Value of the Urinary Stone Promoters/Inhibitors Ratios in the Estimation of the Risk of Urolithiasis

Danica Batinić,*,† Danko Milošević,† Nenad Blau,‡ Paško Konjevoda,§ Nikola Štambuk,⊥ Vesna Barbarić,|| Mirna Šubat-Dežulović,⊗ Ana Votava-Raić,† Ljiljana Nižić,† and Kristina Vrljičak†

Childrens' University Hospital Šalata, Department of Pharmacology, and Clinical Institute for Laboratory Diagnosis, Medical Faculty University of Zagreb, Rudjer Bošković Institute, Zagreb, and University Childrens' Hospital, Medical Faculty of Rijeka, Croatia, and Childrens' University Hospital, Zurich, Switzerland

Received August 16, 1999

An imbalance between urinary-promoting and -inhibiting factors has been suggested as more important in urinary stone formation than a disturbance of any single substance. To investigate the value of promoter/inhibitor ratios for estimation of the risk of urolithiasis, urinary citrate/calcium, magnesium/calcium oxalate, and oxalate/citrate × glycosaminoglycans ratios were determined in 30 children with urolithiasis, 36 children with isolated hematuria, and 15 healthy control children. The cutoff points between normal children and children with urolithiasis, accuracy, specificity, and sensitivity for each ratio were determined and compared with those of the 24-h urine calcium and oxalate excretion and urine saturation calculated with the computer program EQUIL 2. The neural network application (aiNET Artificial Neural Network, version 1.25) was used for the determination of the cutoff points for the classification of normal children and the urolithiasis group. The best test for differentiating stone formers from non-stone formers proved the aiNET determined cutoff values of oxalate/citrate × glycosaminoglycans ratio. The method showed 97.78% accuracy, 100% sensitivity, and 93.33% specificity. Two cutoff points between normal and urolithiasis groups were found showing that the children with urolithiasis had ratio values either above 34.00 or less than 10.16. Increased oxalate excretion was linked to the first cutoff value (34.00), and decreased glycosaminoglycans excretion was typical of the second cutoff value (10.16).

INTRODUCTION

Extensive examination of a number of urinary-promoting and -inhibiting factors was undertaken over a period of years to investigate the risk for stone formation. It has been shown that no single promoter or inhibitor can discriminate clearly enough any particular individual as healthy or sick. A combination of factors seems to provide better separation of stone formers from normal subjects. Several ratios between promoting and inhibiting factors, such as calcium × oxalate/creatinine × magnesium,[1] calcium/citrate,[2] magnesium/calcium × oxalate,[3] oxalate/calcium,[4] citrate/calcium,[5] and oxalate/citrate × glycosaminglycans,[6] as well as more sophisticated methods that take into account the number of urinary components,[7−14] were used to detect the imbalance between the promoting and inhibiting factors leading to stone formation. We examined 11 single urinary factors potentially promoting or inhibiting crystallization and urine saturation with the computer program EQUIL 2 in children with isolated hematuria and overt urolithiasis and compared the findings with the findings of normal healthy children.[15] In our previous report urine saturation was found as the best parameter for the estimation of the relative risk of urolithiasis. However, logistic regression failed to classify correctly 14.59% of the group members.[15]

The aim of the present study was to evaluate the value of promoter/inhibitor ratios for the estimation of the risk of urolithiasis. Those tests are simpler, easier, and cheaper for routine clinical practice than EQUIL 2. Citrate/calcium, magnesium/calcium × oxalate, and oxalate/citrate × glycosaminoglycans ratios were chosen for this purpose because all of them take into account the major urinary stone promoting and inhibiting factors. Neuronal networks analysis by means of aiNET Artificial Neural Network (version 1.25) was used to determine the cutoff points between normal and urolithiasis groups. Accuracy, specificity, and sensitivity were calculated for each of these ratios and compared with those of the 24-h urine calcium and oxalate excretion and urine saturation.

PATIENTS AND METHODS

Patients. Thirty children with urolithiasis and 36 children with isolated hematuria were investigated. A group of 15 healthy sex- and age-matched children without any nephro-urological disease or pathological condition that might influence urine composition served as controls.

In children with hematuria, glomerular diseases, urinary infection, urological anomalies, and coagulopathy were excluded before entering the study. If a checkup of serum

* Corresponding author. Childrens' University Hospital Šalata, Medical Faculty University of Zagreb, Šalata 4, 10000 Zagreb, Croatia.
† Childrens' University Hospital Šalata.
‡ Childrens' University Hospital, Zurich.
§ Department of Pharmacology.
⊥ Rudjer Bošković Institute.
|| Clinical Institute for Laboratory Diagnosis.
⊗ Childrens' University Hospital Rijeka.

10.1021/ci990076i CCC: $19.00
Published on Web 03/01/2000

Table 1. Urinary Promoters and Inhibitors of Crystallization [a]

parameters	normal children			hematuria			urolithiasis		
	md	min	max	md	min	max	md	min	max
urine saturation	1.91	0.76	3.48	4.32	0.74	16.29	19.59	0.72	25.20
calcium/creatinine (mmol/mmol)	0.17	0.08	0.33	0.22	0.11	0.79	0.30	0.08	0.56
oxalate/creatinine (mmol/mol)	46.00	19.00	76.00	51.00	8.00	111.00	75.00	20.00	111.00
citrate/calcium ratio (mmol/mmol)	1.81	0.48	3.59	1.33	0.31	7.74	0.71	0.17	1.38
magnesium/calcium × oxalate ratio (mmol)	1.05	0.13	3.31	0.80	0.09	4.15	0.92	0.06	3.44
oxalate/citrate × glycosaminoglycans ratio (mmol × 10^4)	19.09	7.64	34.80	43.17	7.42	257.85	195.49	7.72	631.77

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

and urine electrolytes revealed hypercalciuria, the known causes of hypercalciuria (renal tubular acidosis, hypercalcemic conditions) were excluded.

In children with urolithiasis, ultrasonography and/or urography established the diagnosis. 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 collections performed on two consecutive days and one urine sample collected from 8 to 10 a.m. on the third day were obtained for analysis. The 24-h urine of the first day served for measuring creatinine, calcium, sodium, potassium, oxalate, phosphate, magnesium, citrate, and sulfate. It was collected in a wide-mouthed plastic bottle containing 10 mL of 6 N hydrochloric acid as preservative. The 24-h urine of the second day was collected in the same way but without the addition of hydrochloric acid in the bottle. It served for measuring chloride, urate, GAGs, and creatinine. The 2-h urine collected on the third day served for ammonium and creatinine measuring. In this sample 500 mg of dipotassium oxalate was added immediately after voiding to prevent ammonium decomposition.

The pH of urine was measured with indicator sticks (Boehringer Mannheim, Germany). Oxalate, citrate, and sulfate were measured using a Dionex Series 4000i gradient ion chromatography system (Dionex Co, Sunnyvale, CA).[16] GAGs were measured by the carbazole method,[17] ammonium by glutamate dehydrogenase (Da Fonseca-Wollheim),[18] and magnesium by atomic absorption spectrophotometry.[19] The following analyses were done on an Olympus AU 800 Analyser: creatinine by a standard kinetic Jaffé procedure,[20] sodium, potassium, and chloride by ion-selective electrodes, calcium by the cresolphthalein-complexon method,[21] phosphate by the molybdate method,[22] and uric acid by the uricase method.[23]

From the values of urinary 24-h volume, pH of urine, calcium, sodium, potassium, chloride, magnesium, phosphate, sulfate, ammonium, urate, oxalate, citrate, and creatinine (mmol/L), the urinary calcium oxalate saturation was calculated by the computer program EQUIL 2.[14,15] Also, 24-h urinary excretion expressed as a ratio to the creatinine was calculated for each of the measured urinary components.

Data Analysis. Data were presented as medians with minimum and maximum values. Cutoff values between normal children and children with urolithiasis were determined using a neuronal network application (aiNET Artificial Neural Network version 1.24, Celje, Slovenia).[24,26]

Neural Network. Artificial neural network aiNET is based on a self-organizing system, called a neural network-like system, and it is very similar to the Kohonen's self-organizing map (SOM) learning algorithm.[24−28] aiNET has an association layer between input and output, i.e., a feature map classifier based on a training method named delta (δ) rule.[24−27,30,31] This method is also known as the least mean-square (LMS) minimization error rule or algorithm.[24−27,30,31] It defines the best solution of the optimal estimator for the conditional probability link between input and output variables.[24−27,30,31] In our case input variables are measured physicochemical parameters (urine tests), and the δ function links them to the observed output vectors defined as the normal children group or urolithiasis group. The calculation of the δ function estimator is approximated by means of the smooth and regular Gaussian function as described by Ritter and Schulten for Kohonen's SOM learning process.[24−31] A detailed mathematical description of the aiNET can be also found at its user's manual Internet address.[24]

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

RESULTS

Table 1 shows median (minimum − maximum) values of the 24-h urinary excretion of calcium and oxalate, urine saturation, citrate/calcium, magnesium/calcium × oxalate, and oxalate/citrate × glycosaminoglycans ratios. Cutoff points between normal children and children with urolithiasis were determined by means of aiNET for all variables except the magnesium/calcium × oxalate ratio, whose data were too dispersed for such discrimination (Table 2). Children with urolithiasis had urine saturation, calcium/creatinine, oxalate/creatinine, and citrate/calcium above 4.70, 0.20, 0.48, and 1.38, respectively. For the oxalate/citrate × glycosaminoglycans ratio, two cutoff points were found. Children with urolithiasis had the ratio values either above 34.80 or less than 10.16. The most accurate method for discriminating normal from sick children was the oxalate/citrate × glycosaminoglycans ratio that showed as 100% sensitive and highly specific with only 6.67% false positive results. Then follow the citrate/calcium ratio and urine saturation, the former with better sensitivity and the later with better specificity. All children with urolithiasis had at least 3 of 5 examined variables in the range of the pathological values, and in 19 out of 30 (63.3%) children all variables showed pathological values (Table 3). On the contrary, all normal children except one, had no more than two variables in the range of the pathological values. In children with hematuria, results were dispersed, although the tendency of having a higher number of pathological variables was noticed.

Table 2. Validity Indexes for Examined Promoters and Inhibitors of Urolithiasis

parameters	cutoff value: normal children	cutoff value: urolithiasis	accuracy	specificity	sensitivity
urine saturation	<4.70	≥4.70	88.89% (75.15−95.84%)	93.33% (71.27−99.67%)	86.67% (70.90−95.62%)
calcium/creatinine (mmol/mmol)	<0.20	≥0.20	82.22% (67.42−91.49%)	73.33% (47.47−90.90%)	86.67% (70.90−95.62%)
oxalate/creatinine (mmol/mol)	<48.00	≥48.00	75.56% (60.14−86.61%)	53.33% (26.68−76.80%)	86.67% (70.90−95.62%)
citrate/calcium ratio (mmol/mmol)	>1.38	≤1.38	91.11% (77.87−97.11%)	73.33% (47.47−90.90%)	100% (90.05−100%)
magnesium/calcium × oxalate ratio (mmol)	*a*	*a*	*a*	*a*	*a*
oxalate/citrate × glycosaminoglycans ratio (mmol × 10^4)	10.16−34.80	<10.16 or >34.80	97.78% (86.77−99.88%)	93.33% (71.27−99.67%)	100% (90.05−100%)

[a] Not possible to determine.

Table 3. Number of Positive Risk Factors in Normal, Hematuria, and Urolithiasis Groups According to Cutoff Values

groups	number of positive risk factors: 0	1	2	3	4	5
normal children	5 (33.33%)	4 (26.67%)	5 (33.33%)	1 (6.67%)	0	0
hematuria	1 (2.78%)	5 (13.89%)	4 (11.11%)	9 (25.00%)	12 (33.33%)	5 (13.89%)
urolithiasis	0	0	0	1 (3.33%)	10 (33.33%)	19 (63.33%)

DISCUSSION

It seems reasonable to consider urolithiasis as a multifactorial disorder with risk of stone formation dependent upon a disturbance in the balance of promoting and inhibiting factors. In our previous study we have shown that urine saturation estimates the relative risk of urolithiasis better than any single urinary constituent.[15] However, the determination of urine saturation may be inconvenient for routine clinical practice, being time-consuming and expensive. Therefore, we tried to find an easier parameter with high sensitivity to discriminate stone formers from healthy children. Saturation can also be expressed in terms of ratios between urine concentration of two or three substances involved in lithogenesis. Among the ratios examined in this study, the best proved to be oxalate/citrate × glycosaminoglycans. Baggio et al. first suggested this ratio as a simple method for detection of the imbalance between promoting and inhibiting factors and found abnormally high ratio values in children with idiopathic urolithiasis.[6] The ratio can differentiate more than 80% of stone formers from non-stone formers. In the present study not only very high but also very low values of the ratio were found in children with urolithiasis in comparison with normal children. The ratio values above the upper normal limit belonged to patients with increased oxalate excretion, while the ratio values under the lower normal limit reflected relatively decreased glycosaminoglycans excretion.

In our previous study[15] standard statistical methods (two-way analysis of variance and Tukey HSD test with correction for unequal *N*) could not detect an influence of glycosaminoglycans on differentiation between normal children and the urolithiasis group.

The method of aiNET analysis was particulary suitable for this type of chemical data analysis in clinical medicine since the LMS algorithm (δ rule) is based on error correction and training by sample.[27−30,31] The significance of this approach is that using this training by sample it is possible to "undo" some of the previous unit training with corrections made on subsequent samples.[31] aiNET is especially good for fast, interactive work. It is designed like a spreadsheet application in the usual Windows enviroment. Its ability to dynamically change the "knowledge basis" was the prime reason aiNET was used to analyze promoters/inhibitors ratios.

The result of neural network classification (Table 2) based on the artificial intelligence method of data analysis implies that decreased glycosaminoglycans values may influence the stone formation in a subpopulation of stone formers. The use of two cutoff points made it possible to increase accuracy of the oxalate/citrate × glycosaminoglycans ratio in differentiation between stone formers and normal children from 80% to 97.78%. Only one healthy child had a pathologically high ratio value owing to unexpectedly high glycosaminoglycans excretion, a finding that is difficult to interpret.

The citrate/calcium ratio has proved to be a very good discriminator between stone formers and normal children, too. Although of somewhat lesser accuracy and specificity than the oxalate/citrate × glycosaminoglycans ratio (Table 2), a citrate/calcium ratio has the advantage of being easily performed in clinical practice.

The study showed once more that the disturbance of more than one of the substances involved in lithogenesis must be present for stone formation. All children with urolithiasis had at least three pathological parameters, while all but one normal child had no more than two pathological parameters.

The neural network analysis of the laboratory tests related to the important and common medical problem of urolithiasis proved to be of potential clinical value.

REFERENCES AND NOTES

(1) Tiselius, H. G. Relationship between the severity of renal stone disease and urine composition. *Eur. Urol.* **1979**, *15*, 5.

(2) Butz, M.; Schulte, P.; Knispel, H. *Neue Gesichtspunkte zur Interpretation harnchemische Befunde beim Harnsteinleiden;* Verh.Ber.34, Tagg Dtsch Ges Urologie, Hamburg, 1982. Springer-Verlag: Berlin, Heidelberg, New York, Tokyo, 1983; p 331.

(3) Robertson, W. G. Physico-chemical aspects of calcium stone-formation in the urinary tract. In *Urolithiasis Research*; Fleisch, H., Robertson, W. G., Smith, L. H., Vahlensieck, W., Eds.; Plenum Press: New York, 1976; p 25.

(4) Robertson, W. G.; Rutherford, A. Aspects of the analysis of oxalate in urine—a review. *Scand. J. Urol. Nephrol*. **1981**, *53* (Suppl.), 85.

(5) Perrone, H. C.; Toporovski, J.; Shor, N. Urinary inhibitors of crystallization in hypercalciuric children with hematuria and nephrolithiasis. *Pediatr. Nephrol*. **1996**, *10*, 435−437.

(6) Baggio, B.; Gambaro, G.; Favaro, S.; Borsatti, A.; Pavanello, L.; Siviero, B.; Zacchello, G.; Rizzoni, G. F. Juvenile renal stone disease: a study of urinary promoting and inhibiting factors. *J. Urol*. **1983**, *130*, 1133−1135.

(7) Marshall, R. W.; Robertson, W. G. Nomograms for the estimation of the saturation of urine with calcium oxalate, calcium pyrophosphate, magnesium amonium phosphate, uric acid, sodium acid urate, ammonium acid urate and cystine. *Clin. Chim. Acta* **1976**, *72*, 253−260.

(8) Robertson, W. G.; Peacock, M.; Marshall, R. W.; Marshall, D. H.; Nordin, B. E. C. Saturation-inhibition index as a measure of the risk of calcium oxalate stone formation in the urinary tract. *N. Engl. J. Med*. **1976**, *294*, 249−252.

(9) Finlayson, B. Calcium stones: some physical and clinical aspects. In *Calcium Metabolism in Renal Failure and Nephrolithiasis*; David D. S., Ed.; Wiley: New York, 1977; Chapter 10, pp 337−382.

(10) Robertson, W. G.; Peacock, M.; Heyburn, P. J.; Marshall, D. H.; Clark, P. B. Risk factors in calcium stone disease of the urinary tract. *Br. J. Urol*. **1978**, *50*, 449−452.

(11) Pak, C. Y. C.; Galosy, R. A. Propensity for spontaneous nucleation of calcium oxalate. Quantitative assessment by urinary FPR-APR discriminant score. *Am. J. Med*. **1980**, *69*, 681−689.

(12) Pak, C. Y. C.; Skurla, C.; Harvey, J. Graphic display of urinary risk factors for renal stone formation. *J. Urol.* **1985**, *134*, 867−870.

(13) Tiselius, H. G. Measurement of the risk of calcium oxalate crystallization in urine. *Urol. Res.* **1985**, *13*, 297−300.

(14) Werness, P.; Brown, C. M.; Smith, L. H.; Finlayson, B. EQUIL II: a BASIC computer program for the calculation of urinary saturation. *J. Urol.* **1985**, *134*, 1242−1244.

(15) Milošević, D.; Batinić, D.; Blau, N.; Konjevoda, P.; Štambuk, N.; Votava-Raić, A.; Barbarić, V.; Fumić, K.; Rumenjak, V.; Stavljenić-Rukavina, A.; Nižić, Lj.; Vrljićak, K. Determination of urine saturation with computer program EQUIL 2 as a method for estimation of the risk of urolithiasis. *J. Chem. Inf. Comput. Sci*. **1998**, *38*, 646−650.

(16) Classen, A.; Hesse, A. Measurement of urinary oxalate: an enzymatic and ion chromatographic method compared. *J. Clin. Chem. Clin. Biochem*. **1987**, *25*, 95−99.

(17) Bitter, T.; Muir, H. M. A modified uronic acid carbazole reaction. *Anal. Biochem*. **1962**, *4*, 330−334.

(18) Da Fonseca-Wollheim, F. Z. Hydrogen Ion Concentration and ADP-Supplementation in Ammonia Determination with Glutamatdehydrogenase *Klin. Chem. Klin. Biochem.* **1973**, *11*, 421−425.

(19) Hansen, J. L.; Frier, E. F. The measurement of serum magnesium by atomic absorption spectrophotometry. *Am. J. Med. Technol.* **1967**, *33*, 158−166.

(20) Bonses, R. W.; Taussky, H. H. On the colorimetric determination of creatinine by the Jaffe reaction. *J. Biol. Chem*. **1951**, *158*, 581−591.

(21) Connerty, H. V.; Briggs, A. R. Determination of serum calcium by means of orthocresolphthalein complexone. *Am. J. Clin. Pathol*. **1966**, *45*, 290−296.

(22) Drewes, P. A. Direct colorimetric determination of phosphorus in serum and urine. *Clin. Chim. Acta* **1972**, *39*, 81−88.

(23) Liddle, L.; Seegmiller, J. E.; Laster, L. Enzymatic spectrophotometric method for determination of uric acid. *J. Lab. Clin. Med*. **1959**, *54*, 903−913.

(24) aiNET Artificial Neural Network, version 1.25, User's Manual; Trubarjeva 42, SI-3000 Celje, Slovenia, 1995 (http://www.ainet-sp.si/).

(25) Grabec, I. Self-Organisation of Neurons Described by the Maximum-Enthropy Principle. *Biol. Cybern.* **1990**, *63*, 403−409.

(26) Grabec, I. Optimization of kernel-type density estimator by the principle of maximal self-consistency. *Neural Parallel Sci. Comput*. **1993**, *1*, 83−92.

(27) Freeman, J. A.; Skapura D. M. *Neural Networks: Algorithms, Applications and Programming Techniques*; Addison-Wesley: New York, 1992; Chapters 2, 7, pp 45−87, 263−289.

(28) Kohonen, T. The "neural" phonetic typewriter. *Computer* **1988**, *21*, 11−22.

(29) Ritter, J. H.; Martinez, T. M.; Schulten, K. J. Topology-conserving maps for learning visuo-motor-coordination. *Neural Networks* **1989**, *2*, 159−168.

(30) Haykin, S. *Neural Networks: A Comprehensive Foundation*; Prentice Hall: Englewood Cliffs: NJ, 1999; Chapter 3, pp 117−154.

(31) Schalkoff, R. J. *Artificial Neural Networks*; McGraw-Hill: Singapore, 1997; Chapter 4, pp 106−109.

CI990076I