ACS occur in patients over 65 years of age[16,17]. Age in AMI-STE patients is not only a risk factor, but also has prognostic significance. Age is known to be an independent risk factor for predicting the clinical outcomes of AMI-STE patients after the procedure. In addition, older age is associated with more comorbidities and risk factors.[17]

Characteristics of research subjects based on gender in this study showed that almost all (92.1%) of the subjects were male. This result was in accordance with various other studies. Research conducted by Duraes et al and N'Guetta et al, found that more than 50% of STEMI patients were mostly men.[18] There are various theories that can explain this finding. The American Heart Association (AHA) states that men have a higher risk of heart attack than women. The low incidence of AMI-STE in women is due to the protective effect of estrogen which slows the progression of atherosclerosis by influencing plaque stability and protecting it from plaque rupture. When entering menopause, women have almost the same risk of heart disease as men. Apart from that, another factor that play a role is the lifestyle of the younger generation which is characterized by work stress, overwork, smoking, drinking alcohol and overeating, which can cause disturbances in the internal organs such as coronary atherosclerosis, increasing the number of heart attacks. Alexander et al.'s study found gender differences in younger

patients, such that smoking was more common in younger men than in women.[19]

Based on infarct location, more than half (52.6%) of the study subjects were diagnosed with anterior AMI-STE. Research conducted by Obeidat et al showed that the anterior region (67%) was the most common location in myocardial infarction patients.[20] It is known that the anterior wall is the most common location with significant morbidity and mortality. The anterior wall of the heart receives blood supply through the Left Anterior Descending (LAD) Coronary artery, which supplies blood to the anterior wall of the left ventricle, the anterior part of the interventricular septum, and the anterior wall of the right ventricle. When rupture occurs in an existing atherosclerotic lesion, it leads to thrombus formation and tissue ischemia. If ischemia continues, the blood supply to the myocardium will be acutely reduced, causing myocardial necrosis. This study showed that the most common risk factors for AMI-STE sequentially were smoking in 30 people (78.9%), hypertension in 23 people (60.5%), diabetes in 17 people (44.7%), and dyslipidemia in 10 people (26.3%). In this study, only one patient had a family history of coronary heart disease.[20]

There are various explanations for these findings. Research by Aminuddin et al in 2023 showed that atherosclerosis is related to the innate immune response and is characterized by a chronic inflammatory process in the walls of blood vessels. Various studies show that in AMI-STE patients, smoking is the most common risk factor found. Smoking is known to increase vascular disease by causing blood vessel inflammation and oxidative stress. Exposure to cigarette smoke can cause excessive matrix metalloproteinase (MMP) activity and inflammation. Increased MMP activity is responsible for activated and unstable plaques in Acute Myocardial Infarction.[21] This theory is in line with this research, it was found that the most common risk factor was smoking with 30 people (78.9%).

As shown in table 2, the mean eNOS level in this study was 47.47 ± 23.88. Median eNOS level was 37,050. Based on the literature, eNOS levels in normal subjects was 48.16 pg/mL[15]. So it was found that the average eNOS level in AMI-STE patients in this study was lower than the eNOS value in healthy people.

Biomarkers of the NO pathway play an important regulatory role in preventing further cardiac damage in AMI-STE patients. eNOS has a major effect on coronary arteries during a heart attack. When there is a blockage in the coronary arteries, blood flow to certain parts of the heart stops. This condition triggers eNOS to produce nitric oxide (NO) which functions as a vasodilator, resulting in widening of the coronary arteries to increase blood flow and oxygen to the heart tissue affected by ischemia. Therefore, eNOS works as protective agent to reduce heart damage caused by a lack of oxygen supply during a heart attack. However, at the same time eNOS can also play a role in producing oxygen free radicals and reactive nitrogen. These substances can trigger oxidative stress conditions and ultimately damage heart cells. Therefore, the role of eNOS in AMI-STE may have a dual impact, both protective and destructive. [22]

Additionally, potential mechanisms through low eNOS levels in cardiovascular risk factors were suspected to cause endothelial dysfunction. Oxidative stress and endothelial dysfunction in the coronary and peripheral

circulation have important prognostic value for the occurrence of AMI. There has not been much research on eNOS levels in AMI-STE patients, but NO levels and oxidative stress are closely related. The research results of Parenica et al showed that NO pathways, such as nitrite/nitrate – (NOx), ADMA, neopterin, and oxidative stress were increased in patients with AMI-STE. Research by Yang and colleagues showed that eNOS levels in AMI-STE patients were significantly reduced compared with normal people, (6.35 ± 0.22 U/ml vs 7.86 ± 0.19 U/ml). Research by Tasolar et al. have findings that support the findings of Yang et al, namely that eNOS levels were reduced in patients with slow coronary flow (SCF) compared to normal subjects, specifically reaching 32.58 ± 21.36 pg/ml in SCF and 48.16 ± 24.35 pg/ ml in normal people.[21,22]

The results of this study showed that overall risk factors were mostly found with low eNOS levels compared with normal ones. eNOS levels had a statistically significant association with diabetes mellitus ($p<0.001$) and hypertension ($p=0.022$) as risk factors for AMI-STE. However, statistical tests showed that there was no statistically significant association between eNOS levels and dyslipidemia ($p=0.135$) and smoking ($p=0.346$) as risk factors for CHD.

In the AMI-STE process, there is a decrease in eNOS function, where eNOS functions as the body's defense mechanism to prevent more severe endothelial damage.[11] In addition, endothelial dysfunction correlates with a decrease in eNOS levels.. This can explain why overall risk factors were found more common in low eNOS levels group compared to normal eNOS group. eNOS expression was found to be decreased in metabolic syndrome.[14] In diabetes mellitus, normal insulin signals will increase eENOS enzymatic activity. On the other hand, conditions of insulin resistance will disrupt the P13K-Akt pathway which will cause reduced eNOS activity, reduced NO formation and consequently cause vasoconstriction in AMI-STE.[15,16]

Activation of eNOS occurs due to phosphorylation of serine 1177 by the protein kinase Akt/PKB. Hyperglycemia-induced mitochondrial superoxide overproduction increases O-linked conversion of N-acetylglucosamine and reduces O-linked phosphorylation of the transcription factor Sp1. In bovine aortic endothelial cells, hyperglycemia inhibited eNOS activity by 67%. Inhibition of hyperglycemia-associated eNOS was accompanied by a twofold increase in eNOS modification by O-linked N-acetylglucosamine and a decrease in O-linked serine phosphorylation at residue 1117. Inhibition of eNOS and changes in post-translational modifications were reversed by inhibition of antisense glutamine:fructose-6-phosphate amidotransferase, the rate-limiting enzyme of the hexosamine pathway, or by blocking mitochondrial superoxide overproduction by releasing protein-1 (UCP-1) or manganese superoxide dismutase (MnSOD). Chronic changes in eNOS activity through this mechanism may explain some of the accelerated atherosclerosis in diabetes.[22]

Likewise with other CHD risk factors, such as hypertension and dyslipidemia, eNOS levels have also been found to be reduced in various studies.[17] Decreased levels of nitric oxide (NO) produced by catalyzed endothelial nitric oxide synthase (eNOS) are associated with higher blood pressure. As is known, eNOS produces the compound nitric oxide (NO) which functions as a vasodilator. A decrease in eNOS levels causes a decrease in NO, so that vasodilation will be

hampered, resulting in an increase in blood pressure.[22] In dyslipidemia, oxidative and non-oxidative LDL will interfere with immunity and regeneration of cells experiencing inflammation. In addition, oxidative LDL inactivates the eNOS/NO pathway.[18,19] Based on latest study, mean endothelial eNOS levels were significantly lower in the group of smokers compared to those who did not.[20] However, in this study no statistically significant differences were found.

Conclusion

The characteristics of the subjects in this study were that the majority of subjects were male by the average age of 62.53 ± 8.2 years. Among the subjects, most of them had anterior AMI-STE, and smoking as the risk factor. There was a statistically significant association between eNOS levels and diabetes mellitus, as well as hypertension as modifiable risk factors for CHD. However, there was no association between eNOS levels with dyslipidemia and smoking as the risk factors for CHD.

Conflict of Interest

The authors declare no potential conflicts of interest or competing interests. The authors received no financial assistance or grants from public, private, or nonprofit funding agencies.

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