Analysis of Calcium, Oxalate, and Citrate Interaction in Idiopathic Calcium Urolithiasis in Children

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The majority of urinary stones in children are composed of calcium oxalate. To investigate the interaction between urinary calcium, oxalate, and citrate as major risk factors for calcium stones formation, their 24-h urinary excretion was determined in 30 children with urolithiasis and 15 normal healthy children. The cutoff points between children with urolithiasis and healthy children, accuracy, sensitivity, and specificity for each risk factor alone as well as for all three taken together were determined. OneR and J4.8 classifiers as parts of the larger data mining software Weka, based on machine learning algorithms, were used for the determination of the cutoff points for differentiation of the children. The decision tree based on J4.8 classifier analysis of all three risk factors together proved to be the best for differentiating stone formers from normal children. In comparison to the accuracy of the differentiation after calcium and oxalate of 80% and 75.6%, respectively, the decision tree showed an accuracy of 97.8%. Even when its stability was tested by the leave-one-out cross-validation procedure, the accuracy remained at a very acceptable percentage of 93.2% correctly classified patients. J4.8 classifier analysis gave a look inside urinary calcium, oxalate, and citrate interaction. Urinary calcium excretion was shown as the most informative in discrimination of the children with urolithiasis from healthy children. However, it was shown that oxalate and citrate excretions might influence the stone formation in a subpopulation of the stone formers. In patients with low urinary calcium, a major role in lithogenesis belongs to oxalate, in some of them alone and in others in conjunction with citrate. Decreased urinary citrate excretion in the presence of increased oxalate excretion may lead to stone formation.

INTRODUCTION

The majority of renal calculi in children are, as in adults, composed of calcium oxalate.[1−4] Urinary calcium, oxalate, and citrate, as major involved promoter and inhibitor factors, are supposed to determine the calcium stone formation. Hypercalciuria is regarded as main etiological factor, although the etiology often remains undetermined.[1−6] Urinary oxalate excretion, that might be as important as calcium excretion, has been only rarely investigated in pediatric stone formers.[7−9] Information about urinary citrate excretion in children with urolithiasis is even more scarce. Hypocitraturia was found by some authors.[10−12]

In our previous report, the children with urolithiasis were found to have significantly higher urinary calcium and oxalate excretions and urine saturation than normal children.[13] No significant difference among the groups in citrate excretion was found. However, there was a substantial overlap between the groups. Therefore the calculation of the relative risk of urolithiasis by logistic regression was done, which showed that for the same value of calcium excretion the risk of urolithiasis rose with the rise of oxalate excretion and vice versa.[13] Such results clearly indicated that the most important factor for development of urolithiasis is interaction between involved urinary factors, all of which when taken separately can be within the normal range.

In the present study the interaction between the three risk factors for urolithiasis development, urinary calcium, oxalate, and citrate, was analyzed. The aim of the study was to see if it is possible to avoid overlapping between stone-formers and normal children and to detect small and not clearly visible disbalance between the risk factors leading to stone formation. OneR and J4.8 classifiers as parts of the larger data mining software, based on machine learning algorithms, called the Waikato Environment for Knowledge Analysis (Weka) were used for the analysis.[14] The decision tree for discrimination of children with urolithiasis from healthy children based on analysis of all three risk factors together was constructed using the J4.8 classifier. The discriminatory ability of the decision tree was compared with the discriminatory ability of the classifications obtained with analysis of each risk factor separately by the OneR classifier. Accuracy, specificity, and sensitivity as well as the 95% confidence interval for determined cutoff values were calculated. The stability of the induced decision tree was tested using the leave-one-out cross-validation procedure.[14]

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Table 1. 24-Hour Excretion of Calcium, Oxalate, and Citrate[a]

	normal children			urolithiasis		
parameters	md	min	max	md	min	max
calcium/creatinine (mmol/mmol)	**0.17**	**0.08**	**0.33**	**0.30**	**0.08**	**0.56**
oxalate/creatinine (mmol/mol)	**46.00**	**19.00**	**76.00**	**75.00**	**20.00**	**110.00**
citrate/creatinine (mmol/mol)	320.00	68.00	697.00	275.00	63.00	685.00

[a] md, median; min, minimum; max, maximum.

PATIENTS AND METHODS

Patients. Thirty children with urolithiasis were investigated. A group of 15 healthy sex- and age-matched children without any nephrourological disease or pathological condition that might influence urine composition served as controls. The median age of healthy children, 8 boys and 7 girls, was 9 years (range 5−18). The median age of children with urolithiasis, 18 boys and 12 girls, was 9.5 years (range 3−18).

The diagnosis of urolithiasis was established by ultrasonography and/or urography.

Children with urinary tract infections and urological anomalies were excluded from the study. If a checkup of serum and urine electrolytes revealed hypercalciuria, the known causes of hypercalciuria (renal tubular acidosis, hypercalcemic conditions) were excluded. Cystinuria and hyperuricosuria were excluded.

The children were enrolled in the study with informed parental consent.

Urine Sampling and Analysis. From each child, 24-h urine collection was obtained for calcium, oxalate, citrate, and creatinine measuring. The children were maintained on a free diet. The samples were collected in a wide-mouthed plastic bottle containing 10 mL of 6 N hydrochloric acid as preservative. A dip-stick test (Combur 9 Test, Boehringer, Mannheim, Germany) was used as a nitrite marker to detect urinary infection. Such samples were excluded. Oxalate and citrate were measured using a Dionex Series 4000i gradient ion chromatography system (Dionex Co., Sunnyvale, CA).[15] Urinary calcium was measured by the cresolphthalein-complexon method,[16] and creatinine was measured by the standard kinetic Jaffe procedure[17] using an Olympus AU 800 Analyzer. Urinary calcium, oxalate, and citrate were expressed as a ratio to the creatinine to ensure completeness of urine collection and correction to the same indicator of metabolic activity.

Data Analysis. Data were presented as medians with minimum and maximum values and compared using the two-tailed Mann−Whitney U-test. p values less or equal to 0.05 were considered as statistically significant.[18]

Cutoff values between normal children and children with urolithiasis were determined using using OneR and J4.8 classifiers inside the Weka software.[14]

Weka. The Waikato Environment for Knowledge Analysis (Weka) is a freeware collection of many state-of-the-art machine learning algorithms, written entirely in Java programming language, and organized into directories containing related classes.[14,19] It is platform-independent, with a command-line interface (stabile versions) or a graphical user interface (development versions). The Weka software has preprocessing routines (filters), classifiers, and metaclassifiers for categorical and numerical learning tasks and evaluation tools.[14] It is well documented and freely available on the World Wide Web (www.cs.waikato.ac.nz/ml/weka). Version 3.3 was used for data analysis.

OneR. All attributes were analyzed using the OneR classifier inside the Weka software. It generates a one-level decision tree, which has the form of a set of rules for one particular attribute.[20] The purpose of OneR analysis was to estimate the discriminatory ability of an individual risk factor and also to estimate the level of improvement in discrimination obtained with the J4.8 classifier which combines all three risk factors.[20]

J4.8 Classifier. J4.8 algorithm is Weka's implementation of landmark C4.5 decision tree program.[21] J4.8 actually implements a later and slightly improved version called C4.5 Revision 8, which was the last public version of this family of algorithms before C5.0, a commercial implementation, was released.[14] C5.0 offers significant improvement in speed of analysis in comparison to C4.5 but only a negligible improvement in the quality of analysis.[14] J4.8 defines the possible decision tree by means of a hill-climbing search based on the statistical property measure called information gain.[21,22] Information gain measure defines how well a given attribute separates the training examples according to their target classification and selects the candidate attribute at each step of the tree.[21,22] The elements of the tree generated by J4.8 are either leafs or decision nodes. The leaf shows a class, and the decision node specifies the test to be implemented on an atttribute value. One advantage of such methods is that they automatically handle nonlinearity and interactions. Output includes a "decision tree" which is immediately useful for prediction.

Accuracy, sensitivity, and specificity as well as the 95% confidence interval for determined cutoff values were calculated.

The stability of induced decision tree was tested using the leave-one-out cross-validation procedure.[14,22]

RESULTS

Table 1 shows median (minimum−maximum) values of the 24-h urinary excretion of calcium, oxalate, and citrate. Standard statistical analysis showed that children with urolithiasis had statistically significant increased urinary calcium and oxalate excretions than normal children (boldface data). There was no significant difference between the groups in urinary citrate excretion.

Cutoff values between normal children and children with urolithiasis determined by the OneR classifier and accuracy, sensitivity, and specificity for determined cutoff values are presented in Table 2. Children with urolithiasis had urinary calcium and oxalate excretions above 0.175 and 44.00, respectively. There was not a statistically significant difference in accuracy of the classification of patients after urinary

Table 2. OneR Classifier Analysis of Urinary Calcium, Oxalate and Citrate Excretions

calcium/creatinine (mmol/mmol)
IF calcium/creatinine < 0.18 THAN diagnosis = normal ELSE IF calcium/creatinine ≥ 0.18 THAN diagnosis = urolithiasis accuracy 80.0% (95% confidence interval 65.0−89.5) sensitivity 90.0% (95% confidence interval 47.4−94.7) specificity 60.0% (95% confidence interval 16.4−76.4)
oxalate/creatinine (mmol/mol)
IF oxalate/creatinine < 44.0 THAN diagnosis = normal ELSE IF oxalate/creatinine ≥ 44.0 THAN diagnosis = urolithiasis accuracy 75.6% (95% confidence interval 60.1−86.6) sensitivity 90.0% (95% confidence interval 28.8−93.6) specificity 46.7% (95% confidence interval 3.5−63.6)
citrate/creatinine (mmol/mol) not possible to determine the cutoff value

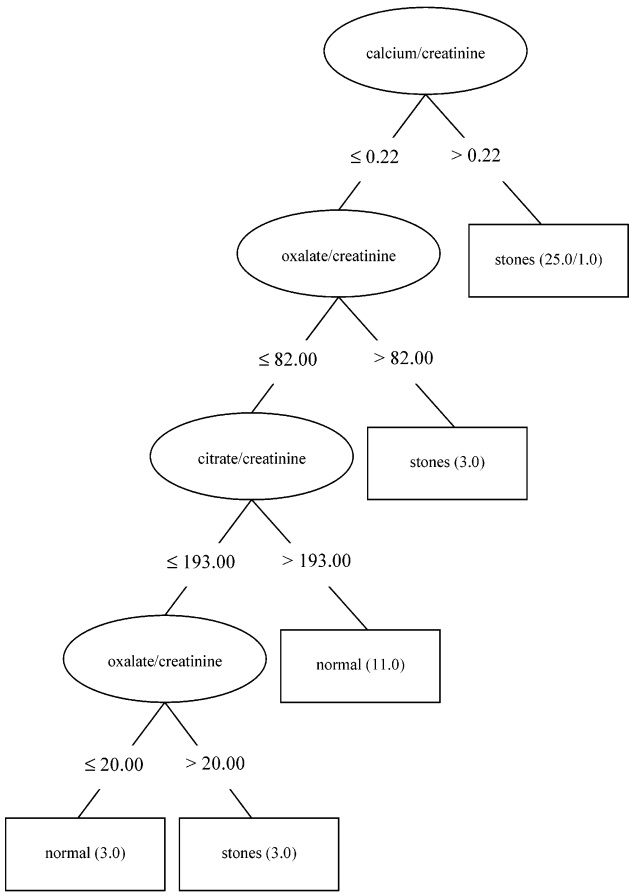

Figure 1. Decision tree for diagnosis of calcium oxalate urolithiasis.

calcium excretion and oxalate excretion (80.0% vs 75.65%). It was not possible to determine the cutoff value for urinary citrate excretion due to considerable overlap between the groups.

The analysis of urinary calcium, oxalate, and citrate excretions together by means of the J4.8 classifier is presented as the decision tree (Figure 1). The decision tree can be read as follows:

1. If the value of urinary calcium excretion is greater than 0.22, a child belongs to class *urolithiasis*.

2. If that condition is not satisfied (i.e. urinary calcium excretion is less than or equal to 0.22), then urinary oxalate excretion must be considered. If urinary oxalate excretion is greater than 82.00, a child belongs to class *urolithiasis*.

3. If that condition is not satisfied (i.e. urinary oxalate excretion is less than or equal to 82.00), then urinary citrate excretion must be considered. If urinary citrate excretion is greater than 193.00, a child belongs to class *healthy*.

4. If that condition is not satisfied (i.e. urinary citrate excretion is less than or equal to 193.00), then urinary oxalate excretion must be considered again. If urinary oxalate excretion is greater than 20.00 a, child belongs to class *urolithiasis.* If that condition is not satisfied (i.e. urinary oxalate excretion is less than or equal to 20.00), then a child belongs to class *healthy*.

Table 3. J4.8 Classifier Analysis of Urinary Calcium, Oxalate, and Citrate Excretions

validity indices under overall prediction
accuracy 97.8% (95% confidence interval 89.5−99.9) sensitivity 100% (95% confidence interval 90.5−100) specificity 93.3% (95% confidence interval 71.3−99.7)
validity indices under leave-one-out cross-validation
accuracy 93.2% (95% confidence interval 82.6−98.2) sensitivity 86.7% (95% confidence interval 62.5−97.7) specificity 86.7% (95% confidence interval 62.5−97.7)

Using the decision tree it was possible to classify patients as stone formers or healthy with 97.78% accuracy, 100% sensitivity, and 93.3% specificity (Table 3). The urinary calcium excretion formed the first and most informative node[22] of the decision tree with 25 patients classified as stone formers, 24 of them correctly and 1 false positive from the group of healthy children. Three next nodes, formed by urinary oxalate and citrate excretions classified 6 children as stone formers and 14 as healthy. Using the leave-one-out cross-validation technique[14,22] to check the stability of the induced decision tree, the accuracy became somewhat lower but still very acceptable with 93.18% correctly classified patients (Table 3).

DISCUSSION

Dynamic ion interaction of various urine promoters and inhibitors determines stone formation. While calcium, as the most explored promoter of crystallization, has an obvious role in calcium stones formation, oxalate as its ionic counterpart is most probably of no less importance.[1−6,7−9,23,24] Citrate, as the most potent inhibitor of crystallization, when decreased could predispose to stone formation.[10−12,25] Clinical experience shows, however, that a number of patients with urolithiasis may not have any risk factor for urolithiasis development. Therefore, it could be supposed that small and not readily detectable disbalance between involved promoters and inhibitors can lead to urolithiasis development.

In the present study each risk factor for urolithiasis development, urinary calcium, oxalate, and citrate, was considered separately and in interaction with others. The analysis of each risk factor separately showed that discrimination of the children with urolithiasis from healthy children is not possible using urinary citrate excretion only (Tables 1 and 2). Also, urinary calcium and oxalate were found as inadequate to discriminate patients exactly enough, showing the accuracy of discrimination of not more than 80% and 75.6%, respectively (Table 2). However, the information of

similar accuracy has a point, implying equal importance of calcium and oxalate in urolithiasis development.

When all three variables, urinary calcium, oxalate, and citrate, were analyzed altogether, the accuracy of discrimination of patients rose to 97.8%. The J4.8 classifier inside the Weka software was shown as particularly valuable for this type of analysis because of its ability to automatically handle nonlinearity and interactions and to extract structural patterns from data.[14,22] The induced decision tree was shown as very stable. Even when its stability was tested by the leave-one-out cross-validation procedure, accuracy remained at a very acceptable percentage of 93.2% correctly classified patients (Table 3). Urinary calcium excretion proved as the most informative[22] in discrimination of the children with urolithiasis from healthy children. The relatively low cut off value of calcium creatinine ratio in our patients when compared to other authors' findings[1−6] probably reflects a specificity and liability of Croatian population to urolithiasis. Nevertheless, the study confirmed hypercalciuria as the most important factor in the genesis of calcium urolithiasis. However, it was shown that oxalate and citrate excretions might influence the stone formation in a subpopulation of the stone formers. In patients with urinary calcium less than the cutoff value, a major role in lithogenesis belongs to oxalate, in some of them alone and in others in conjunction with citrate. The role of urinary citrate, which otherwise could not be detected, has become visible. Decreased urinary citrate excretion in the presence of increased oxalate excretion may lead to stone formation.

The study clearly shows that each risk factor for urolithiasis development is de facto dependent on the level of the others. When considered separately, no one risk factor alone covers the whole population of stone formers.

Machine learning analysis of the laboratory tests related to urolithiasis proved to be of potential clinical value. The decision tree based on urinary calcium, oxalate, and citrate allows a look inside their interaction leading to stone formation and gives a possibility to act preventively, modulating their urinary excretions.

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