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Metabolic Evaluation of Urolithiasis Patients from Eastern Croatia

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A B S T R A C T

Metabolic parameters were determined in fasting blood serum, fasting first morning urine, and 24-hour urine of male patients with recurrent calcium oxalate stones (N=26, age 39.1±6.2 years) as well as in male healthy controls (N=18, age 35.0±7.1 years), recruited from the eastern part of Croatia. The 24-hour urinary calcium excretion was significantly higher ($p<0.01$) for patients (5.6±2.5 mmol) than for controls (3.7±1.9 mmol), but potassium excretion was higher ($p<0.01$) for controls (74.5±33.8 mmol) than for patients (49.2±15.7 mmol). The mean ionic activity product of calcium and oxalate ions, IAP(CaOx), calculated from the fasting first morning urine parameters, was 25% higher for patients than for controls, but the difference was not statistically significant ($p>0.05$). Very strong correlation ($r=0.97$) was obtained between IAP(CaOx) values and calculated Ogawa indices that were recommended for estimating the potential risk for calcium oxalate stone formation.

Key words: *metabolic parameters, urine, calcium oxalate, urolithiasis, hypercalciuria, hyperoxaluria*

Introduction

The formation of urinary stones may be caused by different factors, i.e., metabolic disorders, anatomic abnormalities, infections, and/or environmental factors. Risk factors for stone formation also include[1] a positive family history, nutritio-

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nal factors (i.e., excessive intake of animal proteins, fat, sugar, oxalates, alcohol, caffeine, salt, and vitamin D), nutritional deficiencies (i.e., water, magnesium, calcium, and potassium), lifestyle factors, and associated diseases (i.e., parathyroid problems, osteoporosis, gout). Recurrent stone formation can be expected in about 50 % of patients[2]. Investigations in the field of urolithiasis have revealed that the majority of patients with urolithiasis had some metabolic disorder[3–9]. The extent of the diagnostic procedures for patients with urolithiasis depends mostly on the recurrence rate and stone-related complications.

The major mineral component of calcium containing urinary stones is calcium oxalate monohydrate. The main risk factors for calcium oxalate urolithiasis are high urinary supersaturation (i.e. high ionic activity product of calcium and oxalate ions) and/or insufficient amounts of ions (i.e., magnesium and citrate) and/or macromolecules (i.e., calcium binding proteins, glycosaminoglycans, osteopontine, nephrocalcine-A, etc.) that inhibit precipitation of calcium oxalates[10–13]. Recently, a potent inhibitor (molecular mass between 14.2 and 16.2 kDa), which is closely associated with a chromophore resembling urobilirubin, was identified and it was suggested that this compound could be primarily responsible for high urinary inhibitory activity[14]. In addition, several oxalate specific binding proteins that could either inhibit or promote nucleation, growth, and aggregation of calcium oxalate crystals, were recently identified in the human kidney[15].

Determination of factors responsible for the formation of urinary stones is a prerequisite for efficient medical treatment and prevention of recurrent stone formation. Typical metabolic disorders for adult calcium oxalate stone-formers are urinary hypercalciuria, hyperoxaluria, hypocitraturia, and a low urine volume[5–10]. More over, hypercalciuria was predominant cause in pediatric urolithiasis in Croatia[9]. The recurrence of calcium oxalate stones can be prevented by a high fluid intake, appropriate diet, and pharmacological therapy[16–18]. Recently, phytotherapy (treatment with medicinal plants) was also proposed as a supplement to the conventional therapy[19].

In this paper, the relevant biochemical parameters, as determined in blood serum and in urines of patients with recurrent calcium oxalate urinary stones (26 patients) and healthy individuals (18 controls) from the eastern part of Croatia, are compared and evaluated. Additional parameters (activity product of calcium and oxalate ions, different risk indices) that could indicate a potential risk for calcium oxalate stone formation are calculated and correlated. The inhibitory role of citrate and magnesium ions on calcium oxalate formation is discussed.

Subjects and Methods

The group of patients comprised 26 male stone-formers (mean age 39.1±6.2 years) with recurrent calcium oxalate urolithiasis. Six patients stated, to the best of their knowledge, that they have a family history of stone formation. Urolithiasis was identified by ultrasonography, abdominal X-rays, and by the history of stone passage. Three patients had bilateral urolithiasis. All patients had experienced two or more renal colics with spontaneous elimination of the stone. Eighteen patients were treated earlier by extracorporeal shock wave lithotripsy (ESWL) or percutaneous nephrolithotomy. At the time of the study, two patients had symptomatic renal stones. Patients did not receive medical therapy for stone prevention during the study. The composition of stones was determined by infrared (IR) spectrophotometry (Perkin Elmer Spectrophotometer, Model 882).

The control group comprised 18 healthy male subjects (mean age 35.0±7.1 years) recruited from the hospital staff, who had no history of stone formation or renal diseases. No additional diagnostic procedure was performed to confirm that they did not have renal stones. All subjects gave informed consent to participate in the study, which was approved by the Ethical Committee of the Institute for Medical Research, Zagreb, Croatia.

Estimation of calcium intakes was based on two-day records of food consumption prior to urine collection. Daily calcium intakes were calculated using food composition tables for raw, cooked and local dishes[20]. Recorded intakes were in the range of recommended daily allowances according to age and sex, which are valid in Croatia[21].

The fasting blood and fasting first morning urine samples were collected in the morning (6 a.m. after 12 hours overnight fast). During the 24-h urine collection, the subjects were on their usual diet.

The serum concentrations of creatinine, urea, alkaline phosphatase, calcium, phosphorus, magnesium, sodium, potassium, chloride, cholesterol, triglycerides, proteins, and urate were determined by autoanalyzer (Hitachi 737, Boehringer, Mannheim, Germany) equipped with ion selective electrodes.

The urinary concentrations of calcium, magnesium, phosphorus, urate, creatinine, and citrate[22] were determined spectrophotometrically (autoanalyzer Hitachi 737) using Boehringer kits, and concentration of oxalate[23] using Sigma Diagnostic kit. Urinary pH was determined by glass/calomel electrode (GK 2401 C) connected to a pH meter (all Radiometer, Copenhagen, Denmark), sodium and potassium concentrations by flame photometry (Instrument Laboratory 943, Italy) and chloride concentration by coulometric titration (Chloride Titrator CTM3, Radiometer). The urinary concentration of urea was determined by enzymatic UV method using glutamate dehydrogenase, and concentration of proteins by turbidimetric method using benzoate chloride. Urinary infection was detected by microscopical examination of urinary sediments.

The parameters for the group of patients and the group of controls were expressed as mean value ± standard deviation. The statistical significance of differences in mean values of parameters for patients and controls were tested with the Student's t-test. Values of $p<0.05$ were considered statistically significant. In addition, the non-parametric Mann-Whitney test was used for comparing urine parameters in two groups. Contrary to the parametric t-test, the Mann-Whitney test does not assume that the difference between two independent populations is normally distributed.

The ionic activity product (IAP) of calcium (Ca) and oxalate (Ox) ions calculated by computer program EQUIL[24] from metabolic parameters determined in fasting first morning urine was defined as

$$\mathrm{IAP(CaOx)} = a(\mathrm{Ca^{2+}}) \times a(\mathrm{Ox^{2-}}) \qquad (1)$$

where $a(\mathrm{Ca^{2+}})$ and $a(\mathrm{Ox^{2-}})$ are ionic activities of calcium and oxalate, respectively. Since the chloride ion concentration (important for the calculation of urinary ionic strength) was not experimentally determined, the calculations were performed in two steps. In the first step, the speciation and the ionic charge balance in the urine of each subject were calculated by EQUIL using the experimentally determined parameters listed in Table 4. The calculated value of the charge balance was positive, and so the chloride ion concentration was estimated as the equivalent amount of negatively charged ions required for charge compensation. In the second step, the speciation and IAP(CaOx) were calculated including the chloride

concentration as additional input parameter.

Three differently defined indices, the AP(CaOx)-index and CaOx-risk-index (RI) proposed by Tiselius[25–27], as well as the AP(CaOx)-EQ2-index proposed by Ogawa[28,29] were calculated for each patient and control from the parameters determined in the fasting urine specimens. These indices are essentially simplified estimations of the ionic activity product values, IAP(CaOx), and they could indicate the risk of calcium oxalate stone formation. Tiselius AP(CaOx)-indices[25–27], defined for different urine collection times, require the following five parameters: contents of calcium (Ca), oxalate (Ox), magnesium (Mg), citrate (Cit), and total volume (V) determined at corresponding collection time intervals.

The Tiselius AP(CaOx)-index[26] for the 6.5-h urinary excretion (the first morning urine) was defined as

$$\text{AP(CaOx)-index} = 5.632 \times \text{Ca}^{0.71} \times \times \text{Ox} \times \text{Mg}^{-0.14} \times \text{Cit}^{-0.10} \times \text{V}^{-1.2} \quad (2)$$

where the amounts of Ca, Ox, Mg, and Cit were expressed in mmol, and the urine volume (V) was expressed in liters.

The Tiselius CaOx-risk-index[25] (RI), which requires the concentration of creatinine (Cre) instead of the urine volume, was defined as

$$\text{RI} = (\text{Ca/Cre})^{0.71} \times (\text{Ox/Cre}) \times \times (\text{Mg/Cre})^{-0.14} \times (\text{Cit/Cre})^{-0.10} \quad (3)$$

where Ca, Ox, Mg, and Cit were expressed in mmol and Cre in mol per liter.

The Ogawa AP(CaOx)-EQ2-index[28,29] that requires only four parameters (concentrations of Ca, Ox, Mg, and Cit), was defined as

$$\text{AP(CaOx)-EQ2-index} = 1.496 \times 10^{-8} \times \text{Ca}^{0.78} \times \text{Mg}^{-0.30} \times \text{Ox}^{0.91} \times \text{Cit}^{-0.17} \quad (4)$$

where Ca, Mg, Ox, and Cit concentrations were expressed in mmol/L.

Results

The composition of stones determined by infrared spectrophotometry is listed in Table 1. Eleven patients had pure calcium oxalate (CaOx) stones (calcium oxalate monohydrate (COM), $CaC_2O_4 \cdot H_2O$, and/or calcium oxalate dihydrate (COD), $CaC_2O_4 \cdot 2H_2O$) and fifteen patients had mixed oxalate/phosphate stones with CaOx (COM and/or COD) as major components and apatitic calcium phosphates (Ap) as minor components. The composition and structure of apatitic calcium phosphates (Ap) is similar to stoichiometric calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) in which different ions (i.e., HPO_4^{2-}, CO_3^{2-}, F^-, Mg^{2+}, Na^+) are structurally incorporated.

Patients and controls did not differ significantly in age and anthropometric

TABLE 1
COMPOSITION OF URINARY STONES FOR UROLITHIASIS PATIENTS (N=26)

Stone type	Number (N)	% of total N=26
Calcium oxalate monohydrate (COM)	5	19
COM + Calcium oxalate dihydrate (COD)	6	23
Sum I: Oxalate stones	11	42
COM + Apatitic calcium phosphates (Ap)	9	35
COM + COD + Ap	6	23
Sum II: Mixed oxalate and apatite stones	15	58
Total sum = Sum I + Sum II	26	100

TABLE 2
AGE, ANTHROPOMETRIC PARAMETERS, AND DAILY DIETARY INTAKE OF CALCIUM FOR UROLITHIASIS PATIENTS (N=26) AND CONTROLS (N=18)

Parameter	Patients mean±SD	Controls mean±SD
Age (years)	39.1±6.2	35.0±7.1
Height (cm)	174.5±5.7	180.5±5.8
Weight (kg)	82.4±10.4	83.21±1.3
Dietary intake of calcium (mg/day)	410.2±210.6[a]	644.0±311.5

[a] Significantly different, p<0.01 (t-test).

parameters (Table 2). The dietary intake of calcium was significantly lower in patients than in controls (p<0.01).

The mean concentrations of all parameters determined in the blood serum were in the range of reference values and were not significantly different in the groups of patients and controls (Table 3). Slightly increased levels of calcium and alkaline phosphatase were found in the serums of three patients.

The mean concentrations of all parameters determined in fasting first morning urine (Table 4) were in the range of reference values and were not significantly different for patients and controls. From calcium, oxalate, citrate, and magnesium concentrations in the fasting first morning urines, hypercalciuria (c(Ca) > 3 mmol/L)[30] was determined for seven patients and three controls, hyperoxaluria (c(Ox) > 0.3 mmol/L)[30] for fourteen patients and eight controls, hypocitraturia (c(Cit) < 0.7 mmol/L)[31] for nine patients and six controls, and hypomagnesiuria (c(Mg) < 1 mmol/L)[31] for one patient and two controls. (The concentrations of Ca and Ox, used as criteria for determination of hypercalciuria and hyperoxaluria, respectively, were taken after ref. 30, and concentrations of Cit and Mg for hypocitraturia, and hypomagnesiuria, respectively, were taken after ref. 31).

TABLE 3
SERUM PARAMETERS FOR UROLITHIASIS PATIENTS (N=26) AND CONTROLS (N=18)

Parameter	Patients	Controls
	Mean±SD	Mean±SD
Creatinine (ìmol/L)	96.2±18.1	91.5±7.3
Urea (mmol/L)	5.3±1.7	5.4±1.0
Alkaline phosphatase (U/L)[a]	153.7±53.7	128.6±25.2
Calcium (mmol/L)	2.3±0.1	2.4±0.1
Phosphorus (mmol/L)	0.9±0.2	1.1±0.2
Magnesium (mmol/L)	0.85±0.07	0.84±0.12
Sodium (mmol/L)	141.8±3.1	140.4±4.1
Potassium (mmol/L)	4.3±0.2	4.3±0.3
Chloride (mmol/L)	101.3±5.8	100.1±3.6
Cholesterole (mmol/L)	5.3±1.1	5.8±0.9
Trigliceride (mmol/L)	2.7±1.7	2.2±1.1
Proteins (g/L)	77.7±19.2	70.4±17.7
Urate (μmol/L)	325.0±79.9	338.0±66.0

[a] U is enzyme unit.

TABLE 4
FASTING FIRST MORNING URINE PARAMETERS FOR UROLITHIASIS PATIENTS (N=26) AND CONTROLS (N=18)

Parameter	Patients		Controls	
	Mean±SD	R (parameter/creatinine)[a]	Mean±SD	R (parameter/creatinine)[a]
Volume (mL)	298.2±100.9		349.4±147.8	
pH	5.82±0.52		5.43±0.54	
Creatinine (mmol/L)	15.7±6.0		14.7±5.6	
Calcium (mmol/L)	2.28±1.13	1.39±0.67	1.92±1.32	1.16±0.65
Phosphorus (mmol/L)	17.4±2.8	10.9±3.5	17.7±4.0	11.7±3.7
Magnesium (mmol/L)	2.6±1.6	1.5±0.6	2.8±1.5	1.7±0.8
Sodium (mmol/L)	99.2±40.9	63.7±35.0	100.1±73.9	61.9±39.8
Potassium (mmol/L)	31.2±13.8	18.5±6.7	37.1±17.1	20.8±5.9
Oxalate (mmol/L)	0.37±0.26	0.22±0.12	0.32±0.18[b]	0.22±0.19
Citrate (mmol/L)	1.53±1.22	0.92±0.85	1.56±1.24	0.99±0.74
Urate (mmol/L)	2.57±1.08	1.50±0.46	2.51±1.49	1.44±0.49

[a] Ratio of corresponding parameter and creatinine calculated for each subject and expressed as mean±SD, in mmol/g.
[b] Mean of N=16.

Two patients had three metabolic disorders (hypercalciuria, hyperoxaluria, and hypocitraturia). Four patients had two disorders (two of them had hypercalciuria and hyperoxaluria, and two had hyperoxaluria and hypocitraturia). It is important to note that two of the patients had very low oxalate concentrations (0.05 and 0.07 mmol/L) and another two very low calcium concentrations (0.70 and 0.90 mmol/L) because of their restricted diet, including high fluid intake.

Two controls had three metabolic abnormalities (one of them had hypercalciuria, hyperoxaluria, and hypocitraturia, and one had hyperoxaluria, hypocitraturia, and hypomagnesiuria). Three controls had two disorders (one of them had hypercalciuria and hyperoxaluria, one had hyperoxaluria and hypocitraturia, and one had hypocitraturia and hypomagnesiuria).

The mean values of parameters determined in 24-h urine (daily urine, DU) were inside reference values[32], but patients with urolithiasis had significantly higher calcium excretion ($p<0.01$) and significantly lower potassium excretion ($p<0.01$) compared to the controls (Table 5). All other 24-h urine parameters did not show significant differences between patients and controls. Hypercalciuria (Ca > 7 mmol/DU)[32] was determined for seven patients and two controls and hypomagnesiuria (Mg < 3 mmol/DU)[31,33] for five patients and four controls. (Concentration of Ca used for determination of hypercalciuria was taken after ref. 32, and concentration of Mg for hypomagnesiuria was taken after refs. 31 and 33). The concentrations of oxalate and citrate were not measured in the 24-h urines. Urinary infection was detected in urines of seven patients.

The mean value of IAP(CaOx) calculated for first morning urines (Eqn. 1) was higher for patients than for controls, but the values were not significantly differ-

ent (Table 6). Also, the mean values of Tiselius indexes (Eqns. 2 and 3) and Ogawa index (Eqn. 4) for patients and controls, respectively, were not significantly different (Table 6).

Discussion

Metabolic parameters

The patients and controls were well matched with respect to sex, age, height, and weight (Table 2). As a result of dietary calcium restrictions to which patients were submitted, stone-formers participating in our study had significantly lower calcium intake than healthy controls. However, recent literature data indicate that significantly lower dietary calcium intake could expose the patients to a higher risk for recurrence of calcium oxalate urinary stones[34], because an insufficient supply or low availability of calcium for complexation with oxalate in the intestinal lumen could increase oxalate excretion[35]. Therefore, it has been strongly recommended that dietary calcium intake should not be restricted for calcium oxalate stone-formers[36].

The 24-hour urine is usually used for metabolic evaluation of stone formers, but the long-term storage of 24-h urine and the use of different additives might destroy or alter important urine constituents. The reliability of urines collected in different time intervals (i.e., fasting first morning urine, 8-h urine, and 16-h urines) in the clinical evaluation of stone formers was the objective of several recent studies[37–39]. It was found that 16-h and 8-h urines collected during the day and night, respectively, are reliable specimens for determination of metabolic abnormalities[37]. Moreover, the urinary Ca, Ox, and Cit concentrations, as well as the

TABLE 5
24-HOUR URINE PARAMETERS FOR UROLITHIASIS PATIENTS (N=26) AND CONTROLS (N=18)

Parameter	Patients		Controls	
	Mean±SD	R (parameter/creatinine)[a]	Mean±SD	R (parameter/creatinine)[a]
Volume (mL)	1482.0±545.2		1452.0±154.3	
pH	5.6±0.7		5.5±0.6	
Creatinine (mmol/DU)[b]	16.4±2.4		17.5±4.1	
Calcium (mmol/DU)	5.6±2.5[c]	3.0±1.3[c]	3.7±1.9	2.4±0.8
Phosphorus (mmol/DU)	32.3±10.2	17.3±4.3	33.1±12.2	16.6±4.3
Magnesium (mmol/DU)	4.1±1.7	2.2±0.8	4.2±1.2	2.2±0.6
Sodium (mmol/DU)	190.6±70.6	104.3±38.8	220.0±80.3	109.7±27.2
Potassium (mmol/DU)	49.2±15.7[c]	27.1±9.8[c]	74.5±33.8	36.2±11.2
Chloride (mmol/DU)	196.8±63.6	108.3±37.9	236.7±77.0	118.7±31.5
Urea (mmol/DU)	393.4±75.9	213.8±40.4	419.6±94.4	214.1±37.8
Urate (mmol/DU)	3.6±0.9	1.9±0.5	3.7±1.4	1.8±0.4
Proteins (g/DU)	0.25±0.24	1.14±0.13	0.16±0.04	1.07±0.07

[a] Ratio of corresponding parameter and creatinine calculated for each subject and expressed as mean±SD, in mmol/g.
[b] DU = daily urine (24-h urine).
[c] Significantly different, $p<0.01$ (t-test), from the corresponding value for controls.

TABLE 6
IONIC ACTIVITY PRODUCTS, IAP(CaOx), AND TISELIUS AND OGAWA INDEXES CALCULATED FROM COMPOSITION OF FASTING URINES FOR PATIENTS (N=26) AND CONTROLS (N=16)

Calculated parameters	Patients (mean±SD)	Controls (mean±SD)
10^8 IAP(CaOx)	2.0±1.5	1.6±1.4
AP(CaOx)-index (Tiselius)	18.2±18.3	12.2±19.3
lmult1CaOx-risk-index, RI (Tiselius)	277±214	287±213
$10^8 \cdot$ AP(CaOx)-EQ2-index (Ogawa)	8.7±6.1	6.9±4.9

creatinine-corrected Ca, Ox, Cit, and Mg, showed a significant correlation between early morning spot urine and 24-h urine specimens[39]. The mean values of creatinine-corrected parameters for Ca, P, Mg, Na, and K (parameter/creatinine ratios) determined in fasting first morning urine and 24-h urine (Tables 4 and 5, respectively) in this study, also showed significant correlation; all parameters were significantly higher in 24-h urine than in fasting first morning urine for the group of patients as well as for the group of controls.

The mean concentrations of Ca and corresponding Ca/creatinine ratios in fasting first morning urines and 24-h urines were higher for patients than for controls, but the differences were significant ($p< 0.01$) only in the 24-h urine (Table 5). Higher calcium excretion for calcium oxalate stone-formers is in agreement with previous studies[5–10,37–39].

Significantly lower potassium excretion was determined in 24-h urines of stone-formers (Table 5). This result is in accordance with literature data[1] that indicated nutritional deficiency of potassium as a risk factor for stone formation. In addition, a significant increase in potassium was found in the urines of the recurrent stone formers that were recurrence-free after specific therapy[40].

The lack of statistically significant differences (determined by the parametric t-test) between patients and controls for all urine parameters except calcium and potassium in 24-h urine could not be contributed to the possible non-Gaussian distributions. Namely, the non-parametric Mann-Whitney test that does not assume that the difference between two independent populations has normal (Gaussian) distribution confirmed the t-test data. Therefore, the non-significant differences between urine parameters for patients and controls could be partly explained by (a) the small sizes of patient and control groups, (b) presence of relatively large number of controls with metabolic disorders (i.e., hyperoxaluria and hypercalciuria were determined in fasting first morning urines for seven and three controls, respectively), and (c) presence of patients that were on restricted diet/high fluid regiment (i.e., extremely low calcium and/or oxalate concentrations were determined in fasting first morning urine of four patients).

Influence of Mg and Cit concentrations on IAP(CaOx)

The calculated mean value of IAP(CaOx) in fasting first morning urine, which is an indication of urine saturation with respect to calcium oxalate formation, was higher (but not significantly) for the group of patients. In general, higher concentrations of calcium and oxalates would increase IAP(CaOx) values, whereas higher

concentrations of citrates, magnesium, phosphorus (phosphates), sodium, and potassium would decrease the IAP(CaOx) values. Citrate and phosphate form complex species with calcium, causing a decrease in the free Ca^{2+} activity, whereas magnesium, sodium, and potassium form complex species with oxalates causing a decrease in the activity of free Ox^{2-} (Table 7).

Very high and very low urinary concentrations of citrate and magnesium give different values of IAP(CaOx) (Table 7) calculated for a hypothetical urine having concentrations of all parameters (except Cit and Mg) equal to the mean values determined in the fasting first morning urine of the patients in this study (Table 4). The maximum and minimum Cit and Mg concentrations determined in the fasting urines of the patients were used in the calculations (Table 4) and the calculated Ca and Ox speciation and the corresponding IAP(CaOx) values are listed in Table 7. It is seen that for maximum and minimum Cit concentrations, the mol fractions of $CaCit^-$ complexes are 35.6 % and 9.0 %, and the mol fractions of free Ca^{2+} 49.6 % and 67.4 %, respectively. For maximum and minimum Mg concentrations the mol fractions of $MgOx^0$ complexes are 47.8 % and 9.9 %, and the mol fractions of

TABLE 7
CONCENTRATIONS AND MOL FRACTIONS OF CALCIUM AND OXALATE SPECIES, ACTIVITIES OF CALCIUM AND OXALATE IONS, AND IONIC ACTIVITY PRODUCTS, IAP(CaOx), CALCULATED USING THE MEAN PARAMETERS OF PATIENTS FASTING URINE (LISTED IN TABLE 4) AND (A) HIGH AND (B) LOW CITRATE (CIT) AND MAGNESIUM (MG) CONCENTRATIONS

	(a) High Cit and Mg c(Cit)=5 mmol/L, c(Mg)=9.0 mmol/L		(b) Low Cit and Mg c(Cit)=0.42 mmol/L, c(Mg)=0.80 mmol/L	
Calcium species	Concentration (mmol/L)	Mol fraction (%)	Concentration (mmol/L)	Mol fraction (%)
Ca^{2+}	$1.14 \cdot 10^{-3}$	49.6	$1.55 \cdot 10^{-3}$	67.4
$CaCit^-$	$8.19 \cdot 10^{-4}$	35.6	$2.07 \cdot 10^{-4}$	9.0
$CaOx^0$	$3.37 \cdot 10^{-5}$	1.5	$7.67 \cdot 10^{-5}$	3.3
$CaHPO_4^0$	$1.26 \cdot 10^{-4}$	5.5	$1.87 \cdot 10^{-4}$	8.1
$CaH_2PO_4^+$	$1.80 \cdot 10^{-4}$	7.8	$2.67 \cdot 10^{-4}$	11.6
Oxalate species				
Ox^{2-}	$1.04 \cdot 10^{-4}$	28.1	$1.68 \cdot 10^{-4}$	45.4
HOx^-	$1.47 \cdot 10^{-6}$	0.4	$2.39 \cdot 10^{-6}$	0.7
$CaOx^0$	$3.37 \cdot 10^{-5}$	9.1	$7.67 \cdot 10^{-5}$	20.7
$MgOx^0$	$1.77 \cdot 10^{-4}$	47.8	$3.67 \cdot 10^{-5}$	9.9
$NaOx^-$	$3.33 \cdot 10^{-5}$	9.0	$5.45 \cdot 10^{-5}$	14.7
KOx^-	$1.40 \cdot 10^{-5}$	3.8	$2.20 \cdot 10^{-5}$	6.2
Ca_2Ox^{2+}	$2.73 \cdot 10^{-6}$	0.7	$8.46 \cdot 10^{-6}$	3.0
Ion	Activity		Activity	
Ca^{2+}	$3.67 \cdot 10^{-4}$		$5.09 \cdot 10^{-4}$	
Ox^{2-}	$3.34 \cdot 10^{-5}$		$5.52 \cdot 10^{-5}$	
Ionic activity product				
IAP(CaOx)	$1.23 \cdot 10^{-8}$		$2.81 \cdot 10^{-8}$	

free Ox^{2-} 28.1 % and 45.4 %, respectively. Consequently, for maximum and minimum Cit and Mg, the IAP(CaOx) values are 1.2×10^{-8} and 2.8×10^{-8}, respectively, i.e. about ten times higher concentration of Mg in concert with about ten times higher concentration of Cit reduced the IAP(CaOx) value for about two times only, which indicates that the influence of Cit and Mg ions on the saturation level of urine is not very dramatic. However, the role of Cit and Mg in urines should not be underestimated, since besides reduction of saturation, Cit and Mg are also potent inhibitors of CaOx crystallization.

Correlation between IAP(CaOx) and risk indices

The IAP(CaOx) values calculated for patients and controls were correlated with the values of corresponding indices: AP(CaOx)-index (Tiselius) (Figure 1), CaOx-risk-index, RI (Tiselius) (Figure 2), and AP(CaOx)-EQ2-index (Ogawa) (Figure 3). All correlations were significant. The strongest correlation and the highest correlation coefficient (r=0.97) was determined for IAP(CaOx) vs. AP(CaOx)-EQ-index (Ogawa). This result indicates that the empirical exponents for Ca, Mg, Ox, and Cit concentrations in the equation for AP(CaOx)-EQ2-index (Ogawa) are very well estimated. The values of IAP(CaOx) and Ogawa index were extremely low for two patients and extremely high for three controls (Figure 3). These two patients had very low urinary Ox concentrations and the three controls had very high urinary Ox and/or Ca concentrations.

Determination of IAP(CaOx) or the Ogawa index in different urine samples could be a good indication for the risk of calcium oxalate urolithiasis[35], but determination of other parameters, such as the total inhibitory activity of urines, particularly with respect to macromolecular urine fractions[12,14,15], should improve discrimination between stone-formers and healthy individuals. Some simple, clinically applicable methods for the determination of the inhibitory activity of urines with regard to calcium oxalate precipitation[41–43] and its calcium binding capacity[44] have been proposed and one of them[41] has been successfully used in the follow-up of the therapy of patients with recurrent urolithiasis[45]. Such alternative approach to the prophylaxis and treatment of recurrent urolithiasis seems to have great potential.

Conclusions

The concentration of calcium was significantly higher and concentration of potassium was significantly lower in the 24-h urines of calcium oxalate stone-formers as compared to healthy controls. Very strong correlation (r=0.97) was obtained between IAP(CaOx) values and calculated Ogawa indices that were recommended for estimating the potential risk for calcium oxalate stone formation.

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METABOLIČKA EVALUACIJA ISPITANIKA S UROLITIJAZOM IZ ISTOČNE HRVATSKE

S A Ž E T A K

Metabolički su parametri određeni u krvnom serumu, prvom jutarnjem urinu i 24-satnom urinu 26 muškaraca (dob 39.1±6.2 god.) s recidivirajućim kalcij-oksalatnim mokraćnim kamencima, te u urinu 18 zdravih muškaraca (dob 35.0±7.1 god.), iz istočnog dijela Hrvatske. Izlučeni kalcij u 24-satnim urinima pacijenata (5.6±2.5 mmol) je značajno veći ($p<0.01$) nego u zdravih osoba (3.7±1.9 mmol), dok je izlučeni kalij veći ($p<0.01$) u urinima zdravih osoba (74.5±33.8 mmol) nego u pacijenata (49.2±15.7 mmol). Srednji ionski aktivitetni produkti kalcija i oksalata, IAP(CaOx), računati iz kemijskog sastava prvog jutarnjeg urina, 25 % su veći za pacijente nego za zdrave osobe, ali ta razlika nije statistički značajna ($p>0.05$). Utvrđena je vrlo dobra korelacija izmedju IAP(CaOx) vrijednosti i izračunatih Ogawinih indeksa, koji su pokazatelji rizika za stvaranje kamenaca kalcijevog oksalata.