Determination of Urine Saturation with Computer Program Equil 2 As a Method for Estimation of the Risk of Urolithiasis

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Received November 25, 1997

To investigate the risk for the development of urolithiasis in 30 children with urolithiasis, 36 children with isolated hematuria, and 15 healthy control children, 24-h urinary excretion of calcium, sodium, oxalate, citrate, sulfate, phosphate, magnesium, urate, chloride, ammonium, and glycosaminoglycans was determined and urine saturation for calcium oxalate was calculated with the computer program EQUIL 2. Compared with controls, children with urolithiasis had significantly increased calcium excretion, oxalate excretion, and urine saturation, whereas children with isolated hematuria had significantly increased calcium excretion only. The best estimation of the relative risk of urolithiasis can be made after urine saturation, using logistic regression. The percentage of patients correctly classified after urine saturation is 85.41% in comparison with 80.95% and 73.81% when the estimation was done by calcium excretion and oxalate excretion, respectively. Using the breakpoint value of 4.29 for urine saturation, it was possible to separate children with increased risk of urolithiasis development from the group of children with isolated hematuria.

INTRODUCTION

Urolithiasis is not rare in children, idiopathic calcium stones being among the most frequent. Overt urolithiasis may be preceded for years by microcrystalluria presenting clinically as hematuria.[1−6] A number of factors have been investigated as a cause of stone formation in adults. Calcium, oxalate, and urate are considered the most representative of the promoters, whereas citrate, glycosaminoglycans (GAGs), magnesium, and pyrophosphate seem to be major inhibitors. Information about urinary stone promoters and especially inhibitors are much more scarce in pediatric idiopathic calcium urolithiasis population.[7−14]

Several studies have shown that a combination of factors provides better separation of stone formers from normal subjects than any single factor.[7,15−22] One of the most sophisticated methods that takes into account the number of urinary components is a computer program EQUIL.[22]

To investigate risk factors for stone formation in children, we examined 11 urinary components potentially promoting or inhibiting crystallization and urine saturation with the computer program EQUIL2, in children with isolated hematuria and overt urolithiasis. The findings were compared with the findings of normal healthy children.

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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 nephrourological disease or pathological condition that might influence urine composition served as controls. The children were enrolled in the study with informed parental consent.

In children with hematuria, glomerular diseases, urinary infection, urological anomalies, and coagulopathy were excluded before entering the study. If a checkup of serum and urine electrolytes revealed hypercalciuria, the known causes of hypercalciuria (renal tubular acidosis, hypercalcemic conditions) were excluded.

In children with urolithiasis ultrasonography and/or intravenous urography established the diagnosis. Cystinuria and hyperuricosuria were excluded.

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 sample from the first day was used for measuring creatinine, calcium, sodium, potassium, oxalate, phosphate, magnesium, citrate, and sulfate. The sample was collected in a wide-mouthed plastic bottle containing 10 mL of 6 N hydrochloric acid as preservative. The 24-h urine sample from the second day was collected in the same way but without the addition of hydrochloric acid in the bottle. The sample was used for measuring chloride, urate, GAGs, and creatinine. The 2-h urine collected on the third day was used for ammonium and creatinine measuring. In this sample, 500 mg of dipotassium oxalate was added immediately after voiding to prevent ammonium decomposition.

S0095-2338(97)00108-X CCC: $15.00
Published on Web 05/09/1998

Table 1. Twenty-four-Hour Excretion of Urinary Promoters and Inhibitors of Crystallization and Urine Saturation[a]

	normal children			hematuria			urolithiasis		
parameter	md	min	max	md	min	max	md	min	max
calcium/creatinine (mmol/mmol)	**0 0.170**	**0.080**	**0.0.330**	**0.220**	**0.110**	**0.790**	**0.295**	**0.077**	**0.564**
sodium/creatinine (mmol/mmol)	14.400	9.300	25.400	16.100	8.600	32.800	12.250	8.800	29.800
oxalate/creatinine (mmol/mol)	**46.000**	**19.000**	**76.000**	**51.000**	**8.000**	**111.000**	**75.000**	**20.000**	**110.000**
citrate/creatinine (mmol/mol)	320.000	68.000	697.000	335.000	29.000	688.000	275.500	63.000	685.000
sulfate/creatinine (mol/mol)	1.410	0.720	3.960	1.435	0.500	3.230	1.075	0.370	3.260
phosphate/creatinine (mmol/mmol)	1.460	0.890	2.440	1.465	0.820	3.700	1.375	0.840	3.520
magnesium/creatinine (mmol/mmol)	0 0.450	0.220	1.010	0.540	0.110	1.200	0.560	0.330	0.970
urate/creatinine (mmol/mmol)	0.270	0.140	3.730	0.250	0.100	0.500	0.205	0.130	0.430
chloride/creatinine (mmol/mmol)	16.400	9.000	22.600	16.500	8.600	32.500	13.800	9.400	29.800
ammonium/creatinine (mmol/mmol)	1.980	0.660	6.110	3.090	0.640	8.660	2.950	0.860	8.460
glycosaminoglycans/creatinine (mg/g)	3.863	0.562	13.700	3.199	1.400	5.700	3.312	0.700	5.620
urine saturation	**1.909**	**0.764**	**3.479**	**4.317**	**0.742**	**16.291**	**19.585**	**0.772**	**25.199**

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

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 Company, Sunnyvale, CA).[23] GAGs were measured by the carbazole method,[24] ammonium by the glutamate dehydrogenase method (Da Fonseca-Wollheim),[25] and magnesium by atomic absorption spectrophotometry.[26] The following analyses were done on an Olympus AU 800 Analyzer: creatinine by standard kinetic Jaffé procedure,[27] sodium, potassium, and chloride by ion-selective electrodes, calcium by the cresolphthalein−complexon method,[28] phosphate by the molybdate method,[29] and uric acid by uricase method.[30]

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 with the computer program EQUIL 2.[22] Also, 24-h urinary excretion expressed as a ratio to the creatinine was calculated for each of the measured urinary components.

Statistical Methods. Data were presented as medians with minimum and maximum values. The significance of differences between the groups was evaluated using the two-way analysis of variance and Tukey HSD test, with correction for unequal N.[31] Logistic regression was used for estimating relative risk of urolithiasis.[32]

RESULTS

Table 1 shows 24-h urinary excretions of calcium, sodium, oxalate, citrate, sulfate, phosphate, magnesium, urate, chloride, ammonium, and GAGs as well as the urine saturation in children with isolated hematuria, children with urolithiasis, and healthy control children. Among the parameters examined, a significant difference between the groups was found in three of them; they are, calcium excretion, oxalate excretion, and urine saturation (boldface data).

Daily urinary excretion of calcium was significantly increased with respect to the control group in the hematuria and the urolithiasis group (Figure 1), whereas oxalate excretion (Figure 2) and urine saturation (Figure 3) were significantly increased only in the urolithiasis group. There was, however, an overlap for all three variables between the hematuria and the urolithiasis groups and between both of them with the control group. Therefore, it seemed that a better approach to the problem would be calculation of the

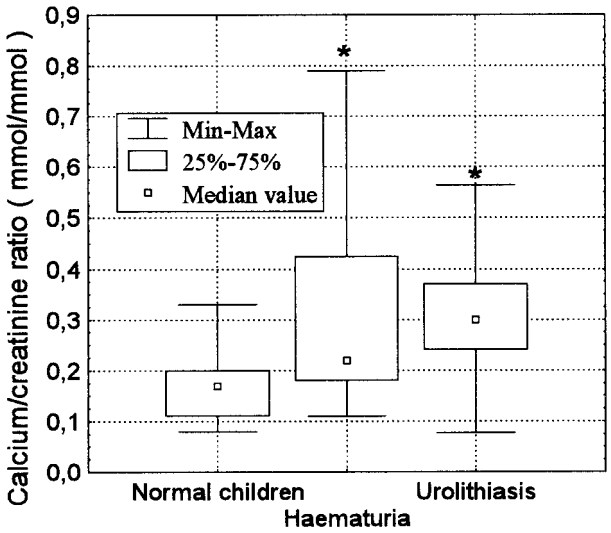

Figure 1.

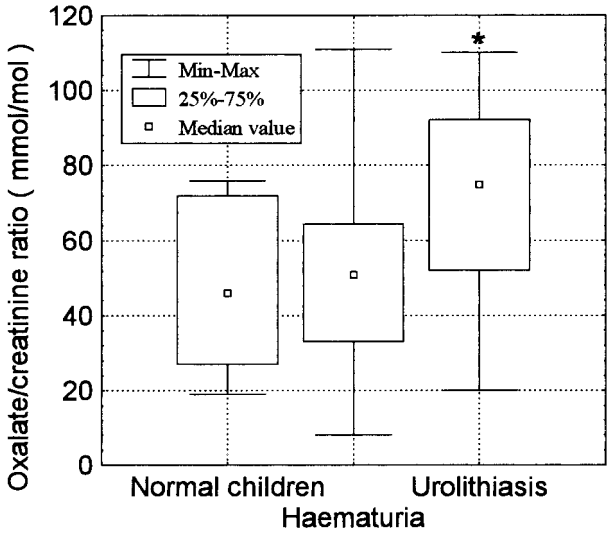

Figure 2.

relative risk of urolithiasis by logistic regression. In the calculation were included the children with urolithiasis and the control children. The results, shown in Figures 4, 5, and 6, indicate that it is possible to estimate the relative risk of urolithiasis for every value of calcium and oxalate excretion and urine saturation. The best estimation can be made after urine saturation. The percentage of patients correctly clas-

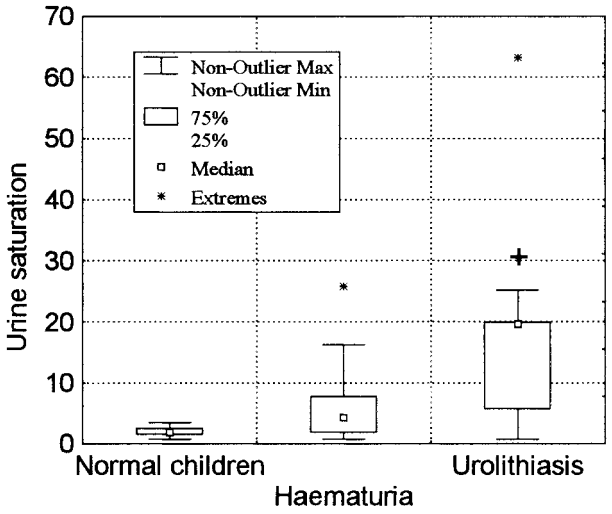

Figure 3.

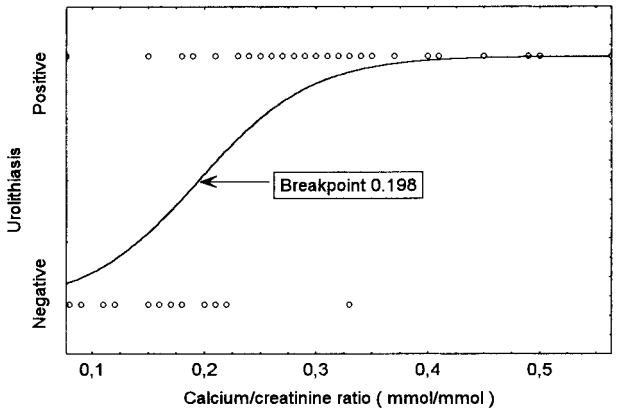

Figure 4.

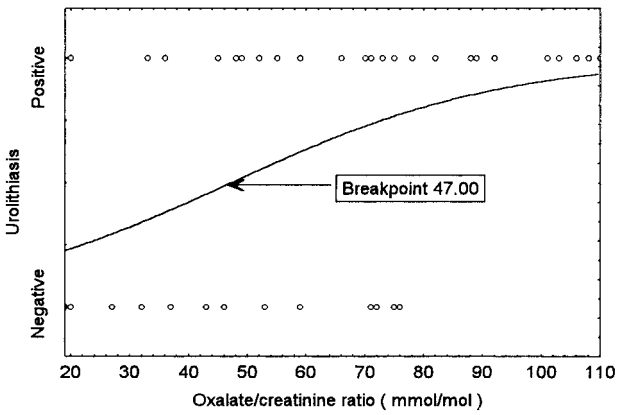

Figure 5.

sified after urine saturation is 85.41% in comparison with 80.95% and 73.81% when the estimation was done by calcium excretion and oxalate excretion, respectively. In addition, the estimation of urolithiasis risk was done with a combined model of logistic regression using values of calcium excretion and oxalate excretion at the same time (Figure 7). Although the percentage of patients correctly classified was somewhat lower than in the model with urine saturation, valuable information about the interaction between calcium excretion and oxalate excretion was obtained. For the same value of calcium excretion, the risk of urolithiasis rose significantly with the rise of oxalate excretion, and *vice versa*.

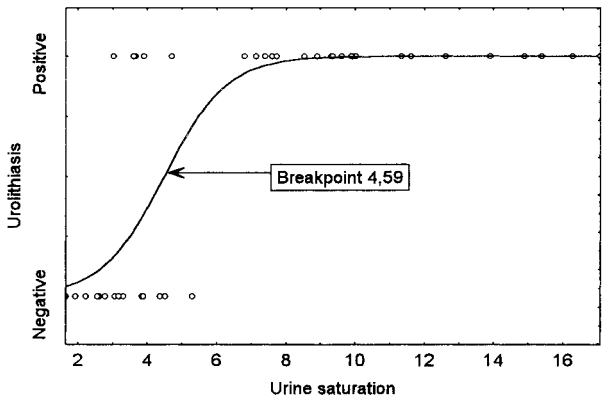

Figure 6.

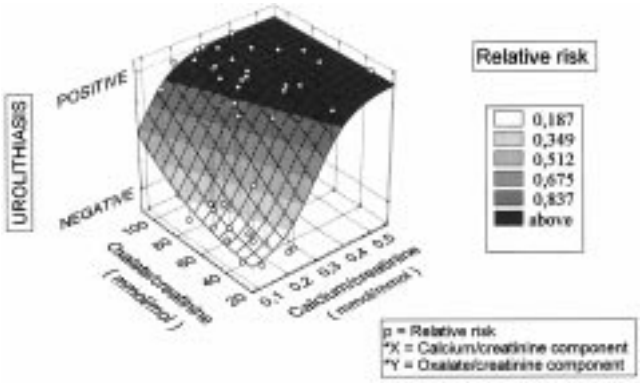

Figure 7.

When these results were extrapolated on the hematuria group, it was possible, using the breakpoint values of 0.189 for calcium excretion, 47.00 for oxalate excretion and 4.29 for urine saturation, to divide this group in two subgroups, one practically undistinguishable from the control group and one with significantly higher values of calcium excretion, oxalate excretion, and urine saturation. Because the highest percentage of patients was correctly classified by breakpoint for urine saturation, it seems best to use this parameter.

DISCUSSION

Substantial efforts have been undertaken over years to explain why some individuals form urinary stones and others do not. However, despite wide investigations pathogenesis of urolithiasis remains unclear. The formation of stone in the urinary tract involves a series of events. Solute excess resulting in urine supersaturation is necessary to trigger this series of events. Hypercalciuria is regarded as one of the major etiological factors in the development of calcium stones,[1,2,4,10,33,34] but recent studies have shown that increased oxalate excretion might be as important as hypercalciuria in the pathogenesis of calcium stone disease.[35,36] Decreased inhibitors of crystallization are frequently pointed out as important in the development of stone disease in adults, but in children their significance is inconclusive as yet. Some authors found decreased urinary GAGs excretion in children with urolihiasis,[13,37] whereas others reported no difference in GAGs excretion between juvenile stone formers and healthy children.[7,9,14]

The results of the present study show that strong promotive factors are necessary for stone formation in children, whereas the significance of inhibitors of crystallization in contrast to

adults is secondary. The children with urolithiasis, as a group, have significantly higher urinary excretion of calcium and oxalate and urine saturation than normal children. The group of children with hematuria had, compared with normal children, increased urinary excretion of calcium too, but not that of oxalate excretion or urine saturation. This result indicates that hypercalciuria, per se, might not be enough to trigger the stone formation.

The present study shows, however, that no single variable can discriminate clearly enough any particular child as healthy or sick. There was a substantial overlap between the groups. Some children with urolitihiasis and some children with hematuria had values of calcium and oxalate excretion and urine saturation in the range of those of normal children, whereas the values of some normal children were found in the range of values of children with haematuria or urolithiasis. So, we adopted a different approach to the problem that enabled us to estimate the relative risk of development of urolithiasis. It was shown that urine saturation can make the best estimation of the risk. Urine supersaturation is indeed a *conditio sine qua non* for the formation of urinary stones. For the calculation of urine saturation for calcium oxalate we used the computer program EQUIL, which employing physicochemical principle of complex equilibria allows the calculation of relative supersaturation for the most important constituent salts of urinary stones. The program is gaining increasing attention in experimental and clinical urolithiasis research and is expanded continuously with respect to chemical species.[38] However, as shown in this study, even the state of urine saturation cannot correctly classify all patients. Influence of some unknown factors, possibly inhibitors such as GAGs, which are not included in EQUIL2, or underestimation of promoters such as oxalate may be responsible for the mistake of estimation. The significance of oxalate in stone formation mirrors in fact that they were found elevated in the urolithiasis group, but not in the hematuria group. Also, the present study shows that for the same value of calcium excretion, the risk of urolithiasis rises substantially with the rise of oxalate excretion.

We believe that the indices of the risk of development of urolithiasis obtained in this study can be useful, in the first place, in discriminating the children with elevated urolithiasis development risk from the group of children with hematuria. The children with hematuria and supersaturated urine should be investigated for the cause of supersaturation and then treated appropriately to prevent the development of urolithiasis.

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CI9701087