Quantitative Mineralogical Composition of Calculi and Urine Abnormalities for Calcium Oxalate Stone Formers: A Single-Center Results

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Purpose: The paper focuses on the relationship of risk factors and metabolic disorders with mineralogical composition of calculi, age and gender of calcium oxalate stone formers.

Materials and Methods: Stone mineralogical composition, 24 hour biochemistry and pH-profile of urine were examined for sixty four stone formers using powder X-ray diffraction, spectrophotometric and potentiometric techniques.

Results: The analysis indicated that 44 % of calculi were composed of pure calcium oxalate monohydrate, whereas other 56 % contained both monohydrate and dihydrate or usually their mixtures with hydroxyl apatite. Hypocitraturia, hypercalciuria and hyperuricosuria were identified as the most frequent disorders. Patients with pure calcium oxalate stones and calcium oxalate mixed with apatite revealed different patterns including age, acid-base balance of urine, calcium, citrate excretion etc.

Conclusions: Our results demonstrate that most patients simultaneously reveal several risk factors. The special attention should be paid to normalize the daily citrate, calcium and urate excretion. High risk patients, such as postmenopausal females or stone formers with a high apatite content require a specific metabolic evaluation towards in highlighting abnormalities associated with stone formation.

Keywords: calcium oxalate calculi; quantitative mineralogical analysis; urine components; metabolic disorders; risk factors

INTRODUCTION

Renal stone formers constitute a major part of everyday patients of urological clinics. Urolithiasis is a highly prevalent and increasingly common disease affecting up to 15 % of population, between 60 up to 90 % of calculi being formed of calcium oxalate hydrates (CaOx) or their mixtures with minor components such as apatities or urates[1-4].

CaOx stones are composed of two major constituents – calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) or frequently their mixtures. The analysis of ten thousand patients with CaOx calculi indicates that COM occurs about twice as frequently as COD, most stones being formed by both mineralogical phases. Stones containing COD as the major component are correlated with permanent hypercalciuria for about 88 % of patients[5]. If the COD content in a mixed COM+COD stone becomes more than 15 mass %, it indicates that the stone is originated by both hypercalciuria and hyperoxaluria[5]. Another important metabolic disorder influencing calcium oxalate stone formation is hypocitraturia affecting up to 60 % of stone formers [6-8]. Since removal of existing CaOx stones does not prevent their further appearance[8] and a recurrence rate reaches up to 30-50 % within 5-year period[9,10], it is essential to find metabolic abnormalities which caused formation of an initial calculus to provide a sound basis to prevent the recurrence. Unfortunately, usual measures mainly devoted to a stone removal are not effective to highlight metabolic abnormalities. Although the 24 hour urine analysis and the quantitative mineralogical study of renal calculi with IR-Fourier spectroscopy or X-ray diffraction became routine procedures for many European centers, these are rarely performed in domestic clinics. The lack of this information significantly contributes to the high recurrence observed.

With this information in mind, we have analyzed the mineralogical composition of calculi and the mean daily excretion of urine components for sixty four patients with calcium oxalate urolithiasis from central Russia. The main goal of this study is to compare the clinical and biochemical patterns with the stone mineralogical composition, gender and age of CaOx stone formers in the hope that this information may provide a deeper insight into etiology of urolithiasis and allow to improve its diagnosis and treatment.

PATIENTS AND METHODS

The calculi or their fragments removed with retrograde intrarenal surgery or usually extracorporeal shock-wave lithotripsy for sixty four CaOx stone formers were accurately examined with X-ray powder diffraction at the

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Received March 2017 & Accepted November 2017

Table 1. The 24 h urine chemistry (in mmol/day), mean age and AP[CaOx] indexes M ± SE for three mineralogical groups of calcium oxalate stone formers.

Parameter	COD [a] patients	COM patients	$CaOx_{AP}$ patients
Ca [b] (<5 mmol/day)	6.97 ± 0.8	5.43 ± 0.5	3.39 ± 1.1
Oxalate, (<0.5 mmol/day)	0.37 ± 0.08	0.38 ± 0.05	0.22 ± 0.03
Citrate, (>2.5 mmol/day)	1.28 ± 0.18	1.65 ± 0.19	1.32 ± 0.26
Phosphate, (<35 mmol/day)	15 ± 2	27 ± 3	37 ± 6
pHmean	5.58 ± 0.15	5.55 ± 0.07	5.94 ± 0.16
AP[CaOx]	1.67 ± 0.47	1.11 ± 0.16	0.39 ± 0.11
Mean age	33.6 ± 5	51.2 ± 2	43.1 ± 8
n	7	50	7

Abbreviations: [a]COM, calcium oxalate monohydrate (whewellite); COD, calcium oxalate dihydrate (weddellite); $CaOx_{AP}$, calcium oxalate with apatite

[b]reference values for medical attention are given in brackets [12]

Institute of Solution Chemistry using the D8 Advance Bruker diffractometer. The stones examined were classified according to their main component (COM or COD) if the mass faction of the mineralogical phase was ≥ 55 mass %. Seven $CaOx_{AP}$ stones contained from 10 to 44 mass % of hydroxyl apatite were also identified.

All patients were routinely asked about possible stone formers in their family, past medical history concerning the last stone episode, physical activity and a normal diet. Height and weight were also recorded to compute a body mass index. A 24 h urine collection was carried out for each patient supported on a random diet. The urine volume and concentration of urine components were determined. Calcium, magnesium, phosphate and urate were measured spectrophotometrically in a clinical laboratory with standard kits. Oxalate and citrate ions were determined with the enzymatic hydrolysis method. The daily pH-profile of urine for each patient was studied independently using the pocket pH-meters pH-009(I) (RoHS) pre-calibrated with standard buffer solutions. The index of urinary supersaturation with respect to calcium oxalate was computed according to the Tiselius equation[11].

Results given in **Tables 1, 2** are presented as means ± SE. Statistical analysis was performed using the Origin 7.0 package. Group differences were studied by the one-way ANOVA test with comparisons according to Bonferroni. For all cases we have used pairwise comparisons which provide for our results a deeper insight into the etiology of stone formation. These comparisons were considered to be significantly different if $P < 0.05$. The ethics board approval number for this study is ISMA EC 2013.12.08.

RESULTS

We have studied sixty four CaOx stone formers (mean age of 48.5 ± 2 years) consisting of 39 females (mean age of 49.8 ± 2 years) and 25 males (mean age of 46.2±3 years). The X-ray diffraction study has indicated that about 44 % of stones contained one COM component, whereas other 56 % of calculi were composed of two (COM+COD) or three (COM+COD+hydroxyl apatite) mineralogical phases. We have found only ten calculi where the COD content was larger than the COM one, none of them contained pure COD.

The relevant parameters associated with stone formation are listed in Table 1 for COM (COM > COD), COD (COD>COM) and $CaOx_{AP}$ (apatite ≥ 10 %) stone formers. The risk factors and reference values for urine components were taken from Straub et al. study [12]. Patients with COD stones were found to be significantly younger than those with COM (P =.006) but not with $CaOx_{AP}$ (P =.11) calculi. The body mass index was not significantly persistent between all three groups. The mean daily oxalate excretion and the daily pH-profile for COM and COD groups were identical. In contrast, the daily pH-profile for $CaOx_{AP}$ stone formers is shifted to more alkaline urine in comparison with COD and, especially, COM (P =.04) patients. The mean daily excretion of Ca and citrate as well as urinary saturation with respect to calcium oxalate differed between COM and COD stone formers. The difference, however, was not yet statistically significant. We have found, however, that patients with $CaOx_{AP}$ stones reveal significantly increased phosphate (P =.006) and simultaneously decreased calcium excretion (P =.02) that COD stone formers. The AP [CaOx] index is also decreased (P =.03). Despite the lower AP [CaOx] index, the shift of the pH-profile to more alkaline urine and elevated phosphate excretion significantly increase the risk of heterogeneous nucleation for such patients.

In order to obviate to possible bias related to sex and age, we have computed the means ± SE values of the biochemical parameters mentioned above for stone formers divided by sex and age (**Table 2**). The excretion of calcium was elevated for old females in comparison with males of the same age (P = .03). The oxalate excretion was surprisingly normal for all groups. The lower level of oxalate in urine for young females in comparison with older ones was statistically insignificant. The daily excretion of citrate was reduced for all CaOx stone formers. Postmenopausal females indicated a lower citrate excretion with urine than premenopausal stone forming women (P = .05) and simultaneously the higher Ca/citrate ratio in comparison with old males (P = .04) and young females (P = .05). The mean daily urate excretion was surprisingly elevated both for males and, especially, females. The difference between these

Table 2. Parameters of the 24 h urine M±SE for CaOx stone formers by gender and age (in mmol/day).

Parameters of urine	Females n = 39	Males n = 25	Patient group Females > 50 n =21	Females < 50 n =18	Males > 50 n =12	Males < 50 n =13
Volume	2.04 ± 0.08	1.78 ± 0.09	1.99 ± 0.07	2.12 ± 0.09	1.74 ± 0.07	1.82 ± 0.17
Ca	5.97 ± 0.49	4.44 ± 0.57	6.36 ± 0.88	5.52 ± 0.51	3.45 ± 0.67	5.34 ± 0.79
Citrate	1.62 ± 0.22	1.49 ± 0.20	1.23 ± 0.27	2.1 ± 0.34	1.56 ± 0.29	1.43 ± 0.28
Ca/Citrate	7.4 ± 1.3	5.3 ± 0.1	9.8 ± 2.1	4.7 ± 1.1	3.4 ± 0.8	6.5 ± 1.7
Oxalate	0.34 ± 0.06	0.39 ± 0.04	0.29 ± 0.04	0.40 ± 0.10	0.42 ± 0.07	0.33 ± 0.05
Phosphate	27 ± 4	25 ± 2	31±7	23 ± 3	24 ± 2	26 ± 4
Urate	6.49 ± 0.67	5.08 ± 0.41	6.32 ± 0.68	6.65 ± 1.28	4.60 ± 0.64	5.53 ± 0.52

Table 3. Frequency of risk factors and metabolic disorders (%) for calcium oxalate stone formers

Disorder or risk factora	Females	Males	Total	Females > 50 years	Males > 50 years	Total > 50 years
Hypercalciuria (Ca excretion >8 mmol/day)	20	6	26	15	2	17
Mild hypercalciuria (Ca > 5 mmol/day)	19	5	24	8	2	10
Hyperoxaluria (Oxalate > 0.5mmol/day)	11	13	24	5	8	13
Hypocitraturia (Citrate < 2.5 mmol/day)	48	30	78	28	18	46
Hypomagnesiuria (Mg < 3 mmol/day)	12	8	20	7	5	12
Hyperuricosuria (Urate > 4 mmol/day)	34	25	59	20	13	33
Hyperphosphaturia (phosphate >35 mmol/day)	15	3	18	7	2	9
Constantly pH ≤ 5.8 (possibly acidic arrest of urine)	20	15	35	13	5	18
Constantly pH > 5.8 (possibly renal tubular acidosis)	11	7	18	3	5	8
Obesity (body mass index > 25 kg/m^2)	33	18	51	28	13	41
Low fluid intake (Diuresis < 1.5 l)	15	20	35	7	7	14

[a] Reference values for medical attention [12]

groups was not yet significant (P = .09).

Table 3 compares the frequency of risk factors and possible metabolic abnormalities associated with calcium oxalate stone formation for the selected group of patients. The most frequent disorders are hypocitraturia, hyperuricosuria and hypercalciuria afflicting 75, 59 and 50 per cent of stone formers, respectively.

DISCUSSION

Introduction of new options such as distant or contact shockwave lithotripsy, retrograde or antegrade endoscopic pyelolithotomy has allowed to achieve a rapid stone removal from the urinary tract. This low invasive treatment appears to be more attractive for many patients than routine metaphylaxis. However, the high recurrence of urolithiasis arising from non-treated metabolic disorders often leads to serious organic lesions both of kidneys and the urinary tract as a whole. Another important point is the high cost of treating such life-threatening complications. This is in times higher than the expenditures for though tedious but really necessary diagnostic and metaphylactic measures. These, however, need further investigations to provide the sound basis to prevent the stone recurrence.

We see from **Table 1** that COD stone formers are significantly younger than COM patients. They also reveal a reduced citrate excretion and a simultaneously elevated calcium level in urine. This result was in a good agreement with the Trinchieri et al. findings[8]. The excess of the promoter of CaOx crystallization and growth with deficiency of citrate ions as inhibitors of stone formation induces urine supersaturation and rapid crystallization of COD.

We have mentioned above that stones containing COD as the main component are correlated with permanent hypercalciuria for the majority of cases. In contrast, COM stones are often associated with hyperoxaluria [5]. This finding is not, however, confirmed by Trinchieri et al.[8] and our results suggesting about a normal oxalate excretion for both groups. As for the mean daily calcium excretion, it is larger for COD stone formers (**Table 1**). Although the difference between COM and COD groups for our patients is not yet statistically significant, this value suggests about mild hypercalciuria for COD patients that is in consistent with the Daudon et al.[5] and Trinchiery et al.[8] findings. It is worthy of note that COM as a component of a renal stone can be formed directly due to the reaction between calcium and oxalate ions in urine or indirectly from thermodynamically unstable COD[13]. Hence, for some mixed COD+COM calculi the COM amount may be overestimated due to the COD to COM transformation[13]. This may artificially increase the percentage of COM stone formers and shift the mean daily calcium excretion for this group to greater values. The recent study[13] supports this idea pointing out the importance of the analysis of stone texture to differentiate stone formers between COM and COD groups.

Table 2 compares the mean daily excretion of urine components for males and females. We see that a calcium excretion is elevated both for young and, especially, old females. This is not the case for males, where the risk arises exceptionally for a younger group. Hence, females over fifty are in a high risk group in comparison with males of the same age.

The mean daily oxalate excretion is surprisingly normal for all groups. Although the oxalate level for selected patients reaches 1 mmol/day and more, it is of 0.2-0.3 mmol/day in most cases. The similar findings have been reported elsewhere[3,8] for Asian and European stone formers. The mean daily excretion of citrate is reduced for all groups. We have mentioned above that for younger females the citrate level in urine is higher than that for menopausal and post menopausal women (**Table 2**). This is in a fair agreement with the observation that premenopausal stone forming females show greater values of a citrate excretion than males or postmenopausal females[7]. It is important that Ca/citrate ratio for the menopausal and post menopausal females is significantly elevated that strongly increases the risk of stone recurrence. Taking into account the fact that the estrogen loss leads to the increase of calcium in urine and the simultaneous decrease of citrate[7], this result is of particular importance for the diagnosis and treatment of urolithiasis.

As for the frequency of metabolic disorders and risk fac-

tors shown in Table 3, our results indicate that the most frequent disorder is hypocitraturia. It afflicts more than 75 % of patients, the significant part of stone formers excreting less than 1 mmol of citrate per day. This observation is in a fair agreement with recently reported results for Chinese stone formers[3]. If we use the reference value of 1.7 mmol/day proposed by Pak[14], the frequency of hypocitraturia reduces to 55 %, which is in consistent with usually reported quantities[7]. In many cases hypocitraturia is accompanied by an elevated calcium and urate excretion that significantly influences the recurrence rate. Hypercalciuria is detected in 26 % of patients and yet 24 % of stone formers with mild hypercalciuria are in a high risk group. The high incidence of hypercalciuria for CaOx stone formers is in a good agreement with the findings given elsewhere[8-12]. Table 3 clearly shows that this disorder afflicts mainly females in the period of menopause and post menopause. Another important abnormality is hyperuricosuria. This surprisingly frequent disorder for our patients is seen to observe both for males and females independently of age. Being structurally similar to COM crystals, uric acid crystallites may induce heterogeneous nucleation and initial aggregation of COM species[15]. Other abnormalities such as hyperoxaluria, hypomagnesuria and hyperphosphaturia are less frequent.

We have also identified several risk factors associated with stone formation. The first and most frequent factor is obesity which is found for many patients (**Table 3**). The patient survey has indicated that an excessive caloric intake with a meal and low physical activity are the major reasons of this pathological condition. Moreover, for many stone formers dietary habits include an excessive intake of sodium chloride, poultry protein and smoked food. It is clear that such a diet significantly contributes to the high incidence of hypocitraturia and hyperuricosuria.

The second risk factor is the abnormal acid-base balance of urine. “Acidic arrest” during the day is observed twice as frequently as urine excessive alkalization (Table 3). Acidic urine may also affect the tubular production of citrate that additionally supports the idea that the high frequency of hypercitraturia arises from a diet.

The third and very important factor is low fluid intake resulting in significant supersaturation of urine with respect to calcium oxalate. It is clear that modern life style, obesity and dietary habits seem to be real promoters in the development of urolithiasis for CaOx stone formers. Hence, all patients should follow at least the basic metaphylactic measures to normalize dietary habits, physical activity and exclude risk factors of stone formation[12,16,17].

CONCLUSIONS

Our results indicate that COD stone formers are younger than patients with COM calculi and reveal higher calcium level in urine. $CaOx_{AP}$ patients demonstrate the lower mean daily calcium and oxalate excretion, the more alkaline pH-profile of urine and the significantly lower AP [CaOx] index. These observations lead to different strategies for diagnosing COD/COM and $CaOx_{AP}$ patients[16]. In particular, we are able to draw a tentative conclusion that for any COD stone former, the first step of a metabolic evaluation should contain the determination of the calcium and citrate excretion with urine. For $CaOx_{AP}$ stone formers the initial step should include the analysis of pH-profile, phosphate, calcium and citrate. Menopausal and postmenopausal females are a high risk group due to the elevated Ca/citrate ratio and need a compulsory metabolic evaluation.

ACKNOWLEDGMENTS

The partial financial support of this work by the Russian Foundation for Basic Researches is gratefully acknowledged (grant N 15-44-03016-reg).

CONFLICT OF INTEREST

The authors report no conflict of interest.

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