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RESEARCH ARTICLE

Risk factors evaluation for urolithiasis among children

Francisco Velásquez-Forero[a,*], Mariela Esparza[a], Alejandro Salas[b], Mara Medeiros[c], Georgina Toussaint[c,†], Francisco Llach[d]

[a] *Laboratorio de Metabolismo Mineral Óseo, Hospital Infantil de México Federico Gómez, Mexico City, Mexico*
[b] *Laboratorio de Metabolismo Mineral Óseo, Hospital Infantil de México Federico Gómez; Servicio de Nefrología, Hospital General de México, Mexico City, Mexico*
[c] *Laboratorio de Nefrología y Metabolismo Mineral Óseo, Hospital Infantil de México Federico Gómez, Mexico City, Mexico*
[d] *Former Director of Clinical Nephrology and Hypertension. Georgetown University Hospital, Washington, DC, USA*

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Abstract
Background: The prevalence of pediatric urolithiasis varies from 0.01–0.03%. Urolithiasis may be caused by anatomical, metabolic and environmental factors. Recurrence varies between 16 to 67%, and it is frequently associated with metabolic abnormalities. The objective of the present work was the identification of risk factors that promote urolithiasis in a child population.
Methods: This study included 162 children with urolithiasis and normal renal function (mean age 7.5 years). Risk factors were investigated in two stages. In the first stage, 24-hour urine, and blood samples were analyzed to assess metabolic parameters and urinary tract infection. During the second stage, the effect of calcium restriction and a calcium load on renal Ca excretion were evaluated. Data were statistically analyzed.
Results: Urolithiasis was observed in 0.02% of children, 50% of them with family history of urinary stones. There were multiple risk factors for urolithiasis including hypocitraturia (70%), hypomagnesuria (42%), hypercalciuria (37%; in 11/102 was by intestinal hyperabsorption, in 13/102 was unclassified. Ca resorption or renal Ca leak were not detected). We also detected alkaline urine (21%), systemic metabolic acidosis (20%), urinary infections (16%), nephrocalcinosis with urolithiasis (11%), oliguria (8%), urinary tract anomalies, hyperuricosemia and hypermagnesemia (7% each one), hypercalcemia (6%), hyperoxaluria (2%) and hypercystinuria (0.61%).
Conclusions: Hypocitraturia and hypomagnesuria were the most frequent risk factors associated with urolithiasis, followed by hypercalciuria. High PTH values were excluded. Children presented two or more risk factors for urolithiasis.

* Corresponding author.
E-mail address: fcovelfor@gmail.com (F. Velásquez-Forero).

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PALABRAS CLAVE
Hipocitraturia;
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Urolitiasis pediátrica;
Acidosis metabólica sistémica;
Factores de riesgo para urolitiasis

Evaluación de factores de riesgo para urolitiasis en niños

Resumen
Introducción: La prevalencia de urolitiasis pediátrica varía de 0.01–0.03%. Las causas de urolitiasis pueden ser anatómicas, metabólicas o ambientales. Las recurrencias varían entre 16 a 67%, y están frecuentemente asociadas con alteraciones metabólicas. El objetivo del presente trabajo fue la identificación de factores de riesgo que promueven la urolitiasis en una población infantil.
Métodos: Se incluyeron 162 niños con urolitiasis y función renal normal, cuya edad media fue de 7.5 años. Los factores de riesgo fueron investigados en dos etapas. En la primera, con la muestras de orina de 24 h y sangre, se investigaron parámetros metabólicos e infecciones del tracto urinario. En una segunda etapa se valoró la calciuria, previa restricción seguida de carga de Ca. Los hallazgos fueron analizados estadísticamente.
Resultados: Se presentó urolitiasis en el 0.02% de los niños con historia familiar en el 50%. Se observó hipocitraturia (70%); hipomagnesuria (42%); hipercalciuria (37%; en 11/102 fue por hiperabsorción intestinal; en 13/102 fue inclasificable; no se observó hipercalciuria por resorción o pérdida renal). También se observó orina alcalina (21%); acidosis metabólica sistémica (20%); infecciones urinarias (16%); nefrocalcinosis con urolitiasis (11%); oliguria (8%); anomalías urinarias congénitas, hiperuricosemia e hipermagnesemia (7% cada una); hipercalcemia (6%); hiperoxaluria (2%); e hipercistinuria (0.61%).
Conclusiones: La hipocitraturia e hipomagnesemia fueron los factores de riesgo con mayor frecuencia, seguidos de hipercalciuria. Se excluyeron los valores de hiperparatiroidismo. Los niños exhibieron dos o más factores de riesgo para el desarrollo de urolitiasis.

1. Introduction

Pediatric urinary stone disease occurs infrequently in developed countries. The incidence of hospital admission due to urinary stones varies from 0.01–0.03%.[1,2] However, in the United States, there has been an increase from 18.4 cases per 100,000 hospital admissions in 1999 to 57 cases per 100,000 in 2008, with an adjusted annual increase of 10.6% ($p > 0.001$). Thus, the incidence of urinary tract stone disease has increased significantly among adolescents since 1996 during a 25-year period.[3,4] Recurrence varies from 16 to 67% and is more frequently present in children with metabolic disorders.[5] The site of localization of the stone varies: 66% of stones are found in the renal parenchyma and the rest are located in the ureter, ureterovesical-junction and bladder.[6]

Factors that enhance stone formation include decreased levels of natural inhibitors of urinary tract stone formation, such as citrate, magnesium, osteopontin,[7] pyrophosphate (PP_i), calgranulin,[8] uromodulin,[9] urokinase,[10] Tamm-Horsfall glycoprotein and albumin.[11] In addition, concentrated urine is a risk factor as it favors an increase of salts, resulting in their precipitation.[12] Other factors include anatomical abnormalities of the urinary tract, metabolic disorders, urinary tract infections, environment and low urine volume.

Urinary tract stones are rarely asymptomatic. Common symptoms include abdominal pain, hematuria and dysuria. Urolithiasis (UL) is observed more frequently in children than in older patients[6]. A family history of urinary tract stones is present in 20–37% of children but it occurs at all ages.[13] Studies suggest that nephrolithiasis (NL) is inherited with a non-Mendelian transmission pattern with multiple genes.[14]

Some studies have described that renal stones are more frequent in males, but recent studies have mentioned that female children are at higher risk.[15] To this day, more than 28 pathogenic factors have been associated with the formation of urinary tract stones; this number is increasing and the frequency changing. Nevertheless, more studies for the evaluation of these factors in the pediatric population are needed. The purpose of this study was to determine the frequency of urinary tract stones and the pathogenic factors associated with their formation in patients from the Hospital Infantil de México Federico Gómez (HIMFG).

2. Patients and methods

This study was conducted according to the recommendations of the World's Medical Association Declaration of Helsinki, and was approved by the hospital's Internal Review Board (IRB) and the Ethics Committee. The IRB considered unwise to submit children for the diagnosis of renal tubular acidosis (RTA) because it requires the sampling of arterial blood, neither children < 5 years for dietary calcium (Ca) manipulation. Parental written consent and assent of the patient were obtained in all cases.

Our data included 775,230 children evaluated from 2009 to 2014, admitted to the hospital outpatient department. The pediatricians in our urology-nephrology clinic initially diagnosed urinary tract stones in 170 children < 17 years

Table 1 Comparison of controls vs. patients results of selected stone-forming risk factors.

Blood (mg/dl)	Control (N = 170)	Patients (N = 162)	*p* value
Ca	9.53 ± 0.68	11.07 ± 0.50 (10)	< 0.001
Mg	2.14 ± 0.31	1.24 ± 0.37 (11)	< 0.001
P	4.11 ± 0.96	4.67 ± 1.22 (162)	< 0.001*
Creatinine	0.67 ± 0.30	0.68 ± 0.27 (162)	> 0.8
Uric acid	4.0 ± 5.8	7.83 ± 1.06 (11)	< 0.001
Albumin	4.4 ± 0.53	2.3 ± 2.4 (21)	< 0.001
PTH (pg/ml)	33.94 ± 12.70	21.0 ± 13.3 (162)	< 0.001*
Urine (24 h)			
Citrates (mg/24 h)	512.66 ± 183.09	204.33 ± 81.13 (113)	< 0.001
Mg (mg/24 h)	87.64 ± 35.12	23.34 ± 10.55 (70)	< 0.001
UCa/BM(mgCa/kg/24 h)	(NV ≤ 4 mg/kg/24h)[19,20]	6.06 ± 2.01 (60)	< 0.001
P (mg/24 h)	651.38 ± 255.84	576.88 ± 116.62 (162)	< 0.001*
Creatinine (mg/24 h)	673.27 ± 655.31	109.2 ± 59.9 (162)	< 0.001*
Uric acid (mg/24 h)	96.04 ± 102.94	141.53 ± 77.92 (162)	< 0.001*
Oxalates (mg/24 h)	15.65 ± 11.58	57.19 ± 6.51 (3)	< 0.001

Values were expressed as mean ± SD (n).
PTH, parathyroid hormone; UCa/BM, urinary calcium-body mass relation; NV, normal values.
* Significant difference but no clinical abnormality (data levels in each children were within the normal range).

of age (mean age 7.5 ± 3.9 years) who were selected to perform this study following the exams according to the research protocol.

Control subjects (170) were also evaluated during this period (2009–2014). Medical charts were reviewed, family history analyzed, and laboratory data collected. Control subjects with normal renal function had a mean age of 8.0 ± 4.7 years. Fifty-two of them (30%) underwent abdominal ultrasound and no UL was found. Blood and urinary biochemical studies were performed and all results were within normal levels. Data of patients with urinary tract stones as compared with controls are included in Table 1.

2.1. Classification criteria

As NL is multifactorial in origin in each children, a minimum scheme diagnostic criteria was used as follows:

Patients (170) were evaluated for urogenital abnormalities, back pain, hematuria, and recurrent urinary tract infections, confirming the diagnosis by ultrasound, plain abdominal X-rays and the spontaneous expulsion of stones. They all resided in Mexico City and the surrounding metropolitan area. A detailed family history suggestive of UL was obtained. The effect of Ca manipulation was evaluated only in children > 5 years. Children with abnormal renal function or receiving therapy that altered Ca, phosphorous (P), magnesium (Mg), uric acid (UA), oxalates, or vitamin D were excluded. Patient nutrition was evaluated via Z-scores from body mass index (BMI), considering the following ranges: normal > −1 to < +1; low ≤ −1; overweight ≥ +1 to < +2; and obese ≥ +2.[16]

An ambulatory protocol divided into two stages was followed:

The first stage (basal data) included two 24-hour urine samples and one blood sample collection. For urine, volume, Ca, oxalates, Mg, uric acid, Na, K, Cl, citrates, P, creatinine, and cystine levels were quantified and urine samples were cultured. Venous blood samples were analyzed for parathyroid hormone ''intact'' molecule (PTHi), HCO_3, PCO_2 and pH, creatinine, Cl, Ca, P, Mg, uric acid, albumin and creatinine clearance.[17,18]

The second stage included a period of Ca restriction, a fasting urinary sample and another sample of Ca load in 102 children > 5 years. The analyses were designed to investigate the origin of hypercalciuria with an indirect assessment of renal handling of Ca after a period of dietary Ca restriction and after a diet Ca load to categorize the hypercalciuria into absorptive, resorptive, renal or unclassified form.[18] Dietary Ca restriction phase consisted of a daily reduced Ca diet (50%) for 7–10 days. On the last day, venous blood and urine samples were collected to test the levels of Ca and Pi in urine, and Ca, P and PTH in blood.[17,18]

Parameters were classified as follows:

- Calciuria. Normal levels of urinary Ca were obtained from international data for children (normal values ≤4 mg Ca/kg/24 h).[19,20] Ca levels were measured in controls and patients with anatomic absorption spectrophotometry.
  - Renal hypercalciuria. A ''fasting urinary sample'' was obtained from children with hypercalciuria on a restricted diet 2 h after the end of the Ca restriction phase. Abnormal fasting urinary Ca on the restricted diet is defined by UCa x SCr/UCr ≥ 0.11 mg/Ca/dlGF (where UCa stands for urinary Ca, SCr for serum creatinine, and UCr for urinary creatinine in mg/dl of glomerular filtrate), accompanied by normocalcemia, normophosphatemia and elevated serum PTH. In this situation, a restricted renal tubular reabsorption of calcium is suggested.[18]
  - Absorptive hypercalciuria (dietary Ca load phase). Patients with hypercalciuria after Ca restricted diet,

with normal ''fasting urinary Ca'' (< 0.11 mg/dlGF) were administered 1 g of Ca (2 tablets, 500 mg each one). Four hours later, a urine sample was obtained for UCa and UCr levels measurement. An increase in urinary Ca after the Ca load is defined by UCa/UCr $\geq$ 0.20 mgCa/mg creatinine, accompanied by normocalcemia, normophosphatemia, and normal PTH. An increased calcium intestinal absorption is suggested.[18]
  - Resorptive hypercalciuria. It occurs if an increased bone turnover leads to urinary loss of bone Ca by primary hyperparathyroidism. Hypercalcemia, hypercalciuria and high serum levels of PTH are present.[17,18]
  - Unclassified hypercalciuria. Hypercalciuria on the restricted diet that cannot be categorized into absorptive, resorptive or renal form is considered as unclassified.[18]
- Hypocitraturia. Urinary citrate was measured with a commercial kit (Boehringer Mannheim, Indianapolis, USA), based on citric conversion to oxaloacetate and the liberation of NADH + H, which is equivalent to the amount of citrate[21] (normal levels from the controls were $\geq$ 306 mg/24 h).
- Hypomagnesuria. Urinary Mg was measured using an atomic absorption spectrophotometry. Hypomagnesuria is determined by levels < 50 mg/d on a random diet, and the absence of a gastrointestinal disorder.[22]
- Systemic metabolic acidosis. It was determined with a gasometer (GEM Premier 3000), recognized by the co-occurrence of blood acidemia (pH < 7.40, low serum bicarbonate concentration < 20 mEq/l and PCO_2 > 34 mmHg in children < 2 years. For children > 2 years, values are pH < 7.39, low serum bicarbonate concentration < 22 mEq/l and PCO_2 > 37 mmHg).[23]
- Low urinary volume. Urinary samples were assessed for volume in ml/m^2 (oliguria $\leq$ 300 ml/m^2 per 24 h).[24]
- Urinary pH. Urinary pH is simple a measure of free H^+ ions in urine. It was measured with an electrode: pH values $\geq$ 6.5 for alkaline urine, and pH $\leq$ 5.518 for acid urine.
- Hyperuricosuria. Hyperuricosuria is defined as the urinary excretion of uric acid > 600 mg/d (measured by enzymatic spectrophotometry procedures), and urinary pH $\geq$ 5.5.[18]
- Hyperoxaluria. It was determined with an oxalate kit purchased from Trinity Biotech (Berkeley Heights, NJ) based on the oxidation of oxalate by oxalate oxidase, followed by the measurement of hydrogen peroxide by oxalate oxidase and a peroxidase-catalyzed reaction (hyperoxaluria > 45 mg/d).[25,26]
- Nephrocalcinosis (NC) and renal calculi. The presence of calcifications in the renal parenchyma (NC) or located within the collecting system (UL) were identified by ultrasound or X-ray. NC and UL may occur simultaneously or individually. NC can be diagnosed mainly when increased echogenicity appears in the renal medulla.[27,28] Familial hypomagnesemia with hypercalciuria and nephrocalcinosis is an autosomal recessive tissue disorder.
- Urinary tract infection. Chronic urinary tract infection may be associated with urease-production bacteria, resulting in alkaline urine, and stones composed of Mg, ammonium and phosphates (struvite stones).[29]
- Urinary cystine. It was detected by Sullivan test, with sodium cyanide-naphto-quinone-1-sulfonate (normal values: >60 mg/1.73/m^2/day).[30]

2.2. Analytical methods

Urinary Na and K levels were measured by flame photometry, and Cl was determined by colorimetric silver precipitation. Albumin was detected using the bromocresol green method (normal values: 3.5–5.0 g/dl).[31] The glomerular filtration rate (GFR) was estimated by Schwartz formula as follows: eGFR/ml/min 1.73 m^2 = kL/SCr (mg/dl), where k is a constant value (0.55 for children and adolescent girls, and 0.7 for adolescent boys), L is the length in cm, and SCr is the serum creatinine levels (normal values: > 60 ml/min/1.73 m^2).[32]

PTHi (normal values: 15.18–53.4 pg/ml) was assessed by radioimmunoassay after labeling with I^{125} (DiaSorin, Inc., Stillwater, MN, USA)[33]. Other reagents were obtained from local distributors.

2.3. Statistical analysis

Data from control children were analyzed to obtain reference values for biochemical parameters. Means and standard deviations were calculated to fix the cut-off points that defined the abnormal values. Subsequently, patients and controls results were compared using the Student's t-test and Fisher's exact test with a 5% of significance level[34]. All data were analyzed using SPSS version 18.0.

3. Results

During a 5-year period (2009–2014), 775,230 children were admitted to the HIMFG outpatient department. The urology/nephrology team detected the presence of UL in 170 children. Thus, the frequency of UL in a 5-year period was 0.02% (1/4560 consults). From this group, eight children were excluded because the lack of compliance. Finally, 162 children with a mean age of 7.5 years, ranging from 6 months to 17 years were included in the study. Sixty children were < 5 years and 102 children were > 5 years. There were 127 male (78%), and the gender ratio male/female was 3:1. A family history of calculi in first and second-degree relatives was present in 49.6%. The mean serum creatinine level was 0.70 $\pm$ 0.3 mg/dl, and the mean creatinine clearance was 109.2 $\pm$ 59.9 ml/min/1.73 m^2. The BMI (children Z-score) was normal in 55%, low in 27%, overweight in 13% and obesity in 5% of the children.

Clinical symptoms included abdominal pain (52%), hematuria (36%), urinary tract infection (22%) and dysuria (18%). The diagnosis of UL was confirmed by renal ultrasonography in 60%, by spontaneous expulsion in 30%, and by plain abdominal X-ray in 10%. The distribution of the stones was pyelocaliceal (52%), ureteral (7%), bladder (8%), or urethra (3%).

Table 1 includes basal serum and urinary values from the 162 patients as compared with controls. Significant changes (p < 0.001) were observed for serum Ca in 10 patients; for serum uric acid, and Mg levels in 11 patients, as well as for albumin in 21 subjects. P, creatinine and basal serum PTHi levels were within the normal levels. In urine, we observed low citrate levels in 113 children; low Mg levels in 70 children; hypercalciuria in 60 patients, and significant hyperoxaluria in three patients. Children with normal renal

Table 2 Main risk factors for urolithiasis observed in children (N = 162).

	N	%
Hypocitraturia	113	70
Hypomagnesuria	70	43
Hypercalciuria	60	37
Ca intestinal hyperabsorption (102 children > 5 years)	11	11
Unclassified hypercalciuria (102 children > 5 years)	13	13
Alkaline urine	34	21
Acid urine	33	20
Systemic metabolic acidosis	33	20
Urinary tract infection	26	16
Nephrocalcinosis with urolithiasis	18	11
Oliguria	14	8
Congenital urinary malformations	11	7
Hyperuricosemia	11	7
Hypomagnesemia	11	7
Basal hypercalcemia	10	6
Hyperoxaluria	3	2
Hypercystinuria	1	0.61

function (170) or lithiasis (162) results exhibited significant differences in blood (P and PTHi) and urine samples (P, creatinine and uric acid), but each one presented laboratory values and clinical behavior within the normal limits.

The frequency of the main risk factors was observed as follows: hypocitraturia in 70%, hypomagnesuria in 43%, and hypercalciuria in 37%. Alkaline urine was present in 21% of the patients; acid urine in 20% and systemic metabolic acidosis in 33 children (10 children < 2 years and 23 children > 2 years). Urinary tract infection (UTI) was noticed in 26/162 children (16%) (Table 2). Fourteen children had positive urine cultures for *E. coli* and *Staphylococcus aureus*, and 12 children for *Proteus sp, Klebsiella sp*, and *Pseudomonas aeruginosa*. UL with NC was noted by ultrasound and with plain abdominal X-rays in 18 children (11%) (Table 2), from which none presented hypomagnesemia with hypercalciuria and NC (Table 3). Ca deposits were predominantly localized in the medullary region and the stones in the collecting tubes. Oliguria was noticed in 8% of these patients; congenital urinary malformations, hyperuricosemia and hypomagnesemia in 7% each; basal hypercalcemia in 6%; hyperoxaluria in 2%; and hypercystinuria in 0.61% (Table 2).

On the second stage (dietary Ca restriction), 102 children were evaluated. Basal Ca, Pi and PTHi levels, and levels after Ca restriction were similar. Basal hypercalciuria was observed in 46/102 (45%) from which 20 patients (19.6%) persisted with hypercalciuria after Ca restriction (Table 4). In the fasting urinary example (Ca > 0.11 mg/dl GF), 13/102 patients exhibited hypercalciuria (12.7%), accompanied by normocalcemia, normophosphatemia and normal PTHi. This findings suggested an unclassified hypercalciuria group (Tables 2 and 4).[18] After dietary Ca restriction and Ca load, 11/102 (10.7%) children > 5 years exhibited an increase in urinary Ca (Ca > 0.20 mg/mg creatinine) accompanied by normocalcemia, normophosphatemia and normal PTHi, suggesting a Ca intestinal hyperabsorption (Tables 2 and 4). We did not identify any children with resorptive hypercalciuria, or renal hypercalciuria. All children presented at least two or more risk factors for the urinary tract lithiasis.

4. Discussion

This study examines the risk factors and frequency of UL in patients from the HIMFG in Mexico City. They were evaluated by the urology and nephrology division. In the past, studies emphasized isolated factors as the cause of UL.[7,8] In the present study (from a single institution), UL frequency was 0.02%, which is similar to those reported in Spain (0.02%), USA (0.057%) and Iceland (0.098%).[1–3] In the present prospective study, more than 28 independent biochemical parameters were evaluated in children > over a 5-year period (2009–2014). Family history of NL was present in 50% of our cases, similar to the percentage reported by Noe, et al. (50%)[35] and lower than those reported by Polito, et al. (73%),[36] and Spivacow, et al. (79%).[6] There were 127 males and 35 females. Male/female ratio was 3:1; other reports have shown that females seem to be at a higher risk.[15] It remains unclear whether gender is a risk factor or not.

Obesity, a risk factor recently associated with NL[37], was observed in 5% of the children. A tendency to the formation of uric acid stones is reported to be increasing in obese people (metabolic syndrome).[38]

Table 3 Serum and urinary parameters in children with nephrocalcinosis compared to those with urolithiasis.

	Nephrocalcinosis (n = 18)	Urolithiasis(n = 162)	*p*
Serum Ca (mg/dl)	9.3 ± 1.19	9.24 ± 0.68	0.73
Serum P (mg/dl)	4.3 ± 0.68	4.8 ± 1.20	0.013
Serum albumin (mg/dl)	4.4 ± 0.53	2.3 ± 2.4	< 0.001*
Serum PTH (pg/dl)	16.6 ± 9.7	18.3 ± 11.7	0.61
UCa/BM (mgCa/kg/24 h)	2.0 ± 1.73	2.6 ± 2.6	0.35
Citric acid (mg/24 h)	148.2 ± 112.3	217.02 ± 319.8	0.96
Urinary pH	6.3 ± 1.21	6.3 ± 0.92	0.09
Hypomagnesemia with hypercalciuria	none	2	
Premature children	No history	4	

PTH, parathyroid hormone; UCa/BM, urinary calcium-body mass relation.
* Student's t-test.

Table 4 Ca restriction biochemical basal values in fasting urinary and after Ca load in 102 children > 5 years.

	Serum basal levels	After Ca restriction	*p*
Ca	9.18 ± 0.80	9.22 ± 0.82	0.3
P	4.68 ± 1.20	4.44 ± 1.24	1.2
PTHi	17.6 ± 10.5	18.3 ± 11.7	0.5
		Urine basal levels (N = 102) n (%)	After Ca restriction n (%)
Basal hypercalciuria		46 (45%)	20 (19.6)
Fasting hypercalciuria (>0.11 mg/dlFG)			13 (12.7)
Hypercalciuria in loaded Ca (>20 mgCa/mgUCr)			11 (10.7)

Hypocitraturia was the most frequent biochemical risk factor identified in 70% of our children (Table 2). Citrate is the most abundant organic anion found in the urine, as well as an important endogenous inhibitor of calcium NL because it binds ionized calcium and reduces calcium saturation preventing the growth of calcium phosphate crystals.[39] Some studies show that males present a lower rate of citrate excretion than females, and that citrate excretion rises with age.[40] Recently, few authors have mentioned the presence of hypocitraturia in children with UL. However, Tefekli, et al.[41] and VanDervoort, et al.[42] reported that hypocitraturia is a predominant risk factor in children with UL (61–52%). The main cause and pathogenesis of hypocitraturia is an increase in the renal tubular reabsorption of citrates, in part due to intracellular acidosis and/or a reduced alkaline load.[43] In our patients, systemic metabolic acidosis (20%) and acid urine (20%) observed may explain partially the hypocitraturia. Urinary citrate deficiency is characteristic of renal tubular acidosis (RTA), particularly distal RTA (dRTA). The correlation of hypocitraturia with RTA[44] was not properly evaluated because samples came from venous blood. High animal-protein diets produce more hydrogen ions that are buffered by calcium released from the bone increasing urinary excretion, which is prone to cause low urinary citrate and stones.[45] Chronic diarrhea produces chronic acidosis and may cause low urinary citrate and NL.[46] Hypokalemia and potassium deficiency decrease urinary citrate probably by an acid-base effect.[46] Thiazide diuretics, often used to lower urinary calcium, decrease citrate excretion, particularly if potassium deficiency is allowed.[47] Intensive exercise has also been reported to cause low urinary citrate.[48] Chronic renal insufficiency reduces daily citrate excretion; this may be secondary to mild chronic metabolic acidosis.[46] All children presented normal renal function. As mentioned before, many patients with calcium NL have low urinary citrate. Although some of these individuals might have unrecognized acid-base problems (chronic diarrhea, high protein diets, or incomplete dRTA), there is no clear reason for the low urinary citrate in others, and the underlying mechanisms are presently unknown. These individuals are considered to have idiopathic hypocitraturia.

Hypomagnesuria was present in 70 children (43%) (Table 2), mostly associated with hypocitraturia. These data are important, since urine magnesium levels are considered as a natural protective factor that inhibit calcium crystal formation.[6,39]

We observed as well that basal hypercalciuria was present in 60 children (37%), from which 43 (72%) were male. This abnormality was not as frequent as previously reported (40–50%).[8,17,18] Naturally occurring inhibitors of calcium crystal formation, anatomical abnormalities, urinary tract infections, environmental factors and low urinary volume enhance the formation of calcium stones. Calcium supersaturation is strongly linked to high urinary calcium excretion.[39] Most urinary stones are composed of calcium oxalate or calcium phosphate.[49]

In the second stage of the analysis, basal serum and urinary Ca before and after dietary Ca restriction in 102 children > 5 years were evaluated. Serum Ca and P results were normal in both groups, with no significant differences. Serum PTHi levels did not increase after Ca restriction due to the absence of hyperparathyroidism (Table 4). However, basal urinary Ca assessment exhibited 46/102 children (45%) with hypercalciuria, and 27/46 of them were associated with hypocitraturia. After Ca restriction only 20/102 children (20%) remained with hypercalciuria, and 11 with hypocitraturia as well. The ''fasting urinary sample'' exhibited hypercalciuria > 0.11 mg/dlGF in 13/102 of the children (13.0%), suggesting an abnormal handle of renal tubular calcium reabsorption. Nevertheless, this group presented normocalcemia, normophosphatemia and PTH levels in serum were also normal. Therefore, it could not be classified as absorptive, resorptive or renal form of hypercalciuria. Levy, et al. termed this as unclassified hypercalciuria.[18] In this group, the excess of PTHi restores serum Ca to normal. Unlike primary hyperparathyroidism, calcemia is normal and the hyperparathyroidism is secondary.[17]

The assessment of Ca levels on the restricted diet and the post Ca load urine sample revealed high levels of Ca (>0.20 mg urinary Ca/mg urinary creatinine) in 11/102 patients (11.0%). Six of them presented hypocitraturia as well, accompanied by normal Ca levels in the fasting urinary sample (>0.11 mg/dlGF). It was associated with normocalcemia, normophosphatemia and normal PTH levels, suggestive of intestinal Ca hyperabsorption.[18]

Alkaline urine (pH $\geq$ 6.5) was observed in 34/162 children (21%). Alkaline urine has been associated with heterogeneous causes, but are well known as *Proteus* sp. UTI and alimentary disorders. $CaHPO_4$ urinary stones belong to an elevated urine pH as a common feature.[50] Acidemia increases calcium phosphatase release from the bone and reduces tubular reabsorption of these

ions. As a consequence, hypercalciuria and hyperphosphaturia favor calcium stone formation and the persistence of alkaline urine.[50]

We found 33 children (20%) with acidic urine (pH $\leq$ 5.5) together with hypocitraturia and hypomagnesuria. Disturbances of this system occur as a reduced renal secretion of H^+ ions, inducing insufficient ammonium with low urinary pH and promoting uric acid stones formation.[50]

Systemic metabolic acidosis was observed in 33/162 children (20%). The main focus on this disturbance leading to UL is the low urine pH with uric acid stones formation.[38,50] Low urinary pH in human urine increases uric acid crystallization and subsequent stone formation, even if the uric acid excretion rate is normal.[38]

Distal renal tubular acidosis (not analyzed because no arterial blood sampling was available) is linked to UL because acidemia increases calcium-phosphate released from the bone, producing hypercalciuria and hyperphosphaturia, two well known risk factors for stone formation. On the other hand, metabolic acidosis reduces urinary excretion of citrate, which delays the crystallization of calcium salts.[46,50]

Twenty-six children (16%) with urinary tract infection (UTI) had metabolic abnormalities, and some had congenital urinary tract malformations (Table 2). The frequency of UTI varied from 10% to 25%, similar to the 16% observed in previous reports.[51,52] Twelve children (12/26) developed urease-producing bacteria (*Proteus* sp, *Haemophilus* sp, *Klebsiella* sp and *Pseudomonas*) with an alkaline urine. Children with infection and stones have underlying metabolic derangements that should be evaluated. Girls are significantly more likely to experience UTI than boys.[53]

Urinary lithiasis (UL) with NC was noticed in 18/162 children (11%). Their age ranged from 1 to 10 years with no significant gender differences ($p < 0.74$). Final incidence rates are not available for UL, or for NC in children[28]. Prematurity is a special risk factor for NC[29]. Metabolic disorders associated to UL and NC, such as renal tubular acidosis, are not uncommon.[50] Patients with paracellin-1 (members of the claudin family) defects develop hypomagnesemia and a striking incidence of hypercalciuria and NC[54]. We observed two children with this biochemical profile, but had UL without NC (Table 3). Hyperprostaglandin E syndrome is a metabolic alteration that causes hypercalciuria and NC by increasing the calcitriol synthesis[55,56]; Dent's disease[57,58], Lowe syndrome[58], tyrosinemia type 1[59], Bartter syndrome[60] and others that we could not identify clinically. To distinguishing between both entities (UL and NC) may be problematic. Papillae endoscopic inspection is better to diagnose NC.[61] Ultrasonography was more sensitive (96%) than computed tomography (85%) in animals for NC diagnosis.[62] Other causes of increased medullary echogenicity are not clear, as the transient medullary hyperechogenicity, or the aggregates of Tamm-Horsfall protein within the renal calyces which may look like nephrocalcinosis.[63]

Children are more likely to have an underlying metabolic disorder for UL/NC and a higher risk recurrence, hence the identification of metabolic disorder is imperative to prevent stone recurrence. Genetic findings with a non Mendelian transmission pattern, involving multiple genes, suggest that tubular fluid supersaturation with calcium and phosphate predisposes to calcium-oxalate stones by triggering cellular mechanisms that lead to Randall's plaque formation on the surface of the renal papillae.[14] Up to date, polymorphisms (SNPs) of 11 genes were associated with idiopathic calcium NL and NC.[14] As shown in table 3, risk factor parameters in patients with NC were similar to those with UL. Interestingly, albumin levels were under 4 mg/dl only in children with UL. These findings are unusual, but an association with NL has been reported.[39] Two children < 3 years had hypomagnesemia with hypercalciuria and UL without NC.[64]

As shown in table 2, oliguria was found in 14 children (8.6%). Children with UL are known to have low urine volumes. Thus, children with a first episode of UL, who were treated with a high-fluid intake for 5 years, had a significantly lower recurrence rate than those who received a low-fluid intake.[13]

Congenital urinary tract anomalies were seen in 11 children (7%): four with ureteropelvic stenosis, two with double ureter, two with a duplication of the upper ureter with a single lower junction, one with unilateral renal agenesis, one with a vesical septation, and one with an anorectal anomaly. Anatomical abnormalities and urinary tract infections are frequently found in most pediatric patients with UL.

Hypomagnesemia was found in 11 children (7%) and was associated with hypocitraturia and hypomagnesuria.[64] Hyperuricosemia was observed in 11 children (7%), and hypercalcemia in 10 (6%); all with normal PTHi values and two or more risk factors associated. Hyperoxaluria was observed in three children (2%) without clinical evidence of any metabolic defect, two of which had moderate inflammatory bowel disease history. Calcium oxalate supersaturation contributes to calcium stone formation.

Hypercistinuria was observed in one patient (0.61%), which had family history of hypercistinuria and frequent urinary tract infection (Table 2). There were no children identified with hyperphosphatemia or hyperphosphaturia.

When the levels of serum P and PTH, and creatinine and P in urine from control children were compared with the same parameters from patients, we found they were similar and normal, although statistically they disclosed differences (Table 1). This is unusual; therefore, some authors make emphasis that not all of the significant statistic results have clinical relevance.[65] None of the children exhibited a single risk factor, but at least two or more risk factors for UL.

In the present prospective study, the incidence of UL in this pediatric population was 0.02% in a 5-year period (2009–2014). The median age was 7.5 $\pm$ 3.9 years. Male/female ratio was 3:1, and a family history of NL was found in 50% of the children. Abdominal pain was the most common symptom detected (52%). Urinary tract stones were diagnosed mainly by ultrasonography (60%). Pyelocaliceal localization was observed in 52% of the patients. Anatomic and metabolic parameters were evaluated. Hypocitraturia was the highest risk factor found in 70%, hypomagnesuria in 43%; hypercalciuria in 37%. After a Ca restriction diet in 102 children > 5 years, hypercalciuria was observed in 20%. After Ca diet followed by a Ca load, 11% of the children exhibited intestinal Ca hyperabsorption. Unclassified hypercalciuria was observed in children > 5 years (13%). Resorptive hypercalciuria and Ca renal leak were not observed. Alkaline and acid urine were present in 21% and 20%, respectively. Systemic metabolic acidosis was observed in 20%.

Urinary infection was observed in 16% of the children. We found 18 patients with nephrocalcinosis with lithiasis (11%), and oliguria in 8%. Hypomagnesemia, hyperuricosemia and congenital urinary malformation in 7% each one. Basal hypercalcemia was observed in 6%, hyperoxaluria in 2% and hypercystinuria in 0.61%. Hyperparathiroidism, hyperphosphaturia, hiperuricosuria, or other additional risk factors were not observed. All children had two or more risk factors for UL. This study identifies urolithogenic factors that are clinically relevant for nephrologists, urologists, and other physicians.

Ethical disclosure

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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Conflict of interest

The authors declare no conflicts of interest of any nature.

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