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Research Article

The Evaluation of the Relationship Between Albuminuria and Serum Asymmetric Dimethyl Arginine Level in Type-2 Diabetes Mellitus

Aysegul Sakin,[1] Mehmet Selim Aslan,[2] Ahmet Behlul,[2] Meltem Gursu,[3] Namik Yigit,[2] Egemen Cebeci,[2] Savas Ozturk[2]

[1]Department of Internal Medicine, University of Health Sciences, Van Training and Research Hospital, Van, Turkey
[2]Department of Nephrology, University of Health Sciences, Haseki Training and Research Hospital, Van, Turkey
[3]Department of Nephrology, Bezmialem University Faculty of Medicine, Istanbul, Turkey

Abstract

Objectives: Asymmetrical dimethyl arginine (ADMA) inhibits nitric oxide synthase causing a decline in nitric oxide levels and so endothelial dysfunction. In our study, we analyzed the relationship between albuminuria and ADMA level in type-2 diabetes mellitus (DM).

Methods: This is a cross sectional study in which 55 type 2 diabetic patients followed up in diabetes outpatient clinic. Patients were divided into three groups according to albuminuria: normoalbuminuric (Group-1), microalbumiuric (Group-2) and macroalbuminuric (Group-3) patients. ADMA level was measured by ELISA method.

Results: A total of 36 (65.45%) patients were female. The median age of patients were 59.9 years. There was no statistically significant difference between groups regarding age, duration of DM, gender, LDL cholesterol and body mass index. The median ADMA level was lower in Group-1 compared to Group-2 and Group-3. ADMA level was significantly correlated with albuminuria. Albuminuria was the only parameter related with ADMA in linear regression analysis.

Conclusion: ADMA level is significantly increased even in microalbumiuric stage among patients with type 2 DM. This may show the increased risk of progressive kidney disease in these patients.

Keywords: Albuminuria, asymmetrical dimethyl arginine, diabetes mellitus, renal disease

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Diabetes mellitus (DM), with its microvascular and macrovascular complications is a major public health problem as well as increasing the health expenditures1. Diabetic nephropathy (DNP) is the leading cause of end stage renal disease and it is the second leading cause of death in patients with DM following cardiovascular causes.[1–3] Microalbuminuria in DM is an important marker for cardiovascular disease (CVD) as well as being the early stage of DNP.[4,5]

Importance of endothelial dysfunction (ED), defined as dysfunction of vascular endothelium to vasodilate, for microvascular and macrovascular complications in patients with DM is clearly known. The cause for ED is thought to be primarily due to low levels of nitric oxide (NO). NO is synthesized in vascular endothelium from L-arginine by the enzyme nitric oxide synthase (NOS).[6]

Asymmetrical dimethyl arginine (ADMA) inhibits NOS causing a decline in NO levels and so ED.[6,7] Although there are

Address for correspondence: Aysegul Sakin, MD. Saglik Bilimleri Universitesi, Van Egitim ve Arastirma Hastanesi, İc Hastaliklari Klinigi, 65030, Van, Turkey
Phone: +90 432 212 19 54 **E-mail:** Mdaysegulsakin@gmail.com

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different results in the literature, previous studies reported elevated ADMA levels in type 2 diabetic patients which was related with both microvascular and macrovascular complications. Krzyzanowska et al.[8] stated that ADMA levels were elevated in type 2 diabetics, while Pavia et al.[9] reported decreased ADMA level that was associated with increased glomerular filtration rate and bad glycemic control.[8–10]

Herein, we aimed to determine the relationship between proteinuria grade and blood ADMA level in type 2 DM patients.

Methods

Patients Data

Type 2 MD patients followed up in nephrology and diabetes outpatient clinics of Haseki Training and Research hospital were screened for our study. American Diabetes Association's (ADA) criteria were used for the diagnosis of type 2 DM.[11] Patients with type 1 DM, those younger than 35 years and older than 80 years, pregnant women, patients having renal disease other than DNP and urinary tract infection, patients having an infection or an acute exacerbation of another disease during the study, those with another chronic inflammatory diseases, acute ischemic heart disease, acute cerebrovascular disease and acute/chronic liver disease were excluded from the study. Among the screened patients, 55 type 2 diabetic patients without the exclusion criteria and who gave informed consent were included. Patients were divided into three groups according to albuminuria levels as Group1, Group-2 and Group-3. Group-1 consisted of normoalbuminuric patients (proteinuria<30mg/day) while microalbuminuric patients (proteinuria=30-300mg/day) and macroalbuminuric patients (proteinuria>300mg/day) were grouped as Group-2 and Group-3, respectively.

Laboratory Analyses

Arterial blood pressure was measured with the same device and operator at rest. Body mass index (BMI) was calculated by the formula: BMI=Weight/(height).[2] Venous blood samples were obtained after 12 hours of fasting. Glucose, HbA1c, total cholesterol, HDL cholesterol, triglyceride, creatinine, sodium, potassium levels were measured by appropriate methods. LDL cholesterol level was calculated by Friedmal formula. Microalbuminuria level was measured in 24-hour urine sample. Fasting plasma sample was collected and stored at -80 °C for measurement of ADMA levels. ADMA level was measured by ELISA method.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) for Windows 14.0 standard version was used for statistical analysis. Intergroup comparisons in numerical parameters were done by paired Student t- test or Mann Whitney U test when necessary. One-way ANOVA or Kruskal Wallis Tests were used for comparison of more than two groups accordingly. For nonnumeric parameters, Fisher's exact test Yates corrected Chi-square test for 2x2 contingency tables when necessary. Correlation analysis of numerical and non-numerical parameters was performed using Pearson and Spearman's rho correlation tests, respectively. P values less than 0.05 or was accepted as statistically significant. A linear regression analysis (enter method) model was used (included parameters: study group, smoking history, age, duration of DM, gender, BMI, history of ischemic heart disease, LDL cholesterol, use of insulin) to find parameters related to ADMA levels.

Results

Of 55 patients included in the study, 19 were male (34.5%) and 36 were female (65.45%). Median age of the patients was 59.9 years (36-80 years). The duration of DM in the whole study group was 12.2 years.

There were 19 patients (male/female ratio: 3/16) in Group-1, 26 patients (male/female ratio: 11/15) in Group-2 and 10 patients (male/female ratio: 5/5) in Group-3. Demographic and biochemical analyses of the groups were presented in Table 1. There was no statistically significant difference between groups regarding age, duration of DM, gender, LDL cholesterol and BMI (Table 1).

Of 34 (%61.81) patients were using insulin while the others were on oral antidiabetic therapy. The number of patients using insulin in Group-1, -2 and -3 were 10 (42.10%), 18 (69.23%) and 8 (80%), respectively.

Median serum ADMA levels for groups were 0.30±0.22 mmol/L, 0.53±0.20 mmol/L and 0.54±0.22mmol/L in Group-1, Group-2 and Group-3, respectively (Table 1). The median ADMA level was lower in Group-1 compared to Group-2 (p=0.001) and Group-3 (p=0.013), whereas Group-2 and -3 were similar (p=0.997) regarding ADMA levels. ADMA levels were not different in smoking and non-smoking groups (p=0.780).

No significant correlation was found between ADMA levels and age (p=0.236), gender (0.53), diabetic age (p=0.110), BMI (p=0.961) and serum LDL cholesterol level (p=0.781) (Table 2).

Serum ADMA levels were similar in patients with or without macrovascular complications (ischemic heart disease, cerebrovascular disease, peripheral vascular disease) (p=0.680), history of smoking (p>0.05) and use of insulin (p>0.05). ADMA level was positively correlated with albuminuria (r=0.32, p=0.016) levels. Study group based on albuminuria

Table 1. Demographic and biochemical analyses of the groups

Characteristic	Group-1 (n=19)	Group-2 (n=26)	Group-3 (n=10)	p
Age (years)	57.5±9.1	57.3±9.4	62.5±12.4	0.356
Duration of DM (years)	10.2±6.0	13.4±9.1	17.1±11.4	0.130
Body mass index (kg/m^2)	31.7±3.21	31.0±5.01	30.4±3.3	0.810
LDL cholesterol (mg/dl)	116.1±32	111±32	117±44	0.845
Albuminuria (mg/day)	14±5	85±66	535±219	**<0.001**
ADMA (mmol/L)	0.30±0.22	0.54±0.19	0.54±0.22	**0.001**

ADMA: Asymmetrical dimethyl arginine; DM: Diabetes mellitus; LDL: Low density lipoprotein.

Table 2. Linear regression analyses of the parameters related to ADMA levels

Characteristic	B	Beta	t	p
Study group	0.146	0.448	2.720	**0.011**
Body mass index	<0.001	-0.008	-0.050	0.960
Gender	-0.003	-0.020	-0.119	0.906
Duration of DM	0.005	0.198	1.130	0.267
Ischemic heart disease	0.021	0.037	0.218	0.829
Insulin use	-0.019	-0.041	-0.240	0.812
LDL cholesterol	<0.001	0.062	0.369	0.714
Smoking	-0.043	-0.069	-0.422	0.676
Age	-0.006	-0.235	-1.459	0.155

ADMA: Asymmetrical dimethyl arginine; DM: Diabetes mellitus; B: Coefficient; LDL: Low density lipoprotein.

level was the only parameter which was found to be significantly related with serum ADMA levels in linear regression analysis (Table 2).

Discussion

Microvascular and macrovascular complications of DM remain as common health problems all over the world and a major cause of mortality. Cardiovascular diseases and DNP are important causes of morbidity and mortality in diabetic subjects. Strict control of glucose, blood pressure and lipid parameters were shown to reduce morbidity and mortality. Herein, we aimed to investigate the relationship between albuminuria and ADMA in patients with DNP and we found that, ADMA level is significantly increased even in microalbumiuric stage among patients with type 2 DM.

Achan et al.[12] reported that intravenous infusion of small dose of ADMA would decrease heart rate and cardiac output, increase arterial blood pressure in healthy individuals. Konukoglu et al.[13] found a significant relationship between BMI and ADMA levels in obese male subjects with high cardiovascular risk1. Thirty-four patients with morbid obesity were examined in a study from our country. It was stated that ADMA levels were elevated in these patients and decreased in parallel with decreasing weight after gastric band surgery. We did not detect any correlation between BMI and ADMA in our study.

Tarnow et al.[14] compared 408 type 1 diabetic patients with DNP and 192 normoalbuminuric diabetics. They found higher ADMA levels in the group with DNP. Besides, they reported higher ADMA levels in the 44 subjects with a history of myocardial infarction or stroke compared to patients without. We did not detect any correlation of ADMA with diabetic complications.

Krzyzanowska et al.[15] compared diabetic patients with normoalbuminuria, microalbuminuria and macroalbuminuria. They found a significant correlation of ADMA levels with renal failure and cardiovascular disease in the groups with significant albuminuria. In the study by Hanai et al.[16] 225 type 2 diabetic patients with varying degrees of albuminuria were followed for five years. The primary end point in the study was the change to the upper albuminuria level. At the end of the study, 27 patients progressed from normoalbuminuria to microalbuminuria while 10 patients progressed from microalbuminuria to macroalbuminuria. ADMA levels in the patients who progressed regarding the stage of DNP were higher than patients who remained in the same stage. In our cross-sectional study, we found

higher ADMA levels in microalbuminuric and macroalbuminuric patients compared to normoalbuminuric patients. But micro- and macroalbuminuric groups were similar regarding ADMA levels.

Caglar et al.[17] studied ADMA and HOMA index in nondiabetic patients with subnephrotic proteinuria (<3.5 gram/day), patient with nephrotic range proteinuria (>3.5 gram/day) and healthy volunteers. All study parameters were highest in the group with nephrotic range proteinuria followed in order by patients with subnephrotic proteinuria and healthy subjects.[17]

So, ADMA level is elevated in patients with nephropathy and renal failure whether or not they are diabetic. Although elevated ADMA level is not specific for DNP, it can be said that ADMA is a specific risk marker for cardiovascular disease in renal diseases.

Our study has some limitations. it is a single-centered study, and our case number is relatively low. Although a direct causal relationship between proteinuria and ADMA was not shown, elevated ADMA level was suspected as a factor in the pathogenesis of renal diseases. The studies conducted for this purpose shown a relationship between proteinuria and ADMA level similar to our study.

In conclusion, serum ADMA level is significantly increased even in microalbumiuric stage among patients with type 2 DM. This may show the increased risk of both CVD and progressive kidney disease in these individuals.

Disclosures

Ethics Committee Approval: This study and all relevant procedures were performed in accordance with the Helsinki Declaration after obtaining the ethical board approval from the Haseki Training and Research hospital Ethics Committee.

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