The Association of Plasma Homocysteine, Cardiac Risk Factors and Serum Nitrite in Patients with Coronary Artery Disease, Cardiac Syndrome X and Healthy Subjects

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Objective: We evaluated the association of plasma total homocysteine (tHcy), cardiac risk factors and total nitrite in coronary artery disease (CAD) patients, cardiac syndrome X patients and in healthy subjects.
Methods: Forty two CAD, 22 cardiac syndrome X patients and 30 healthy subjects, aged 30 to 75 years were included into the study. Blood samples of tHcy, serum total nitrite and cardiac risk factors were studied appropriately. The results were compared between the groups. The independent contributions of tHcy and total nitrite to CAD and cardiac syndrome X and their interactions with cardiac risk factors were evaluated.
Results: After adjusting for age, median values of tHcy and total nitrite were evaluated for their skewness. Coronary artery disease patients had higher median plasma tHcy levels than cardiac syndrome X patients ($p<0.001$) and healthy subjects ($p<0.001$) and lower serum total nitrite levels than patients in the two other groups ($p<0.05$), respectively. Using a univariate linear regression analysis tHcy had a moderately significant positive correlation with age ($\beta=0.34$, $p=0.002$) and a weakly significant inverse correlation with female gender ($\beta=-0.24$, $p=0.032$). Using a partial correlation analysis by controlling for age, gender and clinical situations tHcy had a positive but moderately significant correlation with LDL cholesterol ($r=0.23$, $p=0.01$) and triglycerides ($r=0.27$, $p=0.016$). Total nitrite had a positive but weakly significant correlation with HDL cholesterol ($r=0.23$, $p=0.04$) and fibrinogen ($r=0.24$, $p=0.03$) and an inverse but moderately significant correlation with LDL cholesterol ($r=-0.37$, $p=0.001$). Using a multivariate stepwise regression analysis total nitrite was inversely and significantly associated with tHcy ($\beta=-0.45$) in the control group.The contribution of HDL cholesterol to the association was $\beta=-0.45$, $p=0.044$, $R^2=36.2\%$, HDL cholesterol with fibrinogen - $\beta=-0.45$, $p=0.05$, $R^2=36.6\%$ and HDL cholesterol with LDL cholesterol - $\beta=-0.45$, $p=0.05$, $R^2=36.3\%$. In a forward stepwise logistic regression analysis the age adjusted odds ratio (OR) for coronary artery disease per standard deviation change in log- transformed tHcy concentration was- 0.82, $p=0.013$ and in total nitrite concentration was- 1.08, $p=0.02$. Using the same model neither tHcy nor total nitrite was associated with cardiac syndrome X ($p=0.221$ and $p=0.112$), respectively.
Conclusion: The low nitrite levels can be a marker of endothelial dysfunction in the presence of hyperhomocysteinemia and other cardiac risk factors. Our results might support endothelial dysfunction in CAD but not in cardiac syndrome X patients. *(Anadolu Kardiyol Derg, 2003; 3: 26-34)*
Key Words: CAD, cardiac syndrome X, homocysteine, nitrite

Introduction

Hyperhomocysteinemia is an independent risk factor for the development of atherosclerosis and is associated with a number of other cardiovascular risk factors, including male gender, smoking, aging, high blood pressure, elevated cholesterol and lack of exercise (1).

The increased cardiovascular risk associated with elevated homocysteine levels may result from its direct cytotoxic effects on endothelial cells, its stimulation of increased platelet adhesion and/or its promotion of procoagulant activity and interacti-

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on with lipids by oxidizing low density lipoprotein cholesterol (1-4). The reactivity of the sulfhydryl group of homocysteine has been implicated in molecular mechanisms underlying this increased risk (5).

There is increasingly compelling evidence that thiols react in the presence of nitric oxide (NO) to form S-nitrosothiols (S-NO), compounds with potent vasodilatory and antiplatelet effects. Owing to prolonged exposure of endothelial cells to homocysteine, the adverse vascular properties may result from an inability to sustain S-NO formation because of a progressive imbalance between production of NO by progressively dysfunctional endothelial cells and high levels of homocysteine (5).

This study was aimed to compare cardiac risk factors, plasma total homocysteine (tHcy) and total nitrite levels between coronary artery disease (CAD) patients, cardiac syndrome X patients and healthy controls and to evaluate the independent contributions of tHcy and total nitrite to CAD and cardiac syndrome X and their interactions with cardiac risk factors.

Material and Methods

Subjects: Out of 256 consecutive patients admitted to our hospital with chest discomfort in the last 6 months, 64 patients were included into the study.

Patients with one or more of the following criteria were excluded from the study; chest discomfort not related to cardiovascular origin, deteriorated renal function, high serum uric acid, confirmed macrocytic anemia, alcohol consumption, using medications like nitrate and vitamine preparations, left ventricular ejection fraction (LVEF) of less than 40 percent, metabolic syndrome X (glucose intolerance and/or diabetes mellitus, increased blood pressure, dyslipidemia and obesity, BMI>29 kg/m2) according to Raeven (6) and heavy smokers (twenty cigarettes or more/day) (7, 8).

The study population was composed of 30 healthy controls (group 1), 22 cardiac syndrome X patients (group 2) and 42 coronary artery disease patients (group 3).

Associations between plasma homocysteine concentration and several confounding variables such as age, gender, BMI, serum creatinine, traditional risk factors such as systemic blood pressure, current smoking, lipid profile including triglycerides, serum fibrinogen, uric acid and serum vitamine B_{12}, serum folic acid that are possibly have effects on the causal pathway of plasma tHcy with serum total nitrite levels were selected a priori and evaluated in our study groups of coronary artery disease, cardiac syndrome X patients and healthy controls

Coronary artery disease population: Forty two patients (30 men and 12 women), aged 33 to 75 years (59.8±9.5) had CAD. They had stable angina pectoris with chest pain attributed solely to physical exertion with a normal resting 12 lead ECG, a positive exercise test and angiographically documented stenosis of ≥ 50% in at least one of the major coronary arteries such as the left anterior descending coronary artery with its major diagonal branches or the right coronary artery or the circumflex coronary artery with its major marginal branch according to Nygard (9). Depending on the dominance the posterior descending coronary artery was included as part of the right coronary artery or the circumflex coronary artery. A patient with 50% or greater obstruction of the left main coronary artery was classified as having two vessel disease if the circulation was right dominant and three vessel disease if it was left dominant (9).

Electrocardiogam recordings were analysed by 2 independent cardiologists. All treadmil exercise tests were performed in the morning, according to a symptom limited modified Bruce protocol (10). Three ECG leads (V_1, aVF and V_5) were continuously monitored during the test. A standart 12 lead ECG was printed and blood pressure was measured at the onset of the test, at the end of each stage and at peak exercise as well as at 1 mm ST segment depression, when chest pain occured and when it was clinically indicated. Myocardial ischemia was diagnosed when a horizontal or downsloping ST segment depression of 1 mm at 0.08 seconds from the J-point was observed in at least one lead. All CAD patients had coronary stenosis in at least one main coronary artery, 11 patients had one vessel disease, 31 patients had multivessel disease. No patient had left ventricular hypertrophy, valvular or myocardial disease, mitral valve prolapse, previous MI and heart failure in past medical history and also defined by echocardiography (11-13), 62 % were current smokers of five to fifteen cigarettes daily.

Cardiac syndrome X patients: Twenty two patients (14 men and 8 women), aged 40 to 73 years (51.9±10.8) with normal resting 12-lead ECG but typical exertional angina, reproducible ST-segment depression on exercise testing and totally normal coronary arteries at angiography were included into the study (11-13), 64% were current smokers of three to sixteen cigarettes daily.

Control subjects: Thirty healthy controls (15 men and 15 women), aged 37 to 63 years (50.8±10.6) were screened by 2 physicians for signs of hypertension, diabetes mellitus, dyslipidemia, CAD, dietary habits, exercise and smoking patterns. We could not make an exact match for age and gender but for BMI. All had normal physicial examination, rest ECG, echocardiogram and exercise stress test. None were taking any medications and 73% never smoked, 27% were ex-smokers.

Laboratory measurements: Blood samples for analysis of routine clinical parameters were taken after an overnight fast. For the determination of routine clinical parameters, standart laboratory methods on an automatic analyzer were used (Olympus AU 5200, Olympus Germany). Serum folate and vi-

Table 1: Characteristics of the study population

	Controls n=30	Syndrome X n=22	CAD N=42	Significance Level P1	P2	P3
Age (years)	51 (11)	52 (11)	60 (10)+	P1=0,790	<0,01	<0,01
Sex, n(%Male)	15(50)	14(64)	31(73)	0,622	<0,05	<0,05
Smoking, n (%)						
Ever	73	21	27			
Ex	27	15	11			
Current	0	64	62			
Hemodynamic Data						
SBP (mmHg)	126(9)	123(10)	125(10)	NS	NS	NS
DBP (mmHg)	75(10)	74(8)	74(9)	NS	NS	NS
LVEF (%)	56(9)	54(10)	50(9)	NS	NS	NS
Angiography,n(%)						
One ves, disease	-	0	29			
Multives, disease	-	0	71			
Blood Lipids (mg/dl)						
LDL cholesterol	96.5(17.9)	112,5(18,4)	135,6(19.1)	<0.01	<0.001	<0.01
HDL cholesterol	44.4(4.1)	44,3(8.0)	41,2(7,2)	NS	NS	NS
Triglycerides	123.2(26.4)	137,0(20,5)	152.4(42.4)	<0.05	<0.01	p3=0.159
Clotting factors						
Fibrinogen (mg/dl)	244,9(31)	246.8(67.4)	250.4(74.4)	NS	NS	NS
Dietary Factors						
Vitamin B12(pmol/L)	290(30)	285(39)	287(30)	NS	NS	NS
Folic acid (nmol/L)	9.5(1)	9.6(0.7)	9.1(1)	NS	NS	NS
BMI (kg/m^2)	23(2,4)	23(2.9)	24(2.3)	NS	NS	NS
Uricacid (mg/dl)	5(0,7)	5.1(1.7)	5.6(1.2)	NS	<0.05	<0.05
Creatinine (mg/dl)	1(0.2)	1.2(0.2)	1.2(0.3)	NS	NS	NS
Fasting plasma * tHcy (μmol/L)	14.7 (11.2,15.4)	12.02 (9,17.8)	23.09 (18,4, 30)+	P1 =0.559	<0.001	<0.001
Total nitrite * (μmol/L)	32 (26,3,48)	35.4 (203,49.2)	15.9 (8.6,29.5)+	P1 =0.946	<0.05	<0.05

Values are expressed as mean (SD) for nonskewed distributed data, as median (interquartile range; 25 percentile, 75 percentile) for skewed distributed data and as numbers of subjects (percentages). T test or mann Whitney test with Bonferroni correction was used to determine significance of the differences between the two groups. Thus when Kruskal Wallis test with Bonferroni corretion for the comparisons of the differences between the three groups was positive, a probability value of tp<0.017 was used to determine significance p1: comparison between group 1 and 2; p2: comparison between group 1 and 3; p3: comparison between group 2 and 3. SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, left ventricular efection fraction; LDL, low density lipoprotein; HDL, high density lipoprotein; BMI, body mass index; tHcy, total homocysteine.

tamin B12 were measured by using a commercially available radioisotope protein binding test kit (Amersham, Germany). Fibrinogen was measured using a coagulometric assay according to Clauss on an automatic analyzer Biomerieux-Option B. Uric acid was measured by an enzymatic urikaz PAP method.

Plasma total homocysteine: Blood samples were kept on ice. Serum was separated within an hour and then frozen and stored at below –20°C. Plasma total homocysteine which includes the sum of protein-bound and free homocysteine was measured using Bio-Rad kit by high performance liquid chromatography (HPLC) with fluorescence detection method described by Vester and Rasmussen (14). Total homocysteine levels between 5-15μmol/L were in the normal ranges of the kit used in the study.

Plasma total nitrite: Plasma nitrite levels were determined, by a colorymetric method based on the Griess reaction (15). Nitrate and nitrite levels were measured by the same assay after enzymatic reduction of nitrate to nitrite with nitrate reductase from Aspergillus species (Boehringer Mannheim).

Statistical analysis: Data were analysed using SPSS 8.0 for Windows. Values are expressed as mean (SD) for nonskewed distributed data, as median (range) for skewed distributed data and as numbers of subjects (percentages). We compared normally distributed variables between groups using an unpaired t test or a nonparametric Mann-Whitney test. Kruskal-Wallis ANOVA test was applied to compare skewed variables across two or more groups. If the difference between groups was positive pairwise comparisons with Bonferroni correction was applied. Categorical variables were compared using χ^2 method. The relation between homocysteine, total nitrite concentration and several confounding variables were examined by linear regression analysis and by partial correlation analysis after controlling for age, sex and clinical situations in the control group. Total homocysteine and nitrite values were log-transformed before regression analysis because of a markedly skewed distribution. Logistic regression was used to assess independent contributions of homocysteine, nitrite and other risk fac-

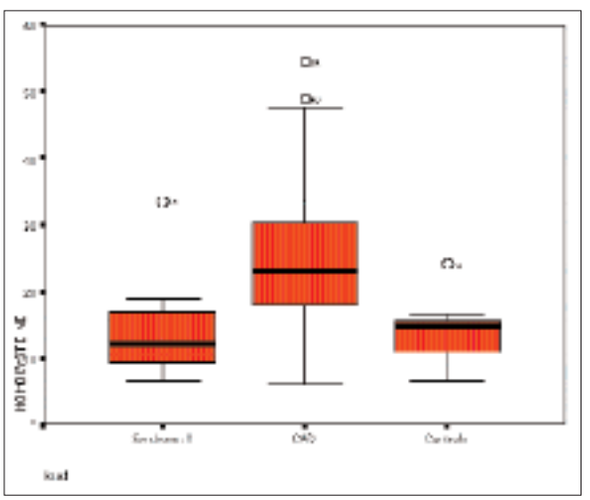

Figure 1: The median levels of plasma homocysteine

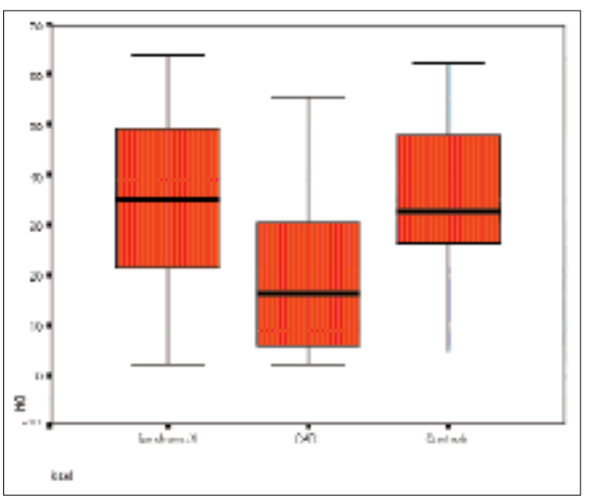

Figure 2: The median levels of serum nitrite

Table 2: Odds ratio (OR) of CAD for one standard deviation (SD) in serum total Hcy and nitrite concentration

		n	OR	95% CI	P
A	tHcy	42	0.82	0.71 to 0.96	0.013
	total nitrite	42	1.08	1.01 to 1.14	0.020
B	tHcy	42	0.74	0.59 to 0.94	0.012
	total nitrite	42	1.06	0.99 to 1.14	0.091
A: Adjusted for age B: HDL cholesterol included in the model					

tors and their interactions to coronary artery disease and cardiac syndrome X.

The study design was approved by the Ethics Committee of Atatürk Research and Training Hospital. All subjects gave written consent to the study.

Results

The general characteristics of the study population are listed in Table 1. In comparison to cardiac syndrome X patients and control subjects patients with CAD were older ($p<0.01$) and had higher proportion of men ($p<0.05$). Current smoking was common in CAD and cardiac syndrome X patients. There were no significant differences among the groups for hemodynamic data, clotting and dietary factors except serum uric acid levels. Although within the normal range of the laboratory the mean serum uric acid was higher in CAD patients than in cardiac syndrome X patients ($p<0.05$) and control subjects ($p<0.05$). Patients with cardiac syndrome X had higher mean LDL cholesterol and triglyceride levels than control subjects ($p<0.01$ and $p<0.05$), respectively. Patients with CAD had higher mean LDL cholesterol levels than patients with cardiac syndrome X ($p<0.01$) and control subjects ($p<0.001$) and also higher mean triglyceride levels than control subjects ($p<0.01$). For the CAD cases, tHcy values were not normally distributed and ranged from 5.93 to 47.7 μmol/L, with a median value of 23.09 μmol/L compared with 12.02 for cardiac syndrome X ($p<0.001$) and 14.7 for control subjects ($p<0.001$) (Fig 1). Total nitrite values were not normally distributed in the entire study population and ranged from 2.10 to 63.8 μmol/L. The median total nitrite value for CAD patients was 15.9 μmol/L compared with 35.4 for cardiac syndrome X ($p<0.05$) and 32 for control subjects ($p<0.05$) (Fig 2). After covariance adjusting for age patients with CAD had higher mean homocysteine level (24.35 μmol/L, 95 % CI 21.51 to 27.20) than patients with cardiac syndrome X (13.7 μmol/L, 95 % CI 10.0 to 17.4; $p<0.001$) and control subjects (14.5 μmol/L, 95 % CI 10.2 to 18.7; $p=0.017$) and had lower mean total nitrite level (20.2 μmol/L, 95 % CI 14.9 to 25.5) than patients with cardiac syndrome X (33.4 μmol/L, 95 % CI 24.5 to 42.3; $p<0.001$) and control subjects (35.0 μmol/L, 95 % CI 27.2 to 42.9; $p=0.011$).

Univarite and multivarite determinants of homocysteine and total nitrite levels: Using an univarite linear regression analysis in the CAD and syndrome X cases and control group tHcy concentration had a positive and a moderately significant correlation with age ($\beta= 0.34$, $p=0.002$) but an inverse and a weakly significant correlation with female gender ($\beta= -0.24$, $p=0.032$). Using a partial correlation analysis by controlling for age, sex and clinical situations, tHcy had a positive and a weakly significant correlation with LDL cholesterol ($r= 0.29$, $p=0.010$) and triglycerides ($r=0.27$, $p=0.016$), total nitrite had a positive and a weakly significant correlation with HDL cholesterol ($r=0.23$, $p=0.040$) and fibrinogen ($r=0.24$, $p=0.030$) but an inverse and a moderately significant correlation with LDL cholesterol ($r= -0.37$, $p=0.001$). Using a multivariate stepwise regression analysis in the control group total nitrite was inversely and significantly associated with tHcy ($\beta= -0.45$). The contribution of HDL cholesterol to the association was - $\beta= -0.45$, $p= 0.044$, $R^2= 36.2$ %, HDL cholesterol and fibrinogen was - $\beta= -0.45$, $p=0.05$, $R^2= 36.6$ % and HDL and LDL cholesterol was- $\beta= -0.45$, $p=0.05$, $R^2= 36.3$ %.

Independent relations of total homocysteine and nitrite to coronary artery disease and cardiac syndrome X: Forward stepwise logistic regression analysis was used to determine whether homocysteine and nitrite lvels were associated with CAD and cardiac syndrome X independent of cardiac risk factors. The age adjusted odds ratio (OR) for coronary artery disease per standard deviation (SD) change in log-transformed tHcy concentration was 0.82 (95 % CI 0.71 to 0.96, $p= 0.013$) and total nitrite concentration was 1.08 (95 % CI 1.01 to 1.14, $p= 0.020$), but the relation was attenuated by inclusion of the confounding variables such as HDL cholesterol (OR: 0.74, $p= 0.012$ and OR:1.06, $p=0.091$), respectively (Table 2). Using the same model neither tHcy nor total nitrite was associated with cardiac syndrome X, but HDL cholesterol (OR: 0.58, $p=0.042$) and LDL cholesterol (OR:0.83, $p= 0.009$) were found to be significantly and independently associated with cardiac syndrome X.

Discussion

Both in retrospective and prospective studies, hyperhomocysteinemia is found to be a common and independent risk factor for cardiovascular disease, and this may be mediated by endothelial

dysfunction (1-4, 16-19). Recently, it has been shown that even mild physiological increments in plasma homocysteine concentrations were sufficient to alter endothelial function thus impairing endothelium dependent vasodilatation in both coronary and peripheral vessels by nitric oxide (20). Endothelium derived nitric oxide is a potent vasodilator in the vasculature, and the balance between nitric oxide and various endothelium derived vasoconstrictors and the sympathetic nervous system that maintains blood vessel tone is impaired in the presence of endothelial dysfunction (20, 21).

In this study the association between traditional risk factors such as age, gender, smoking, blood lipids, fibrinogen and plasma homocysteine and serum nitrite was evaluated a priori. As age (1,18,19,22), serum folate, vitamin B_{12} levels (3,9,18,19,22), serum uric acid level (9), renal function (3,9,18,19,22) and the left ventricular ejection fraction (9) are claimed to be predictors of plasma tHcy concentration. Patients with renal dysfunction, vitamin deficiency, hyperuricemia and low ejection fraction were excluded from the study in order that they might affect the results of the study and adjustment for age was made in the statistical analysis. Total homocysteine had a moderately significant association with age and a weakly significant inverse association with female gender. Fallon (1), Robinson (19) and Glueck (22) found a strong significance between age and tHcy levels in their studies. As gender influences plasma homocysteine levels significantly (19), definitions of hyperhomocysteinemia using mixed-sex control groups like we did, may also be unsuitable for definitions of normality. It is now clear that hyperhomocysteinemia also increases the risk for coronary disease in women (19). Evidence exists for hormonal regulation of plasma tHcy and women in postmenopause have higher plasma homocysteine levels than women in premenopause (23). After controlling for age, sex and clinical situations, total homocysteine and total nitrite were associated with cardiac risk factors particularly with serum lipids and fibrinogen, but the relations were weak. Previous evidence suggests that tHcy is an independent risk factor for cardiovascular disease in patients with hyperlipidemia (22,24). Hyperfibrinogenemia is one of the risk factors for atherosclerosis and clinical trials are under way to assess the potential benefit of decreasing homocysteine and fibrinogen levels in CAD patients with high baseline levels, because a positive correlation between plasma tHcy and serum fibrinogen has been suggested (25). Smoking is another cardiac risk factor and is one of the important causes of impaired endothelial vasodilation as is hyperlipidemia (20). Although Fallon (1), Glueck (22) and Sutton-Tyrrell et al (26) found a strong relation between smoking and tHcy concentration, we did not.

Our results denoted that tHcy was inversely and significantly associated with total nitrite. The contribution of lipids , especially of HDL cholesterol to this association was significant, the contribution of LDL cholesterol to this association was significant only in the presence of HDL cholesterol, as was for fibrinogen. The independent associations of tHcy with CAD and cardiac syndrome X and of total nitrite with CAD and cardiac syndrome X were evaluated. We found that the relative risk for the occurrence of CAD increased by 0.82 for each μmol/L increment in homocysteine concentration and by 1.08 for each μmol/L decrement in total nitrite concentration. In the Framingham Heart Study, a strong association between elevated homocysteine concentrations and occlusive vascular disease that remained even after adjustment for other conventional coronary risk factors was found (3). Robinson (19) reported that even when all patients with vitamin deficiency are excluded, high homocysteine concentrations are still associated with an increased risk of coronary artery disease. Graham et al (27) stated that an elevated plasma homocysteine concentration conferred an independent risk of vascular disease similar to that of smoking or hypercholesterolemia and also had a multiplicative effect on risk among cigarette smokers and patients with hypertension. Nygard et al (9) found a strong graded association between plasma homocysteine concentrations and overall mortality in patients with angiographically documented coronary artery disease. However, total homocysteine concentrations were only weakly associated with the extent of coronary artery disease in this study. Fallon et al (1) stated that their study did not support the hypothesis that homocysteine is a strong independent risk factor for coronary artery disease, while in a recent meta-analysis, Boushey and colleagues estimated that 10

percent of the risk of coronary artery disease in the general population is attributable to homocysteine (3). Ross et al (28) stated that patients with homozygous defects in enzymes necessary for homocysteine metabolism develop severe atherosclerosis in childhood and many have their first myocardial infarction by the age of 20s, because homocysteine is prothrombotic and toxic to endothelium and it increases collagen production and decreases the availability of nitric oxide. We could not find an association between cardiac syndrome X and tHcy and total nitrite concentrations.The only significant and independent association to cardiac syndrome X was found with HDL and LDL cholesterol concentrations. In a recent and convenient study with ours, Desideri and collegues (29) investigated the endothelial activation in patients with cardiac syndrome X by measuring endothelin-1 (ET-1) plasma concentrations which is known to be increased in the presence of endothelial dysfunction and plasma nitrite-plus-nitrate levels, a sharp index of endothelial nitric oxide production and circulating concentrations of the soluble fraction of the endothelial adhesion molecule vascular cell adhesion molecule-1, that is increased in patients with endothelial dysfunction even without overt atherosclerotic lesions. The patients had marked fasting dyslipidemia and plasma glucose levels confirming the previously described link between metabolic and cardiac syndrome X. Their findings showed that baseline levels of ET-1, plasma concentrations of nitrite plus nitrate and soluble VCAM-1 did not elevate in cardiac syndrome X patients compared with control subjects. They concluded that under baseline conditions, no endothelial damage was detected in the patients with endothelium-derived markers. On the contrary Bellamy and collegues (30) measured flow-mediated brachial artery dilatation in 7 syndrome X patients with no known cause of endothelial dysfunction, such as high blood pressure, present or past active or heavy smoking, hypercholesterolemia and hyperhomocysteinemia were in exclusion criterias. The study showed loss of flow-related brachial artery dilatation in the syndrome X patients, compared with the matched normal control group, while endothelium-independent glyceryl trinitrate (GTN) responses were unimpaired. Flow-related dilatation is due predominantly to endothelial nitric oxide (NO) activity and its loss in disease relates to the NO-mediated component. Serum levels of homocysteine, nitrate, nitrite and VWF antigen were unchanged by L-arginin. L-arginin did not increase resting artery diameter or GTN-induced dilatation but only flow-related dilatation, implying that it specifically improved flow-mediated NO production and thus providing support that syndrome X is characterised by endothelial dysfunction.

Failure of the vascular endothelium to elicit NO mediated vasodilation irrespective of the mechanism behind is referred to as endothelial dysfunction and it was stated that in general, the degree of endothelial dysfunction of coronary microvasculature correlates with total serum cholesterol levels, because hypercholesterolemia impaires endothelium-dependent vasodilation of coronary conduit and resistance vessels (20,31,32). Impairment of endothelium-dependent vasodilatation is observed not only in advanced atherosclerotic lesions but also in arteries with minor irregularities or in those with entirely smooth lumen (31,32). These results suggest that endothelial vasodilator function is impaired early in the course of atherosclerosis. Notably, HDL and its efficiency of reverse cholesterol transport in subjects with normal and increased LDL levels may be an important determinant for endothelial function. Indeed, the ratio of LDL/HDL rather than absolute values of LDL determined the degree of endothelial dysfunction in the coronary microcirculation, indicating that HDL exerts a protective effect on endothelial function in patients with hypercholesterolemia (31).

Many of the studies designed to evaluate the relation between plasma total homocysteine concentrations and the extent of CAD used different ranges in accounting plasma tHcy levels. Tokgözoğlu (33) reported that plasma tHcy levels of Turkish population are higher than the European population even in normal subjects and a negative correlation between serum folate and plasma homocysteine levels could be seen. A delay in separation of blood held at room temperature may artificially increase plasma tHcy levels as it can leak from red blood cells into the plasma and this can be a methodological reason for high plasma tHcy levels. In our study blood samples were kept on ice and serum was separated within an hour and then frozen and was stored at

below -20oC until analysis. Our method was appropriate with the method Fallon (1) and colleagues used. Traditional risk factors such as aging, dominance of male gender, current smoking (also high in cardiac syndrome X patients), high levels of serum LDL cholesterol and triglycerides were all related to CAD patients and accompanied by the highest levels of tHcy and the lowest levels of total nitrite in our study

We may conclude that some of the traditional risk factors like hypercholesterolemia and hyperhomocysteinemia may impair NO activity either by increasing destruction of NO alone or by decreasing production of NO synthase activity to result in an endothelial dysfunction during the atherosclerotic process and the low levels of nitrites can be a marker for the ongoing atherosclerosis indirectly. Our results might support endothelial dysfunction in CAD patients but not in cardiac syndrome X patients compared to healthy subjects.

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