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Homocysteine Levels and Catalase A ctivities in Type 2 Diabetic Subjects with Vascular Complications

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The aim of this study was to determine the association between homocysteine levels and macroangiopathic/microangiopathic complications in type 2 diabetes mellitus (DM), folate and vitamin B_{12} levels, antioxidant enzyme activities including superoxide dismutase (SOD) and catalase. This study was performed on 94 type 2 DM patients (56 males, 38 females) and 20 healthy control subjects (12 males, 8 females). The results showed that the catalase and homocysteine levels in the diabetic group were higher than those levels in the control group.

While these data suggest amplified free radical production in patients with macrovascula complications, the role of homocysteine remains uncertain.

Key words: Homocysteine, Catalase and Type 2 Diabetes, superoxide dismutase

Introduction

Type 2 diabetes mellitus (DM) is a common endocrine and metabolic disorder with damage to the vascular system as one of its major complications. Free radical induced oxidative damage may play a role in the pathogenesis of these vascular complications in diabetes. (1). Recently it has been suggested that homocysteine is an independent risk factor for atherosclerosis (2). Homocysteine has a cytotoxic effect on endothelial cells, promotes the generation of reactive oxygen species (3) and, through inhibition of prostacyclin synthesis, it may also contribute to abnormality in the platelet function (4).

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The aims of this study were to determine the possible relationship between homocysteine levels and antioxidant status and to investigate if there is any role of homocysteine in the mechanism of macro- and/or micro-angiopathic complications in type 2 DM.

Materials and Methods

Patients with type 2 DM (n=94) attending the diabetic outpatient clinic at Ege University Hospital, Department of Endocrinology and healthy volunteer control subjects were taken into the study. All patients underwent comprehensive clinical assessment and investigation for the presence of macrovascular or microvascular complications. Investigations included ECG, doppler ultrasonography, ophthalmoscopic examination of optic fundi through dilated pupils, microfilament examination, EMG, measurement of serum urea, creatinine and microalbuminuria.

No subject used vitamins during the 6 months prior to blood sampling. All type 2 DM patients were being treated with a combination of diet and oral

antidiabetic drugs (sulphonylureas, acarbose) during this study.

Blood samples obtained after a 12 hours fast were immediately centrifuged and the serum was frozen at -20°C until analysis. Serum glucose, total cholesterol, tryglyceride, HDL-cholesterol, glycosylated hemoglobin (HbA_{1c}) were measured by auto-analyser. LDL-cholesterol was calculated using the Friedewald formula. Vitamin B_{12} and folic acid were measured by radioimmunassay using commercially available reagents (SimulTRAC-SNB). Serum homocysteine levels were determined by HPLC using a commercially available kit (Bio-Rad). Serum catalase activities and superoxide dismutase (SOD) activities were measured as previously described by Sozmen (5).

Analysis of Variance (ANOVA) was used for comparison of means which was followed by the Newmann-Keuls multiple range test. Student t-test was used for inter group comparisons.

Results

Serum glucose, HbA_{1c}, cholesterol, triglyceride, homocysteine levels and catalase activities were significantly higher in the patient group ($p<0.05$) (Table 1). There was no correlation between homocysteine and age and there was no difference in levels between genders. There were significant group differences for blood glucose and homocysteine levels according to the type of complications (macrovascular and both complications) ($p=0.0112$ $p=0.0232$, respectively). Catalase activity in the patients with complications was higher than in the controls ($p=0.0141$). None of these parameters were affected by the mode of diabetes treatment. There was no correlation between catalase activities and homocysteine levels.

Discussion

Although many studies have shown that hyperhomocysteinemia is a risk factor for macrovascular diseases such as atherosclerosis, stroke and thrombosis (2), a role for homocysteine in the macrovascular complications of diabetes has not been shown. It was previously reported that type 2 diabetic patients had higher homocysteine levels than age-matched controls and that hyperhomocysteinemia was more common in those with macrovascular disease (6). Accordingly, we observed higher homocysteine levels in diabetic patients and particularly in those with micro and macrovascular complications.

Table 1. Clinical characteristics of patients and controls.

	Controls (n = 20)	Type 2 DM (n = 94)	Type 2 DM without complication (n=26)	Type 2 DM with macrovascular complication (n=34)	Type 2 DM with microvascular (n=26)	Type 2 DM with both complications (n=8)
Gender (male/female)	12/8	56/38	16/10	18/16	15/11	5/3
Age	53.3 ± 9.8	53.0 ± 7.2	52.9 ± 8.1	52.8 ± 8.3	53.7 ± 9.2	53.4 ± 8.9
Fasting Plasma Glucose (mg/dL)	90.75 ± 1.80	*155.23 ± 7.32	*122.79 ± 7.52	*138 ± 8.74	*181.73 ± 17.19	*132.38 ± 10.47
HbA_{1c} (%)	3.1 ± 0.11	*7.82 ± 0.26	*7.52 ± 0.61	*7.13 ± 0.28	*9.27 ± 0.52	*7.05 ± 0.36
T-Cholesterol (mg/dL)	186.75 ± 3.82	*208.46 ± 4.73	204.35 ± 7.64	205.15 ± 6.54	214.88 ± 11.17	215 ± 21.60
LDL-Cholesterol (mg/dL)	119 ± 5.6	144 ± 7.3	122.19 ± 7.52	130.85 ± 6.05	127.31 ± 9.42	125.88 ± 16.73
HDL-Cholesterol (mg/dL)	46.60 ± 2.39	46.97 ± 1.53	52.69 ± 4.08	44.21 ± 1.93	45.85 ± 2.15	43.75 ± 4.67
Creatinine (mg/dL)	0.87 ± 0.03	0.91 ± 0.016	0.90 ± 0.029	0.89 ± 0.03	0.92 ± 0.026	0.96 ± 0.037
Trygliceride (mg /dL)	95.05 ± 7.81	*175.81 ± 12.46	162.46 ± 20.33	176.79 ± 21.68	191.46 ± 28.53	164.13 ± 18.40
Homocysteine(µmol/L)	15.06 ± 0.58	*20.98 ± 1.16	17.08 ± 2.01	*24.20 ± 2.29	20.33 ± 1.76	*22.05 ± 2.51
Vitamin B_{12} (ng /mL)	313.90 ± 25.47	341.38 ± 18.32	331.12 ± 25.42	291.03 ± 22.38	362.64 ± 29.05	225.13 ± 34.47
Folic acid (pg/mL)	9.47 ± 0.74	10.85 ± 0.4	12.4 ± 1.01	10.76 ± 0.7	9.17 ± 0.66	11.56 ± 1.95
SOD (U/L)	65.63 ± 3.77	59.88 ± 2.69	68.07 ± 5.43	61.92 ± 4.28	55.49 ± 5.16	49.69 ± 6.29
Catalase (U/L)	9.26 ± 1.07	*15.45 ± 1.32	*14.27 ± 2.33	*17.26 ± 2.76	*14.95 ± 2.22	*13.67 ± 1.30

*$p<0.05$ compared with control. Data are given as mean ± SEM.

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