Urinary calcium excretion in children with vesicoureteral reflux

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Abstract

Background. Renal malformations including vesicoureteral reflux (VUR) are associated with urolithiasis. However, studies on urinary calcium excretion in children with VUR have not been reported. This study was conducted to find out whether children with VUR have a higher prevalence of hypercalciuria and whether their family members are affected by hypercalciuria and/or urolithiasis.

Methods. We studied the prevalence of hypercalciuria and urolithiasis in 46 children (12 males and 34 females) with VUR and in their parents.

Results. Three out of 46 children had renal colic and nine out of 46 exhibited calyceal microlithiasis in the renal sonography. According to Stapleton’s criteria, we found that 27 out of 46 children (58.6%) had hypercalciuria. These children were significantly shorter than children with normal calciuria and showed lower values of maximal urinary osmolality. We found no differences in urinary calcium excretion values related to the VUR grading, or to the presence or absence of renal scars, or to whether VUR was still unresolved or already resolved at the time of study. Seventeen out of 27 children with hypercalciuria (63%) had one or both parents affected by hypercalciuria, and there was a history of urolithiasis in six first-degree relatives and in four second-degree relatives (37%). Besides, 10 out of 19 children without hypercalciuria (52.6%) had one or both parents affected by hypercalciuria and there was a history of urolithiasis in three first-degree relatives and in three second-degree relatives (31.6%). Among the 27 children whose parents had hypercalciuria, four had both parents affected, 19 had only the mother affected and in four patients only the father was affected.

Conclusion. Our results showed that the prevalence of hypercalciuria was greater in paediatric patients with VUR than in the general population. Urolithiasis in patients with VUR had a metabolic origin.

Hypercalciuria was inherited as an autosomal dominant trait although with a higher probability to be inherited from the mother.

Keywords: hypercalciuria; inheritance; vesicoureteral reflux

Introduction

Urolithiasis in children is known to be related to congenital anatomic deformities of the genitourinary tract. The frequency of this association has been estimated between 19.1 and 29.8% [1,2]. The relationship between urolithiasis and malformation is not yet clear. The pathophysiologic mechanism of stone growing has been related to urine infection and to urine stasis, which is more evident in junction obstruction.

The prevalence of vesicoureteral reflux (VUR) in patients with renal stones has been estimated to be between 4.1 and 8.5% [1,3], which is much higher than in the normal population (<1%) [4]. We have studied patients with VUR during infancy who were managed by surgical or prophylactic treatment. Years later some of these patients returned to the hospital with a renal colic. To our knowledge, this is the first study that analyses urinary calcium excretion in children with VUR. This study was carried out to find out if children with VUR had a higher prevalence of hypercalciuria and whether their family members were affected by hypercalciuria and/or urolithiasis.

Subjects and methods

Patients

This study included 46 children (12 males and 34 females) with VUR who were reviewed in our hospital during a 1-year period. The age at diagnosis was 3.9 ± 2.6 years old (range 0.16–9.8 years old), and the age at the study time was 9.6 ± 4.6 years old (range 0.9–21 years old). We did not
include patients with chronic renal failure, or cromosomopathies, or VUR secondary to urological problems (urethral valves, neurogenic bladder). Urine infection was excluded in every child at the urine collection. VUR was diagnosed by voiding cystourethrography and classified in grades I–V according to the International Reflux Classification [5]. One patient was classified as VUR grade V, seven patients as grade IV, 19 as grade III, 16 as grade II and three as grade I. VUR was associated to a bilateral duplex collecting system in three patients. Renal scarring was diagnosed in 28 patients (60.9%) by isotopic renal scanning with 99mTc-dimercaptosuccinic acid. The remaining 18 children exhibited no scars. At the time when the data were collected, VUR had resolved in 41 patients (89.1%) (22 having received antibiotic prophylaxis, 13 having been treated with ureteral reimplantation and six with cystoscopic surgery). In the other five children VUR was still unresolved and they were receiving low-dose antibiotic prophylaxis. The study was approved by the Committee of Clinical Investigations at the Nuestra Señora de Candelaria University Hospital. All parents gave informed consent.

Methods
A retrospective study of the clinical files was carried out to examine the presence of crystalluria, history of renal colic and calyceal mycrolithiasis at the renal sonography. Parathyroid hormone (PTH) and calcitriol levels, urinary excretion of calcium, N-acetyl-β-D-glucosaminidase (NAG), albumin and creatinine was measured as well as maximal urinary concentrating capacity.

Following the criteria reported by Stapleton et al. [6], hypercalciuria was diagnosed when the value of calcium to creatinine ratio (UCa/UCr) in non-fasting single spot urines was >0.5 mg/mg for children under 2 years of age and >0.2 mg/mg for children over 2 years of age, in two consecutive urine collections; this value is exactly 95 percentile in our own control group of 100 healthy children between 4 and 15.3 years old [7].

Calcium to creatinine ratio in non-fasting single spot urine was measured in every patient’s parents without changing their diets. Similarly, hypercalciuria was diagnosed when UCa/UCr was >0.2 mg/mg, according to the average value found previously in our adult population (UCa/UCr = 0.12 ± 0.04 mg/mg) [8]. Every patient was interviewed about family history of renal urolithiasis.

Analytical procedures and renal function tests
Urinary creatinine and calcium were measured by standard techniques using an Hitachi 717 autoanalyzer (Boehringer Mannheim). NAG was determined using the same system by a colorimetric enzymatic assay based on dicitolophenolsulfphate-NAG hydrolysis (Boehringer Mannheim). Urinary albumin was measured by a nephelometric technique (Array). Calcitriol (Incstar) and intact PTH (Nichols) levels were measured by radioimmunoassay.

Renal concentrating capacity was determined after administration of 20 μg of intranasal desmopressin (DDAVP). Three urine samples were collected at 90 min intervals. Urinary osmolality was determined by measuring the freezing point depression in an Osmostat Osmometer (Menarini).

Ultrasound examination was carried out on every patient using an Orion sonolayer (Philips). Calyceal mycrolithiasis was defined on positive finding of hyperechogenic spots in renal calyces. These were usually devoid of shade cone and were <3 mm in diameter.

Statistical methods
Quantitative variables, which follow a Gaussian distribution, are expressed as mean value ± standard deviation. Calcium to creatinine ratio, which does not show a Gaussian behaviour, is expressed as median and interquartile range. Student’s t-test was used to compare pairs of independent quantitative variables. When there were fewer than 15 patients in any of the groups, Mann–Whitney rank sum test was employed. Analysis of variance was applied for more than two groups. Probability values lower than 0.05 were considered as significant.

Results
Intact PTH levels measured in 10 children were normal (22.06 ± 4.95 pg/ml; range 17–31). In eight out of 10 children calcitriol levels were normal, whereas in the other two children, calcitriol levels were >50 pg/ml. Calcium to creatinine ratio mean value and standard deviation in children with VUR was 0.21 ± 0.13 mg/mg (range 0.03–0.56 mg/mg). According to the Stapleton’s criteria [6], 58.6% of the children (n = 27; seven males and 20 females) affected with VUR have hypercalciuria as well.

Children with VUR and hypercalciuria had a height significantly lower than children with VUR and without hypercalciuria (standard deviation score: −0.18 ± 0.84 vs 0.49 ± 1.22, P = 0.02). Maximal urinary osmolality was also lower (Table 1). No differences were found in urinary NAG and albumin excretion between both groups (Table 1). There was no difference in the children’s age (9.0 ± 5.1 vs 10.7 ± 3.5 years). On the other hand, no differences in urinary calcium excretion were found in relation to VUR grading (Table 2), renal scarring presence (Table 3) or VUR state (resolved or unresolved).

Only one of the 27 children with VUR and hypercalciuria had been affected with a renal colic, six out of 27 had calyceal microlithiasis and another four children had exhibited crystalluria in the urinary sediment (calcium oxalate two, urate one and calcium phosphate one).

Within the group of children with VUR and without hypercalciuria (19 out of 46), two had been affected with renal colic at some point, three had calyceal microlithiasis and another had exhibited crystalluria in the urinary sediment sometime (calcium phosphate).

Hypercalciuria prevalence in parents and family urolithiasis history in both subgroups of children are shown in Tables 4 and 5. In summary, hypercalciuria was diagnosed in some members of 37 out of 46 families (80.4%). In 10 families only children were affected, in other 17 families, both children and parents were affected, and in the remaining 10 families, only parents were affected. Parents were affected with hypercalciuria in 27 families (58.7%) (both parents in
Biochemical data in patients with VUR in relation to urinary calcium excretion

<table>
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<tr>
<th></th>
<th>Patients with VUR and hypercalciuria</th>
<th>Patients with VUR and without hypercalciuria</th>
<th>P</th>
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<tbody>
<tr>
<td>Ca/Cr (mg/mg)a</td>
<td>0.29 (0.09) (n = 27)</td>
<td>0.07 (0.04) (n = 19)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>NAG/Cr (U/g)b</td>
<td>4.1 ± 1.1 (n = 8)</td>
<td>3.2 ± 1.2 (n = 13)</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin/Cr (µg/µmol)b</td>
<td>1.7 ± 0.9 (n = 19)</td>
<td>1.1 ± 0.7 (n = 18)</td>
<td>NS</td>
</tr>
<tr>
<td>Uosm (mosmol/kg)b</td>
<td>803.3 ± 197.7 (n = 23)</td>
<td>914.3 ± 126.5 (n = 19)</td>
<td>P = 0.03</td>
</tr>
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</table>

Ca/Cr, urinary calcium creatinine ratio; NAG/Cr, N-acetyl-β-D-glucosaminidase creatinine ratio; Albumin/Cr, albumin creatinine ratio; Uosm, urinary osmolality after administration of desmopressin.

aMedian value and (interquartile range).

Discussion

The prevalence of hypercalciuria in our patients with VUR was much higher than in the normal population. In a previous study, we have reported that the prevalence of hypercalciuria in control children in the island of Tenerife is 3.8% [7]. In other countries hypercalciuria prevalence reported is between 2.9 and 8.6% [8], whereas in other regions of Spain it is probably a little higher, between 7.8 and 13.5%. To our knowledge, the highest prevalence of hypercalciuria (16%) is found in the island of La Gomera, which is very close to Tenerife [7].

Calciuria transiently high has been described in children with *Escherichia coli* urine infection [10], however, none of the children in our study had that infection at the time of the study. Urinary calcium excretion in these children was not related to VUR grade, renal scars (Tables 2 and 3) or reflux status (resolved or unresolved). Maximal urinary osmolality was significantly lower in children with VUR and hypercalciuria than in children with VUR but without hypercalciuria. It is well known that patients with idiopathic hypercalciuria may associate a decrease in concentrating capacity. The mechanism producing this concentrating defect is not yet clear, but it has recently been related to an increased urinary prostaglandin E₂ (PGE₂) excretion, which happens in some patients with idiopathic hypercalciuria [11]. PGE₂ reduces water transport at the collecting duct level, counterbalancing vasopressin effect. Another more complex hypothesis
relates concentrating capacity disturbance to the activation of a calcium receptor in the thick ascending limb [8].

The lower height in children with VUR and hypercalciuria is difficult to understand. There was no significant difference in age between this group and the group with VUR and normal urinary elimination of calcium. Although it has not been reported in the literature that children with idiopathic hypercalciuria are shorter than control ones, some authors have suggested a hypothesis relating both conditions, hypercalciuria and shortness [12].

Renal colic prevalence in this group was 6.5% and calyceal microlithiasis was 19.6%. Hypercalciuria prevalence in this group was much higher than urolithiasis prevalence. It is well known that hypercalciuria remains asymptomatic especially in children, until stones are formed or until it is manifested with other symptoms different from renal colic, such as haematuria, mictional symptoms or urinary tract infections.

The high hypercalciuria prevalence found in the parents and the high prevalence of urolithiasis in other relatives of these children with VUR, point to a genetic origin of the hypercalciuria. One or both parents had hypercalciuria in 27 out of 46 families (58.7%). Moreover, nine out of 92 parents (9.78%) had been affected with renal colic before the study, although urolithiasis prevalence reported in mid-aged people is only between 1.7 and 3.8% [13,14]. In our study, the characteristics of the diet were not investigated. However, the high frequency of hypercalciuria and/or urolithiasis detected in both children and their parents suggests a genetic but not a dietetic origin of our findings.

Our data suggest that the inheritance of hypercalciuria in patients with VUR is autosomal dominant as it has been described for idiopathic hypercalciuria [15], although with a higher probability to be inherited from the mother. This may be due to a mechanism known as genetic imprinting, which has been observed in many autosomal dominant transmitted diseases in which an autosomal gene has a different behaviour depending on the parent from whom has been inherited.

In addition, it is known that VUR is an inheritable disease and it has been reported that 40–50% of siblings of affected children may also have demonstrable VUR early in life. It is generally accepted that VUR inheritance in many families is dominant with varying penetrance and expression [16]. Also, another genetic abnormalities have been reported associated to clinical diseases, which include VUR. Patients with mutations in the gene encoding the PAX2 transcription factor have renal anomalies and VUR [17]. Angiotensin type 2 receptor null mutant mice (Agtr 2) have a high prevalence of structural anomalies of the genitourinary tract including VUR, obstructive megaureter and ureteropelvic junction obstruction [18].

A point of discussion is why some families do not have concordance in the presence of hypercalciuria among their members. In this sense, it is necessary to remember that daily urinary calcium excretion is dependent on several factors; mainly the diet, the intestinal calcium absorption and the bone apposition rate. Any modification in these factors may vary calcium excretion daily. Urinary calcium excretion is modified not only by dietary calcium ingestion, but also by sodium, animal proteins, whole cereals and ω-3 fatty acids intake. Parents who had been affected with renal stones probably had specific diets and this could be varying their calcium excretion. In addition, we have observed some children in our hospital with familial hypercalciuria who exhibit normal transient calciuria near puberty, probably due to an increased need of calcium and later, when puberty is over and growth has finished, calcium excretion increases again. This phenomenon of transient decreased calcium excretion has also been observed in healthy adolescents [9]. In the present study calciuria decreased when renal scars increased (Table 3), although there were no significant differences, probably due to the small size of the sample.

Nevertheless, 8.7% of the families of children with VUR had no relation with renal urolithiasis. Normal calciuria levels in these families could be related to another genetic protective factor that we ignore, as it could be a decrease in number or sensitivity of intestinal or bone vitamin D receptors (VDR). In this sense, it is known that intestinal calcium absorption is lower in patients with an alelic variant BB in VDR [19].

In summary, our data suggest that urolithiasis in children with VUR has mainly a metabolic origin although other factors such as infection or stasis have long been identified and may have some influence. The metabolic origin is hypercalciuria, which is an inheritable disease. This points to a common link between these two inheritable conditions: VUR and hypercalciuria-urolithiasis. Although a prospective study including the diet of the patients is necessary, our data support the idea that adults with hypercalciuria, especially women, symptomatic or not, are at a higher risk of conceiving children affected by VUR. On the other hand, children with VUR are at a higher risk of associated hypercalciuria and urolithiasis along their lives even if the VUR is resolved.

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References


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