Serum Nitric Oxide Levels in Patients with Coronary Artery Disease

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Abstract

Nitric oxide (NO) plays a pivotal role in the pathophysiology of coronary artery disease. The roles of NO are not only physiological but also pathological in the cardiovascular system. An inappropriate release of NO has been linked to the pathogenesis of CAD. The authors investigated whether serum NOx (nitrate and nitrite), a stable end product of NO, level was related to patients with coronary artery disease. The blood chemistry, such as cholesterol, triglyceride, LDL-C, HDL-C and blood sugar, was also measured in comparison with serum NOx. Serum NOx was measured in samples from 20 healthy controls, 20 angina patients without angiographic evidence of coronary lesions (CAG) and 20 angina patients with angiographic evidence of coronary lesions (CAD) by using modified Griess reaction. The mean serum NOx levels in the CAD groups was higher than CAG and control groups (41.3 ± 5.5, 32.7 ± 3.9 and 25.7 ± 3.5 μmol/L, respectively). NOx levels in the CAD group was only significantly higher than the control groups ($p < 0.05$) but not the CAG groups. There were no significant differences of NOx levels in all age groups. In the CAD group, women showed significantly higher NOx levels than men (64.0 ± 7.5 and 29.0 ± 4.7 μmol/L, respectively, $p < 0.05$). Interestingly, the mean serum NOx levels in the CAD groups was significantly higher in a group of abnormal lipid profiles (cholesterol, triglyceride, LDL-C) and blood sugar than in a group of normal profiles. The results suggested that there was an increased NOx levels in patients with coronary artery disease and much higher in patients with multiple underlying conditions such as hyperlipidemia and hyperglycemia. Thus, the measurement of the NOx levels at different times may help to monitor the state and severity of coronary artery disease.

Key word : Coronary Artery Diseases, Nitric Oxide, Serum, Concentration, Chest Pain

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Nitric oxide (NO) plays a pivotal role in the pathophysiology of coronary artery disease[1]. A reduction in NO production contributes to endothelial dysfunction which is a feature of much of cardiovascular pathology[2-4]. NO is one of the most important intracellular and extracellular signalling substances[5]. It initiates host defense homeostasis and developmental functions[6]. Particularly in the cardiovascular system, NO not only modulates blood vessel tone but also affects other functions of the endothelium such as inhibition of platelet adherence and aggregation, reduction of leukocyte adherence to the endothelium, suppressing proliferation of smooth muscle cells, increase in endothelium permeability, and antithrombogenicity[7]. The roles of NO are not only physiological but also pathological in the cardiovascular system such as allograft rejection, induction of lipid peroxidation after myocardial reperfusion injury, vasospasm and thrombogenesis [8]. Thus, an inappropriate release of NO has been linked to the pathogenesis of coronary artery diseases. However, many studies showed that there was either an increase or decrease of NO levels in coronary artery disease patients[9-11]. The differences in results may arise from many factors e.g. phases of the disease or methods of NO measurement. Therefore, the evaluation of the basal NO levels in healthy subjects in comparison with coronary artery disease patients will help to understand the pathogenesis of coronary artery disease. Moreover, the results may be useful for the selection of appropriate treatment and disease progression by using serum NO levels as a follow-up marker.

MATERIAL AND METHOD

Subjects

The study was performed on 60 subjects recruited from Siriraj Hospital. There were three types of subjects. The first group consisted of 20 subjects who displayed a stenotic lesion of larger than 50 per cent in at least one principal coronary artery (CAD group). The second group consisted of 20 subjects who had angina but had no angiographic evidence of coronary lesions (CAG group). The third group consisted of 20 normal healthy subjects who had no history of heart disease (control group). The study was conducted in full conformance with the principles of the "Declaration of Helsinki" with the approval of the local research ethical committee and informed consent was obtained.

Collection of blood

The blood was drawn from peripheral veins of the subjects after 8 h fasting and placed in tubes. Serum samples were centrifuged at approximately 1,000 x g for 10 minutes. Serum were stored at -40°C until assay.

Measurement of serum nitrite and nitrate

Nitrite and nitrate (NOx) are the primary oxidation products formed when NO reacts with oxygen. The concentrations of these anions have been used as a quantitative measure of NO production. This assay determines NO based on the enzymatic conversion of nitrate to nitrite according to the method of Schmidt et al[12]. Briefly, nitrate was stoichiometrically reduced to nitrite by incubation of sample aliquots for 15 minutes at 37°C in the presence of nitrate reductase (5 U/ml), NADPH (1.33 mM) and flavine adenine dinucleotide (FAD, 0.133 mM) in a final volume of 70 µl. When nitrate reduction was complete, the unused NADPH, which interferes with the subsequent nitrite determination, was oxidized by lactate dehydrogenase (1,000 U/ml) and sodium pyruvate (205 mM), in a final reaction volume of 80 µl and incubated for 5 minutes at 37°C. Subsequently, total nitrite in serum was assayed by adding 80 µl of Greiss reagent (1% sulphanilamide and 0.1% naphtylenediamide in 5% phosphoric acid) to 80 µl samples of serum. The determination of nitrite levels was obtained by measuring colored azo-dye product of the Griess reaction that absorbs visible light at 595 nm (reference filter: 655 nm) with a microplate reader. The serum NOx levels were determined from the calibration curve using $NaNO_2$ standard.

Statistical analysis

Statistical analysis performed with SPSS program. All results are shown as mean ± S.E.M from duplicate determinations. Variable in three groups were compared with one way analysis of variance (ANOVA) followed by Turkey's method. Comparison between the two groups was made by unpaired *t*-test. A p-value of less than 0.05 was considered as statistically significant.

RESULTS

Demographic data and laboratory characteristics of all subjects are shown in Table 1. All data were expressed as mean ± SEM. The laboratory data

Table 1. Clinical characteristics of the subjects.

	Healthy controls (n=20)	CAG group (n=20)	CAD group (n=20)
Age, years	51.1 ± 1.6	53.6 ± 1.7	60.1 ± 2.6
(min-max)	(40-63)	(43-71)	(40-77)
Sex			
Male	9	9	13
Female	11	11	7
Lipid profiles			
Chol, mg/dl	213.8 ± 8.5	201.4 ± 9.5	204.2 ± 11.3
TG, mg/dl,	135.2 ± 21.4	121.6 ± 15.1	147.6 ± 15.5
LDL-C, mg/dl	132.3 ± 9.5	123.2 ± 7.5	120.0 ± 9.8
HDL-C, mg/dl	55.6 ± 4.1	46.2 ± 2.3	43.3 ± 2.6
Glucose, mg/dl	97.9 ± 3.1	107.5 ± 4.5	104.5 ± 2.7
Creatinine, mg/dl	1.4 ± 0.4	0.8 ± 0.0	0.8 ± 0.1
BUN, mg/dl	14.2 ± 0.7	15.1 ± 1.0	14.0 ± 1.3
SGOT, U/L	18.6 ± 1.7	17.3 ± 1.6	16.8 ± 1.8
SGPT, U/L	14.3 ± 2.6	9.2 ± 1.7	6.7 ± 0.8

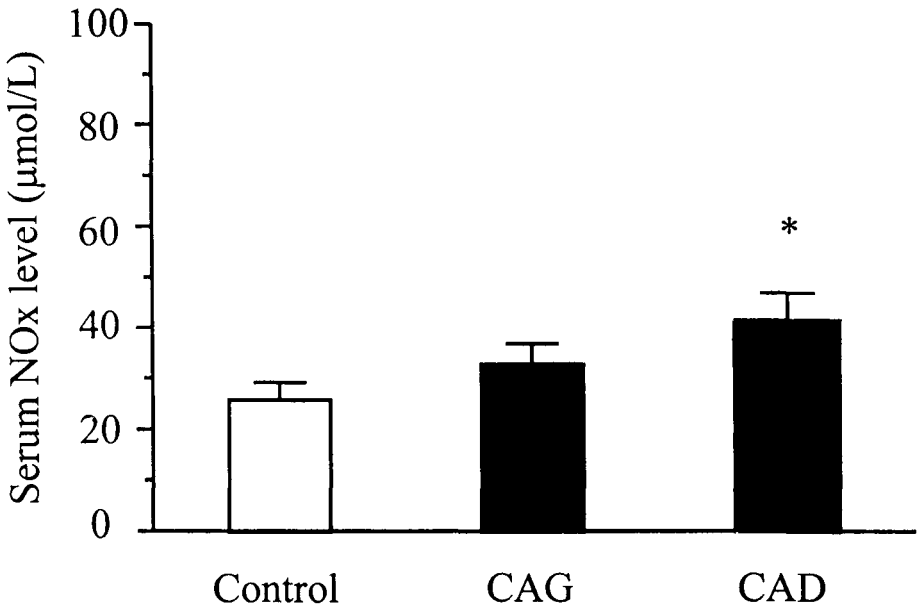

Fig. 1. Serum NOx levels in healthy controls, angina patients without angiographic evidence of coronary lesions (CAG) and angina patients with angiographic evidence of coronary lesions (CAD). Data are expressed as mean ± SEM. * $p < 0.05$ when compared to the healthy controls.

revealed that total cholesterol, triglyceride, LDL-cholesterol, blood sugar, creatinine, BUN and SGOT were not different among the 3 groups. The HDL-cholesterol, SGOT and age showed significant difference in healthy controls, the CAG and CAD group ($p < 0.05$).

Serum NOx levels in coronary artery disease comparison with CAG and control group

The mean serum NOx levels in the CAD group were higher than the CAG and control group (41.3 ± 5.5, 32.7 ± 3.9 and 25.7 ± 3.5 μmol/L, respectively, Fig. 1). NOx level in the CAD group

was only significantly higher than the control group ($p < 0.05$, Fig. 1) but not the CAG group.

The relationship between serum NOx levels and age groups

To investigate the variable effects of serum NOx levels on age, NOx levels were compared in the healthy control, CAG and CAD groups. The data showed no statistically significant difference of NOx levels in all age groups ($p > 0.05$, Fig. 2).

The relationship between serum NOx levels and sex

In the CAD group, women showed significantly higher NOx levels than men (63.9 ± 7.5 and 29.0 ± 4.7 μmol/L, respectively, $p < 0.05$, Fig. 3). Moreover, serum NOx levels increased significantly according to severity of diseases in women (from control, CAG and CAD, respectively, $p < 0.05$, Fig. 3) but did not in men (from control, CAG and CAD, respectively, $p > 0.05$, Fig. 3).

The relationship of serum NOx levels between normal and abnormal cholesterol group

In the abnormal cholesterol group (cholesterol > 200 mg/dl), the mean serum NOx levels of the CAD group were significantly higher than that of the healthy controls (52.4 ± 9.4 and 20.6 ± 3.5 μmol/L, respectively, $p < 0.05$, Fig. 4).

The relationship of serum NOx levels between the normal and abnormal triglyceride group

In both the CAD and healthy control group, the mean serum NOx levels of abnormal triglyceride patients (triglyceride > 150 mg/dl) were significantly higher than that of the normal triglyceride patients ($p < 0.05$, Fig. 5).

The relationship of serum NOx levels between normal and abnormal LDL-C

In the CAD group with an abnormal LDL-C level (LDL-C >160 mg/dl), the mean serum NOx level was significantly higher than that of the healthy control group (60.4 ± 8.5 and 16.6 ± 3.8 μmol/L, respectively, $p < 0.05$, Fig. 6).

The relationship of serum NOx levels between normal and abnormal HDL-C

In the normal HDL-C group (HDL-C > 35 mg/dl), the mean serum NOx levels of the CAD group were significantly higher than the healthy con-

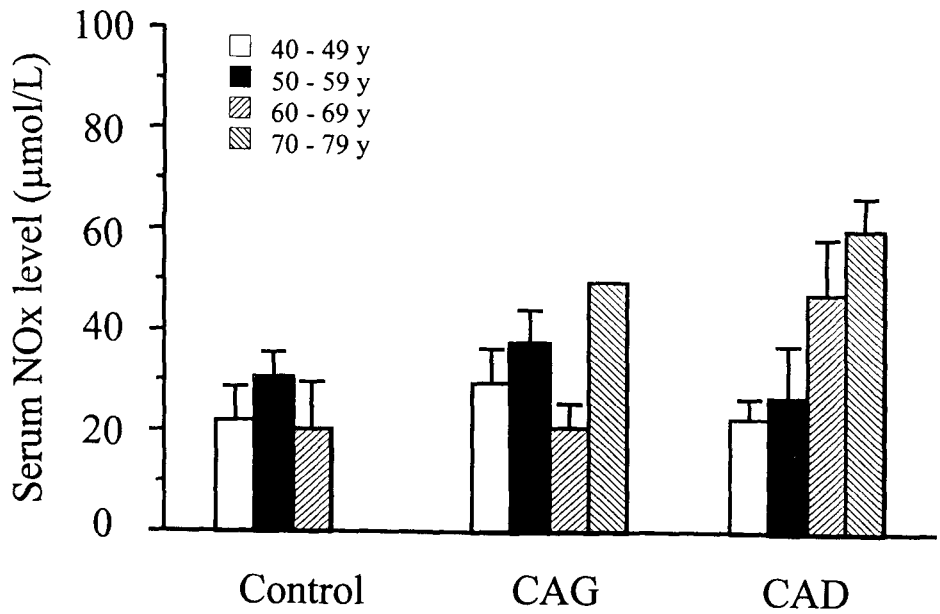

Fig. 2. **The comparison of serum NOx levels between age groups in healthy controls, angina patients without angiographic evidence of coronary lesions (CAG) and angina patients with angiographic evidence of coronary lesions (CAD). Data are expressed as mean ± SEM.**

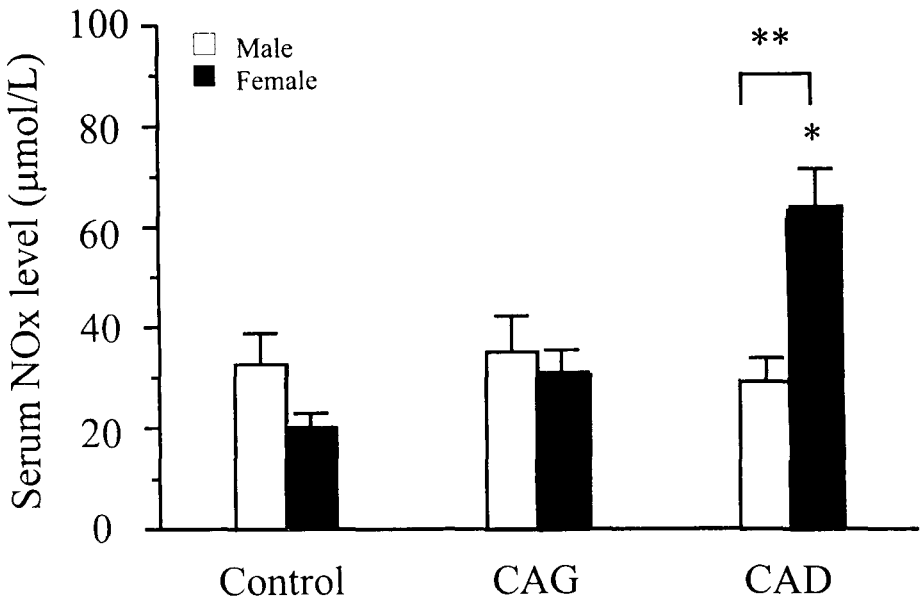

Fig. 3. The comparison of serum NOx levels between male and female healthy controls, angina patients without angiographic evidence of coronary lesions (CAG) and angina patients with angiographic evidence of coronary lesions (CAD). Data are expressed as mean ± SEM. * $p < 0.05$ when compared to females in the control group. ** $p < 0.05$ when compared to males in CAD group.

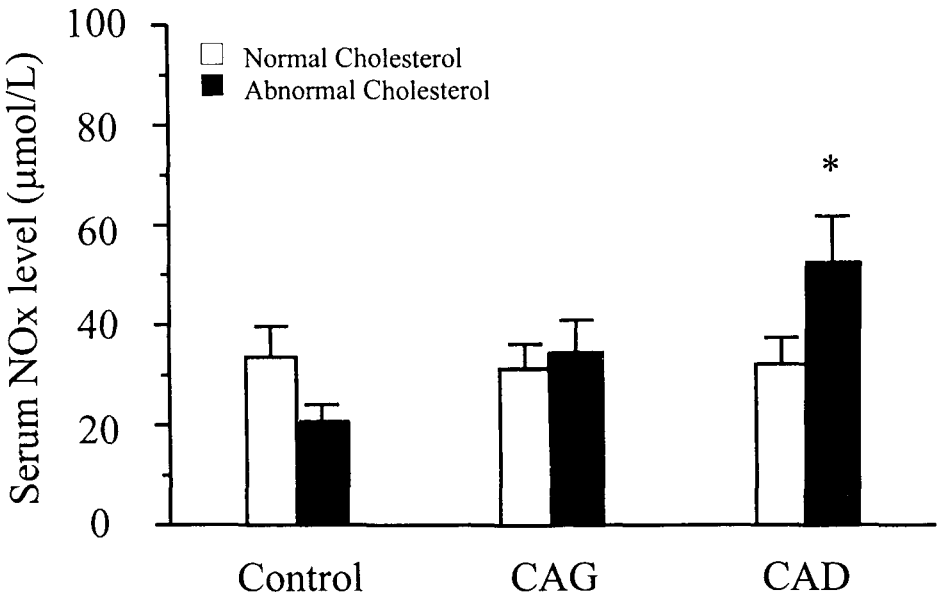

Fig. 4. The comparison of serum NOx levels between normal and abnormal cholesterol in healthy controls, angina patients without angiographic evidence of coronary lesions (CAG) and angina patients with angiographic evidence of coronary lesions (CAD). Data are expressed as mean ± SEM. * $p < 0.05$ when compared to abnormal cholesterol in the control group.

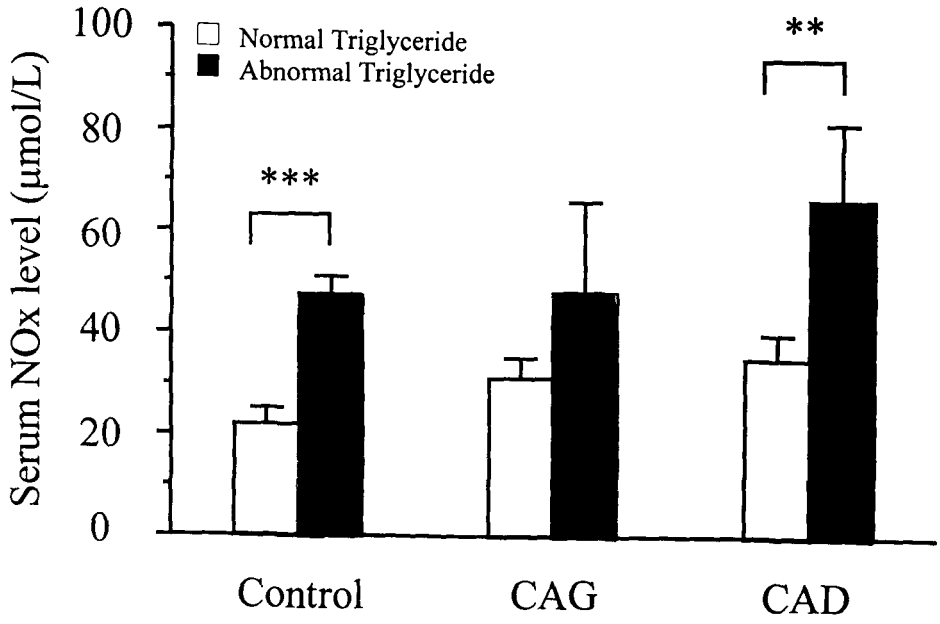

Fig. 5. The comparison of serum NOx levels between normal and abnormal triglyceride in healthy controls, angina patients without angiographic evidence of coronary lesions (CAG) and angina patients with angiographic evidence of coronary lesions (CAD). Data are expressed as mean ± SEM. ** $p < 0.05$ when compared to normal triglyceride in the CAD group. *** $p < 0.05$ when compared to normal triglyceride in the control group.

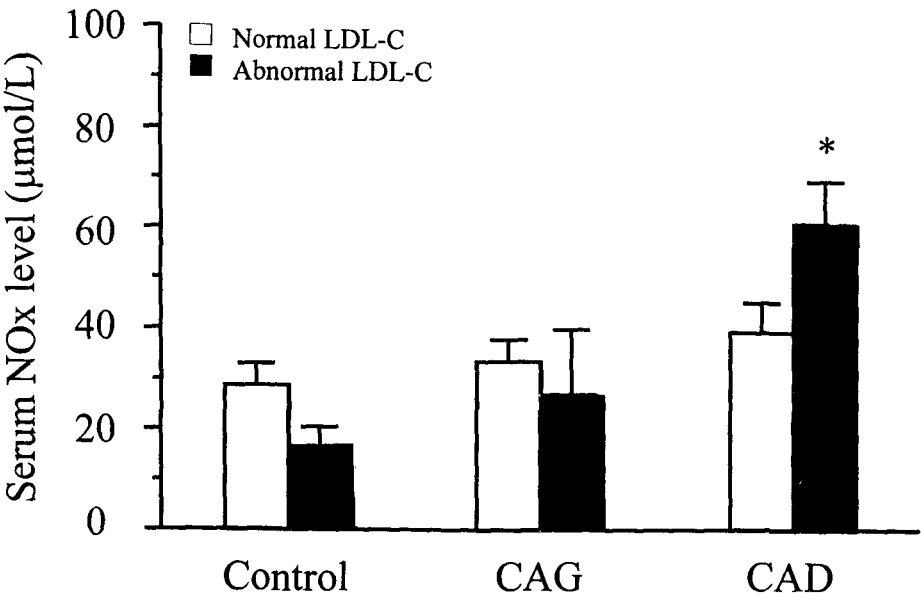

Fig. 6. The comparison of serum NOx levels between normal and abnormal LDL-C in healthy controls, angina patients without angiographic evidence of coronary lesions (CAG) and angina patients with angiographic evidence of coronary lesions (CAD). Data are expressed as mean ± SEM. * $p < 0.05$ when compared to abnormal LDL-C in the control group.

trol group (42.7 ± 5.5 and 24.2 ± 3.5 μmol/L, respectively, $p < 0.05$, Fig. 7).

The relationship of serum NOx levels between normal and abnormal blood sugar

In the CAD group, the mean serum NOx levels of patients with abnormal blood sugar (BS > 110 mg/dl) were significantly higher than patients with normal blood sugar (63.9 ± 10.3 and 31.6 ± 4.6 μmol/L, respectively, $p < 0.05$, Fig. 8).

DISCUSSION

The authors have shown that serum NOx levels in CAD patients were significantly higher than that of the healthy controls. This result is similar to the studies of Lecour et al who found increased NOx levels during prolonged ischemia in an isolated heart rat model[11]. These findings suggested that an increase of serum NOx in the CAD group may be due to a compensatory response according to the severity of the disease. The rise of NOx levels in the CAD group may be caused by induction of the inducible isoform of NOS in the activated macrophages, cardiac myocytes or vascular smooth muscle cells[13] and may result from the acidification occurring during ischemia[14].

In this study, the relationship between serum NOx levels and blood chemistry such as cholesterol, triglyceride, LDL-C and blood sugar were also observed. Interestingly, serum NOx levels were higher in the CAD group with hyperlipidemia than in normal lipidemia and the control group. This might be attributable to the compensatory responses in the diseases. Ferlito and Galina reported that a significantly increased synthesis of the plasma nitrites in hypercholesterol patients means an engagement of NO to neutralize the endothelium damaging molecular substances and particularly the oxidized LDL[10].

In the CAD group with abnormal blood sugar, the NOx levels were significantly different compared with normal blood sugar. This might support a previous study showing that high glucose concentrations induce NOS expression and superoxide anion in human aortic endothelial cell culture [15]. Furthermore, NO mediates increased blood flow to pancreatic islets during hyperglycemia[16]

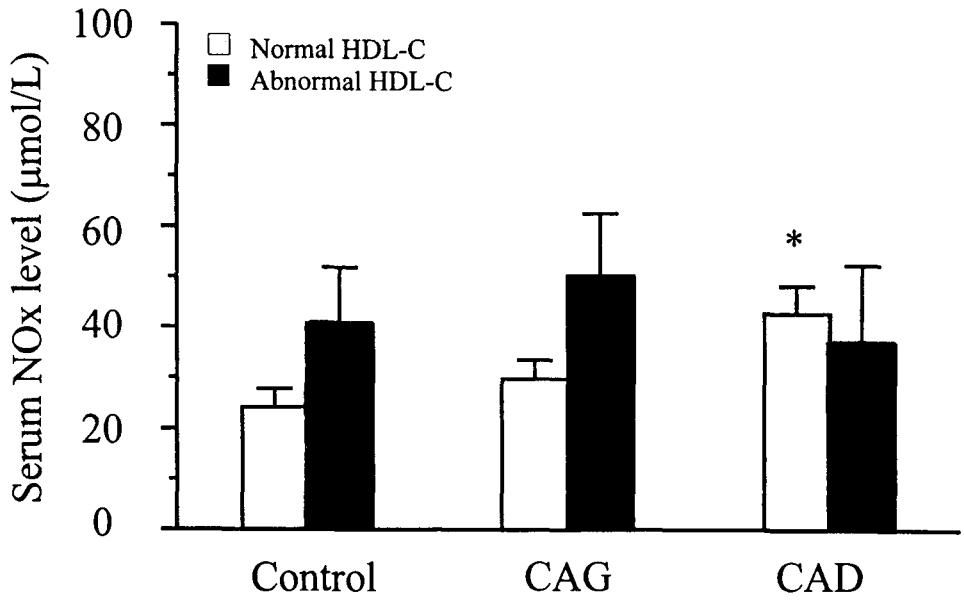

Fig. 7. The comparison of serum NOx levels between normal and abnormal HDL-C in healthy controls, angina patients without angiographic evidence of coronary lesions (CAG) and angina patients with angiographic evidence of coronary lesions (CAD). Data are expressed as mean ± SEM. * $p < 0.05$ when compared to normal HDL-C in the control group.

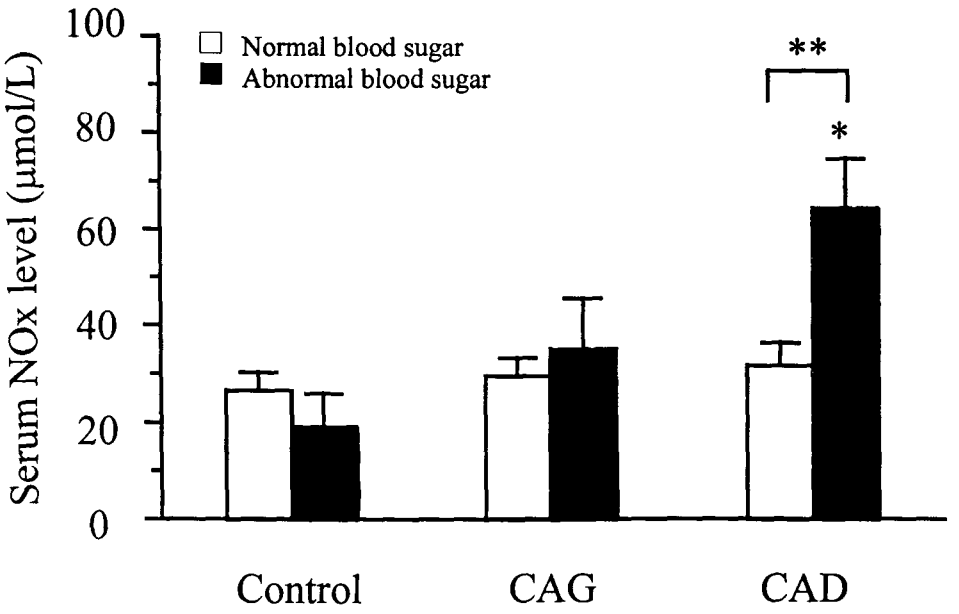

Fig. 8. The comparison of serum NOx levels between normal and abnormal blood sugar in healthy controls, angina patients without angiographic evidence of coronary lesions (CAG) and angina patients with angiographic evidence of coronary lesions (CAD). Data are expressed as mean ± SEM. * $p < 0.05$ when compared to abnormal sugar in the control group. ** $p < 0.05$ when compared to normal blood sugar in the CAD group.

and experimental hyperinsulinemia increases urinary NOx excretion[17].

In the present study, the authors investigated the effects of gender and aging on serum NOx levels in healthy subjects, CAG and CAD groups. However, there was no significant difference among all age ranges. Interestingly, serum NOx levels were increased in women but not in men in the CAD group. Similary, it has been reported that serum NOx levels were increased according to age only in women[18]. Other reports by Colditz et al showed that elevated serum NOx levels were associated in part with estrogen[19]. Estrogen has an anti-atherogenic action which is considered to stimulate NO production by vascular endothelial cells[20]. The protective roles of estrogen on coronary vasculature may in part be related to their complex effects on NOS activity.

In conclusion, the authors demonstrated that NO production may play compensatory and protective roles in coronary artery diseases. The amount of NO production will be much higher when multiple predisposing factors are involved. Therefore, serum NOx levels are expected to be markers for prognosis and severity of coronary artery diseases. Thus, measurement of serum NOx levels at different times in sequence will be helpful for the assessment of coronary artery diseases.

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ระดับซีรั่มไนตริกออกไซด์ในผู้ป่วยโรคหลอดเลือดหัวใจ

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ไนตริกออกไซด์มีบทบาทสำคัญต่อพยาธิสภาพในโรคหลอดเลือดหัวใจ อย่างไรก็ตามไนตริกออกไซด์ยังมีส่วนเกี่ยวข้องในสรีรวิทยาของระบบหลอดเลือดและหัวใจด้วย การศึกษานี้ จะทำการศึกษาระดับซีรั่มไนตริกออกไซด์ (serum NOx, nitrate และ nitrite) ในคนไข้ที่มีอาการของโรคหลอดเลือดหัวใจ ค่าเคมีในเลือด เช่น cholesterol, triglyceride, LDL-C, HDL-C และ น้ำตาล จะถูกวัดและนำมาเปรียบเทียบกับระดับซีรั่มไนตริกออกไซด์ด้วย ระดับซีรั่มไนตริกออกไซด์จะถูกวัดในอาสาสมัครปกติ 20 ราย (กลุ่มควบคุม), ผู้ป่วยที่มีอาการเจ็บหน้าอก (angina) แต่ผล coronary angiogram ปกติ 20 ราย (CAG) และผู้ป่วยที่มีอาการเจ็บหน้าอก (angina) แต่ผล coronary angiogram ผิดปกติ 20 ราย (CAD) โดยวิธี Modified Griess พบว่าระดับซีรั่มไนตริกออกไซด์ในกลุ่ม CAD มีค่าสูงกว่ากลุ่ม CAG และกลุ่มควบคุม โดยระดับซีรั่มไนตริกออกไซด์ในกลุ่ม CAD เท่านั้นที่มีค่าสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ไม่พบความแตกต่างของระดับซีรั่มไนตริกออกไซด์ในระหว่างกลุ่มอายุที่ทำการศึกษาอย่างมีนัยสำคัญทางสถิติ ในกลุ่ม CAD พบว่าผู้หญิงมีระดับซีรั่มไนตริกออกไซด์ที่สูงกว่าผู้ชายอย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบค่าเคมีในเลือด กับระดับซีรั่มไนตริกออกไซด์ พบว่าในกลุ่ม CAD ที่มีค่าเคมีในเลือดผิดปกติได้แก่ cholesterol, triglyceride, LDL-C และน้ำตาล มีระดับซีรั่มไนตริกออกไซด์สูงกว่าในกลุ่มที่มีค่าเคมีในเลือดปกติ ผลการศึกษาบ่งชี้ว่าระดับซีรั่มไนตริกออกไซด์มีค่าสูงกว่าปกติในกลุ่มที่มีโรคหลอดเลือดหัวใจ และจะยิ่งสูงมากขึ้นถ้ามีปัจจัยเสริมมาเพิ่มเช่น ไขมันและน้ำตาลในเลือดสูง ดังนั้นการวัดระดับซีรั่มไนตริกออกไซด์ ที่เวลาต่าง ๆ อาจช่วยในการพยากรณ์พยาธิสภาพและความรุนแรงของโรคหลอดเลือดหัวใจได้

คำสำคัญ : โรคหลอดเลือดหัวใจ, ไนตริกออกไซด์, ซีรั่ม, ความเข้มข้น, เจ็บหน้าอก

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