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An Assessment of the Correlation Between Serum Asymmetric Dimethylarginine and Early Endothelial Dysfunction in Patients with Type 1 Diabetes Mellitus

Çocukluk Çağı Tip 1 Diyabetes Mellituslu Hastalarda Serum Asimetrik Dimetil Arjinin (ADMA) Düzeyi ile Erken Endotel Hasarı Arasındaki İlişkinin Saptanması

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ABSTRACT

Objective: Asymmetric dimethylarginine (ADMA), a methylated L-arginine analog, is a major endogenous competitive inhibitor of Nitric oxide (NO). NO is an important critical vasoactive mediator synthesized by the vascular endothelium, previously referred to as endothelium-derived relaxing factor. ADMA is a novel risk factor and a novel statement of endothelial dysfunction. ADMA levels are clinically increased in several clinical conditions such as diabetes mellitus (DM), hypertension, insulin resistance syndrome, dyslipidemia.

Material and Methods: Thirty-one healthy children and 39 patients with Type 1 DM diagnosis, routinely followed by Keçiören Training and Research Hospital Pediatric Endocrinology Clinic between 2011 and 2013 were included in the study. Gender, age, weight, height, body mass index (BMI), systolic and diastolic blood pressure, fasting blood sugar values and diabetes duration were obtained from patient files. Total cholesterol, HDL cholesterol, triglyceride, ADMA, HbA1c, folate and homocysteine levels were compared between the two groups.

Results: There were no significant differences between the Type 1 DM group and the control group with respect to age, gender, body mass index (BMI), systolic and diastolic blood pressure. Average ADMA level in the Type 1 DM patient group (0.7±0.4 µmol/L) was significantly higher than the control group (0.6±0.1 µmol/L) (p=0.004). The homocysteine and folate levels for the two groups were similar and the differences were not significant (p=0.368 and 0.887, respectively).

Conclusion: The relation between endothelium damage and ADMA may lead to follow up ADMA measurement values being used as a pre marker for vascular damage in type 1 diabetes, and in turn help in preventing vascular complications.

Key Words: ADMA, Homocysteine, Diabetes mellitus, Children

ÖZET

Amaç: Asimetrik dimetil arginin (ADMA), metilenmiş arginin, endotelyal nitric oksit (NO) sentaz inhibitörüdür. NO daha önceden Endothelium-Derived Relaxing Faktörü olarak bilinen, vasküler endotelyumdan sentezlenen önemli vazoaktif mediatördür. ADMA, endotel fonksiyon bozukluğunun yeni bir belirteci ve endotelyal fonksiyon bozukluğu için yeni risk faktörüdür. ADMA'nın insülin direnci, hipertansiyon, diyabetes mellitus, hiperlipidemi gibi birçok kronik hastalıkta arttığı gösterilmiştir.

Gereç ve Yöntemler: Çalışmaya T.C. Sağlık Bakanlığı Keçiören Eğitim ve Araştırma Hastanesi Çocuk Endokrinoloji polikliniğinde 2011- 2013 yılları arasında takip edilen Tip 1 Diyabetes Mellitus tanılı 39 hasta ve aynı yaş grubunda 31 kontrol alındı. Cinsiyet, yaş, ağırlık, boy, vücut kitle indeksi, sistolik ve diastolik kan basıncı, açlık kan şekeri düzeyleri ve diyabet süreleri hastaların dosyalarından kaydedildi. Total kolesterol, HDL kolesterol, trigliserid, ADMA düzeyleri, folat ve homosistein düzeyleri açısından her iki grup karşılaştırıldı.

Bulgular: Hasta grubu ve kontrol grubunda boy, kilo ve VKİ, yaş ve cinsiyet, sistolik ve diastolik kan basıncı arasında istatistiksel olarak anlamlı farklılık saptanmadı (p>0.05). Tip 1 DM grubunda ADMA düzeyi (0.7±0.4 µmol/L) kontrol grubundan (0.6±0.1 µmol/L) anlamlı yüksek saptandı (p=0,004). Hasta ve kontrol grubu arasında ortalama homosistein ve folat düzeyleri benzer olup aralarında istatistiksel olarak anlamlı fark saptanmadı (p=0.368 ve 0.0887, sırasıyla).

Sonuç: Endotelyal hasar ile ADMA arasındaki ilişki tip 1 DM'da ADMA ölçümlerinin vasküler hasarlanmada bir ön belirteç olarak kullanılmasını sağlayacak ve vasküler komplikasyonların önlenmesine yardımcı olabilecektir.

Anahtar Sözcükler: ADMA, Homosistein, Diabetes mellitus, Çocuk

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INTRODUCTION

Asymmetric dimethylarginine (ADMA), a methylated L-arginine analog, is a major endogenous competitive inhibitor of Nitric oxide (NO) (1). NO is an important critical vasoactive mediator synthesized by the vascular endothelium, previously referred to as endothelium-derived relaxing factor. Endothelium derived NO is a powerful endogenous vasodilator and also has an important role in the maintenance of vascular homeostasis (2). ADMA is a novel risk factor of endothelial dysfunction (3). It circulates in the plasma, and is present in various tissues and cells. Major mechanisms that lead to increased ADMA values in various diseases include shear stress, oxidative stress, hyperhomocysteinaemia and high concentration of glucose in the blood (4). ADMA levels are clinically increased in several clinical conditions such as diabetes mellitus (DM), hypertension, insulin resistance syndrome, and dyslipidemia (5). In addition, increasing ADMA levels were associated with target organ damage including retinopathy, nephropathy, cardiac hypertrophy and cardiovascular events in patients with DM (6).

The aim of this study was to show a correlation between increasing ADMA levels and endothelium dysfunction, in a subject group consisting of type 1 DM patients without vasculopathy, followed at the Keçiören Training and Research Hospital Pediatric Endocrinology Polyclinic.

METHODS and MATERIALS

Thirty-one healthy children and 39 patients with a TIDM diagnosis, routinely followed by the Keçiören Training and Research Hospital Pediatric Endocrinology Outpatient Clinic between 2011 and 2013 were included in the study. The study was approved by the hospital's ethics committee. Informed consent was obtained from the patient families.

Patient files were retrospectively studied; clinical and laboratory findings for patient follow up were recorded.

Gender, age, weight, height, body mass index (BMI), systolic and diastolic blood pressure, anti thyroid peroxidase, celiac antibodies, fasting blood sugar values and diabetes duration were obtained from patient files. Additionally, Hemoglobin A1c (HbA1c) levels (for average yearly HbA1c calculations), existence of autoimmune diseases (celiac and autoimmune thyroid disease), 24 hour urine microalbumin measurement, and annual ophthalmologic examination for retinopathy were noted. Following the categorization of Type 1 DM patients as good, moderate and poor based on their HbA1c levels, the correlation with ADMA levels was investigated. Autoimmune diseases (celiac and autoimmune thyroid disease), cardiovascular disease, nephropathy, retinopathy or neuropathy were excluded from the study. Fasting blood samples were obtained between 8.00 and 10.00 am. Serum was separated by centrifuging for five minutes at 5000/min and plasma serums were stored at -80 °C. Total cholesterol, HDL cholesterol and triglyceride were determined using the enzymatic endpoint cholesterol oxidase peroxidase method, non-precipitating enzymatic colorimetric method, and enzymatic-colorimetric glycerol 3 phosphate method, respectively. LDL cholesterol was calculated using the Friedwald formula. Plasma homocysteine levels were determined with the chromatography-mass spectrometry method and folate concentrations were measured using the micro-particle enzyme technique. Determination of Serum ADMA levels was performed with an ELISA kit (immunodiagnostic AG, Stubenwaldallee 8a, D 64625 Bensheim).

Statistical Analyses

All data were analyzed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). The distribution of variables was analyzed using the Kolmogorov-Smirnov test. Normally distributed data are presented as means ± standard deviations. Data with abnormal distributions are expressed as medians (interquartile range), and dichotomous data are presented as percentages. The significance level of the differences between two groups was assessed using independent Student t tests for normally distributed variables and the non-parametric Mann Whitney U test for non-normally distributed variables. The differences between the categorical variables were determined using the chi-square test. Linear association between parametric variables was evaluated using the Pearson correlation test. Correlation analysis of non-parametric data was performed using the Spearman test. Stepwise multivariate logistic regression was analyzed to determine the factors associated with poor SQ (PSQI > 5). The level of statistical significance was accepted as $p<0.05$ for all tests.

RESULTS

A total of 70 children and adolescents consisting of 31 healthy subjects [control group: 15 female (48.4%); 16 male (51.6%)] and thirty-nine patients with Type 1 DM [patient group: 18 female (46.2%); 16 male (53.8%)] were included in the study. There were no significant differences between the Type 1 DM group and the control group with respect to age, gender, body mass index (BMI), and systolic or diastolic blood pressure (Table I).

Diabetes duration time for the Type 1 DM study group was 3.15±27.3 months (6-102 months). During the study, the average HbA1c was 9.4±2.1% (6.5-15.8%) and daily insulin requirement was 0.8±0.3 (0.2-1.3) U/kg/day. Serum creatinine was similar in both groups (p=0.485, Table II). Compared to the control group, cholesterol, triglyceride, HDL-cholesterol and LDL cholesterol levels were higher in the Type 1 DM group but the difference was not significant (p=0.287, 0.057, 0.329, and 0.723, respectively; Table I).

Average ADMA level in the Type 1 DM patient group (0.7±0.4 µmol/L) was significantly higher than the control group (0.6±0.1

μmol/L) (p=0.004). The homocysteine and folate levels for the two groups were similar and the differences were not significant (p=0.368 and 0.887, respectively) (Table I).

Even though average ADMA levels were higher in poorly managed Type 1 DM patients, the difference was not statistically significant (Table II). Comparison of gender and average ADMA levels between the Type 1 DM and control groups showed no significant difference (p>0.05)

No significant correlation between ADMA levels and age, BMI, systolic and diastolic blood pressure, serum homocysteine, cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol was found in the Type 1 DM and control groups (Table III). Type 1 DM group displayed no significant association between HbA1c and diabetes duration (Table III).

Compared to the Type 1 DM group with diabetes duration of less than 3 years, the average ADMA levels and daily insulin requirement (U/kg) were significantly higher in the Type 1 DM group with diabetes duration of 3 or more years (p=0.008 and p=0.029, respectively).

BMI, systolic and diastolic blood pressures, fasting blood sugar, creatinine, cholesterol, triglyceride, HDL-cholesterol, LDL cholesterol, HbA1c, Homocysteine and Folate levels were not significantly different between Type 1 DM groups with diabetes duration of less than 3 years or 3 years or more (Table IV).

DISCUSSION

Endothelium dysfunction is the earliest symptom of vascular complications in diabetes mellitus and the underlying causes are not clearly understood. In type 1 DM, endothelium dysfunction associated with the changes in nitric oxide system (NOS) pathway plays a major role. Chan et al. have shown deteriorated NO secretion in a patient group consisting mostly of subjects with uncomplicated type 1 DM (7). A study by Correa et al. has shown reduced NO metabolite levels proving early endothelium dysfunction in type 1 DM patient group with no microvascular complications (8).

In the study conducted by Altınova et al. on 40 Type 1 DM patients without vascular complications and 35 healthy subjects between the ages 19 and 45, plasma ADMA and L-arginine levels were higher in the diabetic group (9). In a study on 408 patients with diabetic nephropathy as the patient

Table I: Demographic and laboratory characteristics of Type 1 DM and Control Groups.

	Type 1 DM	Control	p
Age (mo)	163.3±29.5	163.8±31.8	0.947
Gender (F/M)	18 / 21	15 / 16	1.000
BMI (kg/m²)	19.7±3.3	19.6±2.1	0.922
Systolic Blood Pressure (mmHg)	106.8±7.5	107.7±8.8	0.629
Diastolic Blood Pressure (mmHg)	68.2±6.8	71.3±5	0.485
Fasting blood sugar (mg/dl)	154.3±43	88.2±6.1	0.001
Creatinine (mg/dl)	0.6±0.1	0.6±0.2	0.485
Cholesterol (mg/dl)	159.4±38.4	151.2±27.8	0.287
Triglyceride (mg/dl)	100.3±61.7	72.8±33.2	0.057
HDL-cholesterol (mg/dl)	50±13.7	47.5±11.3	0.329
LDL-cholesterol (mg/dl)	91±31.8	87.8±24.5	0.723
ADMA μmol/L	0.7±0.4	0.6±0.1	0.004
Homocysteine (mg/dl)	9.2±3.1	9.8±2.5	0.368
Folate (ng/ml)	9.2±3.3	9.8±4	0.887

Table II: The correlation between HbA1c levels and ADMA in Type 1 DM patient group.

		ADMA					Kruskal Wallis H Test		
		n	Mean	Minimum	Maximum	SD	Mean Rank	H	p
HbA1c	6-7.9 (good)	10	0.6	0.4	0.8	0.1	12.7	5.712	0.057
	8-9.9 (moderate)	20	0.7	0.5	1.2	0.2	23.2		
	10 and above (poor)	9	1.0	0.5	2.3	0.7	21.1		

Table III: Comparison of variables with ADMA level in Type 1 DM and Control groups was performed with the Pearson correlation analysis.

	Type 1 DM ADMA µmol/L	Control ADMA µmol/L
Age	r= -0.150 p=0.362	r= -0.275 p=0.134
BMI	r= -0.107 p=0.519	r= -0.078 p=0.675
Systolic BP	r= 0.090 p=0.535	r= -0.158 p=0.395
Diastolic BP	r= 0.085 p=0.606	r= -0.390 p=0.030
Diabetes Duration	r= 0.236 p=0.147	-
HbA1c	r= 0.228 p=0.162	-
Homocysteine	r= -0.022 p=0.178	r= -0,022 p=0.908
Cholesterol	r= 0.037 p=0.821	r= 0. 216 p=0.244
Triglyceride	r= 0.276 p=0.089	r= -0.143 p=0.443
HDL-cholesterol	r= -0.060 p=0.716	r= -0.074 p=0.694
LDL-cholesterol	r= -0.026 p=0.876	r= 0. 217 p=0.242

group and 192 normoalbuminuric type 1 DM subjects as the control group, Tarrow et al. found that plasma ADMA levels in nephropathy patients were significantly higher compared to the control group (10).

In a study conducted by Jehlicka et al. 32 familial hypercholesterolemia patients, 30 type 1 DM patients with a diabetes duration of 4.4±2.1 years and 30 subjects as the control group, with an average age of 14 years were enrolled. Compared to the other two groups, ADMA levels were found to be significantly higher in the familial hypercholesterolemia group. In the type 1 DM group, however, ADMA levels were higher compared to the control group but were not statistically significant (11). When Glowinska- Olszewska B. et al. compared 72 Type 1 DM patients with a diabetes duration between 1 and 14 years with a control group consisting of 41 subjects, no significant difference in serum average ADMA levels were detected (12). In our study, the ADMA levels in patients with 3 or more years of diabetes duration were higher compared to patients with diabetes durations of less than 3 years. This finding supports our view that ADMA, an early symptom of endothelium damage, could increase in children with type 1 DM, or in other words, Type 1 DM may be a risk factor for the development of early vascular damage in childhood before clinical vasculopathy develops

In the study conducted by Altınova et al. in Turkey, even though a significant correlation between ADMA levels and fasting blood sugar was found in 40 patients with Type 1 DM, no association was detected between HbA1c levels and ADMA (9). In a study by Tarnow et al. on Type 1 DM, a correlation between fasting blood sugar and HbA1c was not found. Similarly, in our study

Table IV: Comparison of variables in Type 1 DM patients with diabetes durations of less than 3 years and 3 years or more.

	Diabetes Duration		p
	Less than 3 years	3 years or more	
BMI	19.7±3.6	19.7±3	0.846
Systolic Blood Pressure	106.7±7.1	106.9±8.5	0.934
Diastolic Blood Pressure	68.1±6.3	68.5±8	0.948
Fasting Blood Sugar	151.5±39.4	159.7±50.6	0.777
Creatinine	0.6±0.1	0.6±0.1	0.276
Insulin U/ Kg	0.7±0.3	0.9±0.2	0.008
Cholesterol	161.4±38	155.5±40.5	1.000
Triglyceride	93.9±45.5	113.1±86.5	1.000
HDL- cholesterol	49±11.4	52±17.9	0.881
LDL-cholesterol	92.8±34.3	87.5±27.2	0.777
HbA1c	9±1.6	10±2.9	0.612
Homocysteine	9.8±3.3	8±2.3	0.084
Folate	8.7±3	10.3±3.8	0.180
ADMA	0.6±0.1	0.9±0.6	0.029

on Type 1 DM patients, an association between fasting blood sugar, HbA1C and serum average ADMA levels was not found (10). Classifying the Type 1 DM patients as good, moderate and poorly managed based on average HbA1c values showed that while serum average ADMA levels in the poorly managed group were markedly higher compared to the other two groups, the difference was not statistically significant.

Dyslipidemia prevalence in young diabetes patients was investigated in two large studies: The SEARCH for Diabetes in Youth Study and DPV (German prospective documentation and quality management system) (13,16). The results of these studies show presence of dyslipidemia with high prevalence in young diabetics and is correlated with high HbA1c and cardiovascular system risk factors such as BMI > 90 percentile based on gender and age. In a study conducted by Southern Reh CM. et al., 46 juveniles aged between 12 and 25, were followed for a minimum of 3 years (average 4.2 years) (17). At the onset, 50% of the patients had elevated LDL, and at the end of the follow up this value was determined as 58%. Glycemic management was reported to play a major role in lipid values.t. Similarly, a positive correlation between HbA1c and triglyceride and a non significant negative correlation with HDL was detected. However, we did not find a significant correlation between age, diabetes duration and lipid profile.

There are studies showing a correlation between hypercholesterolemia and ADMA (9,20,21). Huemer et al. detected a positive association between ADMA and HDL in type 1 DM patients (20). In a study on conditions associated with arterial wall thickening, a sign of subclinical atherosclerosis in children, wall thickness was shown to correlate positively with ADMA level and blood pressure, and negatively with HDL cholesterol (20). In the study conducted by Heilman et al., no correlation between ADMA and lipid profile was found. In our study, a correlation between ADMA level and lipid profile was not detected (21) .

In several studies, different correlations between ADMA levels and systolic and diastolic blood pressures have been found (10,20). Tarnow et al. found a positive correlation between ADMA and systolic blood pressure (10). However, in the study on 85 pediatric Type 1 DM patients by Huemer et al., a negative correlation was detected (20). In our study, while no correlation between ADMA and systolic and diastolic blood pressures was detected, a negative correlation with diastolic blood pressure was found in the control group.

In the study conducted by Gruber et al. on 68 pre-atherosclerotic obese juvenile and 68 healthy control subjects, elevated levels of ADMA was detected in the obese group but no correlation between ADMA and obesity related diseases was reported (23). Obesity is thought to increase oxidative stress and leads to free radical associated decrease in nitric oxide levels. Reacting with nitric oxide, the superoxide created in vascular wall may inhibit NO-related endothelial function by producing peroxynitrite, a cytotoxic compound (23,24). In our study we did not find a correlation between ADMA and BMI.

Huemeret et al. investigated 85 type 1 DM patients and 89 healthy subjects as the control group aged 2 to 19 years. ADMA levels in the type 1 DM group were determined to be lower compared to the control group. In the study, higher ADMA levels were detected in younger children and it was noted that elevated ADMA levels in healthy children were not harmful and this was more likely to be a physiological condition to reduce NO production to manage oxidative stress due to excessive NO and peroxynitrite synthesis, and therefore decreased ADMA levels in the type 1 DM group may indicate insufficient defense mechanisms against oxidative stress (20). Decrease in ADMA levels during childhood and adolescence have not been clearly explained but changes, especially in protein turnover associated with somatic growth during puberty, may lead to this condition (25). Studies exhibiting increased ADMA levels with age are also available in literature (26-27). ADMA concentrations were measured in 157 healthy adults and were correlated with their ages. The increased ADMA with age may possibly be explained with increased protein turnover showing decreased sensitivity to insulin (28). Carmann C. et al. investigated 102 type 1 DM and 95 healthy groups and there was no difference n plasma homoarginine, ADMA and the homoarginine/ADMA molar ratio between type 1 DM and healthy control groups (29). In our study, a correlation between age and ADMA was not detected in type 1 DM patients or the control group.

In the literature, varying results have been reported for studies on Type 1 DM and homocysteine correlation (30-32). In the study on 78 patients with an average age of 13.7±2.6 years and 59 control subjects with an average age of 13.4±2.5 years by Wiltshire et al., homocysteine levels were significantly lower in the Type 1 DM group compared to the control group; B_{12} and folate levels were significantly higher in the Type 1 DM group; and it was reported that the difference in homocysteine levels was essentially due to the differences in the B_{12} and folate levels (30). The comparative study conducted by Atabek et al. on 27 children and adolescents with type 1 DM and no complications and 27 control subjects, the measured homocysteine levels in the diabetes group were within normal range (32). Compared to the control group, folate and B_{12} levels were significantly higher and in the diabetes group homocysteine was positively correlated with age, weight, BMI, folate and creatinine levels. In our study homocysteine levels in the patient and control groups were similar. We, also, did not detect a difference in folate levels between the patient and control groups in our study. However, we found a negative correlation between the homocysteine level and folate in patient group. One factor decreasing the homocysteine levels in Type 1 DM group is the effect of insulin on homocysteine metabolism. Insulin stimulates cellular uptake of amino acids, including methionine and homocysteine. Methylenetetrahydrofolate reductase (MTHFR) is regulated by methionine synthase and cystathionine β synthase S-

adenosyl–methionine (33). Increased cellular uptake of methionine results in increased homocysteine transsulphuration and decreased levels. It is possible for insulin to have a direct effect on the activation of these enzymes (29). The fact that we detected a negative correlation between the insulin dose and homocysteine in our study supports the effect of insulin. Even though low homocysteine levels were measured in our study, homocysteine was positively correlated with systolic and diastolic blood pressures, risk factors for cerebrovascular diseases.

In hyperhomocysteinaemia, another mechanism for increasing ADMA is reduced catabolism of ADMA by dimethylarginine (DDA) (34). In their study supporting this mechanism, Cooke et al. observed that homocysteine leads to elevated ADMA levels by inhibiting the dimethylaminohydrolase (DDAH) activity in endothelial cell cultures (35). In our study, a correlation between ADMA levels and homocysteine compared to the control group was not detected.

In conclusion, larger studies are needed to show the relation between endothelium damage and ADMA. Confirmation of this correlation may lead to follow up ADMA measurement values being used as a premarker for vascular damage in type 1 diabetes, and in turn helping to prevent vascular complications.

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