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The Relationship Between Glycemic Control And Asymmetrical Dimethylarginine Levels

[Glisemik Kontrol ve Asimetrik Dimetilarginin Düzeyi İlişkisi]

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ABSTRACT

Objective: The relationship of asymmetrical dimethylarginine (ADMA) and glycemic control in diabetes is not yet fully enlightened. We aim to investigate the association of ADMA and hemoglobin A1c (HbA1c) in normal glomerular filtration rate (GFR) diabetic patients.

Methods: This cross sectional study included 88 diabetic patients whose GFRs were in reference range. 2 different HbA1c values; current (cHbA1c) and mean of 4 successive measurements with 3 months intervals (mHbA1c) were used. The association of ADMA with HbA1c levels and other clinical characteristics of patients were evaluated.

Results: We found significant inverse correlations between ADMA and both current (r =-0,354, p=0,001) and mean HbA1c(r=-0,377, p=0,000) levels. In multiple lineer regression analyses mHbA1c, glucose and duration of diabetes (R^2=0,343, p=0,000) or cHbA1c, glucose and duration of diabetes (R^2=0,318 p<0,001)were predictive variables for ADMA concentrations.

Conclusion: The study indicated that, long term poor glycemic control is associated with decreased ADMA levels.

Key Words: diabetes mellitus, ADMA, HbA1c

Conflict of Interest: Authors declare no conflict of interest.

ÖZET

Amaç: Asimetrik Dimetilarginin (ADMA)'nın diyabet ve glisemik kontrolle ilişkisi henüz aydınlatılamamıştır. Çalışmamızda normal glomeruler filtrasyonu(GFR) olan farklı glisemik kontroldeki hastalarda hemoglobin A1c (HbA1c) düzeyi ile ADMA ilişkisini inceledik.

Yöntemler: Bu kesitsel çalışmaya normal GFR'li 88 diyabetik hasta dahil edildi. 2 farklı HbA1c düzeyi; son HbA1c ve 3 ay aralıklarla ölçülen 4 HbA1c ortalamasını içeren ortalama (ort HbA1c)düzeyleri kullanıldı. ADMA'nın HbA1c düzeyleri ve hastaların diğer klinik durumları ile olan ilişkisi değerlendirildi**.**

Bulgular: ADMA ile ort HbA1c ve son HbA1c düzeyleri arasında negatif yönlü zayıf bir ilişki saptandı (ortHbA1c r=-0.377,p=0.000) (son HbA1c; r=-0.354,p=0.001) Multiple Lineer Regresyon Analizinde ortHbA1c,glukoz ve diyabet süresi (R^2=0.343, p=0.000) ile son HbA1c,glukoz ve diyabet süresinin (R^2=0.318 p<0.001) ADMA için prediktif değişkenler olduğu tespit edildi.

Sonuç: Tip II diyabetli hastalarda uzun dönem kötü glisemik kontrolün daha düşük ADMA düzeyi ile ilişkili olduğu görüldü.

Anahtar Kelimeler: diabetes mellitus, ADMA, HbA1c

Çıkar Çatışması: Katkıda bulunan yazarların hiçbir çıkar çatışması yoktur.

Introduction

Nitric oxide (NO) is the most important endothelium derived vasodilatator and synthesized from L-arginine by nitric oxide synthetase (NOS). ADMA is an endogenous inhibitor of NOS. ADMA reduces the bioavailability of NO by inhibition of NOS. A decrease in NO impairs endothel homeostasis in favour of vasoconstruction and initiates endothelial dysfunction. There are two types of NO synthetase inhibitor, NG-monomethyl-L-arginine and NG-dimetyl-L-arginine (ADMA). ADMA whose circulating concentration is 10 times higher than NG-monomethyl-L-arginine is the major inhibitor of NO synthesis [1]. In several tissues including endothelial cells, ADMA is derived from protein bound arginine residues by means of arginine N-methyltransferases during the routine protein cycle. Renal excretion constitutes %10 of ADMA metabolism and the first ADMA accumulation cases are reported in renal insufficiency. On the other hand, %90 of ADMA is degraded to citrulline and dimethylamine by dimethylaminohydrolase (DDAH) in the liver and an impairment in the activity of this enzyme can contibute to the elevated levels of ADMA [2,3]. The morbidities that impairs DDAH ezyme synthesis such as alcoholic hepatitis or acute liver insufficiency are accompanied with high ADMA levels.

Diabetes Mellitus is associated with elevated atherosclerosis and high cardiovasculatory disease prevalence. Poorly controlled Type 2 diabetic patients have higher risk of cardiovasculatory disease than those with good control [4]. Endothelial dysfunction is an early evidence of the pathogenesis of vasculatory complications which are the most important causes of morbidity and mortality in diabetic patients [5]. Hyperglycemia increases reactive oxidative species (ROS) in endothelial cells [6]. Increased ADMA levels are found in some studies on animals and on patients with diabetic complications. It is assumed that elevated ADMA levels can contribute to endothelial dysfunction and diabetic vasculatory complications [7]. Meanwhile, there are studies reporting low ADMA levels explained by diabetic hyperfiltration status in diabetic patients. The studies that investigate the relationship between HbA1c levels as an indicator of glycemic control and ADMA levels produced varying results. In our study, we aimed to find out the association between diabetes regulation and ADMA concentrations. We used not only the current HbA1c (cHbA1c) levels but also the mean of 4 successive HbA1c measurements in 1 year period (mHbA1c).

Material and Methods

The study subjects were selected from the type 2 diabetic patients diagnosed on the basis of American Diabetes Association criteria and followed by Diabetes Department of our hospital. The patiens with less than 10 years of disease duration and without any diabetic complications and comorbidities such as hepatic, renal, cardiovasculatory, chronic inflamatory or infectious diseases, malignity or evident vasculopathy, were included in the study. The exclusion of cardiovasculatory comorbidity was based on abscence of myocardial infarction, angina, coronary by-pass or stroke history and electrocardiographic changes of ischemia. The neurological and ophtalmological examinations of the subjects didn't show any evidence of neuropathy or ophtalmopathy. Patients with GFR values within the reference range (70-145 ml/min) and who had 2 normoalbuminuric results (<30 mg albumin/g creatinine) out of 3 successive spot urine examinations, were selected to exclude diabetic nephropathy.

Venous blood samples were drawn after 12-hour fasting. Sera obtained after centrifugation were stored at -80°C until the date of analysis. ADMA levels were detected by ELİSA method with Immundiagnostik ELISA reagent (Bensheim, Germany). HbA1c were measured using Bio-Rad Variant II Turbo HPLC (USA). Insulin and C-peptide were analysed by immunoassay technique using Unicel DxI 800 (Beckman Coulter, USA) and Diasorin Liaison (Saluggia, İtaly) respectively. Serum glucose, triglyceride, total and HDL cholesterol, creatinine, microalbumin levels were measured with original reagents, in Modular PP (Roche Diagnostics, Mannheim, Germany). LDL cholesterol was calculated by Friedewald equation.

Blood pressures of the patients were taken after 10 minutes of resting, using automatic sphyngomanometer. Patients taking antihypertensive therapeutics were qualified as hypertensive even their measured blood pressure were within the normal limits. GFR values were calculated by Cockcroft-Gault Formula. mHbA1c values of 4 successive measurements with 3 months intervals were calculated for each patient. The study was approved by our hospital's scientific committee.

Statistical analysis were carried out using SPSS program (Statistical Package for Social Science, version 11.7; Chicago, IL). Kolmogorov-Smirnov test was performed to determine about the distribution of values. Data was expressed as mean±SD or median (2,5 – 97,5 percentiles) Data was evaluated by means of multiple lineer regression , univariate, Spearman correlation analyses. mHbA1c and cHbA1c were evaluated as independent variables in two distinct regression models. Analyses using mHbA1c with other variables is referred as Model 1; analyses using cHbA1c is referred as Model 2 in the study.

$p < 0.05$ was accepted as statistically significant.

Results

General characteristics of patients are given in Table 1. Mean age of study population was 53,19±9,38 years, median time for duration of disease was 84(24-120)

Table 1. General characteristics of the study population Data are expressed as ratios (/), Mean±SD, median (2.5-97.5 percentiles) where appropriate.

N	88
Sex (W/M)	43/45
Age (years)	53.19±9.38
Smokers/nonsmokers	14/74
Duration of diabetes (months)	84 (24-120)
Insulin use (ratio)	44/88
BMI (kg/m²)	28,7±4,6
Hypertension (+/-)	38/50
LDL cholesterol (mmol/L)	2.86 (1.48-4.62)
HDL cholesterol (mmol/L)	1.18 (0.78-1.90)
Glucose (mmol/L)	7.37 (4.16-14.90)
c-peptid(ng/ml)	2.09±1.25
Creatinin (μmol/L)	69,8±15.0
GFR (ml/min)	1.17 (72-144)
mHbA1c (%)	7.1 (5.5-10.4)
cHbA1c (%)	7.0 (5.2-11.4)
ADMA (μmol/L)	0.47±0.9

months. Median values for glucose was 7,37 (4,16-14,9) mmol/L; GFR 117 (72-144) ml/min; mHbA1c (%) 7,15 (5,5-10,4) and cHbA1c (%) 7 (5,2-11,4). ADMA mean of the whole group was 0,47±0,9 μmol/L. The inter-assay CV's of our method were, %6.25 and %5.3 for ADMA levels of 0.25 μmol/L and 0.85 μmol/L respectively

Using simultaneous (enter in SPSS) multiple regression analyses with ADMA as dependent variable and age, duration of diabetes,BMI, HDL cholesterol, LDL cholesterol, glucose, c-peptid, creatinine, mHbA1c or cHbA1c as independent variables was performed. mHbA1c, glucose and duration of diabetes were predictive variables for ADMA (R^2=0,343, F=4.594, P<0,0001) in the first model; and cHbA1c, glucose and duration of diabetes were predictive variables for ADMA (R^2=0,318, F=4.106,P<0,001) in the second model; cHbA1c and mHbA1c levels correlated well (r=0,92, p<0,0001). R^2 values were too small for a precise estimation. Categorical variables (sex, smoking, insulin use, hypertension) didn't have any assosiasion with ADMA levels. Spearman correlation analysis revealed significant correlations between ADMA and duration of diabetes (r = -0,28, p = 0,008); cHbA1c (r = -0,35, P = 0,001); mHbA1c (r = -0,377, P<0,001) ; c-peptid (r = 0,25, P = 0,018) levels. There was also a nearly significant inverse correlation (r = -0,208, p = 0,053) with GFR values even all of them were in physiological range.

Discussion

Our study reveals:

In Type 2 diabetes patients glucose, diabetes duration and HbA1c are independent predictors of ADMA.

There is a negative significant correlation between ADMA and HbA1c levels in Type 2 diabetics.

cHbA1c and mHbA1c levels correlated well (r = 0,92, p<0,0001).

Age, sex,smoking insulin use,BMI, hypertension , HDL cholesterol, LDL cholesterol, c-peptid, creatinine parameters didn't have any assosiasion with ADMA levels.

HbA1c is an indicator of long term glycemic control. There are different hypotheses on how hyperglycemia affects ADMA levels. The authors reporting elevated ADMA levels in diabetes suggest several mecanisms to explain their results. In animal studies, it has been reported that oxidative stress induced by hyperglycemia increases ADMA levels by affecting

ADMA degradation via DDAH enzyme [7]. Sorrenti et al. have shown that exposure of endothelial cells to high glucose concentrations caused elevated oxidative stress, decreased DDAH-2 and NOS imbalance [8]. It has also been reported that ADMA levels were elevated during oxidative stress due to free radicals inhibiting DDAH enzyme activity. ADMA then competed with arginine for the substrate binding sites of NOS which resulted in relative arginine depletion. Arginine defficiency lead NOS to free radical synthesis instead of NO synthesis. It has been supposed that through this stage, NOS perpetuated and aggravated oxidative stress [9]. Another mechanism which may be responsible for ADMA elevation is the hyperglycemia induced elevation of arginine methyltransferase expression, an enzyme of ADMA synthesis [10]. In a study carried out in 40 uncomplicated diabetes patients, Altınova et al. have found high ADMA levels and low L-arginine/ADMA rates (2.6±1.9 vs 1.7±0.7 μmol/L, p<0.01). They have also reported that ADMA levels were similar (2.3±1.0 vs3.0±2.4 μmol/L, p>0.05) in two groups consisted of subjects with HbA1c ≤ 7 and those with HbA1c > 7. However they have not reported whether these two groups were comparable in terms of variables that were thought to influence ADMA concentrations. They have concluded that ADMA levels might be a marker of endothelial dysfunction seen in diabetic subjects [11].

In the studies that have found low ADMA levels, it has been supposed that besides its protective role against oxidative stress, NO could itself be a source of oxidative stress. Pitocco et al., in a study conducted in 99 females with uncomplicated type 1 diabetes and

Table 2. Multiple Lineer Regression analyses using mHbA1c with age, duration of diabetes, BMI, HDL cholesterol, LDL cholesterol, glucose, c-peptid and creatinine as independent variables (Model 1)

Variables	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	b	Std.Error	Beta		
Constant	0.728	0.107		6.830	<0.0001
mHbA1c	-0.039	0.010	-0.464	-4.012	<0.0001
Age	0.000	0.001	0.021	0.198	0.843
Duration of diabetes	-0.001	0.000	-0.270	-2.784	0.007
BMI	0.001	0.002	0.063	0.605	0.547
HDL cholesterol	-0.001	0.001	-0.141	-1.371	0.174
LDL cholesterol	0.000	0.000	0.042	0.420	0.675
Glucose	0,001	0.000	0.288	2.463	0.016
C-peptid	0.000	0.009	0.006	0.054	0.957
Creatinine	0.042	0.058	0.073	0.724	0.471

(R^2=0.343, F= 4.594, ANOVA p<0.0001)

Table 3. Multiple Lineer Regression analyses using cHbA1c with age, duration of diabetes, BMI, HDL cholesterol, LDL cholesterol, glucose, c-peptid and creatinine as independent variables (Model 2)

Variables	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	b	Std.Error	Beta		
Constant	0.678	0.104		6.508	<0.0001
cHbA1c	-0.030	0.008	-0.417	-3.580	0.001
Age	0.000	0.001	0.014	0.129	0.898
Duration of diabetes	-0.001	0.000	-0.268	-2.712	0.008
BMI	0.001	0.002	0.055	0.523	0.603
HDL cholesterol	-0.001	0.001	-0.153	-1.459	0.149
LDL cholesterol	0.000	0.000	0.030	0.290	0.772
Glucose	0.001	0.000	0.251	2.148	0.035
C-peptid	0.002	0.009	0.022	0.186	0.853
Creatinine	0.052	0.059	0.091	0.889	0.377

(R^2=0.318, F=4.106, ANOVA p<0.01)

with less than 10 year disease duration, have reported lower ADMA levels compared to control subjects. They have suggested that NO could affect DNA synthesis and respiratory chain or could react with superoxide to form peroxinitrite, a potent oxidative stress agent. In this model, higher ADMA levels seen in healthy subjects, would be protective against oxidative stress by reducing the production of NO and consequently of peroxinitrite. Therefore, lower ADMA levels in DM1 patients might be interpreted as a sign of impaired protection against oxidative stress rather than a marker of vasculopathy [12]. Huemer et al. have similarly found lower ADMA levels in diabetics than control subjects, in their study conducted in type 1 diabetic patients (2.6±1.9 µmol/L vs 1.7±0.7 µmol/L p<0.01) and also a negative correlation between ADMA and HbA1c results. In the contrary to the theory suggesting that elevated ADMA and decreased NO levels could lead to vasculary damage, these authors have assumed that NO itself might cause oxidative stress [13]. Heilman et al. have found lower ADMA levels in 30 type 1 diabetic children without vasculopathy than those of control subjects [14].

Certain reaserchers have investigated the association of ADMA with GFR. In their study, Paiva et al., have been obtained lower levels of ADMA in type 2 diabetics than in healthy controls (0.29±0.15 μmol/L vs 0.34±0.16 μmol/L $p<0.03$). They have also found a negative correlation between ADMA and HbA1c ($r=-0.28$, $p=0.01$) and a negative correlation between GFR and ADMA ($r=-0.19$, $p=0.008$). They have related the decreased ADMA levels in poorly conrolled diabetics to the renal hyperfiltration. In their study carried out in 330 type 1 diabetes patients, Marcovecchio et al. have found a significant negative correlation between ADMA and HbA1c levels and they have suggested that increased GFR could be responsible for that results. Their study was handicapped by that they have not measured GFR in some subjects and urine ADMA levels in all subjects of the patient group. They have eventually speculated that it would be more appropriate to focus on the liver to explain ADMA levels since the metabolism of ADMA by DDAH in liver was more excessive than its renal clearence [15].

In our study, we constituted the patients group with subjects whose GFR was within the physiological range (70-145 ml/min) to eliminate the possible effect of elevated GFR on long term glycemic control levels and it was found that ADMA levels had significantly decreased with the impairment of the metabolic control of the disease. Therefore, we suggest that the exposure to high concentration of glucose may affect ADMA levels by the course of time and this can occur before the development of the diabetes complications and the impairment of GFR.

Declaration of Interest: Authors declare no conflict of interest.

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