Turkish Journal of Endocrinology and Metabolism, (2003) 4 : 159-164 **ORIGINAL ARTICLE**

Homocysteine Levels in Type 2 Diabetic Patients with Nephropathy and Coronary Artery Disease

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The aim of the study was to investigate the relationship between plasma homocysteine (Hcy) levels and diabetic vascular complications and family history of diabetes mellitus (DM) and coronary artery disease (CAD) in patients with type 2 diabetes. Fasting plasma total Hcy concentrations and uncomplicated[metabolic parameters were investigated in 116 diabetic patients patients (n = 35), patients with nephropathy (n = 41), patients with CAD (n = and 31 healthy control subjects. Family histories of DM and CAD of the]40) patients were also studied. The plasma Hcy levels were significantly higher in 2.9± 6.5 vs. 9.6 ±the diabetic patients than in the control subjects (12.9 mol/l, Pμ<0.001). There were no differences between uncomplicated diabetic ± 3.5 vs. 9.6 ± patients and control subjects with respect to Hcy levels (9.6 0.05). The nephropathy group had higher Hcy levels than the>mol/l, Pμ2.9 mol/l, Pμ 3.5 ± 8.8 vs. 9.6 ±uncomplicated group (14.6 <0.05). Similarly, CAD ± 5.5 vs. 9.6 ±group had higher Hcy levels than the uncomplicated group (13.3 0.05). Hcy did not correlate with lipid parameters and metabolic<mol/l, Pμ3.5 control of diabetes. There was a significant relationship between hyperhomocysteinemia and family history of CAD of patients (38% vs. 12%, P=0.01). The findings suggest that elevated plasma Hcy levels are strongly associated with nephropathy and coronary artery disease in patients with type 2 diabetes. Also the relationship between hyperhomocysteinemia and a positive family history of CAD found in this study suggests that genetic predisposition may be more responsible for the unfavourable effects of hyperhomocysteinemia in macrovascular complications.

Key words: Homocysteine, type 2 diabetes, nephropathy, coronary artery disease

Introduction

It is well known that cardiovascular diseases (CVD), a major cause of death in diabetes mellitus (DM), are more extensive and occur earlier in diabetic patients than in the general population. The cardiovascular mortality rates are 2 to 4 times higher in type 2 diabetic patients than in non-diabetic subjects (1). The vasculopathy in diabetes is not purely dependent on direct diabetes-related metabolic events. This is known to be associated with several other cardiovascular risk factors including hypertension, obesity, dyslipidemia, and cigarette smoking (2).

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It has been recently demonstrated that homocysteine (Hcy) is an independent risk factor for arteriosclerotic vascular disease (3-5). Hcy-mediated vascular disease was first established in the 1960's. Subsequently, many studies have been undertaken to confirm the undesirable effects of Hcy on atherosclerotic process. Many of the potential mechanisms by which Hcy induces vascular damage have been proposed; Hcy may cause damage due to a toxic sulphur-containing amino acid accumulation in endothelial cells, enhance lipid peroxidetion, inhibit nitric oxide synthesis, pose an oxidative stress, decrease anticoagulant endothelial cell properties with suppression of thrombomodulin expression in endothelial cells, and activation of platelets (6-8).

It was suggested that hyperhomocysteinemia contributes to the accelerated atherosclerotic process in diabetes (9). Although a number of studies

have dealt with the association of hyperhomocysteinemia and macro- or microangiopathic complications in diabetes (10-14), the precise mechanisms by which hyperhomocysteinemia is associated with diabetic complications are still being debated. The relationship between Hcy and the metabolic parameters was also studied. Furthermore, association of high levels of Hcy and family history of DM and coronary artery disease (CAD) of diabetic patients were investigated.

Materials and Methods

A total of 147 subjects (91 females and 56 males) were studied. 76 type 2 diabetic subjects were recruited from among those attending the Diabetes Clinics and 40 subjects from Cardiology Clinics with the diagnosis of DM and CAD of Gazi Medical University Hospital. 31 healthy subjects were recruited from the check up unit.

The subjects ranged in age from 44 to 66 years with a mean age of 56 ± 10 and had a mean duration of diabetes of 12 ± 8 years. The diagnosis of DM was established and classified in accordance with the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (15). Proteinuria was recorded following at least two biochemical measurements in 24 h urine specimens (in the absence of urinary infection and hematuria). Nephropathy was diagnosed when abnormal urinary protein concentration (proteinuria >300 mg/day) was recorded. Taking into account that Hcy might be influenced by kidney function (16), patients who had abnormal creatinine levels (normal range of creatinine: 0.50 - 1.30 mg/dl) and glomerular filtration rate (GFR) levels lower than 50 ml/min were excluded from the study. GFR was calculated using the formula (urine volume x urine creatinine) / (1440 x plasma creatinine). CAD was diagnosed with coronary arteriogram. These patients were investigated for the presence of nephropathy.

Hyperhomocysteinemia was defined as a fasting plasma total Hcy level >15.0 μmol/l since increased risk of CVD had been observed with plasma Hcy levels >15.0 μmol/l in a previous report (5). The patients were further divided in two groups according to their plasma Hcy levels: one group had increased Hcy (>15.0 μmol/l, n=26, 22%), whereas the other group had laboratory values within the normal range (5.0 to 15.0 μmol/l, n=90, 78%). The subjects in all groups with hyperhomocysteinemia were also combined and divided according to the presence of a family history of DM and CAD.

Following an overnight fast, blood specimens of all subjects were drawn into vacutainers containing EDTA, and immediately refrigerated at 4°C for Hcy analysis. Within 2 hrs of collection, blood samples were centrifuged, and then the EDTA plasma was separated and stored at – 80°C. Estimation of plasma Hcy was carried out with the IMX analyser (Abbott diagnostics, Chicago, USA). Other measurements were glycemic control, lipid and lipoprotein levels, vitamin status (vitamin B_{12}, folic acid), and renal function tests.

Statistics

Analyses were performed with the SPSS PC (+) statistical package. All data are presented as mean ± SD. A P value of < 0.05 was considered statistically significant. The following statistical tests were performed: parametric statistical tests; two-sided student's *t* test, one-way analysis of variance (ANOVA) and χ^2 test (for categorical variables), nonparametric statistical tests; Mann-Whitney U, and Kruskal Wallis analysis of variance. The analysis of the relationship between plasma Hcy level and the metabolic variables was performed through Spearman's rank correlation test. All variables (independent of whether they were or were not significantly associated with Hcy levels in the univariate statistical analysis) were included in the multiple regression analysis to assess the predictors of hyperhomocysteinemia.

Results

Table 1 shows some of the clinical characteristics of the subjects studied. There were no differences between groups with respect to age and diabetes duration (P>0.05). Table 2 summarises the results of Hcy as well as lipid and lipoproteins and glycemic control parameters measured in the patients and in the control subjects. No significant differences were found in the distributions of fasting glucose, postprandial glucose, HbA_{1c}, triglylcerides, total cholesterol, LDL cholesterol, HDL cholesterol, and VLDL cholesterol in the patient groups (P>0.05).

Table 1. Clinical features of patients in all groups

	Noncomplicated n = 35	Nephropathy n = 41	CAD n = 40	Control n = 31
Age (years)	54.6 ± 8.	58.2 ± 8.0	60.7 ± 10	56.4 ± 6.6
Female/male (no)	27/8	28/13	16/24	16/15
DM duration (years)	9.6 ± 6.7	15.8 ± 6.7	10.1 ± 6.2	0(0)
Smoking (%)	37.1(13)	34.1(14)	22.5(7)	67.7(21)
Hypertension (%)	62.8(22)	73.1(30)	70.9(22)	12.9(4)
Insulin therapy (%)	22.8(8)	87.8(36)	74.1(23)	0(0)
OAA therapy (%)	62.8(22)	9.7(4)	16.1(5)	0(0)

DM: diabetes mellitus, OAA: oral antidiabetic agent.

Table 2. Biochemical features of patients in the groups

	Uncomplicated n=35	Nephropathy n=41	CAD n=40	Control n=31
Hcy (μmol/l)	9.63 ± 3.5	*14.69 ± 8.8	*13.38 ± 5.5	9.66 ± 2.9
HbA_{1c} (%)	6.81 ± 2.1	7.8 ± 1.6	6.9 ± 2.2	3.5 ± 1.1
Creatinine (mg/dl)	0.92 ± 0.2	0.94 ± 0.8	0.80 ± 0.64	0.84 ± 0.1
GFR (ml/min)	96 ± 32	84 ± 12	91 ± 23	88 ± 14
F-glucose (mg/dl)	203 ± 80.6	213 ± 75.1	185 ± 62.5	86 ± 6.1
P-glucose (mg/dl)	247 ± 18.7	229 ± 89.3	240 ± 76.4	80 ± 4.5
Triglyceride (mg/dl)	10.3 ± 12.3	191.2 ± 59.3	96.2 ± 82.0	18.3 ± 7.6
Cholesterol (mg/dl)	14.5 ± 50.1	240.2 ± 40.1	95.2 ± 52.0	62.4 ± 2.6
LDL c (mg/dl)	31.0 ± 38.7	155.2 ± 37.8	09.2 ± 41.0	16.4 ± 9.5
HDL c (mg/dl)	0.3 ± 9.2	38.8 ± 11.2	8.5 ± 9.8	37.8 ± 6.1
VLDL c (mg/dl)	2.8 ± 22.4	38.8 ± 13.6	5.4 ± 14.6	22.3 ± 4.5

F-glucose: fasting glucose, P-glucose: prandial glucose, LDL c: LDL cholesterol, HDL c: HDL cholesterol, VLDL c: VLDL cholesterol.* p<0.05.

Distribution of plasma Hcy level ranged between 3.23 and 30.93 μmol/l (mean ± SD 12.2 ± 6.1 μmol/l; median: 11.6 μmol/l). Mean plasma Hcy levels were 20% higher in males than in females (13.6 ± 7.1 vs. 11.3 ± 5.1 μmol/l; P<0.005). Hcy levels weakly correlated with age (r=0.26; P<0.01), while no significant differences were found in the duration of diabetes (r=0.11; P>0.05).

Plasma Hcy concentrations did not correlate with fasting glucose (r=0.01; P=0.98), postprandial glucose (r=0.01; P=0.90), HbA_{1c} (r=0.05; P=0.68), total cholesterol (r=0.13; P=0.19), triglycerides (r=0.02; P=0.84), LDL cholesterol (r=0.11; P=0.32), VLDL cholesterol (r=0.06; P=0.58), or HDL cholesterol (r=0.01; P=0.91). When the subjects were classified according to their glucose lowering treatments, plasma Hcy concentrations were not significantly different (r=0.09; P=0.33). There was a positive correlation between Hcy and plasma creatinine (r=0.23; P<0.05) but not between Hcy and folic acid, and Hcy and vitamin B_{12} levels.

When the number of stenotic major coronary arteries was compared for Hcy level, plasma Hcy concentrations did not correlate with the number of stenotic major coronary arteries of patients.

The findings of four different groups including hypertensive patients, smoking patients, hypertensive smoking patients, and the patients without hypertension and smoking habit revealed no significant differences for the plasma Hcy level (P>0.05).

As shown in Figure 1, plasma Hcy levels were significantly higher in diabetic patients than in control subjects (12.9 ± 6.5 vs. 9.6 ± 2.9 μmol/l, P<0.001). No differences were found between Hcy levels of uncomplicated and control groups (9.6 ± 3.5 vs. 9.6 ± 2.9 μmol/l, P>0.05). Both the patients with nephropathy and the patients with CAD had significantly higher Hcy levels than the uncomplicated patients (14.60 ± 8.8 vs. 9.6 ± 3.5 μmol/l, P<0.05; 13.3 ± 5.5 vs. 9.6 ± 3.5 μmol/l, P<0.05 respectively).

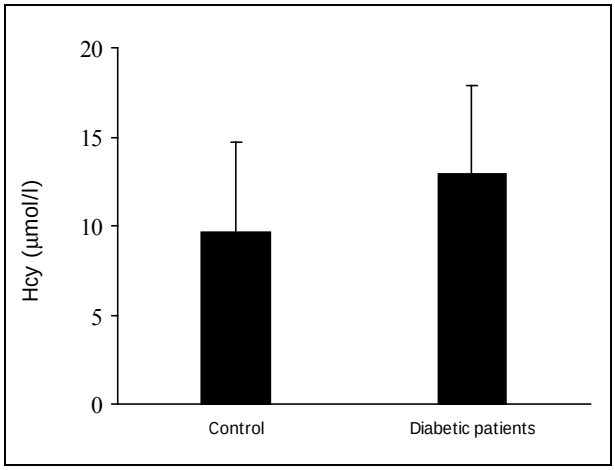

Figure 1. Plasma Hcy values are significantly higher in diabetic patients than in control subjects, P < 0.001.

Multiple linear regression analysis was used to establish the principal determinants of the concentrations of plasma Hcy. Only age (β=0.22, P<0.0001), gender (β=0.25, P<0.001), creatinine (β=0.20, P<0.001) and DM duration (β=0.08, P<0.05) were independently related with plasma Hcy. After adjustment for these variables, the Odds ratio (OR) for hyperhomocysteinemia was 1.24 (95%CI, 1.08-

1.42) in the patients with nephropathy and 1.29 (95%CI, 1.10-1.52) in the patients with CAD.

As shown in Table 3, there was no relationship between hyperhomocysteinemia and family history of DM of the patients (P=0.772) (OR, 1.2; 95%CI, 0.38-3.65). But there was a significant relationship between hyperhomocysteinemia and family history of CAD of the patients (P=0.009) (OR, 4.1; 95%CI, 1.49-11.0).

Table 3. Relationship between hyperhomocysteinemia, family history of DM or CAD

	Patients with hyperhomo-cysteinemia	Patients without hyperhomo-cysteinemia	OR	P value
Patients with family history of DM	5(20%)	15(16%)	1.2	0.772
Patients without family history of DM	21(80%)	75(84%)		
Patients with family history of CAD	10(38%)	12(13%)	4.1	0.009
Patients without family history of CAD	16(62%)	78(87%)		

Discussion

Whereas some studies have shown diabetic patients to have higher a prevalence of hyperhomocysteinemia than control subjects (14,17,18) other studies do not support this finding (12,16,19).

In our study plasma Hcy level was not higher in uncomplicated diabetic patients than control subjects. However, there was a significant difference in the Hcy level between complicated patients and control subjects. There are also controversial studies in the literature regarding the association between plasma Hcy and macrovascular complications (10, 11,20) or microvascular complications (12,13,21, 22). However, the relationship between Hcy and diabetic macrovascular complications seems more stronger than the relationship between Hcy and diabetic microvascular complications.

It has been reported that incipient and overt nephropathy is associated with elevations of plasma Hcy in patients with type 2 diabetes (18). In line with this study, Hofmann et al. (20) found elevated Hcy levels in diabetic patients with nephropathy whereas Robillon et al. (19) found reduced Hcy levels in diabetic patients without overt nephropathy. In our study, we found significant association between plasma Hcy and mild nephropathy but not between various degrees of albuminuria. We especially chose patients without overt nephropathy since we aimed at eliminating the effect of renal clearance on Hcy level.

We found a significant association between plasma Hcy and CAD. It has been shown in Hoogeveen's study (11) that diabetic patients with hyperhomocysteinemia have an increased risk for developing cardiovascular disease than those with normohomocysteinemia, and hyperhomocysteinemia may cause acceleration of vascular damage related to diabetes.

Interestingly, in our study, the relationship between family history of CAD and hyperhomocysteinemia was remarkable. Hyperhomocysteinemia was found 4.1 times higher in patients with family history of CAD than in those without family history of CAD. No data on this topic was available in the literature. We think that genetic factors that lead to hyperhomocysteinemia such as deficiency of cystathione-β-synthase enzyme or other enzymes, or co-factor abnormalities may partly explain this relationship.

A positive correlation of Hcy level with age, consistent with the findings of other studies, was found (5,23,24). It has been shown that the in vitro activity of the rate-limiting enzyme for Hcy metabolism, cystathione-β-synthase, declines with age; thus, declining cystatione-β-synthase activity and glomerular filtration rate with age may explain the age-related increase in plasma Hcy.

Our findings showed no significant relation between plasma Hcy level and the duration of diabetes. This result suggests that regulation of plasma Hcy is mainly based on genetic and environmental factors than on diabetes- related metabolic events.

In contrast to Passaro's study (25), we found no correlation between plasma Hcy level and total cholesterol, LDL cholesterol, HDL cholesterol, VLDL cholesterol, and triglycerides in diabetic patients. This finding is consistent with those of previous studies that showed no relationship between Hcy and lipids (11,26). Drzewoski's study (27) showed correlations between plasma Hcy and glucose or HbA_{1c} , but Araki's study (10) did not. We observed that Hcy was not correlated with glucose or HbA_{1c}.

In conclusion, increases in Hcy in diabetic patients are associated with increased complications especially

CAD and nephropathy. We think that Hcy may not only be a result but also a factor leading to the development of diabetic vascular complications. Since the mechanisms responsible for Hcy-mediated vascular damage still remain obscure, prospective studies are needed to reveal the exact role of hyperhomocysteinemia modified by genetic, dietary and environmental factors in the development of angiopathy in patients with diabetes.

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