An assessment of correlation between serum asymmetric dimethylarginine and glycated haemoglobin in patients with type 2 diabetes mellitus

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Abstract

Studies that investigated an association between asymmetric dimethylarginine (ADMA) and glycated haemoglobin (HbA_{1c}) in type 2 diabetes mellitus (T2DM) have given discordant results. The aim of this study was to determine and compare serum ADMA concentration in patients with T2DM and healthy controls, and to assess correlation between ADMA and HbA_{1c} in patients with T2DM. Serum ADMA concentration was determined by ELISA method with the use of ADMA ® - ELISA kit (DLD Diagnostics, Hamburg, Germany) and HbA_{1c} levels were determined by an immunoturbidimetric method in 60 patients with T2DM and 60 healthy individuals matched for age and sex. Results have shown that mean serum ADMA concentration was significantly higher in T2DM patients (1.54±0.06 μmol/L) compared to mean serum ADMA concentration (0.62±0.02 μmol/L; $p<0.0001$) in healthy subjects. A significant, positive, correlation between serum ADMA concentration and HbA_{1c} levels was observed ($r=0.494$; $p<0.01$) in T2DM patients. Our results suggest that there is an association between endothelial dysfunction and glycaemic control in type 2 diabetes mellitus. Possible explanation for obtained results may be oxidative stress that is increased in conditions of hyperglycaemia and it also promotes endothelial dysfunction. Larger, longitudinal studies are required that will evaluate relation between metabolic abnormalities and increased ADMA levels in patients with type 2 diabetes mellitus.

KEY WORDS: asymmetric dimethylarginine, endothelial dysfunction, glycated haemoglobin, type 2 diabetes mellitus.

INTRODUCTION

Asymmetric dimethylarginine (ADMA) is a novel risk factor of endothelial dysfunction [1]. Its role in the development and progression of type 2 diabetes mellitus (T2DM) is not fully known. ADMA is an amino acid discovered in 1992 and presents non-invasive marker of endothelial dysfunction, as well as novel cardiovascular risk factor [2]. It circulates in plasma, and is present in different tissues and cells. Determined plasma values of ADMA using ADMA ELISA techniques in 500 healthy white individuals 19 to 75 years old were in a range from 0.36 to 1.17 μmol/L [3]. Studies have shown that ADMA increases systemic vascular resistance and mean arterial blood pressure, and decreases cardiac output [4]. It is established that infusion of exogenous ADMA decreases heart rate, and increases pulmonary vascular resistance. Administration of ADMA disturbs renal blood flow, sodium reabsorbtion and decreases cerebral perfusion [5]. One of the most prominent features of ADMA is that it represents inhibitor of nitric oxide (NO) synthesis [6, 7]. Major mechanisms that lead to increased ADMA values in different diseases include shear stress, oxidative stress, hyperhomocysteinaemia, and high concentration of glucose in blood [8, 9]. Studies that investigated an association between ADMA and glycated haemoglobin (HbA_{1c}) in patients with T2DM are scarce and have given discordant results [10-12]. The aim of the present study was to investigate whether serum ADMA is increased in patients with T2DM compared to healthy individuals. Also, we aimed to assess whether there is a correlation between serum ADMA concentration and HbA_{1c} levels in T2DM patients.

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MATERIALS AND METHODS

Subjects

We recruited 60 patients with type 2 diabetes mellitus (30 male, 30 female), as defined by American Diabetes Association [13], who regularly attend the Out-Patient Family Medicine Clinic „Višnjik" in Sarajevo. All patients were receiving antidiabetic and antihypertensive therapies and some were receiving antilipidemic drugs and/or aspirin for at least the previous 6 months. Exclusion criteria were the presence of sustained acute and chronic infections, malignancy, hepatic or renal disease, diabetic retinopathy and nephropathy, and other endocrine dysfunctions. The control group consisted of 60 apparently healthy subjects (30 male, 30 female) with no history of T2DM, other endocrine dysfunctions, hyperlipidemia, hypertension, or coronary heart diseases. None of the control subjects had received any medication (hormone replacement therapy, corticosteroids, vitamin supplements, antioxidant formulations and thiazolidinediones) which may have affected insulin resistance and/or endothelial function and none of the subjects were current smokers and consumers of alcohol.

Blood sampling

Blood was collected in the morning after an overnight fast after a 30-min rest in a semi-recumbent position. Sampling was done without stasis, using the vacutainer technique.

Blood chemistry analysis

Serum ADMA concentration was determined by ELISA method (machine STAT FAX 2100, USA) at the Department of Physiology and Department of Biochemistry, Faculty of Medicine in Sarajevo. ADMA ® - ELISA kit (DLD Diagnostika GmbH, Germany) was used as reagent. ADMA standards range from 0.1 to 5.0 μmol/L and the detection limit for ADMA with the use of this method is 0.05 μmol/L. HbA_{1c} was measured by an immunoturbidimetric method (TINIA; Siemens Healthcare Diagnostics Ltd, Camberley, United Kingdom). Normal range was 4.8-6.0%. The total cholesterol (TC), HDL-cholesterol (HDL-C), and triglyceride levels were measured with the use of standard enzymatic methods. The LDL-cholesterol (LDL-C) levels were calculated using the Friedewald formula. All biochemical tests, except for ADMA determination, were performed at Institute for clinical chemistry and biochemistry University of Sarajevo Clinics Centre. The study was approved by the Ethics Committee of the Medical Faculty University of Sarajevo. Written informed consent was obtained from all subjects. Investigations were carried out in accordance with the Declaration of Helsinki as revised in 2000.

Statistical analysis

The Kolmogorov-Smirnov test of normality was used to test the distribution of variables. An unpaired Student t-test was used to compare the difference between two groups. Since tested variables were normally distributed, correlations were assessed by Pearson's test. A p value of less then 0.05 was considered statistically significant. The software used was SPSS for Windows (version 17.0; SPSS, Chicago, IL, USA).

RESULTS

The baseline characteristics of study participants are reported in Table 1. Age and body mass index (BMI) did not differ between control group of subjects and patients with T2DM. The mean duration of diabetes was 8.27±0.89 years. Statistically significant difference was established in values of systolic and diastolic blood pressure between control group of subjects and patients with T2DM. As it could be expected

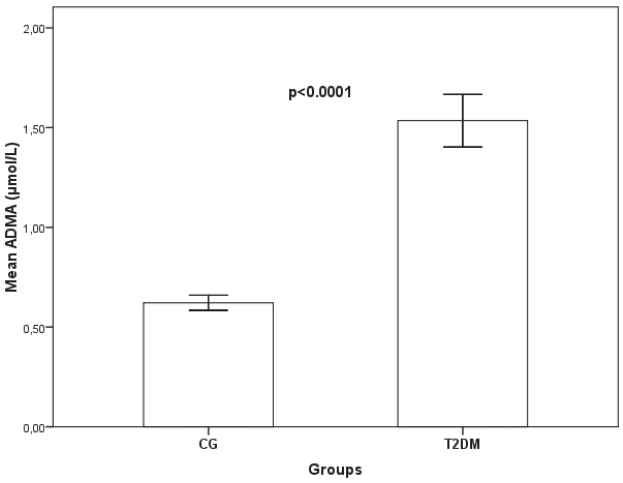

FIGURE 1. Serum asymmetric dimethylarginine concentration in the control group (CG) and type 2 Diabetes Mellitus (T2DM) group.

TABLE 1. Baseline characteristics of the control subjects and patients with type 2 diabetes.

Variables	Control group (n=60)	T2DM group (n=60)	$p<$
Age (years)	54.28±0.98	56.68±0.87	NS
BMI (kg/m^2)	28.36±0.32	30.45±0.65	NS
Diabetes duration (years)	———	8.27±0.89	———
SBP (mmHg)	125.4±2.17	138.66±1.97	0.001
DBP (mmHg)	81.25±0.99	84.58±1.12	0.05
Blood glucose (mmol/L)	5.33±0.10	8.53±0.38	0.001
HbA_{1c} (%)	5.63±0.03	7.40±0.18	0.001
Total cholesterol (mmol/L)	5.49±0.13	5.82±0.11	NS
Triglycerids (mmol/L)	1.64±0.09	2.52±0.15	0.001
HDL-C (mmol/L)	1.34±0.03	1.14±0,03	0.001
LDL-C (mmol/L)	3.54±0.11	3.81±0.12	NS

Data are presented as mean ± SEM; BMI: Body Mass Index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HbA_{1c}: glycated haemoglobin; HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; T2DM group- type 2 diabetes mellitus group.

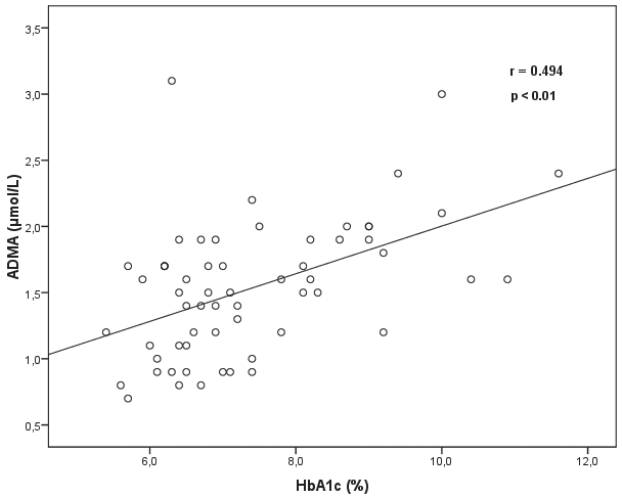

FIGURE 2. Pearson's correlation analysis of serum glycated haemoglobin values and asymmetric dimethylarginine concentrations in patients with type 2 diabetes mellitus.

patients with T2DM had statistically significantly higher concentration of blood glucose and values of glycated haemoglobin then healthy individuals. Likewise, significant difference in values of triglycerides and HDL-cholesterol was determined between subjects of the control group and patients with T2DM. Significant difference in values of total cholesterol and LDL-cholesterol between groups was not observed. Results presented in Figure 1 show that mean serum ADMA concentration in patients with type 2 diabetes mellitus (1.54±0.06 µmol/L) was significantly higher than mean serum ADMA concentration determined in the control group of subjects (0.62±0.02 µmol/L; $p<0.0001$). As shown in Figure 2, there was a significant positive correlation between ADMA concentration and levels of HbA_{1c} in patients with type 2 diabetes mellitus (r= 0.494; $p<0.01$). As presented in Table 2, there was no statistically significant correlation between levels of lipid profile indices and ADMA concentration both in the control and T2DM group.

TABLE 2. Pearson's correlation analysis between levels of serum total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol levels and serum asymmetric dimethylarginine concentration in control subjects and patients with type 2 diabetes mellitus.

	Control group ADMA (µmol/L)	T2DM group ADMA (µmol/L)
Total cholesterol (mmol/L)	r = - 0.175 p = 0.180	r = - 0.131 p = 0.319
Triglycerids (mmol/L)	r = 0.081 p = 0.536	r = - 0.081 p = 0.536
(s)HDL-cholesterol (mmol/L)	r = - 0.070 p = 0.593	r = 0.040 p = 0.759
(s)LDL-cholesterol (mmol/L)	r = 0.095 p = 0.470	r = - 0.204 p = 0.119

ADMA- asymmetric dimethylarginine; T2DM group- type 2 diabetes mellitus group; r- correlation coefficient; p – level of significance

DISCUSSION

Results of our study clearly showed that serum ADMA concentration in patients with T2DM was significantly higher compared to age and sex - matched healthy subjects. Obtained results are in accordance with majority of studies that tested concentration of ADMA in patients with T2DM [14,15]. However, Paiva et al. [10] consider that long-term hyperglycaemia may result in decreased ADMA values in patients with T2DM. Our results are not consistent with the findings of mentioned authors. Contrary to their opinion, we believe that precisely hyperglycaemia may be behind ADMA increase determined in T2DM patients. This metabolic disturbance may induce decreased NO formation and lead to endothelial dysfunction [16]. Furthermore, even though mechanisms that are responsible for ADMA increase in patients with T2DM are still not fully elucidated, it is possible that increased serum ADMA concentration may be a consequence of decreased expression or activity of dimethylarginine dimethylaminohydrolase, which is an enzyme responsible for ADMA degradation [2]. Results of the studies that investigated correlation between ADMA and HbA_{1c} in patients with T2DM have given discordant results. Paiva et al. [10] determined statistically significant negative correlation between ADMA and HbA_{1c} in patients with T2DM. Our results have established highly significant positive correlation between ADMA and HbA_{1c} in our study sample. Results of Anburajan et al. [12] have also observed significant positive correlation between ADMA and HbA_{1c} in patients with T2DM. Results of our study suggest that there is an association between endothelial dysfunction and glycemic control in patients with T2DM. However, since some authors have determined negative, and others positive correlation between ADMA and HbA_{1c} in the conditions of T2DM, we are of the opinion that future studies should give an answer to the question whether good glycemic control is followed by decrease in ADMA concentration or hyperglycaemia *per se* conditions decrease in ADMA values by promotion of NO synthesis. Although it has been demonstrated that hypercholesterolemia is associated with endothelial cell dysfunction [17, 18], results of our study did not show significant correlation between lipid profile indices and ADMA levels in both control subjects and patients with T2DM. Obtained results are consistent with observation of Krzyzanowska et al. [19] that also did not find significant association between serum lipids and ADMA in morbidly obese women. Conversely, Yamamoto et al. [20] reported significant negative correlation between serum ADMA and HDL-cholesterol in patients with T2DM. Furthermore, findings of this study have shown that HDL-cholesterol is

independent determinant of serum ADMA in T2DM patients. However, our results do not support these findings which might be due to limited size of our study sample.

CONCLUSION

In a conclusion it can be stated that serum ADMA concentration is increased in patients with type 2 diabetes mellitus. Among many, one of the possible explanations for the observed ADMA increase may be an axis that consists from hyperglycaemia that induces increased secretion of proinflammatory cytokines such as tumour necrosis factor – alpha (TNF-α), that in turn leads to increased ADMA values in the conditions of type 2 diabetes mellitus. Furthermore, positive correlation between ADMA and HbA_{1c} in patients with type 2 diabetes mellitus is determined. Although in our study indices of oxidative stress were not measured, we find it plausible that possible explanation for the established association between ADMA as a marker of endothelial dysfunction and HbA_{1c} as an index of glycemic control might be this disturbance of oxidant/antioxidant balance since it is known to be increased in conditions of hyperglycaemia and it also promotes endothelial dysfunction. Finally, larger longitudinal studies are required that will further evaluate observed relation between metabolic abnormalities and increased ADMA levels in patients with type 2 diabetes mellitus

DECLARATION OF INTEREST

The authors declare no conflict of interest.

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