CORRELATION BETWEEN PLASMA NITRIC OXIDE LEVEL AND CORONARY ARTERY STENOSIS SEVERITY BASED ON SULLIVAN SCORING SYSTEM IN STABLE ANGINA PATIENTS

Farhanah Meutia, J Nugroho Eko Putranto
Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya

ABSTRAK

Oksida nitrat (NO) merupakan faktor utama anti-aterosklerosis yang dihasilkan oleh endotel. Disfungsi endotel yang menyebabkan gangguan bioavailabilitas NO merupakan penanda awal aterosklerosis. Berbagai sistem penilaian angiografi digunakan untuk mengukur karakteristik lesi arteri koroner aterosklerotik. Sistem penilaian Sullivan adalah salah satu sistem penilaian angiografi yang dapat menunjukkan hubungan antara tingkat keparahan lesi arteri koroner dengan beban plak aterosklerosis. Tujuan utama dari penelitian ini untuk membuktikan korelasi antara kadar NO plasma dan tingkat keparahan stenosis arteri koroner berdasarkan sistem penilaian Sullivan pada pasien angina stabil. Penelitian ini merupakan penelitian observasional analitik, cross sectional, yang melibatkan 25 pasien dengan angina stabil yang menjalani kateterisasi. Kadar NO plasma berkorelasi signifikan dengan tingkat keparahan stenosis arteri koroner berdasarkan Sullivan vessel score (r = -0,75; p = 0,000), dengan tingkat keparahan stenosis arteri koroner berdasarkan Sullivan stenosis score (r = -0,67; p = 0,000), dan dengan tingkat keparahan stenosis arteri koroner berdasarkan Sullivan extent score (r = -0,63; p = 0,001). Semakin tinggi tingkat plasma NO, semakin rendah tingkat keparahan stenosis arteri koroner menurut Sullivan vessel score, stenosis score dan extent score. Simpulan, kadar NO plasma memiliki korelasi yang signifikan dengan tingkat keparahan stenosis arteri koroner berdasarkan sistem penilaian Sullivan pada pasien dengan angina stabil. ***(FMI 2015;51:22-30)***

Kata kunci: *oksida nitrat, Sullivan scoring system, angina stabil*

ABSTRACT

Nitric oxide (NO) is a major antiatherosclerotic factor produced by the endothelium. Endothelial dysfunction that causes impaired bioavailability of NO, is an early marker of atherosclerosis. Various angiographic scoring system are used to quantify the characteristics of atherosclerotic coronary artery lesions. Sullivan scoring system is one of the angiographic scoring system that can demonstrate a link between the severity of coronary artery lesions with atherosclerotic plaque burden. The main objective of this study to prove the correlation between plasma NO level and severity of coronary artery stenosis based on Sullivan scoring system in stable angina patient. This was an observational analytic study, cross sectional, involving 25 patients with stable angina who underwent catheterization. Plasma NO levels significantly correlated well with the severity of coronary artery stenosis based on Sullivan vessel score (r = -0,75, p = 0,000), with the severity of coronary artery stenosis based on Sullivan stenosis score (r = -0,67; p = 0,000), and with the severity of coronary artery stenosis based on Sullivan extent score (r = -0,63; p = 0,001). The higher plasma NO level, the lower the degree of severity of coronary artery stenosis by Sullivan vessel score, stenosis score dan extent score. In conclusion, plasma NO levels has a significant correlation with the severity of coronary artery stenosis based on Sullivan scoring system in patients with stable angina. ***(FMI 2015;51:22-30)***

Keywords: *nitric oxide, Sullivan scoring system, stable angina*

Correspondence: Farhanah Meutia, Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Airlangga, Jalan Prof. Dr. Moestopo 47, Surabaya 60131, Indonesia.

INTRODUCTION

Endothelial dysfunction is an early marker of atherosclerosis. Disruption in the integrity of the vascular endothelial function plays a role in all stages of atherogenesis, ranging from lesion formation to plaque rupture. Nitric oxide (NO) is a major anti-atherosclerotic factor produced by the endothelium. Endothelial injury, both physically and biochemically will disrupt homeostatic production of vascular mediators such as NO, which will lead to the formation of thrombus, impaired vascular tone, and dysregulation of vascular smooth muscle cell growth in the intima. Decreased level of NO can occur due to decreased NO synthesis or increased degradation. This condition causes excessive production of superoxide anion, which results in a decrease in atherogenic and thrombogenic inhibition process and a decrease in the ability of coronary artery dilatation (Yasa & Türkseven 2005).

Coronary heart disease (CHD) is a health problem in both developed and developing countries. CHD patients

were estimated to be 16.8 million people in the United States (U.S.), 9.8 million of them experienced angina pectoris, and nearly 8 million people experience a myocardial infarction. CHD is the leading cause of death in the United States in both men and women in 2005, which occurred in 607 thousand people (about 1 in 5 deaths). Patients were discharged from hospital with a diagnosis of CHD in the U.S., about 1.76 million people in 2006. The major risk factors of CHD include hypercholesterolemia, diabetes, hypertension, and smoking, all of which are strongly associated with impaired NO activity (Cassar et al 2009).

In general, CHD involves a decrease in coronary artery blood flow, which is largely due to the process of atherosclerosis. Partial or total blockage of one or more coronary arteries and multiple branches can be determined by catheterization. Various scoring system are used to quantify the severity of coronary lesions. The purposes of the scoring system, among others, are to assess the relationship between the lesion's severity and the success of coronary revascularization's method, and also with the atherosclerotic plaque burden, and so forth. One of the angiographic scoring system, Sullivan scoring system, can demonstrate a link between the coronary artery lesion's severity with atherosclerotic plaque burden. It was published in 1990. The system consists of Sullivan vessel score, Sullivan stenosis score and Sullivan extent score (Sullivan et al 1990, Neeland et al 2012). Several studies have shown that the production of NO in endothelium contributes to the regulation of vascular tone, blood flow and blood pressure. NO can inhibit platelet aggregation and platelet adhesion to the vascular endothelium. NO can also inhibit leukocyte adhesion to endothelium. Changes in intracellular calcium level is also an important thing in the ischemic heart muscle. NO plays a role in the regulation of calcium, through activation of guanylate cyclase, which can increase cGMP (Garelnabi et al 2011). Past studies have shown the relationship of NO synthesis abnormalities and impaired ability of endothelial vasodilation, but the studies about NO level in patients with CHD who performed angiography are still rare (Napoli et al 2006). This study aims to determine plasma NO level in patients with stable angina who performed coronary angiography, and to prove the correlation between plasma NO level and the severity of coronary artery stenosis as measured by Sullivan scoring system in patients with stable angina.

MATERIALS AND METHODS

This study was an analytic observational study with cross sectional design. The study was conducted in February 2014 until March 2014, in Cardiology Ward, Faculty of Medicine Airlangga University/Dr. Soetomo Hospital Surabaya, Cardiovascular Diagnostic and Intervention Dr. Soetomo Hospital Surabaya, and Central Biomedical Laboratory, Faculty of Medicine Brawijaya University Malang, for the plasma NO measurement.

The study group consisted of 25 consecutive stable angina patients (19 men and 6 women, mean age 58 ± 8 years) undergoing elective coronary angiography. Informed consent was obtained from each patient. All patients were continued their routinely standard medication therapy for CHD, such as statin, ACE inhibitor/ARB, antiplatelet and nitrate, because of safety and ethical reason. All patients had normal renal and liver function test and no evidence of malignant disease and or severe infection. Clinical indications for coronary angiography were stable angina. Exclusion was performed if the patient had acute coronary syndrome, severe infection, severe renal and/or liver function impairment.

Plasma NO measurement

Blood samples were obtained from all subjects before coronary angiography procedure in cardiology ward. Blood samples were placed in EDTA tubes and stored at 4ºC for 24 hours until centrifugation process at 3000 rpm for 15 minutes, and then 1 cc of plasma removal were done and the samples were stored at ≤ -20ºC until the time of assay.

NO was determined by indirect method using the R&D system total NO kit, that measured plasma nitrite plus nitrate, two end stable products of NO metabolism as the half life of NO is very short. This assay determines NO concentration based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by colorimetric detection of nitrite as an azo dye product of the Griess Reaction. The Griess Reaction is based on the two step diazotization reaction in which acidified nitrite produces a nitrosating agent, which reacts with sulfanilic acid to produce the diazonium ion. This ion is then coupled to *N*-(1-naphthyl) ethylene-diamine to form the chromophoric azo-derivative which absorbs light at 540–570 nm. Results were given as µmol/l (Mobarak & Abdallah 2011).

Coronary artery stenosis severity

Diagnostic coronary angiography were performed by a percutaneous femoral approach. Five experienced cardiologist who were unaware of the laboratory data on nitric oxide reviewed all angiograms simultaneously, and were quantified based on Sullivan scoring system

consisting of Sullivan vessel score, stenosis score and extent score.

Sullivan vessel score was defined by calculate the number of vessels with stenosis > 70% luminal diameter reduction (≥ 50% in left main/LM artery). Result score were according to the number of vessels involved: 0 for no vessel disease, 1 for single vessel disease, 2 for double vessel disease, 3 for triple vessel disease. Involvement of LM stenosis only, was considered as single vessel disease. Involvement of LM–left anterior descending artery/LAD stenosis, LM–left circumflex artery/LCx stenosis or LM–LAD–LCx stenosis, were considered as double vessel disease.

Coronary anatomy was devided into 8 segment according to Gensini score coronary segmentation : proximal LAD, mid LAD, distal LAD, proximal LCx, distal LCx, proximal right coronary artery/RCA, mid RCA, distal RCA. The grade of lesions were: 1 for < 50% stenosis, 2 for 50–75% stenosis, 3 for 75–99% stenosis, 4 for 100% stenosis. Sullivan stenosis score was calculated as the sum of the scores given to all segments. The result score ranging from 0 to 32. Sullivan extent score was defined by the percentage of the coronary intimal surface area affected by atheroma, without specific weighting for the degree of luminal narrowing. The percentage involvement of each vessel is estimated and multiplied by a factor representative of the surface area of that vessel in relation to the entire coronary tree: LM 5%, LAD 20%, main diagonal artery 10%, septal perforator artery 5%, LCx 20%, obtuse marginal artery/OM and posterolateral branch artery/PL 10%, RCA 20%, posterior descending artery/PDA 10%. The special notes were if OM/intermediate artery gives the main vascularization to ventricle lateral wall, so the percentage involvement of OM/intermediate artery 20%, LCx 10%. Results of Sullivan extent score ranging from 0 to 100%.

Risk factors for CHD

The study group was grouped as low risk and high risk, evaluating age (> 45 years old for males and > 55 years old for females), male gender, diabetes mellitus (diabetic symptoms with randomized blood glucose ≥ 200mg/dl or fasting plasma glucose ≥ 126 mg/dl or 2h post prandial glucose ≥ 200 mg/dl), hypertension (history of hypertension or blood pressure ≥ 140/90 mmHg on 2 separate examination time in the ward), smoking (smoking history or active smoker), dyslipidemia (total cholesterol ≥ 200 mg/dl or triglycerides ≥ 200 mg/dl or LDL > 100 or HDL-C ≤ 50mg/dl. Patients with fewer than 3 risk factors without diabetes mellitus were defined as belonging to the low risk group (n=2), and patients with 3 and more risk factors or less than 3 risk factors with diabetes mellitus were defined as belonging to high risk group (n=23).

Isosorbide dinitrate therapy

The study group was also grouped based on isosorbide dinitrate drug dosage routinely taken by the patients. The first group (n=13) consisted of patients who taken isosorbide dinitrate at doses greater than or equal to 15 mg per day orally. The second group (n=12) consisted of patients who taken isosorbide de nitrate at doses less than 15 mg per day orally.

Angiotensin converting enzyme inhibitor/angiotensin receptor blocker, antiplatelet and statin therapy

All of the patients (n=25) in the study group were routinely taken antiplatelet drug and statin therapy for secondary prevention of CHD. However, not all of the patients were routinely taken angiotensin converting enzyme inhibitor/angiotensin receptor blocker drug therapy, only some patients in the study group (n=17).

Statistical analysis

Categorical variables were given as counts (percentages) and continuous variables as the mean ± standard deviation (SD). Saphiro Wilk test was used for normalization data. The data analysis began by conducted a multivariate analysis to examine the effect of various confounding variables on the plasma levels of NO and the Sullivan scoring system.

Statistical analysis was performed to assess the correlation between plasma NO levels and severity of coronary artery stenosis based on Sullivan vessel score, stenosis score, and extent score, using Spearman's rank correlation analysis. A p value < 0.05 was considered significant.

RESULTS

Patient characteristics

The study obtained as many as 25 patients with stable angina with details as follows: the mean age of the patients was 58.08 ± 8.108 years old. According to the gender, the study group was consisted of 19 males (76%) and 6 females (24%). Patients who have risk factors for diabetes were 7 patients (28%), hypertension 15 patients (60%), smoking 12 patients (48%), dyslipidemia 16 patients (64%). Standard therapies for stable angina that were routinely consumed by the patients were consisted of isosorbide dinitrate at a dose of ≥ 15 mg/day was found in 13 patients (52%),

whereas isosorbide dinitrate at a dose of < 15 mg/day was found in 12 patients (48%). Angiotensin converting enzyme inhibitors/angiotensin receptor blocker were used by 17 patients (68%), and all patients received anti-platelet therapy and statins.

Plasma NO levels in the study had a mean value of 25.75 ± 24.66 µmol/L, with a minimum value of 1.57 µmol/L and a maximum value of 91.49 µmol/L. The study showed higher plasma NO levels in males than females (p=0.29) (Table 1). Patients with high CHD risk factors, had lower plasma NO levels than patients with low CHD risk factors (p=0.56) (Table 1). Each CHD risk factors had their own characteristic of plasma NO levels. Diabetes mellitus patients had lower plasma NO levels than non-diabetic patients (p=0.15) (Table 1). Patients with risk factors of smoking also had a plasma NO levels were lower (p=0.53), whereas patients with risk factors for hypertension and dyslipidemia, showed slightly different results in this study (Table 1). Plasma levels of NO was higher in hypertensive patients (p=0.67), so does the patients with dyslipidemia (p=0.53) (Table 1). The study also showed that patients who received therapeutic doses of isosorbide dinitrate ≥ 15mg/day orally actually have plasma NO levels lower than those of patients who received therapeutic doses of isosorbide dinitrate < 15mg/day (p=0.26) (Table 1). Patients with angiotensin converting enzyme inhibitor/angiotensin receptor blocker therapy had higher plasma NO levels (p=0.82) (Table 1).

Coronary artery stenosis severity by Sullivan vessel score in the study, a total of 3 patients (12%) had a score of 0 (no vessel disease), 5 patients (20%) received a score of 1 (single vessel disease), 8 patients (32%) with a score of 2 (double vessel disease), while a score of 3 (triple vessel disease) was found in 9 patients (36%). The study showed female patients have higher Sullivan vessel score than men (p=0.118) (Table 2). Patients with high-risk factors for CHD have higher score than patients with low CHD risk factors (p=0.443) (Table 2). Risk factors analysis for diabetes and hypertension, each of them had higher Sullivan vessel score (p=0.212 and p=0.757) (Table 2). In contrast, the study showed lower Sullivan vessel score in patients with dyslipidemia (p=0.339) (Table 2). Patients with or without smoking risk factors showed the same score (p=0.912) (Table 2). Patients who received oral doses ≥ ISDN therapy 15 mg/hari Sullivan vessel has a higher score (p=0.488), whereas patients with ACE inhibitor therapy Sullivan vessel actually have a lower score (p=0.533) (Table 2).

Coronary artery stenosis severity by Sullivan stenosis score has a score range of 0-32, but in the study, the range of variation obtained a score of 0-23. Highest frequency of Sullivan stenosis score obtained in the study by a score of 14 on 4 patients (16%), followed by a score of 10 on 3 patients (12%). The study showed female patients had a higher Sullivan stenosis score than male patients (p=0.155). Patients with high risk factors for CHD, had a higher Sullivan stenosis score than patients with low CHD risk factors (p=0.235) (Table 3). The study also seek to analyze the distribution of each CHD risk factor. Patients with diabetes, hypertension and smoking, had a higher Sullivan stenosis score (p=0.072; p=0.313; p=0.912). In contrast, this study shows that Sullivan stenosis score was lower in patients with dyslipidemia (p=0.472) (Table 3). Patients who received therapeutic doses of oral ISDN ≥ 15mg/day have higher Sullivan stenosis scores (p=0.507), whereas patients with ACE inhibitor therapy actually had a lower Sullivan stenosis score (p=0.609) (Table 3).

Table 1. Distribution of plasma NO levels based on baseline characteristics of the patients

Characteristic	Category	Frequency %	NO plasma (µmol/L)			
			Minimun	Maximum	Mean	Standard Deviation
Gender	Male	19(76)	1.567	91.488	29.274	26.484
	Female	6(24)	4.569	41.604	14.598	14.033
DM	Yes	7(28)	1.567	37.690	12.708	12.335
	No	18(72)	3.344	91.488	30.824	26.597
Hypertension	Yes	15(60)	1.567	91.488	25.794	27.757
	No	10(40)	3.344	58.433	25.688	20.564
Smoking	Yes	12(48)	3.718	68.213	24.609	20.868
	No	13(52)	1.567	91.488	26.806	28.536
Dyslipidemia	Yes	16(64)	3.344	68.213	26.188	22.536
	No	9(36)	1.567	91.488	24.976	29.507
CAD risk factors	High	23(92)	1.567	91.488	25.392	25.477
	Low	2(8)	18.166	41.604	29.885	16.573
ISDN	≥15mg/day	13(52)	1.567	91.488	22.143	26.298
	<15mg/day	12(48)	3.718	68.213	29.660	23.239
ACE inhibitor/ARB	Yes	17(68)	1.567	91.488	26.593	25.669
	No	8(32)	4.659	58.433	23.964	23.942
Antiplatelet	Yes	25(100)	1.567	91.488	25.751	24.657
	No	-	-	-	-	-
Statin	Yes	25(100)	1.567	91.488	25.571	24.657
	No	-	-	-	-	-

Table 2. Distribution of coronary artery stenosis severity based on Sullivan vessel score based on baseline characteristics of the patients

Characteristic	Category	Frequency %	Sullivan Vessel Score			
			Minimun	Maximum	Mean	Standard Deviation
Gender	Male	19(76)	0	3	1.74	1.046
	Female	6(24)	1	3	2.50	0.837
DM	Yes	7(28)	0	3	2.29	1.113
	No	18(72)	0	3	1.78	1.003
Hypertension	Yes	15(60)	0	3	1.93	1.163
	No	10(40)	1	3	1.90	0.876
Smoking	Yes	12(48)	0	3	1.92	0.996
	No	13(52)	0	3	1.92	1.115
Dyslipidemia	Yes	16(64)	0	3	1.75	1.125
	No	9(36)	1	3	2.22	0.833
CAD risk factors	High	23(92)	0	3	1.96	1.065
	Low	2(8)	1	2	1.50	0.707
ISDN	≥15mg/day	13(52)	0	3	2.08	0.954
	<15mg/day	12(48)	0	3	1.75	1.138
ACE inhibitor/ARB	Yes	17(68)	0	3	1.82	1.074
	No	8(32)	1	3	2.12	0.991
Antiplatelet	Yes	25(100)	0	3	1.92	1.038
	No	-	-	-	-	-
Statin	Yes	25(100)	0	3	1.92	1.038
	No	-	-	-	-	-

Table 3. Distribution of coronary artery stenosis severity based on Sullivan stenosis score based on baseline characteristics of the patients

Characteristic	Category	Frequency %	Sullivan Stenosis Score			
			Minimun	Maximum	Mean	Standard Deviation
Gender	Male	19(76)	1	23	9.32	5.667
	Female	6(24)	4	23	13.17	7.167
DM	Yes	7(28)	4	23	13.71	5.936
	No	18(72)	1	23	8.89	5.810
Hypertension	Yes	15(60)	1	23	11.47	6.844
	No	10(40)	3	15	8.40	4.600
Smoking	Yes	12(48)	3	23	10.83	5.702
	No	13(52)	1	23	9.69	6.688
Dyslipidemia	Yes	16(64)	1	18	9.06	5.579
	No	9(36)	3	23	12.33	6.837
CAD risk factors	High	23(92)	1	23	10.70	6.182
	Low	2(8)	4	6	5.00	1.414
ISDN	≥15mg/day	13(52)	3	23	11.08	5.838
	<15mg/day	12(48)	1	23	9.33	6.569
ACE inhibitor/ARB	Yes	17(68)	1	23	9.82	6.136
	No	8(32)	3	23	11.12	6.446
Antiplatelet	Yes	25(100)	1	23	10.24	6.132
	No	-	-	-	-	-
Statin	Yes	25(100)	0	3	1.92	1.038
	No	-	-	-	-	-

Sullivan extent score calculation was made based on the percentage distribution of coronary artery endothelial area with a score range of 0-100%. In the study, the highest frequency was at 40% of Sullivan extent score, obtained at 6 patients (24%), followed by 70% and 80% of Sullivan extent score, which each of them, found in 4 patients (16%), then a score of 20% and 60%, each of them, found in 3 patients (12%). A score of 10% and 50% of Sullivan extent score, respectively found on 2 patients (8%), and only 1 patient (4%) with a score of 25%. The study showed that the female patients have higher Sullivan extent scores than male patients (p=0.161) (Table 4). Patients with high CHD risk factors also have higher Sullivan extent score than patients with low CHD risk factors (p=0.106). Patients with diabetes, hypertension and smoking also have higher Sullivan extent scores (p=0.034; p= 0.174; p=0.346). In contrast, the study showed lower Sullivan extent score in patients with dyslipidemia (p=0.912) (Table 4).

Each of the confounding variables in the study, underwent bivariate analysis test to determine their effects on the plasma levels of NO and on the coronary artery stenosis severity based on Sullivan scoring system. Spearman's rho correlation test result of age, sex, CHD risk factors, isosorbide dinitrate dosage ≥ 15 mg/day orally, and ACE inhibitor/ARB therapy, each variables showed a correlation that was not statistically significant, either with plasma NO levels, nor the Sullivan scoring system.

Correlation between plasma NO level and the coronary artery stenosis severity measured by Sullivan scoring system

Statistical analysis of the study indicated a negative correlation between plasma NO level and coronary artery stenosis severity based on Sullivan vessel score (r=-0.75), and the correlation was statistically significant with p=0.000 ($p < 0.01$). Linear graph shown in figure 1 showed that the higher coronary artery stenosis severity based on Sullivan vessel score, the lower plasma levels of NO will be.

A negative correlation between plasma NO level and coronary artery stenosis severity based on Sullivan stenosis score was showed in the study (r=-0.67), and the correlation was statistically significant with p=0.000 ($p < 0.01$). Logarithmic graph shown in figure 2 showed that the higher coronary artery stenosis severity based on Sullivan stenosis score, the lower plasma levels of NO will be. Correlation between plasma NO level and coronary artery stenosis severity based on Sullivan extent score also showed a negative correlation (r=-0.63), and it was statistically significant with p=0.001 ($p < 0.01$). Logarithmic graph shown in figure 3 showed that the higher coronary artery stenosis severity based on Sullivan extent score, the lower plasma levels of NO will be.

DISCUSSION

Impaired NO bioavailability can be due to the decreased of NO production or the increased of NO degradation, or both. Atherosclerosis is associated with impaired NO bioavailability and endothelial dysfunction, which manifests varying degrees of stenosis.

If the impairment of NO bioavailability is getting severe, it will have an impact on the extent of vascular endothelial damage (Vallance & Chan 2001, Kawashima & Yokoyama 2004, Yasa & Türkseven 2005, Napoli et al 2006). Sullivan scoring system can reflect the involvement of endothelial area in the atheroma formation, so it can be used to assess the correlation between coronary artery stenosis severity and atherosclerotic plaque burden (Sullivan et al 1990, Freedman et al 1998, Bigi et al 2003, Knudtson 2008).

Table 4. Distribution of coronary artery stenosis severity based on Sullivan extent score based on baseline characteristics of the patients

Characteristic	Category	Frequency %	Sullivan Extent Score (%)			
			Minimun	Maximum	Mean	Standard Deviation
Gender	Male	19(76)	10	80	45.79	20.633
	Female	6(24)	20	80	59.17	28.708
DM	Yes	7(28)	40	80	64.29	15.119
	No	18(72)	10	80	43.06	22.954
Hypertension	Yes	15(60)	10	80	54.00	22.928
	No	10(40)	20	80	41.50	21.864
Smoking	Yes	12(48)	20	80	54.17	17.299
	No	13(52)	10	80	44.23	26.914
Dyslipidemia	Yes	16(64)	10	80	48.44	23.361
	No	9(36)	20	80	50.00	23.452
CAD risk factors	High	23(92)	10	80	51.30	22.422
	Low	2(8)	20	25	22.50	3.536
ISDN	≥15mg/day	13(52)	20	80	54.23	23.438
	<15mg/day	12(48)	10	70	43.33	21.881
ACE inhibitor/ARB	Yes	17(68)	10	80	47.35	22.229
	No	8(32)	20	80	52.50	25.495
Antiplatelet	Yes	25(100)	10	80	49.00	22.913
	No	-	-	-	-	-
Statin	Yes	25(100)	10	80	49.00	22.913
	No	-	-	-	-	-

The correlation between plasma NO level and coronary artery stenosis severity based on Sullivan vessel score, in the present study, showed a significant negative correlation, which means, the higher the plasma NO levels, the lower the degree of coronary artery stenosis severity by Sullivan vessel score. This outcome are consistent with previous study conducted by Ekmekci et al (2005) at Turkey, which initially showed a positive correlation between plasma levels of NO in patients with single-vessel disease, but then, the positive correlation disappeared in patients with double-vessel disease, and later, they found a negative correlation in patients with triple vessel disease. Both of these studies reflect that an increase of NO production in the early stages of atherosclerosis, is a compensatory mechanism to withstand endothelial damage, atherosclerotic plaque formation and thrombosis process. NO also has the ability as an antioxidant, preventing lipid peroxidation. The decrease in plasma NO levels in patients with Sullivan vessel score = 3 (triple vessel disease), showed a failure of the compensatory mechanisms in line with the progression of CHD.

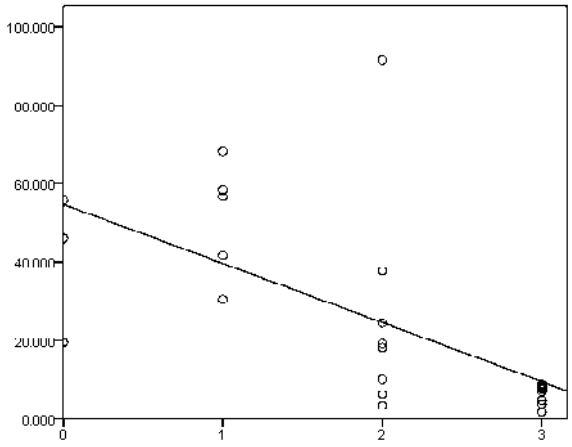

Figure 1. Correlation between plasma NO level and the coronary artery stenosis severity based on Sullivan vessel score

Results of statistical analysis of the correlation of plasma NO levels and coronary artery stenosis severity by Sullivan stenosis scores showed significant negative correlation, which means that the higher plasma NO levels, the lower the degree of coronary artery stenosis severity by Sullivan stenosis score. To the best of our knowledge, this is the first study in which nitric oxide, and Sullivan stenosis score have been studied in an angiographic study. Sahinarslan et al (2006) in Turkey conducted a study about the relationship of asymmetric dimethylarginine (ADMA) level and coronary atherosclerotic score in 98 patients with stable angina. ADMA is one of major NO synthase (NOS) inhibitor. It showed elevated levels of ADMA in the group with coronary lesions, other than that, Sahinarslan et al (2006) found significant correlation between plasma ADMA levels and coronary atherosclerosis score. ADMA, as has been described previously, can inhibit eNOS activity, so that NO synthesis is impaired, which in turn will cause the endothelial dysfunction and atherosclerosis.

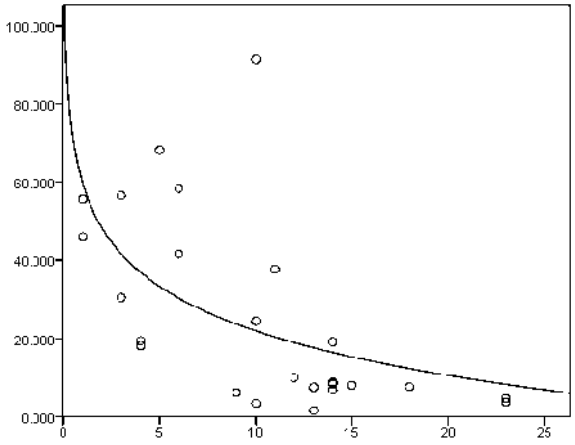

Figure 2. Correlation between plasma NO level and the coronary artery stenosis severity based on Sullivan stenosis score

Our study showed a significant correlation between plasma NO levels with coronary artery stenosis severity by Sullivan stenosis score, which means the higher the plasma NO levels, the lower the degree of coronary artery stenosis severity by Sullivan stenosis score. It proved that a disturbance in NO synthesis or the increased of NO degradation would lead to endothelial dysfunction. The more severe the endothelial dysfunction, the coronary artery obstruction will be more strenuous, and the arterial vasodilation capability will also be decreased. The declining of the capabilities of vasodilation and inhibition of endothelial dysfunction, may be due to a decrease in NO bioavailability.

One thing to note from this study is that all of the subjects are stable angina patients, with chronic coronary artery obstruction. Patients with acute coronary syndrome (ACS) with acute coronary artery obstruction, were excluded, because the pathophysiology of acute coronary syndrome differs from stable angina, mainly due to vulnerable plaque rupture and thromboembolism that occurs within a short onset. Sullivan stenosis score also could not be used to distinguish acute and chronic arterial obstruction, because it does not assess the presence of thrombus on angiography, so the results of this study could not be applied to patients with ACS.

Results of statistical analysis of the present study, the correlation of plasma NO level with coronary artery stenosis severity by Sullivan extent score showed significant negative correlation, which means that the higher plasma NO levels, the lower the degree of coronary artery stenosis severity by Sullivan extents score. Previous study on the correlation of plasma NO levels with Sullivan extent score has never been done. Study that was conducted by Kruszelnicka et al (2013), in Poland, learned about the difference in the relationship between CHD severity and extension based on Sullivan vessel score, Sullivan extent score and Gensini score, with ADMA in male patients with stable angina who did not suffer from diabetes and heart failure, which was already known to have significant coronary artery narrowing (at least 1 segment with the degree of coronary artery stenosis ≥ 70%). The results of the study in Poland showed an increase in ADMA levels in patients with high Sullivan vessel score and Sullivan extent score, but not the Gensini score, when compared with the control group.

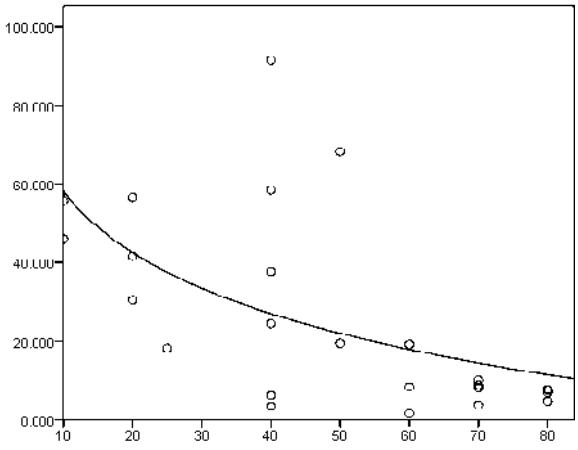

Figure 3. Correlation between plasma NO level and the coronary artery stenosis severity based on Sullivan extent score

This could be potentially caused by the higher Gensini score for proximal lesion than the distal lesion, so it makes the assessment of atherosclerotic plaque burden become less balanced. ADMA as a competitive inhibitor of NOS can inhibit NOS activity, causing a decrease in vascular compliance, increased vascular resistance and decreased blood flow. ADMA blood level is 10xfold higher than L-NMMA, resulting in the increased of ADMA level plays an important role to the decreased of NO level. The decreased of NO level will lead to the failure of NO protective effect on the vascular, such as the effects of vasodilator, anti-oxidant, anti-proliferative, anti-adhesive, and also anti-platelet aggregation.

Both studies results reflected the fundamental problem in endothelial dysfunction is NO bioavailability impairment, which is due to the reduced of NO production or the increased of NO degradation. The increased of NO degradation can be due to the formation of reactive oxygen species (ROS), including excessive superoxide anion and oxidized LDL cholesterol, the decreased of antioxidant mechanisms, and also the accumulation of advanced glycation end-products (AGEs). NO production can be decreased, if there are elevated levels of NOS inhibitors, like ADMA and L-NMMA, and NO cofactor deficiency, such as, BH_4 and NADPH. The increased of NO degradation and decreased of NO production, will impaired NO bio-availability and induce endothelial dysfunction (Vallance & Chan 2001, Kawashima & Yokoyama 2004, Yasa & Türkseven 2005, Napoli et al 2006). Endothelial dysfunction is an early marker of the atherosclerotic process that eventually continued to atherosclerotic plaque formation (Herman & Moncada 2005, Poredos 2011). Several mechanisms are considered to explain the endothelial dysfunction in atherosclerosis include endothelial signal transduction disruption, impaired of L-arginine bioavailability, eNOS and its cofactors changes, inhibition of NO by ROS, impaired response of vascular smooth muscle to NO and thickening of the intima layer that makes the diffusion processes in blood vessels become inhibited (Yasa & Türkseven 2005, Napoli et al 2006, Kinlay & Ganz 2007, Dobutovic et al 2011).

High Sullivan extent score indicates extensive coronary artery lesions, thus indicating the complex process of atherosclerosis, a combination of endothelial dysfunct-ion, vascular inflammation, accumulation of lipids, cholesterol, calcium, and cellular debris within the blood vessel's intima. This process can lead to plaque formation, vascular remodeling, acute and chronic luminal obstruction, abnormalities of blood flow and a decrease in O2 supply to the target organ. NO bioavailability impairment in the coronary artery lesions will cause a series of changes in the function, such as, increased vasoconstriction, increased platelet and monocyte adhesion and migration and proliferation of smooth muscle cells (Schwartzman et al 1998, Vallance & Chan 2001, Yasa & Türkseven 2005, Soydinç et al 2007).

CONCLUSION

Lower NO plasma levels are significantly linked to the coronary atherosclerosis severity process. Despite being investigated in a relatively small number of samples, acute coronary syndrome patients were excluded, and also without control group of normal subject so the

normal range of NO plasma level has not available, our study implies that a failure in nitric oxide compensation may be important in the progression of atherosclerosis.

REFERENCES

Bigi R, Cortigiani L, Colombo P, Desideri A, Bax JJ, Parodi O (2003). Prognostic and clinical correlates of angiographically diffuse non-obstructive coronary lesions. Heart 89, 1009-1013

Cassar A, Holmes DR Jr, Rihal CS, Gersh BJ (2009). Chronic coronary artery disease: diagnosis and management. Mayo Clin Proc 84, 1130-1146

Dobutovic B, Smiljanic K, Soskic S, Düngen HD, Isenovic ER (2011). Nitric oxide and its role in cardiovascular diseases. The Open Nitric Oxide Journal 3, 65–71

Ekmekci H, Ekmekci OB, Sonmez H, Ozturk Z, Domanic N, Kokoglu E (2005). Evaluation of fibronectin, vitronectin, and leptin levels in coronary artery disease: impacts on thrombosis and thrombolysis. Clinical & Applied Thrombosis/Hemostasis 11, 63-70

Freedman JE, Ting B, Hankin B, Loscalzo J, Keaney JF Jr, Vita JA (1998). Impaired platelet production of nitric oxide predicts presence of acute coronary syndromes. Circulation 98, 1481-1486

Garelnabi M, Gupta V, Mallika V, Bhattacharjee J (2011). Platelet nitric oxide signaling system in patients with coronary artery disease. Ann Vasc Dis 4, 99–105

Herman AG and Moncada S (2005). Therapeutic potential of nitric oxide donors in the prevention and treatment of atherosclerosis. Eur Heart J 26, 1945-1955

Kawashima S and Yokoyama M (2004). Dysfunction of endothelial nitric oxide synthase and atherosclerosis. Arterioscler Thromb Vasc Biol 24, 998-1005

Kinlay S and Ganz P (2007). Endothelial vasodilatory dysfunction: basic consepts and practical implementation. In: de Caterina R and Libby P (eds). Endothelial Dysfunctions and Vascular Disease. Singapore, Blackwell Publishing Inc, p 179–189

Knudtson M (2008). Coronary Scoring Systems, A Historical Perspective. Available from http://www.approach.org/pdfs/tutorials. Accessed January 18, 2013

Kruszelnicka O, Surdacki A, Golay A (2013). Differential associations of angiographic extent and severity of coronary artery disease with asymmetric dimethylarginine but not insulin resistance in non-diabetic men with stable angina: a cross-sectional study. Cardiovasc Diabetol 12, 145

Mobarak EH and Abdallah DM (2011). Saliva nitric oxide levels in relation to caries experience and oral hygiene. Journal of Advanced Research 2, 357–362

Napoli C, de Nigris F, Williams-Ignarro S, Pignalosa O, Sica V, Ignarro LJ (2006). Nitric oxide and atherosclerosis: an update. Nitric Oxide 15, 265-279

Neeland IJ, Patel RS, Eshtehardi P, Dhawan S, McDaniel MC, Rab ST, Vaccarino V, Zafari AM, Samady H, Quyyumi AA (2012). Coronary angiographic scoring systems: an evaluation of their equivalence and validity. Am Heart J 164, 547-552

Poredos P (2011). Markers of preclinical atherosclerosis and their clinical relevance. The Open Atherosclerosis & Thrombosis Journal 4, 1-10

Sahinarslan A, Cengel A, Biberoglu G, Hasanoglu A, Turkoglu S, Timurkaynak T (2006). Plasma asymmetric dimethylarginine level and extent of lesion at coronary angiography. Coronary Artery Disease 17, 605-609

Schwartzman RA, Cox ID, Poloniecki J, Crook R, Seymour CA, Kaski JC (1998). Elevated plasma lipoprotein(a) is associated with coronary artery disease in patients with chronic stable angina pectoris. J Am Coll Cardiol 31, 1260-1266

Soydinç S, Çelik A, Demiryürek S, Davutoğlu V, Tarakçıoğlu M, Aksoy M (2007). The relationship between oxidative stress, nitric oxide, and coronary artery disease. Eur J Gen Med 4, 62-66

Sullivan DR, Marwick TH, Freedman SB (1990). A new method of scoring coronary angiograms to reflect extent of coronary atherosclerosis and improve correlation with major risk factors. Am Heart J 119, 1262-1267

Vallance P and Chan N (2001). Endothelial function and nitric oxide: clinical relevance. Heart 85, 342–350

Yasa M and Türkseven S (2005). Vasoprotective effects of nitric oxide in atherosclerosis. FABAD J Pharm 30, 41-53