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Relationship Between Serum Asymmetrical Dimethylarginine Level and Urolithiasis

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ABSTRACT

In the present prospective clinical human study, we focused on researching whether or not there is a difference between metabolic syndrome (MS) positive/negative and renal stone disease (SD) positive/negative patients with regard to asymmetrical dimethylarginine (ADMA) serum levels to clarify the possible effect of endothelial cell dysfunction on renal SD.

We included 76 patients (17 males and 59 females) who were admitted to the endocrinology and urology outpatient clinic between December 2014 and February 2018. Patients were segregated into 4 groups; group 1; MS (–) SD (-), group 2; MS (–) SD (+), group 3; MS (+) SD (-) and group 4; MS (+) SD (+). Patients' age, sex, medical history, and anthropometric measurements were recorded. Endothelial dysfunction was assessed with serum ADMA levels.

The mean age was 40.1 ± 11.4 years (range: 18–59). There was a statistically significant difference between groups for mean homocysteine, uric acid and BMI values. However, there was no statistically significant difference between groups for mean CRP, age and creatinine values. The mean ADMA value was 152 ± 77 (range: 51–445). There was no statistically significant difference between groups for the mean ADMA values.

These results showed that studies must focus on MS components separately from each other and sex distribution between patients must be homogeneous or different sexes must be examined separately. In addition, stone compositions of patients enrolled in the study must be known to arrive at more trustworthy and worthwhile results.

Key Words: asymmetrical dimethylarginine, metabolic syndrome, urolithiasis

Introduction

Urinary system stone disease (SD) is one of the most common urological diseases (1). According to a recent epidemiological study about SD in our country, urolithiasisis commonly seen in hot and humid regions in accordance with the literature and has a prevalence of 11.1% throughout the country (2,3). In addition, renal SD is a chronic disease which usually relapses during the first ten years after the first episode, it has also been connected with many chronic illnesses such as obesity, diabetes mellitus (DM), hypertension (HT) and metabolic syndrome (MS) (4-6).

Even though supersaturation is the crucial step for crystal formation, stone generation never forms unless crystals adhere to renal tubular epithelial cells. It is obvious that calcium oxalate crystals favor adhesion to damaged renal tubular epithelium rather than to healthy epithelium (7). Also, the close relationship between renal vascular endothelium and proximal tubular epithelium, with respect to regulating the effect of endothelium on epithelium ion transport, leads the researchers to suggest the possible effect of vascular endothelium on stone formation (8). At this point, in a rat model study which firstly showed the reasonable effect of endothelium on calcium oxalate (CaOx) stone formation, it was indicated that hyperoxaluria causes a significant rise in asymmetrical dimethylarginine (ADMA; an endothelial dysfunction marker) in renal tissue after administration of ethylene glycol (9).

In light of this knowledge, in this present prospective clinical human study, we focused on researching whether or not there is a difference

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between "MS positive/negative" and "SD positive/negative" patients with regard to ADMA serum level to clarify the possible effect of endothelial cell dysfunction in SD.

Materials and Methods

The ethics committee of Dr. Lutfi Kirdar Training and Research Hospital approved this prospective clinical human study. We included patients (all patients were older than 18 years of age and we obtained written informed consent from all patients) who were admitted to the endocrinology and urology outpatient clinics between December 2014 and February 2018. Patients who were reluctant to participate in the study and who had additional disease, other than urinary system stone disease and MS,were excluded from the study. All of the included patients were drug naive or did not use any drugs affecting glucose metabolism or endothelial dysfunction in the three months prior to enrollment. Patients who had MS were called MS (+) and those who did not have MS were called MS (-). Similarly, patients who had a history of kidney stone or kidney stone,who were diagnosed by unenhanced computed tomography (CT) or urinary ultrasonography (USG) or kidney, ureter and bladder radiography (KUB), were called SD (+) and patients who hadnone of these featureswere calledSD (-). The patients were segregated into 4 groups with 19 patients in each:Group 1; MS (–) SD (-), group 2; MS (–) SD (+), group 3; MS (+) SD (-) and group 4; MS (+) SD (+).

Patients' age, sex, medical history including smoking and alcohol habits, anthropometric values such as height, weight and waist circumference (abdominal circumference at umbilical level at the end of expiration) and body mass index (BMI) were recorded. Also, c-reactive protein (CRP; inflammatory marker), homocysteine (potential marker of elevated risk for endothelial dysfunction), uric acid, and creatinine values were evaluated. Endothelial (dys)function was evaluated with serum ADMA levels that inhibits nitric oxide synthesis by competing with the substrate of nitric oxide, L-arginine, leading to endothelial dysfunction.

We diagnosed MS according to the modified National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria, if at least three of the following five features were present: waist circumference higher than 88 cm in females and 102 cm in males, serum HDL-C lower than 50 mg/dl in females and 40 mg/dl in males, serum triglyceride higher than 150 mg/dl, systolic blood pressure higher than 130 mmHg and/or diastolic higher than 85 mmHg or specific treatment of previously diagnosed HT, and fasting blood glucose higher than 100 mg/dl (10).

After overnight 12-h fasting, blood samples of patients were taken in the morning between 8 am and 9 am and tested for total cholesterol profile, glucose, insulin, (mmol/L), homocysteine, CRP, uric acid, creatinine and ADMA level. The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as glucose (mg/dL) x insulin (μIU/mL)/405 (11).

The blood samples were centrifuged at 4000 rpm for 15 minutes at 4 ºC, and transferred into fresh polypropylene tubes and stored at -80 ºC. Total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) concentrations were measured by using enzymatic calorimetric kits with intra- and inter-assay coefficients of variation of <10% (Roche Diagnostics GmbH). We calculated the low-density lipoprotein cholesterol (LDL-C) according to the Friedwald formula. Plasma glucose concentration was determined by the glucose oxidase method (Olympus AU 2700; Olympus America Inc). Serum ADMA levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit in accordance with the manufacturer's instructions, with intra- and inter-assay coefficients of variations of <10% (EIAab Science Co. Ltd, Wuhan, China).

We analyzed the statistical data by SPSS® 19.0 for Windows®.

Since the data in our study did not show a normal distribution, Mann Whitney-U test was used for two independent group comparisons and Kruskal Wallis test was used for more than two independent group comparisons. Groups which were compared using the Kruskal–Wallis test were also analyzed by post-hoc Scheffe's test if needed. Statistically significant difference was accepted as $p < 0.05$.

Results

A total of 76 patients (17 males and 59 females) were included in the study. The mean age was 40.1 ± 11.4 years (range: 18–59) and the mean BMI was 34.4 ± 7.6 kg/m^2 (range: 16.6 ± 50.4). The mean homocysteine, CRP, uric acid, and creatinine values of all patients were 2.4 ± 1.7 mmol/L (range: 0.3 - 7.3), 6.4 ± 4.6 mg/dL (range: 1.2 - 23.7), 4.9 ± 0.9 mg/dL (range: 2.5 - 8.1) and 0.71 ± 0.14 (range: 0.41 - 1.2), respectively. There were

Table 1. Laboratory Results

Parameters	Group-1 MetS (–) SD (-) n:23	Group-2 MetS (–) SD (+) n:23	Group-3 MetS (+) SD (-) n:23	Group-4 MetS (+) SD (+) n:23	p values
BMI (kg/m2)	33,8 ± 5,2	37,4 ± 5,3	30,1 ± 5,1	35,8 ± 5,6	0,004a and 0,005b
Blood uric acid (mg/dL)	4,1 ± 0,61	5,66 ± 0,76	5,44 ± 1,21	5,65 ± 1,42	0,04c
Blood creatinine(mg/dL)	0,66 ± 0,11	0,67 ± 0,14	0,68 ± 0.12	0,71 ± 0,17	0,43
CRP (mg/dL)	6,3 ± 3,2	6,2 ± 2,4	8,1 ± 5,3	5,5 ± 3,3	0,49
Blood homocysteine (mmol/L)	1,57 ± 0,81	2,91 ± 1,37	2,51 ± 1,41	2,04 ± 1,32	0,02d

MetS: Metabolic syndrome; SD: Stone disease; BMI: Body mass index; CRP: C-reactive protein.
[a] Between group 2 and 3. [b] Between group 3 and 4.
[c] Between group 1 and 2.
[d]Between group 1 and 2.

Table 2. Mean ADMA Values of Groups (Kruskal Wallis Test)

Groups	Mean ADMA value	Std. deviation	p
1	177	100	0.315
2	152	77	
3	150	76	
4	132	43	
Total groups	152	77	

ADMA: Asymmetrical dimethylarginine

statistically significant differences between the groups for mean homocysteine values (group 1: 1.57 ± 0.81, group 2: 2.91 ± 1.37, p= 0.02), uric acid values (group 1: 4.1 ± 0.61, group 2: 5.66 ± 0.76, p=0,04) and BMI values (group 2: 37.4 ± 5.3, group 3: 30.1 ± 5.1, p = 0.004 and group 3: 30.1 ± 5.1, group 4: 35,8 ± 5.6, p= 0.005). However, there were no statistically significant differences between groups for mean CRP, age and creatinine values (data were summarized in Table 1).

The mean ADMA value was 152 ± 77 (range: 51–445). There was no statistically significant difference between groups for the mean ADMA values (p= 0.31) (Table-2).

Discussion

MS, a major consequence of obesity, is on the rise (12). According to the Third National Health and Nutrition Examination Survey (NHANES III), the prevalence of MS in the United States is 23% and 40% for people ≥20 and 60 years, respectively (13). Also, urinary system SD is on the rise. Though SD is a common disease and has a cumulative incidence of 5% –10% during whole length of life, its prevalence has increased worldwide recently (14,15). Possibly, such high prevalence is because of the non-randomized nature of the study population in which diabetic and hypertensive patients were over-represented.

Regarding the epidemiological trial NHANES III, there was a significant correlation between traits of the metabolic syndrome and a self-reported history of kidney stones. The prevalence of kidney stoneswas 3%, 7.5% and 9.8% for no traits, three traits and five traits ofMS, respectively (16). In another study, it was reported that while thenumber of MS components increased, the prevalence of stones also progressively increased (17). In the same study, it was shown that most important component of MS for kidney stone formation was HT. Increased age and waist circumference, and being male were other important risk factors for kidney stone formation. However, serum TG or HDL-C levels were not found to be risk factors (17). Then, a smaller study from Japan reported that there were significant differences in insulin, HOMA-IR, systemic vascular tension, and anthropometric values between women who had stones and did not have stones (18). Also, a large adult population-based study from Korea revealed that being MS (+) and male and having high blood pressure for both genders were very important risk factors for

becoming SD patients according to multivariate analysis. They also emphasized that as the number of MS components increased, the presence of SD was higher in male subjects with a statistically significant difference (19). In another study, it was reported that while uric acid stones, which is already known to be associated with several metabolic disorders, was significantly associated with MS (OR, 1.82; 95% CI 1.19 – 2.79); calcium stones, which are observed in about 80% of SD cases, were not associated with the presence of MS (20).

In our study, we found that there was no statistically significant difference between the groups for ADMA values. According to the results of our study, we can say that behavior of study groups with respect to endothelial dysfunction was possibly different even between patients in the same group due to different metabolic syndrome components and sex distribution. This situation is also viable for stone composition,which was not defined which causes challenges for understanding. For example, formation of some stones in group 4, which was defined as MS (+) SD (+), was not related to MS; but patients had MS and they were collected in group 4. This is also applicable to group 2, which was defined as MS (-) SD (+).In short, as we do not know the MS components and stone composition of patients, this is a possible reason why we did not find differences in ADMA values between the groups. Furthermore, we can consider that different sex distributionsin the groups also influenced the results of the study.Similarly, other parameters like homocysteine, uric acid, BMI, creatinine, age, and CRP which were examined must be evaluated in this manner. In addition to situations mentioned above, the small sample size in our study is another limitation. Besides these limitations, the prospective nature of the study increases its power.

We did not find any statistically significant difference for ADMA values between groups; however, results showed that studies must focus on MS components separately from each other. Also, sex distribution between patients must be homogeneous or different sexes must be examined separately.Stone compositions of patients enrolled in the study program must also be known to arrive at more trustworthy and worthwhile results. Future studies should be designed in accordance with the inferences we have reached.

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