

AVALIAÇÃO DA CITRATURIA E DA PROPORÇÃO CÁLCIO/CITRATO EM PACIENTES COM NEFROLITÍASE. A SAGA CONTÍNUA VERSUS INTERVALADA

EVALUATION OF CITRATURIA AND CALCIUM/CITRATE RATIO IN NEPHROLITHIASIS PATIENTS. THE CONTINUOUS VERSUS INTERVAL SAGA

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RESUMO

Introdução: Apesar do avanço no conhecimento fisiopatológico da nefrolitíase, a avaliação do risco individual de formação e recorrência de cálculos renais por mensuração de fatores de risco na urina de 24 horas é muitas vezes difícil. Nosso objetivo é avaliar fatores de risco urinários associados à nefrolitíase, particularmente citraturia e a proporção cálcio/citrato em pacientes com cálculos renais recorrentes e naqueles sem litíase urinária. **Método:** 103 pacientes com nefrolitíase recorrente (30 homens e 73 mulheres) e 32 pacientes sem doença (11 homens e 21 mulheres) foram estudados retrospectivamente. Dados clínicos e laboratoriais foram colhidos de todos os pacientes. **Resultados:** Não houve diferença de idade entre os grupos (44,4±11,8 anos vs 43,3±17 anos). Na urina, houve aumento de volume, cálcio (189,6±98,7 vs. 150,7±107,3, p=0,029), sódio e oxalato (33,7±28,2 vs. 22,2±15,3, p=0,041) no grupo dos litíasicos, comparados ao grupo controle. A excreção urinária de ácido úrico e citrato foi similar entre os grupos, sem diferenças significativas. Houve correlação positiva entre a excreção de cálcio e citrato na urina dos pacientes litíasicos (r=0,41, p<0,001). Não foi possível utilizar a proporção cálcio/citrato para diferenciar os grupos, pois diferenças não foram encontradas. **Conclusão:** Em pacientes com nefrolitíase recorrente, os fatores de risco em urina de 24 horas necessitam ser interpretados como variáveis contínuas, e não intervaladas. Estudos prospectivos são necessários para determinar valores normais diários de citraturia e para avaliar o papel da hipocitraturia isolada na incidência, prevalência e curso clínico na formação de cálculos renais.

Palavras-chave: citrato; nefrolitíase; cálculo renal; hipocitraturia.

ABSTRACT

Introduction: Despite the progress in the pathogenesis of nephrolithiasis, the individual risk assessment of formation and recurrence of kidney stones by measuring risk factors in 24-hour urine is often difficult. Our objective was to evaluate urinary risk factors associated with nephrolithiasis, particularly citraturia and the calcium/citrate ratio in recurrent kidney stones formers and in subjects without nephrolithiasis. **Methods:** 103 kidney stone formers (30 men and 73 women) and 32 non-stone subjects (11 men and 21 women) were retrospective studied. Clinical and laboratory data were collected from every subject. **Results:** There was no difference in age between the groups (44.4±11.8 years and 43.3±17 years). In urine, volume, calcium (189.6±98.7 vs. 150.7±107.3, p=0.029), sodium and oxalate (33.7±28.2 vs. 22.2±15.3, p=0.041) were higher in kidney stones when compared to control subjects. Urinary excretion of uric acid and citrate was similar in both groups, with no significant differences. For the cohort of stone formers patients, but not for healthy subjects, there was a positive correlation between calcium and citrate excretion in urine (r=0.41, p<0.001). When utilizing the calcium/citrate ratio to differentiate stone and non-stone formers subjects, we could not find any difference. **Conclusion:** For the kidney stone formers, the assessment of risk factors by 24-hour urine needs to be categorized as continuous and not as interval variables. Prospective studies are necessary to determine the normal range of daily citraturia and to evaluate the role of isolated hypocitraturia in the incidence, prevalence and clinical course of kidney stone formation.

Keywords: citrate; nephrolithiasis; kidney stone; hypocitraturia.

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INTRODUCTION

The occurrence of urolithiasis is high and is increasing worldwide. The lifetime risk of symptomatic kidney stones is approximately 13% in men and 7% in women¹. In addition, its recurrence rate is also elevated. Once diagnosed, 50% of adult urolithiasis patients recurred in 5–10 years and 75% in 20 years².

Despite the progress in the pathogenesis of nephrolithiasis, the precise assessment of the risk of calcium oxalate urinary stones and the detection of patients at risk of recurrent stones are often difficult³. Hypercalciuria, the most common metabolic abnormality found in calcium stone formers, even being often familial and idiopathic, is mainly influenced by diet⁴. Hypercalciuria increases urine supersaturation and promotes crystal formation and growth. Reducing urine calcium with thiazide diuretics decreases the number of stone recurrences and stone formation rate⁵.

Urinary citrate also plays an important role in reducing the formation and recurrence of kidney stones by chelating calcium, inhibiting spontaneous nucleation and aggregation of oxalate crystals⁶ and interacting with Tamm-Horsfall protein to inhibit calcium oxalate crystallization⁷. Idiopathic hypocitraturia, usually defined as urinary citrate excretion less than 320 mg/day for adults, is a common metabolic abnormality occurring in 20% to 60% of stone formers^{3,8}. Citrate excretion is under influence of multiple factors and thus may influence by different ways the formation of kidney stones⁸.

Analysis of risk factors for nephrolithiasis by 24-hour urine is essential to prevent kidney stone recurrence. However, some publications that studied large cohort of patients found that traditional values need to be reassessed, as a substantial proportion of controls would be defined as “abnormal”. Furthermore, the association of several urinary parameters with risk of stone formation may be continuous rather than dichotomous⁹. Other authors suggested indexes (for example, the calcium/citrate ratio in urine) to improve the clinical diagnosis accuracy along with the criteria currently in use^{10,11}.

Our objective was to evaluate the urinary excretion of common risk factors associated with nephrolithiasis, with particular attention to citraturia and to the calcium/citrate ratio in recurrent kidney stones formers and in subjects without nephrolithiasis.

PATIENTS AND METHODS

We conducted a retrospective study from recurrent stone-formers who attended our Nephrolithiasis Clinic. The diagnosis of recurrent urolithiasis was based on

at least two episodes of spontaneous or surgical elimination of a stone or demonstration of more than one Rx-opaque kidney stone.

No patient had any systemic or renal disease as cause or consequence of stones and all had normal blood calcium. Sarcoidosis, vitamin D excess, malignant neoplasm, renal tubular acidosis, calcium or citrate supplement use at the time of the study evaluation was excluded. A control group of non-stone forming and without any known medical disorder was selected at the same time and in the age range of the first consultation of the case patient, in a proportion of ~1:3, respectively.

Data on each patient was collected, including age at first visit, gender, body mass index (BMI) and blood pressure (BP). Using our usual laboratory protocol [12], blood samples were taken after an overnight fasting to measure serum creatinine and calcium. 24-hour urine samples were collected with an interval of at least 30 days after ESWL or an acute pain episode, with patients in their usual diet. The following parameters were analyzed: pH, volume, creatinine, calcium, sodium, oxalate, citrate, and uric acid. Creatinine clearance was calculated as the ratio of 24-hour creatinine excretion to the serum creatinine and calcium/citrate ratio by the ratio of urine calcium to citrate in mg/day. The urinary parameters represent the average of two or three 24-hour urine collections for each subject.

Normally distributed continuous data were expressed as mean \pm SD and inter-group differences were assessed by independent Student's *t* test. Skewed continuous data were reported as median and interquartile range (25-75 percentiles), and differences between the groups were evaluated by the Mann-Whitney rank-sum test. Categorical data were assessed by Yates corrected chi-square test. Correlations between urinary parameters were assessed using simple linear regression. A multivariable linear regression model was used to evaluate the citrate excretion after adjusting for other variables. Statistical analysis were performed with Sigmastat software version 3.5 (Systat Software Inc, San Jose, CA) for data analysis. A *p* value of less than 0.05 was considered statistically significant and all reported *p* values are two-tailed.

The study protocol was approved by the local ethics committee.

RESULTS

A total of 103 kidney stone formers (30 men and 73 women) and 32 non-stone subjects (11 men and 21 women) were included in this study. The mean age for the urolithiasis group was 44.4 \pm 11.8 years and 43.3 \pm 17 years for the control subjects (*p*=0.656). Systolic (122 \pm 13 and 119 \pm 13 mmHg) and diastolic (82 \pm 10 and 77 \pm 12 mmHg) BP were within normal ranges for kidney stone formers and

controls, respectively. Both groups were categorized as overweight according to the mean BMI. However, subjects without kidney stones had a higher BMI (27.8 ± 5.7) than stone formers (25.4 ± 5 , $p < 0.025$).

Creatinine clearance, serum calcium and 24-hour urine collection are listed in Table. Glomerular filtration rate, as estimated by creatinine clearance, was similar between groups. Serum calcium has higher in the kidney stone formers, but within the reference range values (8-10

citrate excretion as the dependent variable. Only calcium was likely to be independently related to citrate excretion ($p < 0.02$).

DISCUSSION

About 80% of kidney stones are composed of

Table - Serum and 24 hour-urine biochemistry*

| | Stone Formers (n=103) | Controls (n=32) | p value |
|-----------------------|-------------------------|---------------------|---------|
| GFR** (ml/min) | 123 ± 34.87 | 123.38 ± 44.97 | 0.347 |
| Serum Calcium (mg/dl) | 9.4 (8.9 - 9.9) | 9.0 (8.8 - 9.3) | 0.008 |
| Volume (ml/24 hr) | 1920 ± 690 | 1550 ± 584 | 0.012 |
| pH | 5.96 ± 0.65 | 5.72 ± 0.57 | 0.238 |
| Creatinine (mg/24 hr) | 1490 ± 1097 | 1487 ± 618 | 0.995 |
| Calcium (mg/24 hr) | 189.6 ± 98.7 | 150.7 ± 107.3 | 0.029 |
| Sodium (meq/24 hr) | 160.8 (122.37 - 221.75) | 116.5 (79 - 172.80) | 0.013 |
| Oxalate (mg/24 hr) | 33.7 ± 28.2 | 22.2 ± 15.3 | 0.041 |
| Citrate (mg/24 hr) | 579.4 ± 423.5 | 542.1 ± 381.3 | 0.828 |
| Uric Acid (mg/24 hr) | 484.16 ± 193.29 | 508.57 ± 266.01 | 0.922 |

* Data showed as mean ± SD or median (25-75%); **GFR: Glomerular filtration rate as estimated by Creatinine Clearance

mg/dL). In urine, volume, calcium, sodium and oxalate were higher in kidney stones when compared to control subjects. Forty percent of stone formers (41/103) had urinary calcium levels higher than 200 mg/day compared with 15% (5/32) of controls ($\chi^2 - 0.09$). Urinary excretion of uric acid and citrate was similar in both groups, with no significant differences (Table). A total of 29% stone formers and 32% of controls subjects had urinary citrate levels less than 320 mg/day. Female stone formers had greater citraturia compared with men with nephrolithiasis (median 500.75 vs. 351.3 mg/day, respectively, $p < 0.03$). When utilizing the calcium/citrate ratio to distinguish stone and non-stone formers we could not find any significant difference ($p = 0.27$, Figure 1A). The same was true when we analyzed the calcium/citrate ratio among men or women with or without kidney stones (Figure 1B).

For the entire cohort of kidney stone patients, but not for the subjects without kidney stones, there was a positive correlation between calcium and citrate excretion in urine ($r = 0.41$, $p < 0.001$, Figure 2). In this group, multiple linear regression was performed using urinary volume, sodium, oxalate and calcium as significant variables with

calcium oxalate with variable amounts of calcium phosphate. Evaluation and medical management of recurrent kidney stone formers is cost-effective and can help decrease further recurrence¹³. Metabolic abnormalities, as identified by 24-hour urine collection, are found in the majority of patients.

In this study, urinary volume and excretion of calcium, sodium and oxalate were higher in this select population of recurrent kidney stone formers when compared to healthy, control subjects.

Imbalances between excretion of calcium, oxalate, and water create supersaturation. Idiopathic hypercalciuria is the most important metabolic abnormality found in patients with recurrent calcium stones. It is usually a familial disorder, although strongly influenced by diet⁵. Hypercalciuria often is defined as an excretion of more than >300 mg/day for men and >250 mg/day for women⁴ or sometimes as greater than 200 mg daily with no gender differences¹⁴. Our stone formers group excreted more calcium compared to controls (189.6 ± 98.7 vs. 150.7 ± 107.3 mg/day, $p = 0.029$) and 40% of them had urinary calcium levels higher than 200 mg/day compared

with 15% of controls ($\chi^2 = 0.09$). We believe that urinary calcium is continuously distributed and it is likely that values below those cited before are still relevant and should not be ignored¹⁵. Pak et al., by receiver operating characteristic curve analysis showed that the optimal cutoff point for calciuria was 172 mg/day, even if the patients were in a restricted diet¹⁶. In one study with large cohorts of patients, the relative risk of stone formation was increased more than 4 times with urine calcium ≥ 200 mg/day. Additionally, in this study the percentage of hypercalciuria in subjects without kidney stones was similar to our findings, ~15%⁹.

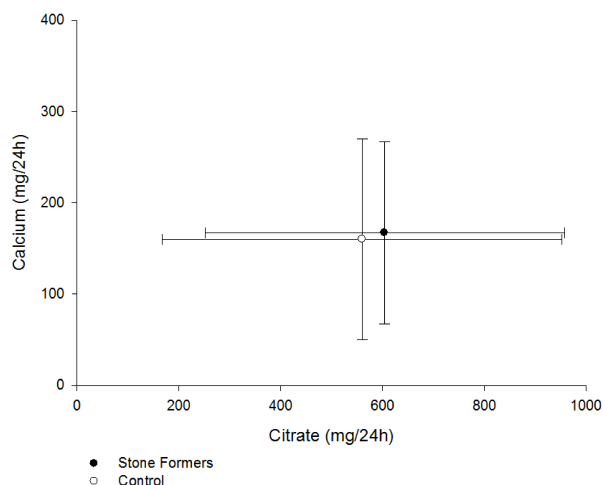


Figure 1A - Calcium/citrate ratio in stone formers and control subjects

Three findings of our work are relevant and related to pathogenesis of hypercalciuria and deserve a brief comment. First, we found a higher urinary volume in stone formers compared to control subjects (1920 ± 690 vs. 1550 ± 584 ml/day, $p=0.012$). Although the stone formers were selected for this work after the first or second visit to our clinic, the most plausible explanation for this finding is the so called stone clinic effect, the act of encouraging a high intake of fluid and diet counseling in the treatment of kidney stone disease^{17,18}. Another hypothesis, albeit not clinically tested, is that higher urinary calcium diminishes urinary concentration as an adaptation to prevent calcium stone disease. This assumption is based in animal studies that found reductions of medullary collecting duct water permeability with higher luminal calcium concentrations due to activation of calcium sensing receptors¹⁹. Second, both groups showed elevated 24-hour urinary sodium excretion, with higher levels in the kidney stone subjects (median 160.8 vs. 116.5, $p=0.013$). A high salt intake increases stone risk by reducing tubular calcium reabsorption and increasing urinary calcium, decreasing citruria and elevating urinary saturation of monosodium urate¹⁴. Finally, excretion of oxalate was significantly higher in stone forming group, although in levels

below the commonly accepted definition of hyperoxaluria, i.e. greater than 40 mg daily (33.7 ± 28.2 vs. 22.2 ± 15.3 , $p=0.041$)⁴. In a cross-sectional study of 3348 stone forming and non-stone-forming participants in the Health Professionals Follow-up Study (men), the Nurses' Health Study (older women), and the Nurses' Health Study II (younger women), Taylor and Curhan found similar median urinary oxalate of 39 mg/day in men, 27 mg/day in older women, and 26 mg/day in younger women²⁰. In normal people, the impact of dietary oxalate on urinary oxalate appears to be small. The amount of oxalate available for absorption throughout the intestine is highly dependent on the state of oxalate in the food ingested, and in the intestinal contents at each section of the intestinal tract since only the soluble form of oxalate can be absorbed²¹.

In our point of view, the most relevant topic to be discussed in this work is the failure of urinary citrate to differentiate stone formers from the control group and the high percentage of hypocitraturia, defined as <320 mg/day, in healthy subjects. Neither daily citruria (579.4 ± 423.5 vs. 542.1 ± 381.3 , $p=0.828$), the percentage of hypocitraturia, (29% vs. 32%, $\chi^2 = 0.9$), or the calcium/citrate ratio (Figure 1B) were different between the groups. We could only demonstrate that female stone formers had greater citruria compared with men with nephrolithiasis [median 500.75 vs. 351.3 mg/day, respectively, $p<0.03$] and that stone formers showed a positive correlation between calcium and citrate excretion in urine ($r=0.41$, $p<0.001$, Figure 2).

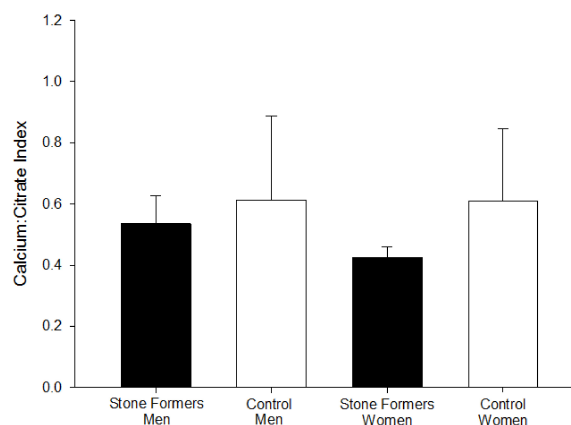


Figure 1B - Calcium/citrate ratio between men and women with or without kidney stones.

It is well known that urinary citrate inhibits calcium oxalate stone formation by lowering urinary calcium and by preventing agglomeration and growth through its ability to bind to the crystal's surface⁸. Citrate also improves inhibitory activity of Tamm-Horsfall protein, leading to formation of smaller and less aggregated crystals⁷. Additionally, randomized trials have shown

significant reductions in stone recurrence among hypocitraturic patients treated with potassium citrate⁴.

However, few studies compare citrate excretion between stone formers and healthy controls. In a work with a large number of subjects, the authors found no gender or case-control differences in citraturia. The percentage of hypocitraturia (<320 mg/day) varied from 3 to 9% in the control group and from 5 to 11% in the kidney stone formers and citraturia was not inversely associated with the risk of stone formation⁹. Other works also found overlap in the urinary citrate excretion between normal subjects and stone-formers²², similar values between normal subjects and first time stone formers, but not recurrent calcium stone formers²³ and even findings of 70% of kidney stone formers and 72% of controls presenting with citraturia <320 mg/day²⁴. There are also divergent findings when different works considered the balance between calcium and citrate (the calcium/citrate ratio) and not the absolute excretion of citrate in mg/day. Some studies considered the index useful^{10,11}, others could not find any difference between stone formers and controls²⁵.

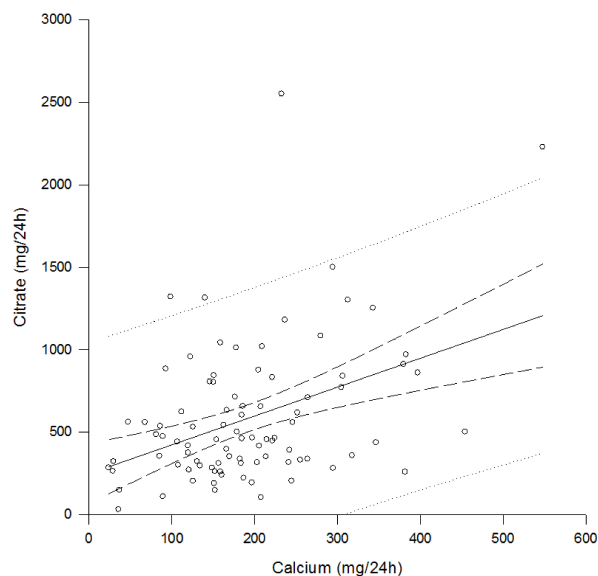


Figure 2 – Positive relationship between calcium and citrate urine excretion rate in stone formers group ($r=0.41$, $p<0.001$).

A number of aspects can influence citraturia and partially explain the large variation of hypocitraturia prevalence reported in the literature. Determination of citrate levels in urine can be affected by factors such as intra-individual variation²⁶ or even acidification of the samples²⁷. Laboratories can use different methods to measure citrate (for example, high-performance liquid chromatography (HPLC) or enzymatic citrate lyase-based) and results can be difficult to compare^{28,29}. Reference values of citraturia can vary substantially, ranging from a

cutoff of 115 mg/day in males and 200 mg/day in females³⁰ to >450 mg/day for males and >550 mg/day for females⁴. In addition, considering the complex nature of urinary citrate metabolism, genetic influences^{31,32} and diverse dietary and medical conditions can influence excretion of citrate^{2,6,8}.

Limitations of this work include the small sample size and its retrospective design. It is clear that we need more prospective studies to determine the normal range of daily citraturia and to evaluate the role of hypocitraturia in the incidence, prevalence and clinical course of kidney stone formation.

In summary, the present study showed that excretion of calcium, sodium and oxalate were higher in recurrent kidney stone formers when compared to healthy subjects and that neither the usual definition of hypocitraturia (>320 mg/day) or the calcium/citrate ratio were able to differentiate both groups.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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