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Homocysteine and nitric oxide in patients undergoing diagnostic coronary angiography.

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Abstract

We evaluated the plasma homocysteine (tHcy) and nitric oxide metabolites (nitrite plus nitrate; NOx) data of consecutive patients undergoing diagnostic coronary angiography (n=79) with respect to the presence and severity of coronary artery disease (CAD), the presence of acute coronary syndromes (ACS), and the risk status of patients. Hyperhomocysteinemia (>15 micromol/L) was detected in 11% of the controls (n=19) and 37% of CAD patients (n=60) (p=0.03). Plasma tHcy in CAD patients was not significantly different from controls, but those with 3-vessel disease had a significantly higher tHcy concentrations than did controls (p=0.049). The patients with 3-vessel disease and ACS had the highest concentrations of tHcy (16.9 +- 4.4 micromol/L), and the difference from the ACS patients with 1- and 2-vessel involvement was significant (p=0.03). In patients with 1-vessel involvement, tHcy was correlated with NOx (r=0.62, p=0.005); in patients with 2- and 3-vessel disease this correlation could not be observed. The high-risk patients (n=51) had a higher mean number of vessel involvement and tHcy ($p<0.001$, $p<0.05$, respectively) but lower NOx ($p<0.05$) when compared to the low-risk patients (n=28). It appears that in the early stages of atherosclerosis hyperhomocysteinemia causes an increase in NOx production, but with progression of the disease this compensatory increase disappears.

KEYWORDS: homocysteine, nitric oxide, coronary angiography

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Original Article

Homocysteine and Nitric Oxide in Patients Undergoing Diagnostic Coronary Angiography

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We evaluated the plasma homocysteine (tHcy) and nitric oxide metabolites (nitrite plus nitrate; NOx) data of consecutive patients undergoing diagnostic coronary angiography (n = 79) with respect to the presence and severity of coronary artery disease (CAD), the presence of acute coronary syndromes (ACS), and the risk status of patients. Hyperhomocysteinemia (> 15 μmol/L) was detected in 11% of the controls (n = 19) and 37% of CAD patients (n = 60) ($p = 0.03$). Plasma tHcy in CAD patients was not significantly different from controls, but those with 3-vessel disease had a significantly higher tHcy concentrations than did controls ($p = 0.049$). The patients with 3-vessel disease and ACS had the highest concentrations of tHcy (16.9 ± 4.4 μmol/L), and the difference from the ACS patients with 1- and 2-vessel involvement was significant ($p = 0.03$). In patients with 1-vessel involvement, tHcy was correlated with NOx ($r = 0.62$, $p = 0.005$); in patients with 2- and 3-vessel disease this correlation could not be observed. The high-risk patients (n = 51) had a higher mean number of vessel involvement and tHcy ($p < 0.001$, $p < 0.05$, respectively) but lower NOx ($p < 0.05$) when compared to the low-risk patients (n = 28). It appears that in the early stages of atherosclerosis hyperhomocysteinemia causes an increase in NOx production, but with progression of the disease this compensatory increase disappears.

Key words: homocysteine, nitric oxide, coronary angiography

Homocysteine is an amino acid with a sulphydryl group, formed by demethylation of methionine. It is converted to cystathionine by B_6-dependent cystathionine β-synthase and remethylated to methionine by B_{12}-dependent 5-methyl tetrahydrofolate-homocysteine methyltransferase. Major causes of hyperhomocysteinemia are age, renal failure, excessive coffee consumption, drugs including methotrexate, phenytoin, and theophylline, dietary deficiencies of vitamin cofactors (folic acid, vitamin B6, and vitamin B12), or abnormalities in the enzymes required for homocysteine metabolism (cystathionine β-synthase, 5,10-methylenetetrahydrofolate reductase) [1-3].

In the last decade, moderate elevation of homocysteine has been shown to be associated with coronary, cerebral, and peripheral vascular diseases. Moreover, hyperhomocysteinemia has been accepted as an independent risk factor for cardiovascular dis-

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ease [4–8]. Although there is considerable epidemiologic and clinical evidence for these associations, angiographic studies have shown contradictory results regarding the relationship between homocysteine and the severity of atherosclerosis [9–11]. In addition, the exact mechanism through which homocysteine exerts its effects on the arteries is still not clear. Although previous reports have shown an association of hyperhomocysteinemia with abnormalities in nitric oxide synthesis and impaired endothelium-dependent vasodilatation, studies investigating both homocysteine and nitric oxide in angiographically evaluated patients are lacking [12, 13]. Thus in this study, in patients undergoing diagnostic coronary angiography, we determined homocysteine and nitric oxide metabolites (nitrite plus nitrate; NOx) [14] in plasma and evaluated the data with respect to the presence and severity of coronary artery disease (CAD), to the presence of acute coronary syndromes (ACS), and also to the risk status of patients determined by the number of other major coronary risk factors present.

Materials and Methods

Study groups. The study protocol was approved by the local Research Ethical Committee, and an informed consent was obtained from each patient. The study group consisted of 79 consecutive patients (51 men and 28 women, mean age 56 ± 10 years) undergoing selective coronary angiography. All patients had normal renal and liver function and no evidence of malignant disease, and they also did not take vitamin supplements or medications such as intravenous nitrates that could interfere with the analysis of either homocysteine or NOx.

Clinical indications for coronary angiography were ACS (myocardial infarction or unstable angina pectoris) for 40 patients (51%) and diagnosis of CAD for 39 patients (49%).

Coronary angiography. Diagnostic left heart catheterization and coronary angiography were performed by a percutaneous femoral approach with the Judkins procedure. Three experienced cardiologists who were unaware of the laboratory data on homocysteine and nitric oxide reviewed all angiograms simultaneously. Significant CAD was defined as a stenosis of more than 50% of the vessel diameter. Severity of CAD was assessed according to the number of vessels with significant stenosis. Patients were divided into 2 groups: 1) Control group, CAD (−), consisting of patients with normal coronary arteries (n = 19), 2) Coronary artery disease group, CAD (+), consisting of patients with significant stenosis in at least one epicardial coronary artery (n = 60). The CAD (+) patients were further grouped according to the number of vessels involved: 1-vessel disease (n = 19), 2-vessel disease (n = 21), or 3-vessel disease (n = 20). These 1-vessel, 2-vessel, and 3-vessel patients were further grouped according to the presence or absence of acute coronary syndrome, as ACS (+) and ACS (−).

The study group was also grouped as low-risk and high risk, evaluating male gender, age (> 45 years for males and > 55 years for females), family history of coronary artery disease, diabetes mellitus, hypertension (> 140/90 mmHg), smoking status, and hypercholesterolemia (total cholesterol higher than 200 mg/dl) as major risk factors. Patients with fewer than 3 risk factors were defined as belonging to the low-risk group, and patients with 3 and more risk factors or with diabetes mellitus were defined as belonging to the high-risk group.

Biochemical Measurements. Blood samples were withdrawn from the antecubital veins after an overnight fast, and the plasma samples were stored at − 70 °C until analysis.

Plasma total homocysteine (tHcy) was measured by using an EIA kit (BioRad, Norway). Nitric oxide was determined by spectrophotometric measurement of nitrite plus nitrate (NOx), which are stable oxidation products of nitric oxide (Roche-Boehringer Mannheim, Germany) [15].

Total cholesterol and LDL cholesterol were determined by the CHOD-PAP enzymatic method (Diasys Diagnostic Systems GmbH & Co. KG., Germany) adapted to an Olympus AU 800 autoanalyzer. Serum triglyceride concentration was measured by the enzymatic GPO-PAP method (Diasys Diagnostic Systems GmbH & Co.). HDL cholesterol was determined after precipitation of apoB-containing lipoproteins with phosphotungstic acid and magnesium chloride by the Olympus AU 800 autoanalyzer.

Statistical Analysis. Categorical variables are given as counts (percentages) and continuous variables as the mean ± standard deviation (SD). For

comparisons of controls and ACS (−) and ACS (+) patients or high-risk and low-risk patients, we used an unpaired Student's *t*-test (or Mann-Whitney *U*-test when necessary) to examine continuous variables and a chi-square test for categorical variables. Differences between controls, 1-, 2-, and 3-vessel involvements were examined by one-way variance analysis. Correlations of homocysteine and NOx with age and serum lipid concentrations were examined using Spearman's rank correlation analysis and with angiographic vessel involvement by linear regression analysis. A p value < 0.05 was considered significant.

Results

Patient characteristics. On evaluation of the coronary artery disease patients according to the severity of atherosclerosis, 19 (32%) had 1-vessel, 21 (35%) had 2-vessel, and 20 (33%) had 3-vessel disease. ACS was the indication for coronary angiography in 51% of the patients (n = 40).

Major risk factors for CAD (age, male gender, hypertension, family history, diabetes mellitus, hypercholesterolemia, and smoking) were assessed in the patients, who were then grouped according to their total risk status. There were 51 high-risk patients and 28 low-risk patients.

Major risk factors for CAD are compared between controls and patients in Table 1. Significant differences were noted for age, male gender, and total number of risk factors. Total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride concentrations of the study groups are shown in Table 2.

Plasma Homocysteine and NOx. Hyperhom ocysteinemia, defined as a plasma level of higher than 15 μmol/L, was present in 11% of the controls and 37% of the coronary artery disease patients ($p = 0.03$). Half of the patients with 3-vessel disease had hyperhomocsyteinemia.

The tHcy and NOx concentrations of plasma were not observed to be significantly different between patients with and without significant coronary lesions (Table 3). However, patients with 3-vessel disease had significantly higher homocysteine concentrations when compared to the patients with normal coronary arteries ($p = 0.049$).

Homocysteine concentration was higher in patients with an acute coronary syndrome compared to patients without an acute event, but the difference did not achieve statistical significance (Table 4). However, the patients with 3-vessel disease and ACS had the highest concentrations of plasma homocysteine (16.9 ± 4.4 μmol/L), and the difference from the acute coronary syndrome patients with 1- and 2-vessel involvement was statistically significant ($p = 0.03$).

Table 1 Major risk factors in the study groups

	CAD (−)	CAD (+)			
	Control (n=19)	1-vessel Disease (n=19)	2-vessel Disease (n=21)	3-vessel Disease (n=20)	Total (n=60)
Major risk factors					
Age	52 ± 9	59 ± 10	55 ± 11	61 ± 10	58 ± 10[a]*
Male gender (%)	37	79	67	75	73[a]**
Hypertension (%)	53	32	48	50	43
Diabetes mellitus (%)	11	11	14	35[b]*	20
Family history (%)	18	6	39	37	28
Hypercholesterolemia	41	38	47	60	48
Smoking (%)	26	47	52	35	45
Number of risk factors	2.4 ± 1.6	3.0 ± 1.1	3.5 ± 1.3	4.0 ± 1.3[b]*	3.5 ± 1.3[a]**

Values are means ± SD.
CAD (−): patients with normal coronary arteries.
CAD (+): patients with significant coronary artery stenosis.
[a]: vs CAD (−), [b]: vs 1- and 2-vessel disease.
*$p < 0.05$, **$p < 0.01$

Table 2 Cholesterol and triglyceride concentrations of plasma in the study groups

	CAD (−)	CAD (+)			
	Control (n=19)	1-vessel Disease (n=19)	2-vessel Disease (n=21)	3-vessel Disease (n=20)	Total (n=60)
Parameters					
Total chol. (mg/dl)	197 ± 45	189 ± 35	211 ± 48	209 ± 43	204 ± 131
HDL-chol. (mg/dl)	43 ± 12	42 ± 14	48 ± 11	41 ± 12	44 ± 13
LDL-chol. (mg/dl)	127 ± 38	114 ± 27	140 ± 39	137 ± 33	131 ± 35
Triglycerides (mg/dl)	161 ± 102	152 ± 70	137 ± 65	163 ± 58	150 ± 64

Values are means ± SD.
CAD (−), patients with normal coronary arteries; CAD (+), patients with significant coronary artery stenosis.

Table 3 Homocysteine and NOx concentrations of plasma in the study groups

	CAD (−)	CAD (+)			
	Control (n=19)	1-vessel Disease (n=19)	2-vessel Disease (n=21)	3-vessel Disease (n=20)	Total (n=60)
Homocysteine (μmol/L)	11.5 ± 3.9	12.7 ± 5.5	12.7 ± 3.3	15.4 ± 7.2*	13.6 ± 5.6
NOx (μmol/L)	40.2 ± 18.9	45.8 ± 18.3	49.3 ± 19.3	49.5 ± 23.9	48.3 ± 20.4

Values are means ± SD.
CAD (−), patients with normal coronary arteries; CAD (+), patients with significant coronary stenosis; NOx, Nitrite plus nitrate.
*$p < 0.05$ vs CAD (−).

Table 4 Homocysteine and NOx concentrations of plasma in the coronary artery disease patients with and without acute coronary syndrome

	1-vessel disease		2-vessel disease		3-vessel disease		Total	
	ACS (−) (n = 5)	ACS (+) (n = 14)	ACS (−) (n = 6)	ACS (+) (n = 15)	ACS (−) (n = 9)	ACS (+) (n = 11)	ACS (−) (n = 20)	ACS (+) (n = 40)
Homocysteine (μmol/L)	10.4 ± 5.6	13.1 ± 5.9	11.4 ± 2.7	12.7 ± 3.4	14.6 ± 9.5	16.9 ± 4.4*	12.9 ± 7.37	14.1 ± 4.9
NOx (μmol/L)	40.1 ± 8.7	46.1 ± 21.6	54.1 ± 15.9	45.3 ± 17.4	51.9 ± 24.2	44.3 ± 23.6	50.5 ± 19.8	45.3 ± 20.2

Values are means ± SD.
ACS (−), absence of acute coronary syndrome; ACS (+), presence of acute coronary syndrome; NOx, Nitrite plus nitrate.
*$p < 0.05$ vs 1-vessel and 2-vessel disease patients with acute coronary syndrome.

As evident from Table 4, no significant differences were observed for nitric oxide when coronary artery disease patients were evaluated with respect to the presence of ACS.

The patients with higher risk had higher homocysteine concentrations compared to the low-risk group patients ($p = 0.049$) (Table 5). Patients with higher risk had significantly lower NOx concentrations compared to low-risk group patients ($p = 0.04$). Mean numbers of vessel involvement were significantly higher in the high-risk group than in the low-risk group ($p < 0.001$).

The tHcy and NOx levels showed a moderate positive correlation in patients with 1-vessel involvement

($r = 0.62$, $p = 0.005$) (Fig. 1). This positive correlation disappeared in patients with 2-vessel disease and, though nonsignificant, the correlation became negative in patients with 3-vessel involvement ($r = -0.28$, $p = 0.23$).

On further evaluation of tHcy and NOx correlation with respect to ACS (−) and ACS (+) classification in 1-vessel, 2-vessel, and 3-vessel patients, we observed a positive correlation only in the ACS (+) 1-vessel disease patients ($r = 0.566$, $p = 0.044$) and not in the ACS (+) patients with 2- or 3-vessel disease.

Plasma homocysteine was significantly correlated only with age in the whole study group ($r = 0.30$; $p = 0.008$). Plasma NOx was not correlated with any of the risk factors. Total number of risk factors was the most significantly related factor to the number of vessels involved ($r = 0.29$; $p = 0.002$) when adjusted for age, LDL-cholesterol, HDL-cholesterol, level of homocysteine, and NOx.

Table 5 Homocysteine, nitric oxide, and number of vessels involved of plasma in low- and high-risk coronary angiography patients

	Coronary Angiography Patients	
	Low risk (n = 28)	High risk (n = 51)
Total number of risk factors	1.75 ± 0.52	4.04 ± 1.07***
Number of vessels involved	0.89 ± 0.96	1.9 ± 1.1***
Homocysteine (μmol/L)	11.6 ± 5.1	13.9 ± 5.3*
NOx (μmol/L)	53.7 ± 23.1	46.3 ± 19.2*

Values are means ± SD.
NOx: Nitrite plus nitrate.
*$p < 0.05$, ***$p < 0.001$.

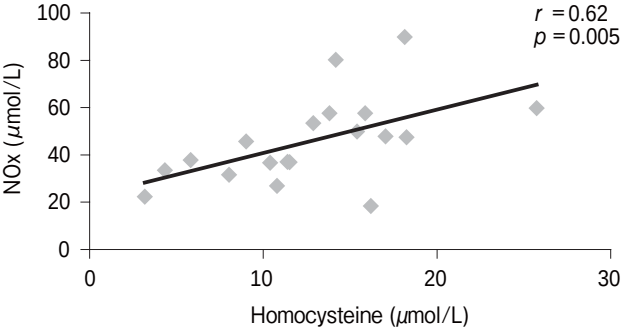

Fig. 1 Correlation between plasma homocysteine and NOx (nitrite plus nitrate) in patients with 1-vessel disease.

Discussion

Hyperhomocysteinemia has been accepted as an independent risk factor for cardiovascular disease [4–8]. Even mildly elevated plasma homocysteine concentration has been associated with increased risk of cardiovascular disease [11, 16]. Phenotypically, hyperhomocysteinemia-associated vascular disease is characterized by vascular lesions, endothelial dysfunction, smooth muscle cell proliferation, and platelet hyperreactivity [17, 18]. These phenotypic changes have largely been replicated in experimental models of hyperhomocysteinemia [19, 20]. Previous reports have shown an association of hyperhomocysteinemia with abnormalities in nitric oxide synthesis and impaired endothelium-dependent vasodilatation [14, 18, 21, 22]. However, to the best of our knowledge, this is the first study in which homocysteine, nitric oxide, and coronary atherosclerosis have been studied in an angiographic study.

In the present study, we observed no statistically significant difference in plasma total homocysteine levels between controls and patients with coronary artery disease. However, the frequency of hyperhomocysteinemia was significantly higher in coronary artery disease patients, with half of the 3-vessel disease patients having hyperhomocsyteinemia. Among 3-vessel disease patients, those with ACS had the highest concentrations of plasma homocysteine. Patients with a recent history of ACS have also been previously reported to have higher homocysteine levels [22]. Thus our findings reflect that higher levels of plasma homocysteine are significantly linked to the extent of coronary atherosclerosis.

Importantly, we observed a moderate significant positive correlation between homocysteine and NOx in 1-vessel disease patients. Since NOx concentration is considered to be a measure of NO production [15], this correlation suggests that in the early stages of atherosclerosis NO production increases to serve as a compensatory mechanism for the restriction of the endothelial damage, atherosclerotic plaque formation, and thrombotic events. NO also exhibits an antioxidant action with respect to lipid

peroxidation [18]. The disappearance of this positive correlation in the 2-vessel disease patients and, though non significant, the tendency to a negative correlation in the 3-vessel disease patients suggest the failure in this compensation mechanism with the progression of the disease. Consistent with these earlier findings, we observed lower NO production in patients with high risk. These high-risk patients had both significantly higher homocysteine concentration and a higher mean number of vessel involvement. We observed that only age, and not the other risk factors, was significantly correlated with homocysteine, which in turn is in accordance with the fact that hyperhomocysteinemia has an independent effect on coronary atherosclerosis [22, 23].

Angiographic studies have shown contradictory results with regard to the relationship between homocysteine and the severity of atherosclerosis. Tsai *et al.* showed a positive correlation between angiographic score and homocysteine in patients without ACS [23]. Different from Tsai *et al.*, we used simple scoring with number of vessel involvement and ACS. We observed that hyperhomocysteinemia is associated with number of vessel involvement in high-risk group patients, and this was more evident in patients with ACS. The mechanism by which homocysteine promotes atherothrombosis is not yet clear.

Homocysteine has been shown to be associated with impairment in both production and bioavailability of endothelial-derived NO, which has a protective effect as both a potent vasodilatator and a platelet inhibitor [14, 18]. Furthermore, homocysteine itself also promotes the formation of thrombin [22]. A commonly held view is that oxidative stress may be an important contributing factor in the promotion of atherothrombosis by homocysteine. Homocysteine autooxidation in hyperhomocysteinemia causes the generation of reactive oxygen species (ROS) and mediates endothelial damage [24]. Superoxide anion radicals interact with nitric oxide, causing the formation of peroxynitrite and thus the loss in NO bioavailability [18, 21].

Homocysteine also inhibits cellular glutathione peroxidase expression in endothelial cells, an effect that would induce oxidative stress by itself [14, 21]. Superoxide anion radicals and peroxynitrite also cause oxidation of sulphydryl groups of endothelial nitric oxide synthase (eNOS), leading to a lower NO output [18]. Through a process known as eNOS "uncoupling" eNOS can also generate superoxide. This process appears to occur under conditions typically associated with vascular disease and in relative deficiency of tetrahydrobiopterin. On the other hand, homocysteine has been shown to upregulate inducible nitric oxide synyhase (iNOS) enzyme activity by NF-κB-dependent transcriptional activation in rat aortic smooth muscle cells and cause a dose-dependent increase in nitric oxide content of cells [25]. This suggests the contribution of homocysteine to the inflammatory response that characterizes early atherosclerosis.

It has been suggested that NO production by vascular smooth muscle cells may in part compensate for the absence of endothelial NO synthesis and may suppress the atherothrombotic risk of a hyperhomocysteinemic state [15]. On the other hand, NO attenuates the pathogenicity of homocysteine by reacting with its thiol group and the product, S-NO-homocysteine, has potent vasodilatory and antiplatelet effects. The progressive imbalance between NO production and homocysteine resulting in decreased S-NO formation has been suggested to account for the adverse vascular properties of homocysteine [26]. The positive correlation that we observed between homocysteine and nitric oxide production in 1-vessel disease patients but not in 2- and 3-vessel disease patients may be due to the above-mentioned compensatory NO production by iNOS or the existence of such a protective mechanism in the early stages of atherosclerosis.

To conclude, our study investigating a patient group who underwent diagnostic coronary angiography shows that higher levels of plasma total homocysteine aresignificantly linked to the extent of coronary atherosclerosis. Despite being investigated in a relatively limited number of patients, our study shows a positive correlation between nitric oxide production and homocysteine in-vivo for the first time and implies that a failure in nitric oxide compensation may be important in the progression of atherosclerosis and acute coronary events.

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