Enhancing factors of urinary stones and evaluation of the incidence of various urinary stone types in hospitalized patients and outpatients

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Summary

Purpose: Urinary stones’ formation depends on various factors, we studied some of these in 153 patients with lithiasis compared to 62 controls during 5 years. Materials, methods: We obtained information on lifestyle and medical history. Macro-and microscopic examination of stones was performed and chemical analysis. GraphPad InStat was used for data processing. Results: Average age of patients with lithiasis was 52.66 years +/-13.94 (SD). Calcium oxalate was found in 96% of the stones, phosphate in 66%, xanthine/uric acid 17%, cysteine in 3%, carbonate in 1%, the composition is mixed in 73% of the cases. Compared to the controls, patients with urolithiasis consume significantly less liquids, incidence of urinary infection is significantly higher. Significantly more patients are taking medications in the study group compared to the control group, some products favor lithiasis. Average pH of urine was significantly lower in the study group compared to controls. The highest incidence is in case of calcium oxalate stones. Conclusions: Patients with urolithiasis present numerous factors favoring stones: ingestion of low liquid amounts, decreased urinary pH, high frequency of urinary infections. Occurrence of relapses could be prevented by elimination of favoring factors, having an appropriate diet, adequate fluid intake and treatment of urinary infections.

Introduction

The development of urinary stones depends on a number of factors: genetic predisposition, diet, diseases of the urinary tract and hormonal influences. Metabolic degradation products and excess of ions are eliminated by the body through the urinary tract, and under certain circumstances these can precipitate, leading to the formation of urinary stones (Yagisawa et al, 2000). Each stone contains an inorganic part formed by
different types of crystals, and 1-5% of organic substances (Brener et al, 2011), Shakhaee et al, 2012).

The type of stone depends on the surrounding environment, such as the pH of the urine, the chemical composition and the concentration of the different dissolved substances in the urine. The main important stone types, their chemical composition, properties and the pH of the urine favoring their formation are included in table 1.

Table 1. Classification of urinary stones (Simon et al, 1993).

<table>
<thead>
<tr>
<th>Stone types</th>
<th>Chemical composition</th>
<th>Properties</th>
<th>pH of the urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whewellit (calcium oxalate monohydrate)</td>
<td>Ca(COO)₂H₂O</td>
<td>Dark brown very hard crystals, smooth surface</td>
<td>5.6-6.5</td>
</tr>
<tr>
<td>Weddellit (calcium oxalate dihydrate)</td>
<td>Ca(COO)₂ 2H₂O</td>
<td>Pale, shiny, glass-like crystals</td>
<td>5.5-6.5</td>
</tr>
<tr>
<td>Apatite</td>
<td>Ca₅(PO₄,CO₃)₂</td>
<td>White, soft crystals</td>
<td>6.3-7.5</td>
</tr>
<tr>
<td>Brushit</td>
<td>CaHPO₄ 2H₂O</td>
<td>Bone-like, compact, hard crystals</td>
<td>6.0-6.5</td>
</tr>
<tr>
<td>Struvit</td>
<td>MgNH₄PO₄ 6H₂O</td>
<td>White, gel-like crystals</td>
<td>6.8-8.6</td>
</tr>
<tr>
<td>Uric acid</td>
<td>H₂C₅H₂O₃N₄</td>
<td>Brownish-purple-colored, compact crystals</td>
<td>4.6-5.5</td>
</tr>
<tr>
<td>Ammonium urate</td>
<td>NH₄HC₅H₂O₃N₄</td>
<td>White, compact crystals</td>
<td>6.0-7.0</td>
</tr>
<tr>
<td>Cystine</td>
<td>(C₃H₆O₂NS)₄</td>
<td>Yellow, pale, resinous, wax-like crystals</td>
<td>5.5-7.0</td>
</tr>
</tbody>
</table>

The factors that favor urinary stone formation can be classified in three groups:
1. Prerenal causes: exogenous (environmental factors and diet) and endogenous factors (metabolic disorders, endocrine diseases like hyperparathyroidism, tumors, gastric hypoacidity, etc.)
2. Renal causes: kidney malfunction, tubular disorders (such as tubular acidosis)

Urinary lithiasis is a very common disease in Romania, with an increasing incidence worldwide, it affects around 5% of the population. The peak age for developing is between 30 and 50 years and recurrence is common. Prevention of stone recurrence in patients predisposed for this disease is a main important aspect.

The aim of the study

In our study we followed the results of urine analysis (including associated urinary tract infections) and assessment of lifestyle habits in patients with urinary lithiasis compared with a control group, we determined the frequency of different types of stones based on microscopic examination and chemical analysis.

Materials and methods

The study was conducted between September 2009 and April 2014, the study group included 153 patients with urinary stones belonging to the Marmed and Procardia Medical Units, the Clinic of Urology and the Extracorporeal Shock Wave Lithotripsy Unit.
in Tîrgu Mureș. In the control group consisting of patients without urinary stones we included 62 people.

We obtained data on lifestyle, diet and medical and family history using a special questionnaire designed by our research team members, which contained 28 questions. We were interested in the type of food and the liquids consumed by each individual and the quantity of the intake, the associated diseases and type of medication, the presence of urinary lithiasis in the family history and information about urine analysis (pathological elements determined by strips and sediment, and uroculture). In case of the patients admitted to the hospital, we also obtained information for our study from medical records. We compared the data of questionnaires and urine analysis between the two groups.

We performed the macro-and microscopic examination (Elerom photomicroscope, 3,4X) of the urinary stones followed by their chemical analysis performed in glass test tubes or porcelain capsules, using the powder obtained by smashing the stones in a mortar using a pestle.

The presence of oxalate was determined by adding 1 mL of 5% H2SO4 solution and 2-3 drops of 1‰ KMnO4 to a small amount of the stone powder. After heating the violet solution, if oxalate is present, it reduces KMnO4 causing discoloration.

The presence of phosphate was determined by adding a few drops of concentrated HNO3 (d = 1.42 g/mL) and 1-2 mL of 5% ammonium molybdate solution to a small amount of the stone powder. After heating a yellowish color or precipitate of ammonium phosphomolybdate indicates the presence of phosphate in the renal stone.

If carbonate is present, dissolving a small amount of stone powder in 2N HCl solution CO2 gas is released.

Urate (murexid reaction) is performed in a porcelain capsule, by adding 2-3 drops of concentrated HNO3 (d = 1.42 g/mL) to the stone powder and boiling the content till it evaporates. The reddish color which appears indicates the formation of purpuric acid through the oxidation of uric acid by HNO3. By adding a drop of 25% NH3 and a drop of 10% NaOH a violet color appears due to formation of murexid (ammonium purpurate).

For the identification of xanthine the same reaction is performed in a porcelain capsule as in case of the identification of urate, with concentrated HNO3. The presence of xanthine determines the formation of a yellow residue which doesn’t change color after adding NaOH solution. By adding NH3 solution to it the color changes to red.

The presence of cystine was determined in a test tube by adding 1 mL 10% NaOH and 2-3 drops of Pb(CH3COO)2 solution to the stone powder. After boiling the content of the test tube for 1-2 minutes, the presence of cystine causes the formation of PbS (black precipitate) (Hobai et al, 2010).

The GraphPad InStat program was used for statistical data processing, using unpaired Student’s t test and contingency tables.

Results and discussions

The mean age of the patients with urolithiasis was 52.66 +/- 13.94 (SD) years, 53% of them being men, and the control group’s was 54.89 +/-15.81 (SD) years (p>0.05).

Some urinary stones were removed by surgery, the majority of them were eliminated by the patients spontaneously or after extracorporeal lithotripsy.

Each stone was measured and examined at a photomicroscope. After the chemical analysis, we selected a few stones based on their composition and the type of the crystals to be examined with a stereomicroscope (Traveler, 200X). Some of the obtained images are presented below.
Fig. 1. Urinary stone examined by the stereomicroscope.

Fig. 2. Kidney stone containing calcium oxalate and uric acid crystals (Stereomicroscopy, 200X).

Fig. 3. Kidney stone containing calcium oxalate dihydrate crystals (Stereomicroscopy, 200X).

Fig. 4. Kidney stone containing phosphate and carbonate crystals (Stereomicroscopy, 200X).

Fig. 5. Kidney stone containing calcium oxalate, phosphate and cystine crystals (Stereomicroscopy, 200X).

Fig. 6. Kidney stone containing calcium oxalate monohydrate and phosphate crystals (Stereomicroscopy, 200X).

Regarding the chemical composition of urinary stones calcium oxalate could be found in 96% of the cases, phosphate in 66%, xanthine/uric acid in 17%, cystine in 3%.
carbonate in 1%, the composition is mixed in 73% of the cases.

**Fig. 7.** Chemical nature of the mixed urinary stones.

Based on the data obtained from the questionnaire, patients with urolithiasis consume significantly less liquids, under 2 liters per day (p = 0.018) compared to the control group.

Significantly more patients are taking medications in the study group compared to the control group (p = 0.0004), a part of them (certain diuretics, antacids, steroids, etc.) favouring lithiasis.

The mean pH value of the urine was significantly lower in the patients with urinary stones compared to the control group (p=0.0473), but this parameter is prone to important variations in time.

**Fig. 8.** Urine pH in the study group and the control group.

The incidence of urinary infection was significantly higher in the group presenting urolithiasis compared to the control group (p<0.0001).

**Fig. 9.** Incidence of urinary infections in the study group and the control group.

Urinary infection occurred in 38% of the stone patients. The most frequent microorganisms were: *Escherichia coli*, responsible for 26% of the infections, coagulase negative *Staphylococcus*, which caused 26% of the infections, *Pseudomonas aeruginosa* was present in 20% of the infections, Klebsiella pneumoniae caused 6% of the infections and *Proteus mirabilis* was present in 6% of the infections.

**Discussions**

Percentage of different types of urinary stones obtained after chemical analysis corresponds to data in the literature (Foley & Boccuzzi, 2010), (Jawalekar et al, 2010), (Manglaviti et al, 2011).

An important etiological factor of urinary stones containing calcium is primary hyperparathyroidism. The main biochemical change in these patient’s blood is the elevated calcium level, associated with high parathormone (PTH) levels. Unfortunately we don’t have data regarding calcium and PTH values of our patients included in the study, so we cannot evaluate the incidence of this disease in case of our study group.

The study needs to be continued, to include larger number of patients for a final conclusions based on processing the data of the questionnaires regarding diet and lifestyle habits and the incidence of hormonal disorders. Each patient included in the study received a document containing general and special recommendations depending on the result of chemical stone analysis.

In case of calcium oxalate stones, which is the most representative crystal type in our study
group, a low-calcium diet is recommended, patients are instructed to avoid large amounts of milk, yogurt and cheese. Bread also contains important quantities of calcium, so it should be consumed in small amounts. On the other hand these patients are also advised to avoid consuming large amounts of oxalate-rich foods such as walnuts, spinach, rhubarb, parsley and chocolate. Carbohydrates also promote oxalate formation so carbohydrate-rich foods should also be avoided especially in obese patients. Some studies demonstrated that in certain cases, in patients with idiopathic hypercalciuria, a diet with a normal amount of calcium but with reduced amounts of animal protein and salt can be more effective than the traditional low-calcium diet in reducing the risk of recurrent stones (Borgi et al., 2002), (Pearle et al, 2014).

Several researchers have used the magnesium/calcium ratio as an index of susceptibility of urine to form kidney-stones in patients. In general, patients with a urinary magnesium/calcium ratio of 0.7 is normal, whereas a value lower than 0.7 may be considered as stone-forming (Bastian & Vahlensieck, 1975).

It would have been interesting to evaluate the body mass index for our patients (which we couldn’t do because of missing data), obesity being another risk factor that favors urinary stone formation (Nicola et al, 2012), (Rendina et al, 2014), (Shavit et al, 2015), (Torricelli et al, 2015).

Other bacteriological studies found the same Escherichia coli as the most frequent isolated species both from urine and urinary stones (Tudor et al, 2013), (Tudor et al, 2011).

Conclusions

Calcium oxalate is the most frequent crystal type in our study group, followed by phosphate, and most patients present crystals with mixed composition.

Patients with urolithiasis presented numerous factors favoring stone development compared to the control group: ingestion of too low amounts of liquids, decreased pH of the urine favoring especially oxalate stone formation, high frequency of urinary tract infections.

The occurrence of relapses could be prevented by elimination of favoring factors, having an appropriate diet, adequate fluid intake and treatment of urinary tract infections.

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References

Nicola Th, Dobreanu Minodora, Buda Brânduşa, Oşan V., Gliga Florina, Sátán Edit: Obesity and Calcium Oxalate Renal Stones, Acta Medica Marisiensis, 58(6), 422-425, 2012
Rendina, D., De Filippo, G., D'Elia, L., & Strazzullo, P. Metabolic syndrome and nephrolithiasis: a systematic review and meta-analysis of the

Shakhaee K., Malaouf N.M., Sinnott Bridget: Kidney stones: Pathogenesis, Diagnosis and Management, J Clin Endocrinol Metab., 97(6), 1847-1860, 2012


