Chemical analysis of stone in patients with nephrolithiasis

ABSTRACT

Introduction: Nephrolithiasis is a multifactorial disease and it has relation with genetic disorders and environmental factors. Stones are most common in adults and are associated with several metabolic disorders. Calcium oxalate is the most common type of stone. The objective of this study is to evaluate chemical analysis of calculi in our region. Methods: We made a retrospective study on 1,342 patients with evidence of recent formation of renal stones. Laboratory investigation included chemical analysis when the stones were available. Results: 1,342 patients with nephrolithiasis were consulted, among whom only 109 (8.1%) were submitted to chemical analysis of stones. Mean age of those patients were 38.9±13.4 years, and 55 (50.5%) were male. Familial history occurred in 65% of the cases. Calcium oxalate stones were found in 87% of the cases. Hypercalciuria and hyperuricosuria were the most associated metabolic disturb in patients with calcium oxalate and uric acid in the stones (60%). Conclusions: Chemical analysis has demonstrated that calcium oxalate is the most common component found in our region, according to the literature.

Keywords: nephrolithiasis, stone composition.

INTRODUCTION

Nephrolithiasis is a high prevalence disease affecting mainly adults aged between 20 and 60 years. It is estimated that up to 12% of the population will suffer an episode of nephrolithiasis during lifetime and, depending on the type of stone, more than 50% of them will have a recurrence within 10 years. Caucasians are more affected. Stone formation is related to hereditary factors, as well as to environmental factors such as geographical area, climate, dietary habits, and socioeconomical situation. Regarding the gender, it is believed that males are more affected, as there was a proportional decrease in the prevalence of nephrolithiasis compared to female gender.

The composition of urine is a major factor in crystal formation. An imbalance between promoters and inhibitory factors is responsible for nucleation and further development of the stone. States of known supersaturation are: hypercalciuria, hyperphosphatemia, hyperoxaluria, hyperuricosuria, and cystinuria. Hypocitraturia is the main condition of crystallization inhibitor deficiency. Other factors that may influence in the stone formation are pH changes and/or a reduction in urinary volume.

The main chemical component found in renal calculi was calcium oxalate, as shown by several authors. Calcium oxalate and uric acid calculi are more frequent in males, while calcium phosphate and struvite calculi are more frequent in females. The chemical analysis of the renal calculus is diagnosed in cystinuria events and calculi secondary to urinary infection. The aim of this study was to demonstrate the prevalence of the main chemical components found in urinary calculi in patients with nephrolithiasis in the west region of Paraná.

MATERIALS AND METHODS

The present study was approved by the Ethics Committee for Research in Humans at the UNIOESTE. Hospital charts from patients with evidence of urinary lithiasis within the last six months were evaluated from December 2001 to December 2008, and the following laboratory tests were performed: urinalysis, qualitative cystinuria, determina-
tion of oxalate, citrate, calcium, sodium and uric acid in a 24-hour urine sample, in addition to serum creatinine, calcium, uric acid, and parathormone. Laboratory methods used and adopted reference range adopted for 24-hour urine samples were: Calcium: Atomic absorption spectrophotometry method (<4.0 mg/kg); Uric acid: uricase enzymatic method (>15 mg/kg); citrate: citrate enzymatic method (>320 mg). For plasma determinations the methods used were as follow: Calcium: colorimetric method (8.5–10.5 mg/dL), uric acid: uricase colorimetric method (2.0 to 7.0 mg/dL); creatinine: alkaline picrate method (0.7 to 1.4 mg/dL); and parathormone: intact molecule assay. For qualitative cystinurina test: Sodium nitroprusside test. Chemical analysis of renal calculi was performed when it was available.

The method used for calculi chemical analysis, determined by the manufacturer of reagent Bioclin®️, is described as follows:

1. Preparing the sample being analyzed: Spraying the calculus. Transfer a small amount (40–50 mg) of the homogeneous powder into a test-tube of 13×100 mm and add 10 drops of reagent # 5+10 drops of distilled or deionized water. Heat under immersion in water at 56°C for 5 minutes, and shake the tube 2 or 3 times during this period. Centrifuge to 3,000 rpm for 3 minutes. Transfer all supernatant to another test-tube of 13x100 mm; Mark with letter S (to be utilized on phase 3) and mark the letter P for the other tube with the precipitated (to be utilized on phase 2).

Precipitated analysis: Carbonate: In the tube marked with letter P add 10 drops of reagent # 1, simultaneously observing if there was any gas separation. In affirmative case, the test is positive for carbonate. Add then 10 drops of distilled water and homogenize. Heat the tube in direct flame up to the first sign of boiling. Wait to be cold. This solution will serve as sample (sample P) for oxalate, calcium and magnesium tests described below. Oxalate: 0.1 ml of sample P+3 drop of reagent # 2. The formation of an intensive turvation or a white precipitate indicates the presence of oxalate. Calcium: 0.1 mL of sample P+5 drops of reagent # 6. The formation of white precipitation indicates the presence of calcium. Magnesium: Transfer 0.02 mL of sample P to an Erlenmeyer and add 20 mL of distilled or deionized water. Add to this solution 1 drop of reagent # 5. Homogenize. This will be the diluted sample. Use a test-tube 12x75 mm and add 7 drops of reagent # 7+10 drops of reagent #8. Homogenize. Add 0.05 mL of the diluted sample and homogenize. The appearance of violet color indicates the presence of magnesium.

Supernatant analysis: Urate: Transfer 0.1 mL of the supernatant from stage B.1 to a test-tube of 12x75 mm. Add 5 drops of reagent # 10 and 5 drops of reagent # 11. The appearance of an intense blue color indicates the presence of urate. Cystine: Transfer 0.1 mL of supernatant from stage B.1 to a test-tube of 12x75 mm. Add 1 drop of reagent # 1 and 1 drop of reagent # 13. Wait for 5 minutes. Add 2 drops of reagent # 14. The presence of an intense red color indicates the presence of cystine. Note: The color formed quickly disappears. Remember to take care while manipulating reagent # 13. It contains cyanide. Ammonium: Transfer 0.1 ml of supernatant, from B1 stage to a test-tube of 12×75 mm. Add 10 drops of distilled or deionized water. Homogenize. Add 5 drops of reagent # 9. The appearance of orange-yellow color indicates the presence de ammonium. Note: The color formed may acquire a dark tone if there is a high level of ammonium in the sample. Phosphate: Transfer 0.1 mL of supernatant, from stage B.1 to a test-tube of 12x75 mm. Add 1 mL of distilled or deionized water + 1 drop of reagent # 1. Homogenize. Add two drops of reagent # 3. Homogenize. Add 2 drops of reagent # 4. Let it rest for 2 minutes and add 2 drops of reagent # 5. The appearance of a blue color indicates the presence of phosphate.

Data was stored in a Microsoft Excel database and analyzed using descriptive statistics: Arithmetic means, standard deviation, minimum and maximum values, raw and percentage frequencies.

RESULTS

One hundred and nine (109) patients underwent an investigation of the chemical analysis of calculi, corresponding to 8.1% of the total cases considered for metabolic investigation of nephrolithiasis. The mean age of these patients was 38.9±13.4 years (ranging from 5 to 77 years). In relation to the gender, 55 patients (50.5%) were male. Regarding family history we obtained that information from 100 patients, and it was positive in 65 (65%) of the cases.

Regarding the chemical analysis of the stone, 95 patients (87.2%) had calcium oxalate, and this was the most frequent composition found. Table 1 shows the results of the chemical analysis performed in these stones. In relation to the results of metabolic investigation in patients who performed the chemical analysis, among those with calcium oxalate calculi or uric acid calculi, hypercalciuria and hyperuricosuria were the most common metabolic disorders.

DISCUSSION

In this study, 1,342 patients with nephrolithiasis were assisted; of these 109 performed the chemical analysis of their calculi. Calcium oxalate was the most prevalent chemical component, which is consistent with findings from other regions and corroborated by the literature.2,3
Urinary calculi formation may affect 5% to 12% of population. Several metabolic changes have been identified in the urine of patients with idiopathic recurrent nephrolithiasis, particularly hypercalciuria, hyperoxaluria, hypocitraturia, and hyperuricosuria, among others. These changes depend on a complex relationship between genetic and environmental factors. Data from the literature has demonstrated that, among patients with urinary lithiasis, 40% of them presented hypercalciuria as the ethiopathogenic factor.

Calcium oxalate calculi are more frequent than calcium phosphate calculi. The most important physiopathologic factor in formation of these calculi is hypercalciuria, which is a defect in at least one of the following organs: kidney, bones, or gout. Hypercalciuria increases the saturation and crystallization of calcium salts, reducing citraturia. In previous studies from our group, hypercalciuria was the most prevalent metabolic disorder in our region, but, as far as we know, no study evaluating the prevalence of chemical components of urinary calculi have been performed in the west region of Paraná.

The main chemical component in renal calculi is calcium oxalate. Baker et al. have found a 68% prevalence of calcium oxalate in urinary calculi studied in their series. Calcium oxalate calculi and uric acid calculi are more frequent in males, while calcium phosphate and struvite are more common in females.

Urinary composition is an important factor in crystal formation. An imbalance between promoter and inhibitory crystallization factors is responsible for nucleation and posterior development of the calculus. States of known supersaturation are: hypercalciuria, hyperphosphatemia, hyperoxaluria, hyperuricosuria, and cystinuria. Hypocitraturia is the main condition of crystallization inhibitor deficiency. Other factors that may influence the calculus formation are pH changes and/or reduction in urinary volume.

The interaction among renal tubular cells, calcium oxalate crystal and oxalate ions are an early event in lithogenesis. Urine contains ions, glucoproteins and glucosaminoglicans, which inhibit the crystallization process, protecting the kidney against lithogenesis. Renal epithelial tubular cells are the main target to the effects induced by hyperoxaluria- and by calcium oxalate crystal incorporation. Since tubular cells damage predisposes to nucleation, maybe an increase in glucosaminoglicans synthesis may protect the tubular epithelium against crystal adhesion and toxic effect of hyperoxaluria, what could limit the formation of renal calculi.

This study was the base to the knowledge of chemical components of urinary calculi in patients with nephrolithiasis in the west region of Paraná.

### Table 1: Chemical Analysis of Urinary Stones in 109 Patients

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium oxalate</td>
<td>95</td>
<td>87%</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>44</td>
<td>40%</td>
</tr>
<tr>
<td>Uric acid</td>
<td>23</td>
<td>21%</td>
</tr>
<tr>
<td>Ammonia</td>
<td>18</td>
<td>17%</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>8</td>
<td>7%</td>
</tr>
<tr>
<td>Cystine</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Struvite</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Total of chemical constituents</td>
<td>190</td>
<td></td>
</tr>
</tbody>
</table>

**REFERENCES**


