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Is There a Correlation Between Plasma Levels of Asymmetric Dimethylarginine (ADMA) Levels and Atherosclerosis in Type 2 Diabetes Patients in Turkey?

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ABSTRACT:

Is there a correlation between plasma levels of asymmetric dimethylarginine (ADMA) levels and atherosclerosis in type 2 diabetes patients in Turkey?

Objective: Atherosclerosis is very common in patients with diabetes mellitus (DM). We aimed to investigate the relationship between asymmetric dimethylarginine (ADMA) which is known as 'an endogenous inhibitor of nitric oxide synthase' and atherosclerosis among patients with a diagnosis of type 2 DM.

Material and Methods: A total of 85 patients with a diagnosis of type 2 DM (48.2% [n: 41] male) with a mean age of 55.73±8.78 years were enrolled in this trial. Plasma levels of ADMA, and laboratory parameters such as lipid profile and HbA1c were evaluated. Carotid intima-media thickness (IMT), a marker of atherosclerosis was measured. The patients were classified into two groups as cases with high and low ADMA levels.

Results: No statistically significant correlation was found between serum ADMA levels and total cholesterol (r=0.045; p=0.684), triglyceride (r=-0.067; p=0.544), LDL cholesterol (r=0.142; p=0.194) and HDL cholesterol (r=0.085; p=0.085). A statistically significant correlation was determined between serum ADMA level and HbA1c (r=0.376; p=0.001) and between serum ADMA level and carotid-intima media thickness (r=0.321; p=0.003).

Conclusions: Serum ADMA level is correlated with carotid IMT. Evaluation of ADMA levels in type 2 DM patients may be helpful in predicting atherosclerotic diseases such as coronary artery disease.

Keywords: Asymmetric dimethylarginine, atherosclerosis, type 2 diabetes mellitus

ÖZET:

Türkiye'deki tip 2 diyabet hastalarında serum asimetrik dimetilarjinin düzeyleri ile ateroskleroz arasında bir korelasyon var mıdır?

Amaç: Ateroskleroz diabetes mellitus (DM) hastalarında çok yaygındır. Biz tip 2 DM hastalarında 'endojen nitrik oksit sentaz inhibitörü' olarak bilinen asimetrik dimetil arjinin (ADMA) ile ateroskleroz arasında bir ilişki olup olmadığını araştırdık.

Gereç ve Yöntemler: Tip 2 DM tanısı alan, yaş ortalaması 55.73±8.78 yıl olan toplam 85 hasta (%48.2 [n:41] erkek) çalışmaya dahil edildi. ADMA plazma seviyeleri, lipid profili, HbA1c gibi laboratuvar parametreleri değerlendirildi. Aterosklerozun bir göstergesi olan karotis intima-media kalınlığı (IMT) ölçüldü. Hastalar, yüksek ve düşük ADMA düzeyleri olarak iki gruba ayrıldı.

Bulgular: İstatistiksel olarak serum ADMA düzeyleri ile total kolesterol (r=0.045; p=0.684), trigliserid (r=-0.067; p=0.544), LDL kolesterol (r=0.142; p=0.194) ve HDL kolesterol (r=0.085; p=0.085) arasında anlamlı ilişki saptanmadı. ADMA düzeyi ile HbA1c arasında (r=0.376; p=0.001) ve serum ADMA düzeyi ve karotis-intima media kalınlığı arasında (r=0.321; p=0.003) istatistiksel olarak anlamlı bir ilişki saptandı.

Sonuç: Serum ADMA düzeyi karotis IMT ile ilişkilidir. Tip 2 DM hastalarında ADMA düzeylerinin değerlendirilmesi koroner arter hastalığı gibi aterosklerotik hastalıkları öngörmede yararlı olabilir.

Anahtar kelimeler: Asimetrik dimetil arjinin, ateroskleroz, tip 2 diyabet

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INTRODUCTION

Prevalence of diabetes mellitus (DM), which is accepted worldwide as equivalent to coronary artery disease (CAD), is gradually increasing (1). The prevalence rates of premature and subclinical cardiovascular disease among diabetic cases is reported as ≥70%, regardless of blood glucose levels of patients (2).

Endothelial dysfunction was indicated as a major cardiovascular risk factor in various trials (3). Endothelial dysfunction is defined as a decrease in bioavailability of nitric oxide (NO) which inhibits the adhesion and aggregation of platelets, vascular smooth muscle cell proliferation and low density lipoprotein (LDL) oxidation, and adhesion of monocytes and leukocytes to the endothelium (4). Asymmetric dimethylarginine (ADMA) is essentially a competitive inhibitor of endothelial nitric oxide synthase (eNOS). ADMA regulates the production rate of NO. Plasma ADMA concentration has been shown to increase during the course of diseases associated with endothelial dysfunction such as diabetes mellitus, peripheral artery disease, hypertension and cardiovascular diseases (5-9).

Carotid artery intima-media thickness (IMT) is an indicator of generalized atherosclerosis in adults. It's correlated with severity of CAD and is a significant indicator of cardiovascular disease, primarily of early atherosclerosis (10-12).

In DM patients, CAD usually presents with a subclinical course, as compared to non-diabetic cases (13) and is associated with a high incidence of mortality (14). Hence, early diagnosis is of utmost importance. Although a significant correlation was reported between serum ADMA level and carotid IMT in various studies (12,13), some studies didn't find a correlation between plasma ADMA levels and coronary endothelial function, and cardiovascular diseases (15,16). In previous studies, association of ADMA with diabetes mellitus has also remained controversial and not clear. These data are unsatisfactory and new trials conducted in different countries and patients groups are required to confirm the results. There is no study in our country in this direction. The purpose of this trial is to investigate the relationship between serum ADMA levels and carotid IMT, lipid parameters and HbA1c in type 2 DM patients.

MATERIAL AND METHODS

Enrollment of patients: A total of 85 patients with type 2 DM (41 male, 44 female), previously diagnosed and currently treated in Diabetes Outpatient Clinic were enrolled in this study. Patients with blood pressure >140/90 mm Hg or being treated with antihypertensive agents were regarded as hypertension positive. Exclusion criteria were as follows: ischemic heart disease, history of treatment with lipid lowering agents, triglyceride level >400 mg/dl, history of familial dyslipidemia, history of nephropathy (urea, elevated creatinine levels, microalbuminuria), thyroid dysfunction and alcohol consumption. Demographic data and anthropometric measurements of patients were recorded. Based on height and weight, body mass index (BMI) [weight (kg)/ height^2(m^2)] was calculated for each patient.

Measurement of ADMA levels with biochemical laboratory tests: Blood samples were obtained in the morning after a fasting period of at least 12 hours. Routine examinations were performed in Clinical Biochemical Laboratory and Clinical Microbiology Laboratory. Fasting blood glucose, total cholesterol, triglyceride, HDL-cholesterol and hemoglobin A1c levels were determined in Aeroset 2.0 analyzer (Abbott Laboratories). LDL-cholesterol levels were calculated by Friedewald formula (total cholesterol – [HDL cholesterol + (triglyceride /5)]).

In measurement of plasma ADMA levels; blood was placed in 6 mL vacuum tubes with EDTA (Becton Dickonson, lot no: 8169826) to be centrifuged within 1 hour (2.500 gr 10 minutes); the resultant plasma samples were kept at -80°C. In the ELISA method (Immundiagnostik AG, Germany), tetramethylbenzidine (TMB) was used as a substrate for peroxidase enzyme, after competition with polyclonal ADMA-antiserum. Plasma ADMA levels were determined as inversely proportional to absorbance values obtained at 450 nm wavelength (17,18).

Ultrasonographic determination of carotid IMT: To provide standardization in carotid IMT measurements, evaluations were performed in Department of Radiology with LOGIQ-7 GE Ultrasound device by the same physician, who was blinded to patient data. The patient was kept at supine position during the examination. Neck was kept at mild extension, head facing the opposite side being examined. Gray scale examination was performed with 10 MHz probes. Gray scale examination was initiated at transverse projection. Procedure was performed by covering the whole area of cervical artery, starting with bilateral supraclavicular indentation to mandibular angle. In the current trial, measurements were performed in both carotid arteries. IMT was defined as the area from the margin of lumen-intima to margin of media and adventitia. Measurements in B-mode US examination of extracranial vascular structures were performed in plaque-free areas. In bilateral carotid arteries, IMT was measured in the thickest region. Mean values of measurements were calculated and recorded.

Ethical Aspect: Informed consent was obtained from each patient prior to initiation of the trial. Ethical approval of this trial was granted by local ethical committee of our hospital.

Statistical Analysis

SPSS (statistical package for social sciences, for Windows, release 12.0.0 standard version) software was used for statistical evaluations. Scale variability was expressed as mean±standard deviation (mean±SD). A p value <0.05 was considered to be statistically significant. Student's test, Mann Whitney u test and chi-square test were used in comparison of variables. Pearson's method was utilized in correlation analysis. p<0.05 was accepted as significant.

RESULTS

Among all enrolled patients, 48.2% (n=41) were male and 51.8% (n=44) were female. Hypertensive patients constituted 50.6% (n=43) of all cases.

Table-1: General characteristics of all patients

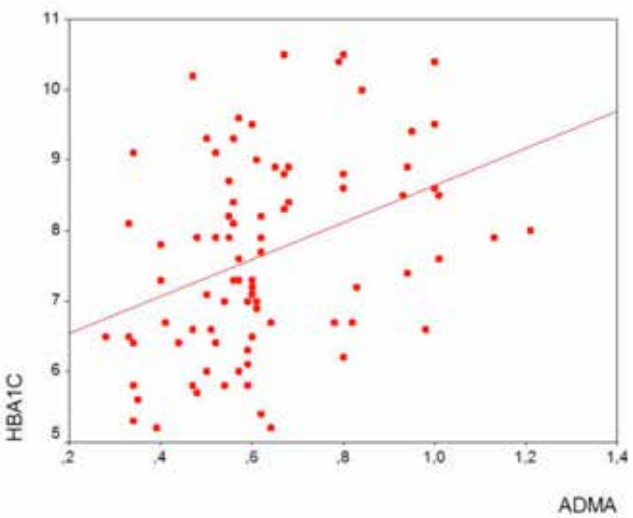
ADMA (μmol/L)		0.6288±0.2000
Gender (n,%)	Women	41 (48.2)
	Men	44 (51.8)
Age, years		55.73±8.78
BMI (kg/m^2)		29.09±4.02
DM duration, years		6.52 ±4.68
Treatment (n,%)	OAD	58 (68.2)
	İnsulin	27 (31.8)
HT (n,%)	No	41 (49.4)
	Yes	43 (50.6)
Smoking (n,%)	No	36 (42.3)
	Yes	49 (57.7)
HbA1c		7.670±1.395
Total cholesterol (mg/dL)		208.60±30.45
Triglyceride (mg/dL)		187.46±79.14
LDL (mg/dL)		131.48±26.02
HDL (mg/dL)		40.16±7.46
TSH (mU/L)		1.98±1.09
IMT (mm)		0.934±0.200

ADMA: Asymmetric dimethyl arginine, BMI: Body mass index,
HT: Hypertension, OAD: Oral antidiabetic DM: Diabetes Mellitus, FT4: Free T4
HbA1c: Hemoglobin A1c, HDL: High density lipoprotein
IMT: Intima-media thickness, LDL: Low density lipoprotein
TG: Triglyceride, TSH: Thyroid stimulating hormone

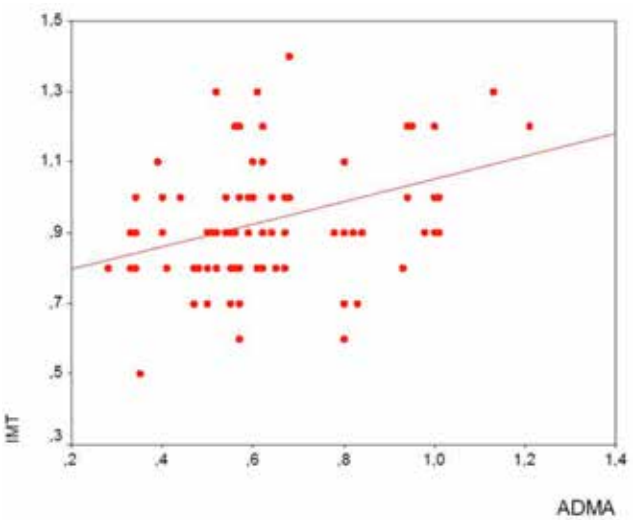
Among all cases, 68.2% (n=58) were using oral antidiabetic agents and 31.8% (n=27) were treated with insulin. A total of 57.7% (n=49) of all patients were smokers. Mean age of patients was 55.73±8.78 and other parameters were shown in Table-1.

Correlation of serum ADMA levels with laboratory and clinical data was evaluated statistically. No statistically significant correlation was determined between serum ADMA levels and total cholesterol (TC) (r=0.045; p=0.684), triglyceride (TG) (r=-0.067; p=0.544), LDL cholesterol (r=0.142; p=0.194), HDL cholesterol (r=0.085; p=0.085), TSH (r=-0.046; p=0.674), free T4 (r=-0.19; p=0.864), creatinine (r=0.052; p=0.639) and urea (r=0.046; p=0.674). A statistically significant correlation was found between serum ADMA levels and HbA1c levels (r=0.376; p<0.001) (Graph-1). A statistically significant correlation was determined between serum ADMA levels and carotid intima-media thickness (r=0.321; p=0.003) (Graph-2).

According to results of the trial defined in kit insert (n= 70), expected values for plasma levels of ADMA in healthy individuals were indicated as 0.26 - 0.64 μmol/L. In trials conducted by HPLC (High Performance Liquid Chromatography) and LC-MS



Graph-1: The correlation between ADMA level and Hb1c
Linear positive correlation between serum ADMA level and HbA1c observed in regression analysis



Graph-2: The correlation between ADMA level and carotid intima-media thickness
Positive statistical correlation between serum ADMA level and carotid intima-media thickness observed in regression analysis

(Liquid Chromatography – Mass Spectrophotometry), normal range of ADMA was expressed as 0.225-0.485 µmol/L (14,15). We classified our patients in two groups, as patients with ADMA levels below 0.6 µmol/L and over 0.6 µmol/L and assessments were performed in these two groups. Comparison of general demographic characteristics of cases with serum ADMA levels below and over 0.6 µmol/L is shown in Table-2. No significant difference was found between cases with ADMA levels below and over 0.6 µmol/L in terms of gender distribution, treatment modality, smoking, hypertension, BMI, age and duration of DM (p>0.05).

Comparison of laboratory and radiological data of cases with ADMA levels below and over 0.6 µmol/L is shown in Table-3. No significant difference was determined between patients with ADMA levels below and over 0.6 µmol/L in terms of TC, TG, LDL,

Table-2: Comparison of general demographic characteristics of cases with serum ADMA levels below and over 0.6 µmol/L

		ADMA levels		p values
		< 0.6 µmol/L (n=43)	≥ 0.6 µmol/L (n=42)	
Gender (n,%)	Women	23 (53.5)	21 (50)	0.748
	Men	20 (46.5)	21 (50)	
Treatment (n,%)	OAD	32 (74.4)	26 (61.9)	0.215
	Insulin	11 (25.6)	16 (38.1)	
Smoking (n,%)	No	27 (62.8)	22 (52.4)	0.180
	Yes	16 (37.2)	20 (47.6)	
HT (n,%)	No	21 (48.8)	22 (52.4)	0.744
	Yes	22 (51.2)	20 (47.6)	
Weight (kg)		81.44±12.48	78.76±11.61	0.309
Height (cm)		163.11±26.39	164.93±9.31	0.675
BMI (kg/m^2)		29.09±4.02	28.97±3.91	0.887
Age, years		55.74±8.55	55.71±9.50	0.988
DM duration, years		5.86±4.51	7.19±4.81	0.192

ADMA: Asymmetric dimethyl arginine, BMI: Body mass index, HT: Hypertension, OAD: Oral antidiabetic

Table-3: Comparison of laboratory and radiological data of cases with ADMA levels below and over 0.6 µmol/L (mean±SD)

	ADMA levels		p values
	< 0.6 µmol/L (n=43)	≥ 0.6 µmol/L (n=42)	
Total Chol (mg/dl)	207.74±26.73	209.50±34.14	0.792
TG (mg/dl)	188.67±68.02	186.21±89.94	0.887
LDL (mg/dl)	128.19±23.05	134.86±28.63	0.240
HDL (mg/dl)	41.63±7.59	38.67±7.10	0.067
TSH (mU/L)	2.14±1.24	1.83±0.90	0.204
FT4 (ng/dL)	1.28±0.36	1.25±0.16	0.597
Urea (mg/dl)	29.95±9.90	30.24±4.95	0.866
Creatinine (mg/dl)	0.87±0.15	0.82±0.18	0.103
HbA1c (%)	7.24±1.33	8.11±1.34	0.003
IMT (mm)	0.87±0.15	1.00±0.22	0.003

BMI: Body mass index, DM: Diabetes Mellitus, FT4: Free T4, HbA1c: Hemoglobin A1c, HDL: High density lipoprotein, IMT: Intima-media thickness, LDL: Low density lipoprotein, TG: Triglyceride, TSH: Thyroid stimulating hormone

TSH, FT4, urea and creatinine levels (p>0.05). HbA1c levels of cases with ADMA values over 0.6 µmol/L were significantly higher than cases with levels below 0.6 µmol/L (p<0.01). Intima media thickness values of patients with ADMA levels > 0.6 µmol/L were significantly higher, as compared to cases with levels below 0.6 µmol/L (p<0.01).

Plasma ADMA levels (µmol/L) were determined as 0.6456±0.2137 in male patients (n=41) and 0.6131±0.1877 in female paients (n=44), with no statistically significant difference between the groups (p=0.460). No statistically significant difference was found between smokers and non-smokers (p=0.920) and between hypertensive and normotensive patients (p=0.830).

DISCUSSION

In the present study, we investigated whether serum ADMA levels had any association with markers of atherosclerosis in type 2 diabetic patients. Our study found a positive correlation between ADMA levels and carotid IMT and HbA1c in type 2 DM patients. Serum ADMA concentrations did not correlate with BMI, lipid parameters, gender, duration of diabetes and age or other risk factors of atherosclerosis.

As one of the most common diseases in our country and around the world, significance of type 2 diabetes mellitus is substantially increasing day by day. Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in patients with type 2 diabetes (19). The main purpose of trials related to this issue is to determine the risk of CVD prior to development of the disease and to prevent actual manifestation of CVD in patients with diabetes. For this purpose, studies primarily concentrated on ADMA, a molecule which inhibits nitric oxide synthase responsible for synthesis of nitric oxide from arginine, a substance indispensable in maintaining normal endothelial function (20,21).

In the current trial conducted in patients with type 2 DM, we determined a significant correlation between ADMA and carotid IMT. In previous trials, carotid IMT was shown to be correlated with severity of CAD and was specified as a significant indicator of early atherosclerosis (10,11). Miyazaki et al. (22) showed that a significant correlation exists between carotid IMT and ADMA. Kocak et al. (23) determined a strong correlation between carotid IMT and ADMA levels in peritoneal dialysis patients. Ari Hasan et al. (24) conducted a trial on 104 patients and reported that ADMA levels have a predictive value in terms of CVD, irrespective of diabetes, hypertension, LDL and total cholesterol. In Athero Gene study, a prospective and large scale trial, high ADMA levels were stated as a strong indicator of cardiovascular risk, independent from conventional risk factors (25). In another trial, risk of development of CVD was reported to increase approximately 2.35 times, as per each 1 µmol/l increase in ADMA levels (26). In a

separate trial, high ADMA levels were specified as a strong and independent indicator for fatal and non-fatal myocardial infarction and for all causes of cardiovascular mortality after acute coronary syndrome (27). On the other hand, in another trial, the results showed that the cases with a high level of ADMA could have cardiovascular complications of diabetes mellitus in the future within five years (28). Results of recent trials conducted on DM patients suggested that high ADMA levels may have a predictive value in cardiovascular diseases (29-31). Based on our current trial and literature data, we may conclude that serum ADMA level has a predictive value for CAD in type 2 DM patients.

HbA1c, which indicates the mean blood glucose levels during the previous 2-3 months, was recently adopted as a novel diagnostic criterion of diabetes (32). In the current trial, we found a positive correlation between HbA1c and serum ADMA levels. Eliana et al. conducted a trial on prediabetic women and determined a correlation between ADMA and HbA1c (33). Hsu et al. (34) found no significant correlation between plasma ADMA level and HbA1c level. On the other hand, Marcovecchio et al. (35) reported an inverse correlation between HbA1c and ADMA in type 1 DM patients. Yasuda et al. (36) showed that in hospitalized type 2 DM patients, intensive glucose lowering significantly decreased ADMA levels, compared to standard treatment. In conclusion, correlation between ADMA and HbA1c is not as prominent as the correlation between carotid IMT and ADMA.

In the current trial, we were unable to find a correlation between smoking and ADMA. Contradictions regarding this issue is available in literature. While Zhang WZ et al. (37) found higher ADMA levels in smokers in their trial, opposite results were reported in another trial conducted on 563 elderly male patients (38). In their case-control study, Maas et al. (39) observed that high ADMA levels are significant in prediction of coronary artery disease among non-smokers while results in smokers were reported to be insignificant. This finding was suggested to be related to an ADMA-metabolizing component in cigarette smoke.

In the current trial, we were unable to find a significant correlation between ADMA levels and variables of HT, BMI, lipid panel, gender, duration of diabetes and age. Xia et al. (40) conducted a trial on 72 type 2 diabetes patients in 2012 on correlation of systemic endothelial dysfunction and atherosclerosis with ADMA and came up with the following results; a strong correlation was determined between plasma ADMA level and carotid intima-media thickness (IMT) while no significant correlation was found with diabetic age, HbA1c, lipid levels, hypertension and creatinine clearance. Meinitzer et al. (20) were unable to detect a statistically significant correlation between ADMA and HT, BMI and LDL cholesterol. A significant correlation was found between ADMA and triglyceride and HDL cholesterol levels. Markus Juonala et al. (41) conducted a trial on 2096 young Finnish patients and found significantly lower levels of HDL, triglycerides and isolated systolic pressure in the group with higher ADMA levels, compared to group with lower ADMA values. No significant correlation was determined between LDL and total cholesterol. In a trial conducted by Altınova et al. (44) on patients with type 1 diabetes, elevated ADMA levels and correlation of elevated ADMA levels with BMI, fasting blood glucose, LDL cholesterol (positive correlation) and with HDL cholesterol (negative correlation) were reported. In study of Onat et al. (43) from Turkey, no significant difference was observed between female and male patient groups in terms of plasma ADMA concentrations. Similarly, no significant difference was found between plasma ADMA levels and mean age of total population. Results of the same trial revealed a positive correlation between plasma ADMA levels and HDL cholesterol, LDL cholesterol, total cholesterol. No significant correlation was observed between smokers and non-smokers. Contradictory data was obtained in various trials regarding the correlation of ADMA with HT, BMI, lipid parameters, gender, duration of diabetes and age.

In the current trial conducted in type 2 DM patients, we found a positive correlation of ADMA levels with carotid IMT and HbA1c. No significant correlation was determined with BMI, lipid parameters, gender, duration of diabetes and age. Upon evaluation of previous trials conducted on

ADMA levels, we can say that there is a relationship between ADMA level and atherosclerosis in type 2 DM patients.

Study limitations

Our study has some limitations. In the study, only carotid IMT for atherosclerosis was evaluated. It could have assessed other parameters of atherosclerosis. Monitoring of the patients could be continued after the study, with a higher number of patients. In addition, there was no control group.

Conflict-of-interest issues regarding the authorship or article: None declared.

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