Urinary glycosaminoglycan excretion in urolithiasis

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Abstract
Urinary glycosaminoglycan (GAG) excretion was measured in children with idiopathic urolithiasis (15 girls and 10 boys; mean (SD) age 6.2 (2.4) years) and in healthy controls (10 girls and 14 boys; mean (SD) age 6.8 (3.8) years). GAG excretion was expressed as a GAG/creatinine (mg/g) ratio and was evaluated using dimethylmethylen blue. In healthy control children, the mean (SD) GAG/creatinine ratio was 31.67 (12.76) and it was similar in girls and boys. The children with idiopathic urolithiasis had significantly lower mean (SD) GAG/creatinine ratios than controls (22.59 (7.35)). Therefore, urinary GAG excretion may be important in the disease process in children with urolithiasis, as it is in adults.

Keywords: glycosaminoglycans; urolithiasis; renal stones

The urinary inhibitors of crystal nucleation, growth, and aggregation play an important role in urolithiasis. Such inhibitors are presumed to afford protection against the formation of stones in normal individuals. Their deficiency in, or absence from, the urine of patients with stones is thought to predispose these individuals to the disease.

In vitro studies—except those of Grases and Costa-Bauza, who reported that pentosan polysulphate promoted calcium oxalate dihydrate crystal formation in synthetic urine—have shown that glycosaminoglycans (GAGs) are potent inhibitors of crystal growth and aggregation.

There have been few studies on urinary GAG excretion in various forms of childhood nephrolithiasis. Therefore, we have attempted to establish whether the differences that we described previously between controls and adults with renal stones are also present in children with urolithiasis.

Subjects and methods
We studied 15 girls and 10 boys, mean (SD) age 6.2 (2.4) years, with idiopathic renal stone disease. The calculi were composed of calcium oxalate (n = 18) or calcium phosphate (n = 7). The children had taken no medication for at least two weeks, and were not suffering from malabsorption, either as a result of tubular acidosis or malformations of the urinary system. The control group consisted of 24 healthy children (10 girls and 14 boys; mean (SD) age 6.8 (3.8) years).

Written consent was obtained from parents. All subjects had normal renal function, blood pH, and serum concentrations of parathyroid hormone, uric acid, sodium, potassium, chloride, magnesium, calcium, and phosphorus. Routine urine examinations were normal and urine was sterile in all subjects.

Patients and controls were placed for three days on a standard diet containing predetermined amounts of calories, proteins, and mineral salts in proportion to age. At the end of the third day, 24 hour urine specimens were collected at 4°C without preservatives. On arrival at the laboratory, the total volume and relative density of each sample were measured and general urine analysis tests were carried out.

Calcium and magnesium measurements were performed by atomic absorption spectrophotometry. Uric acid, oxalate, and phosphate concentrations were measured by means of conventional enzymatic kits.

For GAG measurement, the GAGs were precipitated with cetylpyridinium chloride and then reacted with dimethylmethylen blue to produce a complex with the polyanionic molecule of sulphated GAGs. The GAG results were expressed as a GAG/creatinine (mg/g, respectively) ratio. All chemical materials were purchased from Sigma Chemicals.

Statistical analysis was performed using the Student’s t test for unpaired data.

Results
Table 1 lists the concentrations of urinary calcium, magnesium, phosphate, oxalate, and uric acid, and pH values. Figure 1 shows the GAG/creatinine ratios.

There were no significant differences in the excretion of calcium, magnesium, phosphate, oxalate, and uric acid between the patients and controls.

Table 1. Urinary calcium, magnesium, oxalate, phosphate, and uric acid concentrations, and pH values in controls and patients with urolithiasis

<table>
<thead>
<tr>
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<th>Controls (n = 24)</th>
<th>Patients (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mmol/l)</td>
<td>2.70 (1.23)</td>
<td>2.93 (1.32)</td>
</tr>
<tr>
<td>Magnesium (mmol/l)</td>
<td>2.43 (0.90)</td>
<td>2.41 (1.20)</td>
</tr>
<tr>
<td>Oxalate (mmol/l)</td>
<td>0.40 (0.13)</td>
<td>0.42 (0.16)</td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
<td>41.5 (8.1)</td>
<td>37.6 (10.5)</td>
</tr>
<tr>
<td>Uric acid (mmol/l)</td>
<td>4.45 (1.69)</td>
<td>4.70 (1.50)</td>
</tr>
<tr>
<td>pH</td>
<td>6.90 (0.30)</td>
<td>7.20 (0.30)</td>
</tr>
</tbody>
</table>

Concentrations are mean (SD) in mmol/l.

Figure 1 Mean (SD) urinary GAG/creatinine ratio (mg/g) in controls and in patients with urolithiasis.

*Comparison with controls (p < 0.005).
The control subjects had a mean (SD) GAG/creatinine ratio of 31.67 (12.76). We found no differences between girls and boys in mean (SD) urinary GAG/creatinine ratio (33.34 (9.43), 30.39 (14.69), respectively). In patients, the mean (SD) urinary GAG/creatinine ratio was significantly lower (22.59 (7.35)) than in control subjects (p < 0.005).

**Discussion**

In adults with calcium stones a higher urinary excretion of calcium, oxalate, and urate as well as a deficiency in inhibiting substances has been reported repeatedly. There have been numerous reports showing that urinary GAGs are low in adult patients with nephrolithiasis. However, although Michelacci et al suggested that urinary GAG excretion in children with urolithiasis was significantly lower,6 these data have not been confirmed by others. These data have not been confirmed by others.6

The mean (SD) urinary GAG concentrations in 15 healthy children were reported as 17.00 (15.60) mg/day by Lama et al. However, Baggio et al reported that the mean (SD) GAG concentrations in healthy children were 61.26 (17.94) mg/l and found no significant difference in urinary GAG excretion between children with idiopathic urolithiasis and healthy controls. Michelacci et al reported a mean (SD) GAG/creatinine (mg/g) ratio of 24.33 (1.91) in healthy children; this result is close to our findings of 31.67 (12.76). Discrepancies between data from different studies might be caused by the different methods used to measure GAGs. In the literature, the measurement of urinary GAG concentrations is commonly performed by the borate–carbazole method or other procedures involving the basic metachromatic dye, alcian blue. The borate–carbazole method measures the hexuronic acid residues of GAG molecules and, therefore, cannot detect keratan sulphate because the hexuronic acid residues are replaced with galactose in keratan sulphate. In addition, other procedures involving the use of alcian blue are not specific for urinary GAGs because negatively charged molecules, other than sulphated GAGs, interfere with the assay. Finely dispersed precipitates obtained with alcian blue are often difficult to harvest. On the other hand, Hesse et al indicated that there was a diurnal rhythm in urinary GAG excretion. As GAG excretion was highest in the morning and lowest at night, 24 hour urine collection would avoid falsely high results. Therefore, we performed the GAG measurements in 24 hour urine specimens and we also calculated total GAG concentration by using the GAG/creatinine ratio in 24 hour urine samples, which gives more reliable results.

A procedure involving the use of the basic metachromatic dye dimethylene blue, which we used for the first time to determine GAG concentrations in the urine of children with stones, is reported to be more sensitive than the other methods used to analyse urinary GAG concentrations in patients with urolithiasis. Our data lead us to propose that urinary GAGs may play an important role in the prevention and reduction of calculi in children, as has been found in adults.

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