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Original Article / ***Klinik Çalışma - Araştırma***

Plasma Levels of Asymmetric Dimethylarginine and Total Homocysteine in First Degree Relatives of Type 2 Diabetic Patients

Tip 2 Diyabetik Hastaların Birinci Derece Yakınlarında Total Homosistein ve Asimetrik Dimetilargininin Plazma Düzeyleri

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Objectives: Cardiovascular diseases are more common among first degree relatives of type 2 diabetic patients than healthy subjects without a family history of diabetes. Plasma asymmetric dimethylarginine (ADMA) and homocysteine (Hcy) levels are markers of endothelial dysfunction and cardiovascular disease. The objective of this study was to evaluate levels of ADMA, Hcy and their association with cardiovascular risk factors in first degree relatives of type 2 diabetic patients.

Patients and Methods: The circulating ADMA and Hcy levels were measured in 15 first degree relatives of type 2 diabetic patients and 15 control subjects without a known family history of diabetes.

Results: No statistically significant differences were found in plasma levels of ADMA and Hcy between the two groups (p>0.05). Plasma ADMA levels correlated significantly with waist circumference (p=0.02), fasting insulin levels (p=0.03), insulin resistance (p=0.01), total cholesterol (p=0.04) and HDL-cholesterol (p=0.03) levels in the first degree relatives of type 2 diabetic patients.

Conclusion: These results suggest that plasma ADMA levels do not directly contribute to the development of endothelial dysfunction in first degree relatives of type 2 diabetic patients with cardiovascular risk factors.

Key Words: Diabetes mellitus, type 2/genetics; N,N-dimethylarginine; homocysteine.

Amaç: Tip 2 diyabetik hastaların birinci derece yakınlarında, ailesinde diyabet öyküsü olmayan sağlıklı olgulara göre kardiyovasküler hastalıklar daha sık görülmektedir. Asimetrik dimetilarginin (ADMA) ve homosistein (Hcy) plazma düzeyleri kardiyovasküler hastalıklar ve endotel disfonksiyonuyla ilişkili göstergelerdir. Bu çalışmada, tip 2 diyabetik hastaların birinci derece yakınlarında ADMA ve Hcy plazma düzeyleri ile bu göstergelerle kardiyovasküler risk faktörleri arasındaki ilişkilerin incelenmesi amaçlandı.

Hastalar ve Yöntemler: Dolaşımdaki ADMA ve Hcy düzeyleri 15 tip 2 diyabet hastasının birinci derece yakınında ve ailesinde diyabet öyküsü olmayan 15 kontrol olgusunda ölçüldü.

Bulgular: Her iki grup arasında ADMA ve Hcy plazma düzeyleri açısından anlamlı farklılık saptanmadı (p>0.05). Asimetrik dimetilarginin plazma düzeyi tip 2 diyabetik olguların birinci derece yakınlarında, bel çevresi (p=0.02), açlık insülin düzeyi (p=0.03), insülin direnci (p=0.01), total kolesterol (p=0.04) ve HDL kolesterol (p=0.03) ile ilişkiliydi.

Sonuç: Bu sonuçlara göre, kardiyovasküler risk faktörlerine sahip olan tip 2 diyabetik olguların birinci derece yakınlarında, ADMA plazma düzeylerinin doğrudan endotel disfonksiyonunun gelişimine katkıda bulunmadığını düşünmekteyiz.

Anahtar Sözcükler: Tip 2 diabetes mellitus/genetik; N,N-dimetilarginin; homosistein.

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Atherosclerosis has been reported to be more common among first degree relatives of type 2 diabetic patients than healthy subjects without a family history of diabetes.[1] Several mechanisms play important roles in the pathogenesis of endothelial dysfunction, which is observed in the early stages of atheroscleosis. Nitric oxide (NO) provides endothelial cell-dependent vasodilatation, and antiplatelet, antiproliferative, antiinflammatory and antioxidative effects in the vascular wall.[2] It has been shown that asymmetric dimethylarginine (ADMA), an endogenous NO synthase inhibitor, reduced NO synthesis during the atherosclerotic process.[3] In addition, it has been reported that hyperhomocysteinemia (Hcy) augments the generation of oxygen-derived free radicals and reduces the activity of the enzyme dimethylarginine dimethylaminohydrolase (DDAH) which metabolizes ADMA.[4] Consequently, all these atherogenic reactions may lead to decrased bioactivity of endothelium-derived NO and impair endothelial-cell dependent vasodilatation. In the present study, our aim was to compare plasma ADMA and Hcy levels of the first degree relatives of type 2 diabetic patients with subjects without a family history of diabetes, which were taken as a control group. Furthermore, the relationship between circulating ADMA levels and risk factors for cardiovascular disease was evaluated in the first degree relatives of type 2 diabetic patients.

PATIENTS AND METHODS

Fifteen first degree relatives of type 2 diabetic patients and 15 healthy subjects with no family history of diabetes were enrolled in this study. All patients gave informed consent to participate in the study. The two groups were carefully matched for age, sex, and body mass index (BMI). First degree relatives of type 2 diabetic patients which were recruited for the study had either one or both parents with type 2 diabetes. None of the participants had endocrinologic or metabolic disease, hypertension, cardiovascular disease, or any other systemic disease. None of the subjects were current smokers. Subjects taking medications (antidiabetics, hormonal replacement therapy, antihyperlipidemics, corticosteroids), which might have affected insulin resistance or endothelial function were excluded. Diabetes mellitus was excluded by a 75 g oral glucose tolerance test.[5]

All participants underwent physical examination. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements were performed by a mercury shygmomanometer after a 10-min rest. Height, weight, waist circumference (WC) and hip circumference were recorded by a standardized protocol. Body mass index (kg/m^2) and waist-hip ratio (WHR) were calculated.

Blood samples were collected from the antecubital vein without the use of a tourniquet after an overnight fast. Serum glucose was measured by the glucose oxidase technique (Beckman Coulter LX20). Spectrophotometric method (Beckman Coulter LX20) was used to measure serum triglyceride, total cholesterol and high density lipoprotein-cholesterol (HDL-C) concentrations. Low density lipoprotein-cholesterol (LDL-C) levels were calculated by the Friedewald formula. Serum insulin levels were determined by chemiluminescent enzyme immunoassay (Immulite DPC 2000, CA). Insulin resistance was assessed according to homeostasis model assessment (HOMA-IR= fasting serum glucose mmol/L x insulin μIU/mL/22.5).[6]

Plasma Hcy and ADMA samples were frozen at –80 °C until analysis. Plasma total Hcy concentrations were measured by ELISA (Axis-Shield, UK). Plasma concentrations of asymmetric dimethylarginine (N^G, $N^{G'}$-dimethylarginine, ADMA) were measured by high-performance liquid chromatography (HPLC) by precolumn derivatization with o-phthaldialdehyde as described by Teerlink et al.[7] HPLC was performed on a computer-controlled Waters chromatography system consisting of an Alliance 2690 XE separations module and a Model 474 fluorescence detector. Plasma samples and standards were extracted on Oasis MCX cation-exchange solid phase extraction cartridges (Waters). L-homoarginine (1 mM) was used as an internal standard. Chromatography was per-

formed on a Symmetry C18 column (3.9 x150 mm; 5 µm particle size; 100 Å pore size) with a 3.9x20-mm guard column (Waters). Mobile phase A consisted of 50 mM potassium phosphate buffer (pH 6.5), which contained 8.7% acetonitrile, and mobile phase B was acetonitrile/water (50/50, v/v). Separation was carried out under isocratic conditions with 100% mobile phase A at a flow rate of 1.1 ml/min, and at 30°C column temperature. Strongly retained compounds were eluted by a strong solvent flush (50% B from 20 to 22 min), after elution of the last analyte. Between 22 and 23 minutes the gradient returned to initial conditions and the column was equilibrated for an additional 7 minutes, resulting in a total run time of 30 minutes. An injection volume of 20 µl was used. Fluorescence was measured at excitation and emission wavelengths of 340 and 455 nm, respectively. After elution of arginine, the gain of the detector was switched to a 10-fold higher sensitivity.

Statistical analysis

Statistical analysis was performed with the SPSS 11.5 for Windows. Results are presented as mean±SD. Mann-Whitney U test was used for comparisons between the groups. All correlations were analyzed with Spearman's non-parametric correlation coefficient. Differences were considered statistically significant at a p level less than 0.05.

RESULTS

Baseline clinical and metabolic characteristics of first degree relatives of type 2 diabetic patients and control group were similar (Table 1). No significant differences in plasma levels of ADMA (0.4±0.08 µmol/L, 0.4±0.07 µmol/L) and Hcy (23.5±35.7 µmol/L, 18.6±16.5 µmol/L) were observed between the two groups (Fig. 1).

Spearman's correlation coefficient analysis was performed for further evaluation of the relationship between ADMA and risk factors of insulin resistance in first degree relatives of type 2 diabetic patients. ADMA concentrations correlated significantly with WC (p=0.02), fasting plasma insulin levels (p=0.03), HOMA-IR (p=0.01), total cholesterol (p=0.04), and HDL-C levels (p=0.03) in first degree relatives of type 2 diabetic patients. Plasma hcy concentrations in the first degree relatives of type 2 diabetic patients did not correlate with ADMA levels and cardiovascular risk factors (Table 2).

Table 1. Characteristics of the first degree relatives with type 2 diabetic patients and control group

	First degree relatives of type 2 diabetic patients (n=15)	Control group (n=15)
Sex (female/male)	7/8	6/9
Age (year)	38.1±7.1	40.3±5.1
Body mass index (kg/m^2)	27.4±4.2	28.3±7.1
Waist circumference (cm)	91.3±10.0	89.7±12.0
Waist-hip ratio	0.86±0.88	0.81±0.09
Systolic blood pressure (mmHg)	123.5±7.5	126.8±6.5
Diastolic blood pressure (mmHg)	76.4±5.5	80.1±5.9
Fasting plasma glucose (mg/dL)	91.6±6.5	87.1±7.5
Fasting insulin (µIU/mL)	10.0±6.8	9.2±4.1
HOMA-IR	2.29±1.70	1.9±0.89
Total cholesterol (mg/dL)	172.2±43.8	173.1±33.5
Triglyceride (mg/dL)	98.6±68.9	80.0±39.7
LDL-C (mg/dL)	106.2±39.3	104.6±33.3
HDL-C (mg/dL)	52.1±9.4	53.1±6.4

HOMA-IR: Homeostatis model assessment of insulin resistance; p>0.05 for all characteristics between the groups.

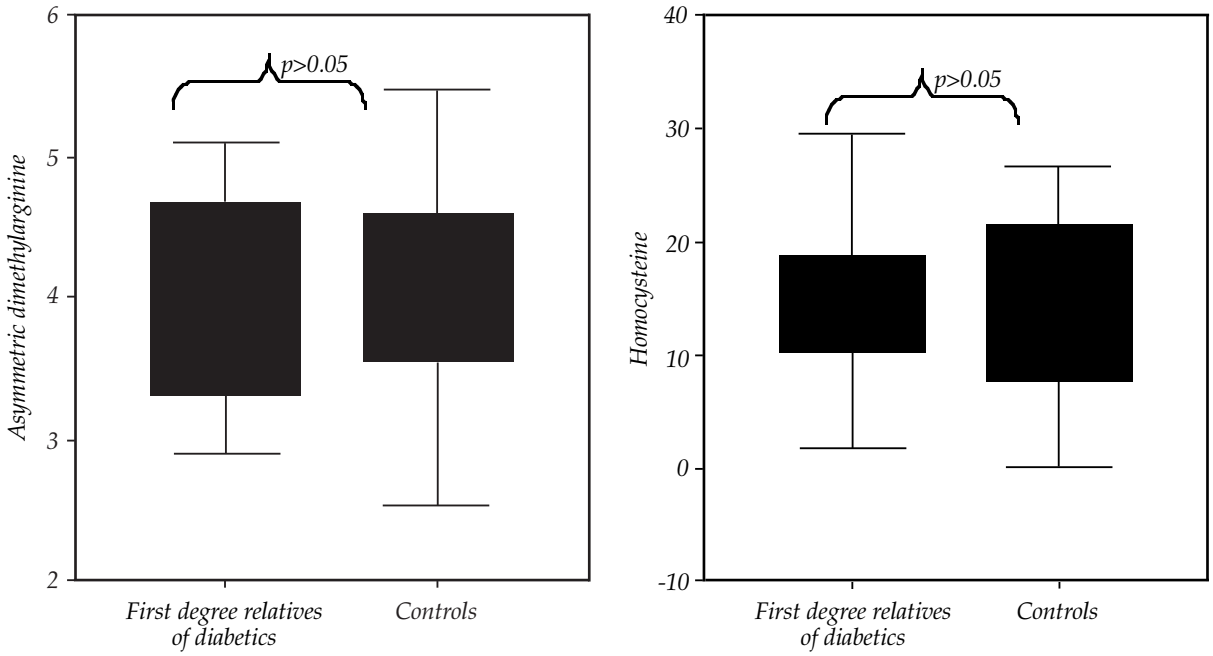

Fig. 1. *Plasma levels of asymmetric dimethylarginine and homocysteinemia in the two groups.*

DISCUSSION

Diabetes mellitus is a heterogeneous, multifactorial and polygenic disease, characterized by insulin secretory defect of beta cell and insulin resistance in peripheral tissue which lead to elevated glucose levels.[8] Several investigators suggested that non-diabetic first degree relatives of type 2 diabetic patients also show clinical and metabolical abnormalities in contrast to the subjects without a family history of diabetes.[9-11] For this reason, evaluation of the markers of endothelial function in the first degree relatives of type 2 diabetic patients is important in determining their susceptibility to cardiovascular disease. The results of this cross-sectional study demonstrated that non-diabetic first degree relatives of type 2 diabetic patients have similar circulating ADMA concentrations and other risk factors associated with metabolic syndrome compared with the well-matched controls. However, we found a significant correlation between plasma ADMA levels and cardiovascular risk factors, including increased WC, dyslipidemia, and indicators of insulin resistance (fasting insulin level and HOMA-IR) in first degree relatives of type 2 diabetic patients.

Elevated ADMA level is accepted as an early marker of the initial stages of atherosclerosis.[12] It has been demonstrated that ADMA plasma concentrations were elevated in patients with cardiovascular and metabolic diseases, including hypertension,[13] lipid disorders,[14] and diabetes mellitus[15] which are the components of metabolic syndrome. The main common pathophysiological mechanism for all these metabolic disorders is insulin resistance. Stühlinger et al.[16] reported a significant relationship between

Table 2. Correlations between ADMA, plasma Hcy levels and cardiovascular risk factors in the first degree relatives of type 2 diabetic patients

	ADMA		Hcy	
	r	*p*	r	*p*
WC	0.685	0.02	0.439	0.20
Fasting insulin	0.622	0.03	0.120	0.72
HOMA-IR	0.699	0.01	0.214	0.43
Total cholesterol	0.587	0.04	0.445	0.17
HDL-C	–0.617	0.03	–0.467	0.06
Hcy	0.427	0.16	–	–

ADMA: Asymmetric dimethylarginine; Hcy: Homocysteinemia; WC: Waist circumference; HOMA-IR: Homeostatis model assessment of insulin resistance

insulin resistance and plasma ADMA levels in a population of healthy, normotensive, non-diabetic volunteers even before the onset of clinically overt diabetes. They also reported that plasma ADMA concentrations were not increased in patients with cardiovascular risk factors, unless they were insulin resistant. Additionally, treatment of insulin resistance with rosiglitazone[16] and metformin[17] were found to cause a reduction of ADMA levels in insulin resistant patients. In our study, we hypothesized that the increment in plasma levels of ADMA could contribute to impaired endothelial function in the first degree relatives of type 2 diabetic patients. However, we did not find an increment in their plasma ADMA concentrations. The absence of insulin resistance in these subjects may contribute to this result. Balletshofer et al.[11] determined impaired endothelium-dependent and -independent vasodilatation in insulin resistant first degree relatives of type 2 diabetic patients compared with insulin sensitive relatives and controls. In this context, the correlation between ADMA and insulin resistance in this group suggested that ADMA levels in first degree relatives of type 2 diabetics might be elevated in the insulin resistant-stage.

Another molecular mechanism underlying the elevation of plasma ADMA levels in 2 diabetes mellitus may be the hyperglycemia that impairs DDAH activity and causes ADMA accumulation in vascular smooth muscle and the endothelium.[18] It has been reported that a minor elevation of plasma glucose levels in non-diabetic sibling pairs of type 2 diabetics could increase cardiovascular risk.[19] In this study, first degree relatives of type 2 diabetic patients were normoglycaemic and we did not find any association between fasting glucose and ADMA levels.

Elevated levels of Hcy which contribute to atherosclerosis by damaging the inner lining of arteries and promoting blood clots is associated with increased risk of cardiovascular disease.[20] In addition, homocysteine inhibits DDAH enzyme activity and causes ADMA accumulation.[4] Hcy levels in first degree relatives of type 2 diabetic patients were similar to those found in the controls. Furthermore, no correlation between ADMA and Hcy was found. We speculated that ADMA levels might be influenced by other unknown factors associated with endothelial function. However, because of the small size of the study population, these results must be interpreted with caution.

In conclusion, in this study, no significant difference in plasma ADMA levels was found between normoglycemic first degree relatives of type 2 diabetic patients and healthy controls. However, a positive correlation between ADMA and insulin resistance in first degree relatives of type 2 diabetic patients was observed, suggesting that ADMA accumulation could contribute to the development of impaired endothelium-dependent vasodilatation in first degree relatives of type 2 diabetic patients with reduced insulin sensitivity.

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