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Determination of Urinary Calcium and Citrate Ratios in Children with Urolithiasis

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Abstract:

Objective: The urine calcium-creatinine ratio (UCa/Cr) varies significantly with age; therefore, age-specific reference ranges are required for accurate interpretation. Using different markers in differential diagnosis may improve diagnostic accuracy. Therefore, we compared the spot urinary calcium-citrate ratio (UCa/Cit) in children with urolithiasis and in a healthy control group. We examined the relationship between the UCa/Cit ratio with age and gender and evaluated its discriminatory performance in differentiating children with urolithiasis from healthy controls.

Methods: This case-control study included 121 children aged 1-18 years with urolithiasis and 107 healthy controls. UCa/Cr and UCa/Cit ratios were calculated in the spot urine sample. Differences between groups were compared, age-related trends were evaluated, and receiver operating characteristic (ROC) analyses were performed to determine diagnostic performance.

Results: The UCa/Cit ratio did not show a statistically significant association with age or gender in this cohort. However, the UCa/Cit level was higher in the patient group compared to the control group (1.28±2.87 vs. 0.63±1.51 mg/mg; P<0.0001). In the ROC analysis, a value of 0.390 mg/mg was determined as the optimal cutoff point for UCa/Cit to distinguish children with urolithiasis from healthy controls (sensitivity, 66.3%; specificity, 65.2%; AUC, 0.675).

Conclusion: Although UCa/Cit was significantly elevated in children with urolithiasis and was not statistically associated with age or gender in this cohort, it had limited discriminatory performance. Therefore, UCa/Cit should be considered a supportive metabolic parameter rather than a standalone diagnostic or screening test. Additional studies with standardized sampling, dietary assessment, and multicenter validation will be required to define its clinical utility in pediatric stone disease.

Keywords: Pediatric Urolithiasis, Urinary Calcium-to-Citrate Ratio, Hypercalciuria, Hypocitraturia

Although urolithiasis has long been considered a disease affecting adults, it is now recognized in all pediatric age groups, including infancy. Pediatric stone disease not only causes acute symptoms such as pain and hematuria, but also leads to urinary obstruction and recurrent urinary tract infections. This condition can cause renal parenchymal damage in the long term, resulting in clinically significant morbidity.

Additionally, due to high recurrence rates and the

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frequent occurrence of underlying metabolic abnormalities in these patients, long-term follow-up and comprehensive metabolic evaluation are mandatory [1]. Disease epidemiology reveals significant differences between various geographic regions, socioeconomic conditions, climates, and dietary habits. While prevalence is reported to be in the range of 1-5% in developed countries, it has been reported to be as high as 15% in developing countries [2]. Geographically, certain parts of the Middle East and Southeast Asia are frequently referred to in the literature as the "stone belt," and the prevalence of urinary tract stone disease is higher in these regions. Turkey is also considered among the countries with a significant clinical burden in terms of pediatric stone disease and associated metabolic risk factors [3-6].

The etiology of urinary system stones in childhood is multifactorial, and underlying metabolic abnormalities are detected in a significant proportion of cases. The most common stone components in the pediatric age group are calcium oxalate and calcium phosphate [3, 7].

Hypercalciuria is one of the primary metabolic risk factors for the formation of calcium stones [6-8]. However, decreased urinary inhibitors, particularly the presence of hypocitraturia, significantly contribute to the pathogenesis of stone formation [6-9].

Citrate in urine is a potent inhibitor that suppresses crystal formation by forming soluble complexes with calcium. A decrease in the concentration of citrate in urine significantly increases the risk of calcium stone formation [10].

The gold standard method for metabolic evaluation in children with urolithiasis is 24-hour urine collection and calculation of solute excretion levels [4-6]. However, collecting a 24-hour urine sample completely and correctly is difficult, especially in infants and young children who have not been toilet-trained. This situation presents both technical limitations and challenges related to patient/parent compliance. For this reason, solute/creatinine ratios calculated from spot urine samples are more commonly used in clinical practice. However, since creatinine excretion is mainly dependent on muscle mass, interpreting these ratios can pose additional difficulties, particularly in the pediatric age group, where growth and development are rapid.

The most commonly used ratio in spot urine samples is the urine calcium-creatinine ratio (UCa/Cr). However, the UCa/Cr ratio shows significant age-related differences in childhood. Therefore, age-specific reference ranges must be used to evaluate this parameter, which complicates standardization and interpretation [11-14].

Given these limitations, there is a need for a biomarker that is less affected by age-related creatinine variability, produces more consistent results between individuals and over time, and has high applicability in the clinical decision-making process. This would enable more reliable patient control differentiation and contribute to a more accurate interpretation of changes occurring during follow-up. Some studies report that the urine calcium/citrate ratio (UCa/Cit), calculated from spot urine, may be a clinically beneficial alternative and is less affected by age- and gender-related variability compared to UCa/Cr [13, 15].

No studies have been conducted in our country on calculating the urine calcium/citrate ratio in children. For these reasons, we hypothesized that the spot UCa/Cit ratio would be higher in children with urolithiasis than in a healthy control group, and that the age-related variability of UCa/Cit would be lower than that of the UCa/Cr ratio. To this end, we aimed to compare spot urine UCa/Cit levels between children with urolithiasis and a healthy control group, evaluate the relationship of these parameters with age and gender, and determine the diagnostic performance of the UCa/Cit ratio in the differential diagnosis of pediatric stone disease.

METHODS

Study Design

This prospective case–control study was conducted between October 2009 and February 2010 at the Department of Pediatric Nephrology, Faculty of Medicine, Uludağ University, Bursa. The study protocol was approved by the local ethics committee on June 9, 2009 (decision no. 2009-11/100). Written informed consent was obtained from the parents/legal guardians of all participants, and the study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Children aged 1-18 years undergoing outpatient

follow-up with a diagnosis of urolithiasis were included in the study. All patients' diagnoses were confirmed by ultrasound. The control group consisted of healthy children matched for age and sex who had no history of known acute or chronic kidney disease, urolithiasis, urinary symptoms, active urinary tract infection, or a family history of urolithiasis. Control group participants were selected from children who presented for routine outpatient examinations and were assessed as clinically healthy based on medical history, physical examination, and routine urinalysis. No systematic imaging-based screening was performed in the control group to rule out occult urolithiasis. The exclusion criteria for both groups were active febrile illness, urinary tract infection, presence of chronic systemic disease, and use of drugs that could affect mineral metabolism.

If there were no contraindications in the patient group, current treatments (e.g., citrate supplements, diuretics) were discontinued one week prior to urine sample collection to reduce the possibility of affecting the amount of solute excreted in the urine. Participants were instructed to maintain their usual dietary patterns during this period.

Sample Collection and Biochemical Analysis

The morning urine sample was defined as the second urine sample collected after the first morning urine was discarded, and these cases were collected in this manner. Routine urinalysis (chemical analysis and/or automated sediment evaluation) was performed on non-centrifuged specimens using an automated analyzer (UriSed–LabUmat 77, 77 Elektronika Kft, Hungary). Samples intended for citrate measurement were centrifuged (3000 rpm, 5 min) and stored at -80°C until analysis. All other specimens were kept at room temperature and analyzed within 1 hour of collection. Sodium (Na), Potassium (K), Calcium (Ca), and Creatinine (Cre) levels in spot urine samples were measured using an autoanalyzer (Aeroset-1, Abbott). Urinary citrate was measured using an enzymatic method with spectrophotometric detection (BEN-Biochemical Enterprise, Code CI8820). After incubation at 37°C, absorbance was measured at 340 nm using a UV–Visible spectrophotometer (Shimadzu UV-1601), and citrate concentration was calculated according to the manufacturer's instructions.

Calculations

The amount of solute measured in urine samples was reported as a ratio to urinary creatinine. Accordingly, the UCa/Cr and UCa/Cit ratios were expressed in mg/mg, the UCit/Cr ratio in mg/g, and the UNa/Cr and UK/Cr ratios in mEq/mg.

Statistical Analysis

All analyses were performed using SPSS (version 16.0) and MedCalc. Continuous variables are presented as mean±standard deviations (SD), and categorical variables are presented as counts and percentages. Distributional assumptions were evaluated using histograms, Q-Q plots, and the Shapiro–Wilk test. Although descriptive values are presented as mean±SD, inferential comparisons for right-skewed urinary ratios were performed using log-transformed data. Between-group comparisons were performed using the independent-samples t-test on transformed data (Welch's correction was applied when variances were unequal). Categorical variables were compared using the chi-square test (or Fisher's exact test, as appropriate). Associations with age were assessed using Pearson correlation on transformed variables. ROC (Receiver Operating Characteristic) analysis was used to calculate the sensitivity, specificity, and cutoff values of the UCa/Cit ratio. A P-value <0.05 was considered statistically significant.

RESULTS

Demographic Characteristics

A total of 228 children were included in the study, comprising 121 patients with urolithiasis and 107 healthy controls. The mean age of the patient group was 8.86±5.01 years; 53.7% (n=65) were female, and 46.3% (n=56) were male. The mean age of the control group was 9.12±4.61 years; 46.7% (n=50) were female, and 53.3% (n=57) were male. There were no significant differences between groups in age or sex distribution (P>0.05) (Table 1).

Comparison of Biochemical Data

As expected, the UCa/Cr ratio was significantly higher in the patient group compared to the control group (0.17±0.14 vs 0.10±0.11 mg/mg; P<0.001). The

TABLE 1. Demographic Characteristics of the Patient and Control Groups

Characteristics	Patient group (n=121)	Control group (n=107)	P-value
Age (years)	8.86±5.01	9.12±4.61	0.693
Gender (female/male)	65/56	50/57	0.292

Data are shown as mean±standard deviation or n.

UCa/Cit ratio, the main parameter of the study, was higher in patients (1.28±2.87 mg/mg) compared to the control group (0.63±1.51 mg/mg; P<0.001) (Table 2). No significant difference was observed between the groups for UNa/Cr, UK/Cr, or UCit/Cr (all P>0.05).

Relationship of Parameters with Age and Gender

When evaluating age-related changes in urinary solute excretion, a decrease in UCa/Cr was observed with advancing age in both patients (r = -0.262, P=0.004) and the control group (r = -0.371, P<0.001). Similarly, the UCit/Cr ratio decreased with advancing age in both the patient group (r = -0.283, P=0.002) and the control group (r = -0.296, P=0.002). In contrast, the UCa/Cit ratio did not show a significant correlation with age in either the patient group (r = -0.061, P=0.509) or the control group (r = -0.137, P=0.161). This result indicates that the UCa/Cit ratio remains constant across different age groups (Table 3).

When examining the effect of gender, another important factor, on solute excretion, no significant difference was observed between girls and boys in the patient and control groups in terms of UCa/Cr, UCit/Cr, or UCa/Cit excretion (Table 4).

ROC Analysis

ROC analysis showed that the UCa/Cit ratio could distinguish children with urolithiasis from healthy controls with limited discriminatory power (AUC=0.675; 95% CI: 0.605-0.745; P<0.001) (Figure 1). A cutoff value of 0.390 mg/mg for UCa/Cit was determined to be optimal, with a sensitivity of 66.3% and a specificity of 65.2% (Table 5, Figure 1).

DISCUSSION

Urolithiasis is a clinically significant disease with a high risk of recurrence in childhood. If not diagnosed in time and treated appropriately, the obstruction and recurrent urinary tract infections it causes can lead to damage in the renal parenchyma. If this condition persists, it can lead to end-stage renal failure [1, 2, 16]. The accurate identification of metabolic risk factors and initiation of medical prophylaxis in these children directly affects the success of treatment. Hypercalciuria is one of the most common metabolic abnormalities detected in children, and the gold standard for its evaluation is a 24-hour urine analysis [7, 17].

TABLE 2. Comparison of Urine Solute-to-Creatinine Ratios and Urinary Calcium-to-Citrate Ratios Between Patients and Controls

Parameter	Patients (n=121)	Controls (n=107)	P-value
UCa/Cr (mg/mg)	0.17±0.14	0.10±0.11	**<0.001**
UNa/Cr (mEq/mg)	0.21±0.14	0.20±0.16	0.568
UK/Cr (mEq/mg)	0.08±0.06	0.08±0.06	0.933
UCit/Cr (mg/g)	368.76±494.26	388.53±383.90	0.231
UCa/Cit (mg/mg)	1.28±2.87	0.63±1.51	**<0.001**

Data are shown as mean±standard deviation. UCa/Cr, urinary calcium-to-creatinine ratio; UNa/Cr, urinary sodium-to-creatinine ratio; UK/Cr, urinary potassium-to-creatinine ratio; UCit/Cr, urinary citrate-to-creatinine ratio; UCa/Cit, urinary calcium-to-citrate ratio. Statistically significant P-values are shown in bold.

TABLE 3. Correlations of Creatinine-Normalized Urinary Ratios with Age in Patients and Controls

	Patients (r)	Patients (P-value)	Controls (r)	Controls (P-value)
UCa/Cr	−0.262	**0.004**	−0.371	**<0.001**
UCit/Cr	−0.283	**0.002**	−0.296	**0.002**
UCa/Cit	−0.061	0.509	−0.137	0.161

UCa/Cr, urinary calcium-to-creatinine ratio; UCit/Cr, urinary citrate-to-creatinine ratio; UCa/Cit, urinary calcium-to-citrate ratio. Statistically significant P-values are shown in bold.

Our findings, consistent with the literature, show that the spot urine UCa/Cr ratio is significantly related to age. The UCa/Cr ratio decreased significantly with increasing age in both healthy children and cases with urolithiasis [7, 10-12]. This finding is related to the increase in urinary creatinine excretion with age, parallel to the increase in muscle mass during the growth process [18-20]. The data reported from our region also supports this pattern. Çalışkan *et al*. [18] and Ceran *et al*. [12] reported higher upper limit values for UCa/Cr in younger age groups. Similarly, reference ranges from different geographical locations also indicate that UCa/Cr changes with age; Sargent *et al*. [21] and Esbjörner and Jones [20] confirmed this age-related variability in their respective populations. However, accurate interpretation of UCa/Cr requires age-specific reference ranges. This is a significant disadvantage that makes standardization difficult and limits its applicability in routine clinical practice.

Among environmental factors associated with stone formation, dietary sodium intake is particularly relevant. High sodium intake is known to induce hypercalciuria by reducing tubular calcium reabsorption [22–24]. In our patient group, we found a positive correlation between urinary calcium and sodium, supporting this pathophysiological mechanism. This result also reinforces the importance of salt restriction in the diet, in addition to pharmacological approaches, in children with urolithiasis. Given the limitations imposed on the use of current biomarkers, it is evident that clinical practice requires more consistent measures that are not affected by age and gender. In this context, one of the prominent findings of our study is that while UCa/Cr varies with age, the UCa/Cit ratio remains stable and does not show a significant relationship with age or gender. The number of studies on this topic in the childhood age group is quite limited. In one of the first studies in the literature, Srivastava *et al*. reported that, similar to our findings, the Ca/Cit ratio in children was not affected by age or muscle mass [13].

In our study, we determined the UCa/Cit threshold value to be 0.390 mg/mg, which differentiates children with urolithiasis from a healthy control group. Srivastava *et al*. [13] reported a similar value of 0.326 mg/mg. The difference between the two results may stem from the demographic and clinical characteristics of the studied populations, variability in dietary habits, and methodological differences in the analytical approaches used. The cutoff value we found for the

TABLE 4. Sex-based Comparison of Spot urine Calcium and Citrate Ratios in Patients and Controls

Parameter	Patients (Female)	Patients (Male)	P-value	Controls (Female)	Controls (Male)	P-value
UCa/Cr (mg/mg)	0.18±0.14	0.15±0.13	0.162	0.10±0.13	0.10±0.09	0.446
UCit/Cr (mg/g)	411.13±609.36	319.59±310.38	0.123	414.75±360.62	365.53±405.01	0.248
UCa/Cit (mg/mg)	0.97±1.20	1.64±4.01	0.909	0.65±1.85	0.61±1.15	0.136

Data are presented as mean±SD. SD, standard deviation; UCa/Cr, urinary calcium-to-creatinine ratio; UCit/Cr, urinary citrate-to-creatinine ratio; UCa/Cit, urinary calcium-to-citrate ratio.

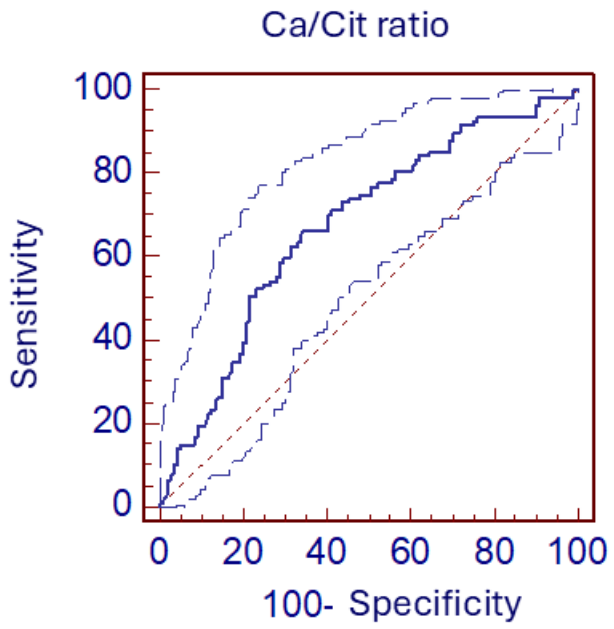

FIGURE 1. Receiver operating characteristic (ROC) curve illustrating the discriminatory performance of the urinary calcium-to-citrate ratio (UCa/Cit) for differentiating children with urolithiasis from healthy controls. The area under the curve was 0.675 (95% CI: 0.605-0.745; P<0.001), indicating limited discriminatory performance.

TABLE 5. Diagnostic Performance of the Urinary Calcium-to-Citrate Ratio (Uca/Cit) for Discriminating Patients from Healthy Controls (ROC Analysis)

Cut-off (mg/mg)	Sensitivity (%)	Specificity (%)
0.381	64.4	74.7
0.386	65.4	74.7
0.390	**66.3**	**65.2**
0.394	66.3	66.4
0.396	66.3	63.4

UCa/Cit, urinary calcium-to-citrate ratio; AUC, area under the ROC curve; CI, confidence interval.

The area under the ROC curve was 0.675 (95% CI: 0.605-0.745; P<0.001), indicating limited discriminatory performance.

UCa/Cit ratio in our study had limited performance in clinical use. Therefore, values above 0.390 mg/mg should be considered not as a diagnostic measure alone, but as an auxiliary measure supporting clinical evaluation. Furthermore, independent and multicenter studies are necessary to support the clinical application of this proposed threshold value.

Strengths and Limitations

This study has several strengths. First, it included a pediatric urolithiasis cohort and a healthy control group matched for age and gender, thereby enabling a direct comparison of urinary calcium to citrate ratios in children with and without stone disease. Second, and this is the study's strongest point: the independence of the UCa/Cit ratio from age and sex was demonstrated in both the patient and control groups, and it was directly compared with the well-known age-dependence of the UCa/Cr ratio within the same cohort; this reinforces the internal consistency of this observation. Additionally, urine sampling and biochemical measurements in the study were performed using clearly defined laboratory methods and a standardized protocol. Medications that could potentially affect urinary excretion of soluble substances were discontinued prior to sampling when clinically appropriate, thereby minimizing treatment-related confounding factors. Finally, to the best of our knowledge, this study is one of the first to evaluate the spot urine UCa/Cit ratio in children in our country. It provides preliminary regional data that may inform future research. However, this study has several limitations. First, no systematic imaging based screening was performed in the control group; therefore, occult and asymptomatic urolithiasis could not be completely ruled out. Second, dietary intake was not standardized prior to urine sample collection. Since urinary calcium and citrate excretion can be influenced by dietary calcium, sodium, and protein intake as well as hydration status, this may have contributed to the variability in the urinary indices. Third, the study relied on a single spot urine sample, so intraday variability could not be assessed.

CONCLUSION

In conclusion, our study found higher urine calcium-to-citrate ratios in children with urolithiasis compared to a healthy control group. Although UCa/Cit did not show a statistically significant association with age or gender in this cohort, its discriminatory performance remained limited. Therefore, UCa/Cit should be

interpreted not as a standalone diagnostic or screening test, but as a supportive metabolic parameter in the evaluation of pediatric urolithiasis. To clarify its clinical utility in pediatric stone disease, multicenter studies utilizing standardized dietary assessment, repeated urine sampling, and validated cutoff values are required.

Ethics Approval and Consent to Participate

This study was approved by the Bursa Uludağ University Healthcare Institutions Medical Research Not Involving Drug Review Ethics Committee. (Decision No: 2009-11/100; date: 09.06.2019). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from the parents/legal guardians of all participants prior to enrollment.

Data Availability

All relevant data supporting the findings of this study have been included in the article. Data with identifying information removed may be obtained from the corresponding author upon reasonable request, subject to the approval of the ethics committee and, where necessary, institutional permissions.

Authors' Contribution

Study Conception: OA, OD; Study Design: OA; Supervision: OA, OD; Funding: OA; Materials: OA; Data Collection and/or Processing: OA; Statistical Analysis and/or Data Interpretation: OA; Literature Review: OA; Manuscript Preparation: OA; and Critical Review: OA.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

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Generative Artificial Intelligence Statement

Grammarly was used in parts of the manuscript for English language editing. No AI-assisted tools were used for data collection, statistical analysis, result generation, or the creation of tables and figures. All scientific content and conclusions are the responsibility of the authors.

The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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