Urolithiasis (2017) 45:545–552
DOI 10.1007/s00240-016-0941-2

ORIGINAL PAPER

Systemic endothelial function measured by flow-mediated dilation is impaired in patients with urolithiasis

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Received: 17 June 2016 / Accepted: 10 November 2016 / Published online: 23 November 2016

Abstract Some in vitro and animal studies have shown endothelial dysfunction in hyperoxaluria models indicating its role in pathogenesis of urolithiasis and relation to CVD. The aim of this study was to investigate endothelial function in patients with urolithiasis in relation to urinary stone risk factors and metabolic parameters. A total of 120 subjects without any known CVD (60 with urolithiasis and 60 healthy subjects) were included into study. Fasting blood and 24-h urine samples were collected to study metabolic parameters (glucose and lipids) and urine stone risk factors (oxalate, citrate, uric acid, and calcium, pH). Endothelial function was assessed as flow-mediated dilation (FMD) at the brachial artery. Age, sex, and body mass index were similar in patients and controls. Of urine stone risk factors, oxalate and citrate were higher in patients than controls. Fasting blood glucose, total LDL cholesterol, and triglyceride were higher, and HDL cholesterol was lower in patients than controls. Although within normal limits systolic blood pressure was higher in patient group, patients with urolithiasis had a lower %FMD than controls. Percent FMD was negatively correlated with urinary oxalate/creatinine ratio ($p = 0.019$, $r = -0.315$), calcium/creatinine ratio ($p = 0.0001$, $r = -0.505$) age ($p < 0.001$, $r = -0.694$), BMI ($p < 0.001$, $r = -0.838$), total cholesterol ($p < 0.001$, $r = -0.559$), and triglyceride ($p < 0.001$, $r = -0.529$). Urine oxalate/creatinine ratio was positively correlated with age ($p = 0.01$, $r = 0.327$) and calcium/creatinine ratio with BMI ($p = 0.001$, $r = 0.410$). This is the first study demonstrating endothelial dysfunction in human subjects with urolithiasis. This indicates a possible predictive role of urolithiasis in future development of cardiovascular diseases.

Keywords Urolithiasis · Endothelial dysfunction · Flow-mediated dilation · Cardiovascular risk

Introduction

Urolithiasis is a common disease with an increasing in prevalence throughout the world [1]. Studies have well-documented close association of urolithiasis to certain systemic disorders, such as obesity, diabetes mellitus, metabolic syndrome, and hypertension all of which are characterized by increased cardiovascular risk [2–5]. Urolithiasis by itself was also found to be associated with increased risk of cardiovascular diseases. It was reported in a longitudinal study that myocardial infarction risk was increased by 31% in patients with a history of kidney stones [6] and increased cardiovascular disease and mortality risk in patients with calcium oxalate stone disease in another study [7].

Endothelial dysfunction is the early key element in the development of atherosclerotic cardiovascular diseases [8]. It is widely used to show increase in cardiovascular risk

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in various diseases. Endothelial dysfunction was also proposed in the pathogenesis of urolithiasis. Animal model and cell culture studies demonstrated that hypeoxaluria causes local and systemic endothelial dysfunction which results with proximal renal tubular cell apoptosis [9, 10]. We studied circulating endothelial cells which was also accepted as a reflection of endothelial dysfunction and found to be increased in a rat model of hyperoxaluria (unpublished data). There is no human study on this subject.

The primary aim of this study is to investigate endothelial function in patients with urolithiasis in comparison with healthy subjects. The secondary aims were to study correlation of endothelial dysfunction with urine stone risk factors and metabolic parameters.

Materials and methods

Subjects

Patients, aged 20–65 years were recruited from the outpatient urology clinic at the Yeditepe University Hospital. Sixty patients with the diagnosis of calcium oxalate urolithiasis were included in the study. All patients were evaluated and treated appropriately for their urinary calculi.

Each subject was screened by clinical history, thorough physical examination, and routine chemical analysis for the evidence of any disease. Subjects with any history or evidence of metabolic, cardiovascular, respiratory, hepatic, renal, endocrine, or any other chronic disease and pregnancy were excluded. Patients on treatment with drugs that can directly or indirectly affect endothelial function or calcium metabolism were not also included in the study.

The controls were 60 healthy age and sex-matched subjects recruited among 150 individuals who visited our hospital for an annual check-up and had not been observed any acute or chronic systemic diseases. Each subject was screened by clinical history, thorough physical examination, and routine chemical analysis for the evidence of any disease. Subjects with history or evidence of any chronic systemic disease, pregnancy or a history of heavy smoking, drug, or alcohol abuse were excluded. Subjects taking or on treatment with any drug that can interfere with study parameters prior to the start of study were also not included. None of the subjects had a history of urinary stone disease. Abdominal ultrasonography which is a normal part of check-up program did not reveal urinary stone in any of the control subjects. They had no history of predisposing diseases for renal stone formation.

This study was conducted in accordance with the guidelines proposed in the Declaration of Helsinki and was approved by the ethical committee of Yeditepe University. All subjects gave informed consent before entrance into the study.

Study design

After anthropometric measurements and collection of demographic data, blood samples were collected on 12-h of fasting, 24-h urine samples were collected as described in the following, and ultrasonographic measurements were done for endothelial function.

Anthropometric measurements

Body weight and height were measured with the patient in light dress. Body mass index (BMI) was calculated as body weight divided by square of the height.

Assays

Serum glucose levels were analyzed with the glucoxidase method (Roche Diagnostics GmbH, Mannheim, Germany). Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels were measured by enzymatic colorimetric assays (Roche Diagnostics GmbH, Mannheim, Germany). High-sensitivity C-reactive protein (hsCRP) levels were measured by an immunoturbidimetric assay (Roche Diagnostics GmbH, Mannheim, Germany). Insulin concentrations were quantified with an immulite analyzer using an immunometric method (DPC, Los Angeles, CA). Insulin resistance was detected using homeostasis model assessment of insulin resistance (HOMA-IR) which was calculated with the known formula of HOMA-IR = plasma glucose × plasma insulin/22.5.

The risk factors for renal stone disease

Stone-forming risk factors have been assessed in 24-h collected urine of each patient in specially prepared bottles obtained from the laboratory. Urine collections were preserved with thymol in isopropanol and kept refrigerated at 4 °C during collection. Samples were acidified to adjust the pH to 1.5–2 with 6 M HCl (approximately 10 ml HCl/24-h sample based on normal adult output of 1000–2000 ml/24 h) to ensure complete dissolution of any CaO*x* crystals formed and to prevent spontaneous conversion of ascorbate into oxalate and kept below −20 °C until evaluation. The analysis of the urine specimen included the assessment of the urinary risk factors, namely, oxalate, calcium, and citrate. Urinary citrate was assessed enzymatically (BEN-Biochemical Enterprise S.r.l., Milano, Italy); urinary oxalate was determined by ion chromatography

(BEN-Biochemical Enterprise S.r.l., Milano, Italy); and calcium using atomic absorption method (Roche Diagnostics GmbH, Mannheim, Germany). To ensure complete urine collection in an individual basis, daily urine collection was based on the normal range of creatinine contents of urines in men and women. No specific diet program was ordered to patients prior to urine collection. The 24-h urine output values were recorded in each patient.

Endothelial function

Endothelial function was assessed with high-resolution B-mode Doppler ultrasonography (GE Logic 700 with an 8.5-Hz linear-array transducer) by examination of the brachial artery using the protocol described by Celermajer et al. by a blinded radiologist [11]. All subjects were studied between 8 am and 11 am after a 12-h overnight fast. They were asked not to smoke on the morning of study day and to avoid alcohol for 48 h. The brachial artery was scanned longitudinally 5–15 cm above the antecubital fossa. A blood pressure cuff was then inflated around the forearm to suprasystolic level for 5 min. Measurement of the maximal postischemic diameter was taken 45–60 s after cuff release. After 20 min, further measurement was taken at rest and at 4 min after sublingual spray of 400 μg of GTN. All measurements were taken at the end of diastole coincident with the R-wave on an electrocardiogram (ECG) monitor. The difference in lumen diameter between rest and reactive hyperemia, expressed as percent change was regarded as endothelium-dependent (%FMD), and GTN% as endothelium-independent vasodilatation.

Ten-year risk for development of coronary heart disease

The Framingham risk-scoring table was used for calculating the 10-year risk for developing coronary heart disease [12]. The risk factors included into the scale were age, TC, HDL-C, systolic blood pressure (SBP), hypertension whether treated or not, and cigarette smoking. The total score was yielded with the sum of points for each risk factor using the gender-specific tables. The risk of the probability of experiencing a cardiac event over a 10-year period was defined in terms of three categories (low <10%, intermediate 10–20%, and high >20%).

Statistical analysis

Statistical analysis was performed using the SPSS software package (version 15.0). Differences between groups were tested by student's t test. Correlation analysis was performed by the Pearson test. Multiple linear regression analysis was performed to determine independent predictors of %FMD. Level of significance was set at $p < 0.05$. Data were shown as mean ± standard deviation (SD).

Results

Baseline characteristics

Demographic characteristics of study groups are shown in Table 1. Patients and control subjects were age and sex matched. Majority of the patients were young and below the age of 45 years. Men dominated the study with a 2/3 preponderance. Anthropometric measurements were similar in both groups. Maximum body mass index in study group was 29.3 kg/m^2, but two patients in control group had a body mass index above 30 kg/m^2. Less than a quarter of subjects in both group were smoker. There were no differences in any parameters between men and women.

Cardiovascular risk factors

Cardiovascular risk factors were presented in Table 2. Patients with urolithiasis had higher TC ($p < 0.0001$), lower HDL-C ($p = 0.002$), higher triglyceride ($p < 0.0001$), and higher LDL-C ($p < 0.0001$). Although normoglycemic in whole study group, blood glucose was higher in patients with urolithiasis compared to control subjects ($p < 0.0001$). Fasting serum insulin level was not different between groups. HOMA-IR was again higher but within normal ranges in study group compared to controls. Serum hsCRP levels were higher in patients with urolithiasis ($p < 0.0001$). There was no difference either in systolic or diastolic blood pressure between groups. Ten-year cardiovascular risk calculated by Framingham risk score tables indicated low risk

Table 1 Demographic features of study subjects

	Patients	Controls	p
Age (years)	37 ± 8	37 ± 6	ns
Sex (female/male)	20/40	20/40	ns
Height (cm)	167.5 ± 5.5	165.4 ± 7.9	ns
Weight (kg)	74.3 ± 8.3	72.6 ± 8.9	ns
BMI (kg/m^2)	26.4 ± 2.1	26.4 ± 2.1	ns
Waist circumference (cm)	92.3 ± 10.7	91.0 ± 8.7	ns
Hip circumference (cm)	109.8 ± 11.1	109.1 ± 8.1	ns
Waist/Hip ratio	0.84 ± 0.06	0.83 ± 0.07	ns
Smoking (%)	20	23.3	ns
Systolic blood pressure (mmHg)	123.9 ± 15.6	120.6 ± 11.3	ns
Diastolic blood pressure (mmHg)	75.9 ± 15.6	72.8 ± 8.3	ns
Framingham risk score	5.6 ± 6.7	1.75 ± 1.66	<0.0001

Table 2 Laboratory measurements of study subjects

	Patients	Controls	*p*
Glucose (mg/dl)	91.6 ± 6.4	82.4 ± 9.2	<0.0001
Insulin	6.1 ± 1.9	5.5 ± 2.8	ns
HOMA-IR	1.3 ± 0.4	1.1 ± 0.6	0.013
Total cholesterol (mg/dl)	229.1 ± 41.7	184.6 ± 32.3	<0.0001
Triglyceride (mg/dl)	163.7 ± 45.3	131.1 ± 58.2	0.0009
HDL cholesterol (mg/dl)	43.3 ± 8.6	49.6 ± 13.0	0.0026
LDL cholesterol (mg/dl)	153.0 ± 36.5	108.8 ± 28.6	<0.0001
hsCRP (mg/l)	2.7 ± 1.5	1.5 ± 0.8	<0.0001
Urine volume (ml)	2514 ± 1060	2539 ± 930.8	ns
Urine creatinine	1399 ± 489.1	1481 ± 291.1	ns
Urine calcium/creatinine	0.15 ± 0.07	0.08 ± 0.03	<0.0001
Urine oxalate/creatinine	0.02 ± 0.01	0.01 ± 0.00	<0.0001
Urine uric acid/creatinine	0.36 ± 0.12	0.34 ± 0.12	ns
Urine citrate/creatinine	0.28 ± 0.26	0.20 ± 0.15	0.04
Urine pH	5.6 ± 0.74	5.4 ± 0.6	ns

(<10%) in both study and control subjects but was statistically higher in patients with urolithiasis ($p < 0.0001$). There were no differences in any parameters between men and women.

Urinary stone risk factors

Urine stone risk factors were presented in Table 2. Twenty-four hour urine volume, creatinine, and pH were similar between study group and controls and between women and men. Both 24-h urine calcium excretion and calcium/creatinine ratio were higher in patients with urolithiasis compared to healthy controls and women than men ($p < 0.0001$ and $p = 0.01$, respectively). Similarly, 24-h urine oxalate excretion and oxalate/creatinine ratio were also higher in study group compared to control group and women compared to men ($p < 0.0001$ and $p = 0.002$, respectively). On the other hand, there were no difference in 24-h urine excretion of uric acid and citrate and uric acid/creatinine ratio. Urine citrate/creatinine ratio was higher in patients with urolithiasis compared to control subjects.

Endothelial function

Parameters about endothelial function were demonstrated in Table 3. All the parameters except percent increase in lumen diameter after glyceryltrinitrate were lower in patients with urolithiasis. Basal lumen diameter ($p = 0.004$), final diameter ($p < 0.0001$), and percent increase in flow-mediated dilatation ($p < 0.0001$) were higher in controls than patients. There was no difference between women and men.

Table 3 Endothelial function parameters of study subjects

	Patients	Controls	*p*
Basal lumen diameter (mm)	3.09 ± 0.54	3.30 ± 0.50	0.0046
Lumen diameter after FMD	3.26 ± 0.52	3.70 ± 0.50	<0.0001
FMD (% increase)	6.49 ± 3.52	10.50 ± 5.10	<0.0001
NTG (% increase)	19.7 ± 8.77	21.30 ± 13.90	ns

FMD flow-mediated dilation, *GTN* glyceryl trinitrate

Correlations

Endothelial function

Percent FMD was negatively correlated with age ($p < 0.0001$, $r = -0.694$), body weight ($p < 0.0001$, $r = -0.649$), BMI ($p < 0.0001$, $r = -0.838$), TC ($p < 0.0001$, $r = -0.559$), TG ($p < 0.0001$, $r = -0.529$), and LDL-C ($p < 0.0001$, $r = -0.511$). There were no correlations between FMD and fasting blood glucose, fasting serum insulin, and HOMA-IR. In addition, neither systolic nor diastolic blood pressure had any correlation with FMD. Similarly, there was no significant correlation between FMD and 10-year cardiovascular disease risk measured by Framingham risk score tables.

Among urinary stone risk factors, 24-h urine oxalate excretion ($p = 0.005$, $r = -0.360$), oxalate/creatinine ratio ($p = 0.019$, $r = -0.315$), 24-h urine calcium excretion ($p < 0.0001$, $r = -0.559$), and calcium/creatinine ratio ($p = 0.0001$, $r = -0.505$) were correlated with %FMD (Fig. 1). There were no correlations between %FMD and 24-h urine excretion of uric acid and citrate, uric acid/creatinine ratio, and citrate/creatinine ratio and urine pH.

Urinary stone risk factors

Urine calcium excretion (calcium/creatinine ratio) was positively correlated with age ($p = 0.02$, $r = 0.282$), body weight ($p = 0.02$, $r = 0.296$), and BMI ($p = 0.001$, $r = 0.410$). It was also positively correlated with urine oxalate/creatinine ratio ($p < 0.0001$, $r = 0.553$).

Age was the only demographic parameter positively correlated with urine oxalate excretion (oxalate/creatinine ratio) ($p = 0.01$, $r = 0.327$). Among other urinary stone risk factors, calcium/creatinine ratio ($p < 0.001$, $r = 0.553$), uric acid/creatinine ratio ($p = 0.03$, $r = 0.274$), and oxalate/creatinine ratio ($p = 0.04$, $r = 0.264$) correlated with urine oxalate excretion. There was also a correlation between hsCRP ($p = 0.02$, $r = 0.282$) and oxalate excretion and %FMD ($p = 0.02$, $r = -0.298$).

Urine citrate and uric acid excretion rate did not have significant correlations. They only correlated with urine

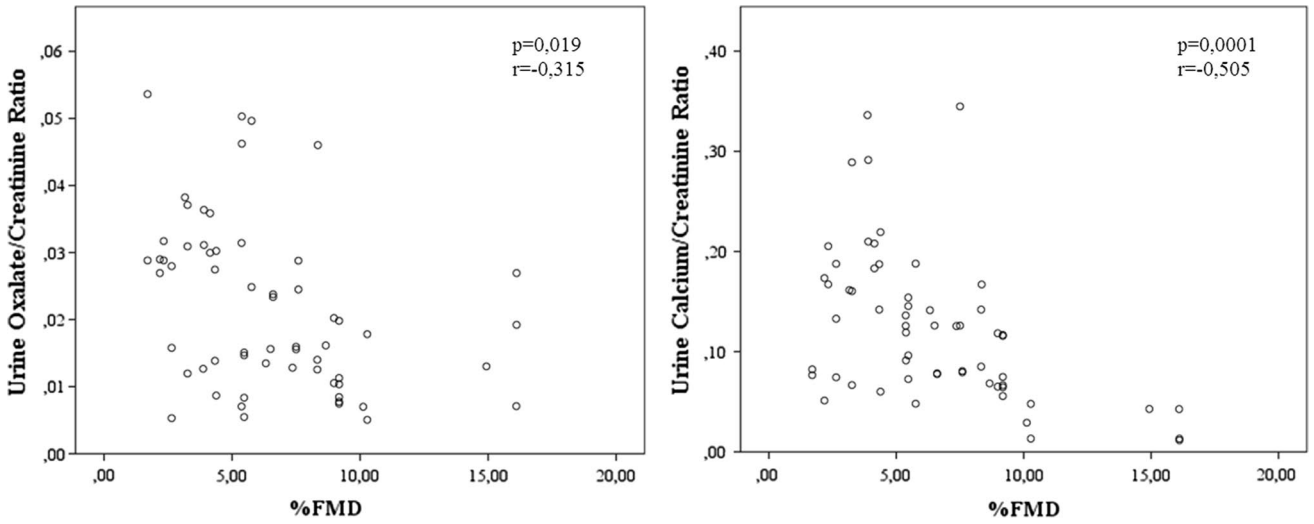

Fig. 1 Urine oxalate and calcium excretion is negatively correlated with %FMD

oxalate/creatinine ratio ($p = 0.04$, $r = 0.264$, and $p = 0.03$, $r = 0.274$, respectively).

Urine pH did not have any significant correlations with any of the study parameters.

Ten-year cardiovascular risk assessed by Framingham risk score tables was only correlated with hsCRP ($p = 0.01$, $r = -0.303$).

A stepwise multiple linear regression analysis was performed to assess the independent determinants of %FMD in patients with urolithiasis. Data were presented in Table 4. %FMD was selected as dependent variables in the model. Among independent variables, age ($p < 0.0001$, $R^2 = 0.473$), body weight ($p = 0.003$, $R^2 = 0.542$), BMI ($p = 0.005$, $R^2 = 0.732$), triglyceride ($p = 0.0001$, $R^2 = 0.769$), systolic blood pressure ($p = 0.03$, $R^2 = 0.783$), and urine calcium excretion rate ($p = 0.01$, $R^2 = 0.801$) were the determinants of FMD in patients with urolithiasis.

Discussion

The results of this study showed that the presence of urolithiasis was associated with endothelial dysfunction and related to some of the urinary stone risk factors. After controlling confounding factors, the association persisted. These findings are in accordance with previous report of Glybochko et al. which they reported an endothelial dysfunction in surgical urolithiasis patients [1].

Endothelial dysfunction is a systemic pathological state of the endothelium resulting from some systemic disorders or local factors produced by or acting on endothelium [3]. Oxidative stress is the initiating event altering the endothelial cells capacity to maintain homeostasis and leads to the development of endothelial dysfunction [4, 13]. Several disease processes, such as hypertension, hyperlipidemia, diabetes mellitus and inflammatory, or infectious diseases or environmental factors, such as smoking, and air pollution, can result in endothelial dysfunction [5, 14]. It is widely accepted that endothelial dysfunction is an early key element in the pathophysiology of atherosclerosis and cardiovascular disease states [6, 15]. Testing for endothelial function was proposed to have role in prediction of future atherosclerotic diseases, such as coronary artery disease or stroke [8, 16].

Urolithiasis is closely linked to atherosclerotic conditions. Coronary artery disease was more prevalent among urolithiasis patients [17]. Both past history and presence of urolithiasis carry high risk of developing coronary heart disease [18]. Patients with prior history of kidney stones had 14–43% increase in risk of coronary heart disease [6], and Rule et al. reported an increased risk for myocardial infarction in kidney stone formers in a 9-year prospective study [17]. Atherosclerosis prone diseases, such as diabetes mellitus, hypertension, hyperlipidemia, and metabolic syndrome, were frequently accompanied by urolithiasis in the population [2–5]. All these disorders are characterized by endothelial dysfunction. In urolithiasis, there is only one study up to now documenting relationship of endothelial dysfunction in patients with urolithiasis [19]. Only article published was tested circulating endothelial dysfunction markers, such as endothelin-1, interleukin-6, and vascular endothelial growth factor, in urolithiasis patients just after shockwave lithotripsy followed by surgery and found them to be increased with a recovery after surgery [19]. However, it is not obvious in this study whether increase in these cytokine and growth factor levels was due to lithotripsy and surgery or urolithiasis by itself. Recovery after

Table 4 Stepwise multiple regression analysis for variables with the %FMD in patients with urolithiasis

	*B**	Beta†	95% CI	*p*
Independent variables				
Age	−0.473	0.473	−0.347 to −0.198	<0.0001
Body weight	−0.353	0.542	−0.244 to 0.053	<0.0001
BMI	−1.400	0.697	−1.640 to −1.160	<0.0001
Systolic blood pressure	0.033	0.734	0.007 to 0.065	0.015
Triglyceride	−0.016	0.717	−0.028 to −0.004	<0.0001
Urine calcium/creatinine	6.851	0.754	0.180 to 12.982	<0.0001
Excluded variables				
Sex		0.051		0.473
Total cholesterol		−0.126		0.121
HDL cholesterol		0.007		0.924
Smoking		−0.006		0.938
Framingham risk score		−0.024		0.731
Glucose		0.015		0.828
Insulin		0.068		0.374
HOMA-IR		0.059		0.434
hsCRP		0.043		0.933
Urine pH		−0.063		0.346
Urine volume		0.011		0.873
Urine creatinine		−0.068		0.314
Urine oxalate/creatinine		−0.069		0.360
Urine uric acid/creatinine		0.061		0.358
Urine citrate/creatinine		0.046		0.497

Multiple coefficient of determination (R^2) = 0.175, $p = 0.021$

* Unstandardized regression coefficient

† Standardized coefficient

surgery makes to think that it was the result of intervention not the disease state.

There are some studies in the literature reporting a cause and effect relationship between endothelial dysfunction and urolithiasis. First study was on animals showing that induction of hyperoxaluria by ethylene glycol resulted in local (renal tissue) and systemic increase in asymmetrical dimethylarginine which is a marker for endothelial dysfunction [9]. Another experimental study had shown more direct relationship. In this cell culture study, hyperoxlauric condition was created with a minimal toxic effect on renal proximal tubular epithelial cells and human umbilical endothelial cells. Co-culture of both cells caused further apoptosis of renal proximal tubular epithelial cells which was prevented by endothelial function preservative, pyrrolidine dithiocarbamate [10]. The results indicated the role of endothelial dysfunction on the development of renal tubular cell apoptosis which is accepted as an initial step in the development of urolithiasis. To further explain the relationship, we did another animal study in which hyperoxaluria induced by ethylene glycol caused an increase in circulating endothelial cell count which is another indicator of endothelial dysfunction (unpublished data). Finally, Taylor et al. proposed a vascular theory in the development of kidney stones. According to hypothesis, event initially takes place in vascular area such that vascular insult causes development of Randall's plaque which is a nidus for stone development. Renal papilla's vasculatures proneness to hypoxia, hyperosmolar surroundings, and turbulent blood flow is thought to be the main reason [20]. On the other hand, similarity between vascular calcification and urolithiasis is another clue for the participation of vascular tissue to stone development [21]. However, the exact pathophysiological mechanism could not be explained with known literature. In this study for the first time, we have shown that endothelial dysfunction is present in patients with urolithiasis. The findings may indicate that urolithiasis is a systemic disorder causing atherosclerosis prone environment in whole body.

There are different methods to measure endothelium functional capacity [22]. Percent increase in FMD is the most accurate one [23]. The method was studied in various atherosclerotic conditions or disease states [24]. This is the first study on determination of endothelial dysfunction by %FDM in urolithiasis. Results indicated dysfunctional endothelium in urolithiasis in a subgroup of patients with low cardiovascular risk. Since endothelial dysfunction is accepted as increase in cardiovascular risk, the results of this study are in accordance with previous studies with increased cardiovascular risk in urolithiasis [6, 17, 18].

The mechanism underlying endothelial dysfunction in urolithiasis is not obvious yet. Vascular hypothesis might be one of the explanations. Studies found that history of kidney stones has been independently associated with coronary artery disease even after multivariable analysis for risk factors [6, 17, 18]. This raised the idea that kidney stones may be the manifestation of a systemic disorder [25]. Frequent association of stone formation in patients with cardiovascular risk factors, such as diabetes mellitus, hypertension, obesity, and hyperlipidemia, is supportive. The interesting question would be whether urolithiasis is a subcomponent of metabolic syndrome or not which has to be searched further. We did a subanalysis to our patient population and found a non-significant number of patients that fulfill metabolic syndrome criteria. However, subcomponents of MetS were found to be associated with percent FMD, such as lipid parameters and systolic blood pressure. Our findings also support urolithiasis to be a systemic disorder. Insulin resistance is the core component of metabolic

syndrome. Schwille et al. found an association between postprandial insulinemia and increased urinary calcium and phosphorus excretion in patients with kidney stones [26]. However, we did not detect any significant correlation between stone-forming risk factors and either plasma insulin level or insulin resistance index. This may be due to selection of our study population, since none of the subject had a real high insulin resistance index and none of the patients were obese. Another finding that supports urolithiasis to be a systemic disorder is lack of effective calcification inhibitors in calcium oxalate stone formers which was also found to be a mechanism in coronary artery calcification [27, 28]. Pyrophosphates are the inhibitors of calcification and found both in blood and urine [29]. The deficiency of pyrophosphates which was reported both in urolithiasis and CAD might help to explain the association between CAD and kidney stone formation. We did not measure calcification inhibitors in our study population, but it would be an interesting finding.

Association between stone formation and development of cardiovascular diseases or equivalents may be the result of certain common pathophysiological features. Cardiovascular risk factors exert their deleterious effects of vascular system through oxidative stress and vascular inflammation [30, 31]. Similarly, both of these processes play also role in the development of kidney stones [32–35]. It is well documented in tissue culture and animal model studies that the interaction between CaOx crystals and renal epithelial cells produces reactive oxygen species and clear evidence was also handled from clinical studies which showed increased oxidative stress markers in kidney stone formers [36]. Association of endothelial dysfunction with low-grade inflammation and oxidant stress was also extensively studied. Decreased synthesis, release, or bioactivity of nitric oxide by any predisposing factor either as in these two conditions by cardiovascular risk factors or urolithiasis results in dysfunctional endothelium [37]. Vascular NO bioavailability and increased oxidative stress were supposed to be mediated by inflammation-induced widespread endothelial dysfunction [38]. Considering previous studies in urolithiasis it can be speculated here that stone formation starts in the systemic circulation which goes in two ways: local effect on renal tissue results in stone formation through vascular insult and renal tubular cell ischemia causing development of Randall's plaque and stone nidus. Systemically, it triggers many cascades which we know little up to now and results in atherosclerosis and cardiovascular diseases. It is also not obvious whether urolithiasis is an isolated systemic disorder or part of a syndrome. Studies until now indicate it to be part of a systemic syndrome and may be metabolic syndrome.

Although the results of this study indicated endothelial dysfunction, there are some limitations in this study. First, study population included only low-risk young patients. It might be better to choose a wide range of patients with low and high cardiovascular risk. Second, study population was mostly men. The number of women is not enough to make an inference. However, this was because of randomization not a direct selection. Instead, certain number of men and women could be included.

In conclusion, this is the first study demonstrating endothelial dysfunction in patients with calcium oxalate stone-forming patients with low cardiovascular risk. The results are important on two aspects: first, it may help to explain systemic effects of co-morbidities of stone disease and second, help to explain previous studies on pathogenesis which indicated endothelial dysfunction in stone formation. Further studies will clarify these findings.

Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interests. There is no financial support to disclose here.

Research involving human participants and/or animals This study was conducted in accordance with the guidelines proposed in the Declaration of Helsinki and was approved by the ethical committee of Yeditepe University.

Informed consent All subjects gave informed consent before entrance into the study.

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