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Oxalate status in stone-formers

Two distinct hyperoxaluric entities

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Summary. A retrospective analysis of oxalate status in 115 stone-forming individuals revealed hyperoxaluria in 30%. These individuals could be divided into two distinct groups according to urinary oxalate excretion patterns and plasma oxalate levels. The cause of hyperoxaluria in one group may be explained on the basis of increased absorption of dietary oxalate and decreased renal clearance. Hyperoxaluria in the other group appears to be a consequence solely of enhanced endogenous production of oxalate. These two entities can be distinguished from one another in the context of a routine metabolic evaluation of calcium stone disease when urine and plasma oxalate measurements are included.

Key words: Calcium oxalate stone formation – Hyperoxalemia – Hyperoxaluria – Urolithiasis

The physiological aspects of oxalate handling and their interactions have not been clearly defined in the context of oxalate stone disease, and many questions remain unanswered. With the exception of certain hyperoxaluric conditions such as primary hyperoxaluria types I and II and enteric hyperoxaluria the etiology of hyperoxaluria in stone disease is unknown. The search for the underlying cause of hyperoxaluria and/or hyperoxalemia ultimately requires the determination of urinary and serum/plasma oxalate concentrations. Many reliable techniques are available for the measurement of oxalate in urine and most methods have established that a 24-h excretion of oxalate in excess of 0.45 mmol defines hyperoxaluria [2, 9]. Reliable determination of serum/plasma oxalate involves greater technical difficulty, primarily because of the low levels present. Currently, several techniques are being used which yield comparable results and the consensus is that mean serum/plasma oxalate in normals is approximately 2 μmol/l [3, 10].

This paper presents information regarding oxalate status in 115 individuals forming calcium-containing stones. Two distinct entities of hyperoxaluria emerged from this retrospective analysis of oxalate excretion patterns and plasma oxalate concentrations which were determined in the context of a routine outpatient metabolic evaluation of calcium stone disease.

Materials and methods

The data presented here were derived from the metabolic evaluation of renal stone disease in 115 adult patients (72 male and 43 female) between 19 and 75 years of age. All of these patients were known to be calcium stone-formers; however, stone analysis reports were not available in all cases to determine the exact composition of the stones. Mean creatinine clearance was 128 ml/min for the 115 patients and mean serum creatinine was 91 ± (SE) 2 μmol/l in the fasting state.

The outpatient metabolic evaluation is routinely performed at the New York Stone Center and has been described in detail elsewhere [8]. The evaluation required that each patient make two 24-h urine collections while on a random diet, and following an overnight fast a calcium-load study was performed according to the protocol described by Drach et al. [4]. Briefly, on the morning of the study urine was collected for a 2-h period and a fasting blood specimen was drawn. The patient then ingested a synthetic drink made with distilled water (Calcitest, General Clinical Research Center, Tex.) containing 1 g calcium (Neocalglucon), and urine was collected for another period of 4 h. At the end of this time another blood specimen was drawn. These urine and blood specimens were analysed for a variety of compounds involved in renal stone disease.

Urinary volume was defined "low" in the context of a stone-forming patient if it was less than 2 l/24 h. Although average urine volume for a non-stone-forming individual may be less, a minimum volume excretion of 2 l by a stone-former ensures an adequate dilution effect on the excretion of stone-forming salts [17].

The normal ranges for plasma and urinary electrolytes and organic solutes have been established by the hospital laboratories and these tests were performed as part of a multichannel screen. Urinary citrate was measured using the Boehringer Mannheim (FRG) citric acid kit and hypocitraturia was defined by a 24-h excretion < 1.67 mmol. Plasma and urinary (concentrated HCl-acidified collections) oxalate determinations are routinely performed in the author's laboratory using previously published methodology [2, 6]. The current plasma oxalate assay incorporates modifications in sample handling and preparation as suggested by Costello and Landwehr [3]. The details of these modifications have been described elsewhere [7]. Briefly, plasma was separated from

Table 1. Percentage of stone-forming patients in the four subgroups with major risk factors for stone formation identified in 24-h urine collections

	Group				All
	1 ($n=64$)	2 ($n=16$)	3 ($n=22$)	4 ($n=13$)	($n=115$)
Low volume	68	63	82	77	72
Hypocitraturia	44	38	18	38	35
Absorptive calciuria	18	13	9	38	19
Renal leak calciuria	26	31	14	15	24
Hyperuricosuria	9	6	18	0	9

blood immediately after collection and in order to avoid time-dependent oxalogenesis the plasma was promptly ultrafiltered into an acidified container. Oxalate was precipitated (at pH 5) as the calcium salt prior to extraction and assay of the citrate extracts. The cumulative losses of oxalate incurred throughout the sample preparation were corrected for by the addition of radiolabeled oxalic acid to the initial plasma sample. In our laboratory, plasma oxalate was determined to be 2.5 ± 0.1 μmol/l (mean ± SE) in 48 healthy individuals (range 0.5–4 μmol/l), which agrees well with recently published values [3, 10]. Patients were classified as hyperoxaluric, hypercalciuric and hyperuricosuric when 24-h excretion exceeded 0.45, 7.5, and 4.5 mmol, respectively.

The results of a calcium-load study in each case permitted classification of hypercalciuria. Fasting hypercalciuria was defined by a calcium/creatinine ratio of greater than 0.11 in the fasting 2-h urine collection, while absorptive hypercalciuria was defined by a ratio of greater than 0.2 in the 4-h urine collection following the ingestion of the calcium load [4, 12].

The results obtained from a group of 15 healthy, non-stone-forming individuals (8 males, 7 females) who followed the same outpatient protocol as the patients permitted comparisons for the purpose of statistics. Data from 73 of the 115 patients who had formed calcium-containing stones were included in the earlier report which focused on the classification of stone-formers in the New York Metropolitan area [8]. The data from an additional 42 patients who were also evaluated for their stone disease are included here.

Data analyses

The data obtained from the laboratory analyses of urine specimens collected before and after the calcium-load study were analysed in two ways. First, since the absolute concentration of solutes is important in the process of stone formation, data expressed in terms of concentration were evaluated. Secondly, the laboratory data were computed on a temporal basis (i.e. mmol/h) because urinary volume was highly variable amongst individuals and particularly variable in the 2-h and 4-h collection periods for any one individual. This data transformation also permitted comparisons between urinary solute excretion in the 2-h (fasting) and 24-h (random diet) collections which provided useful information regarding possible dietary contributions to urinary solute excretion.

Statistical methods

Statistical analyses of the data obtained from patients and controls was performed using Student's *t*-test (two-tailed) to establish the significance of the difference between means. A one-way analysis of variance was employed, in conjunction with Duncan's multiple range test, to establish the significance of difference among the means of three or more patient subgroups. All results were expressed as means ± SE and differences were considered significant if $P \leq 0.05$.

Results

The 115 patients were divided into four subgroups on the basis of their 24-h and fasting urinary oxalate excretion patterns; this was done in an attempt to determine differences in their status of oxalate metabolism. Group 1 included all patients who had a 24-h urinary oxalate excretion within normal limits. A separate group, group 2, included patients with a 24-h oxalate excretion at the high end of the normal range (0.39–0.45 mmol/24 h). All patients identified as hyperoxaluric on the basis of the 24-h collection (> 0.45 mmol/24 h) were included in groups 3 or 4; however, urinary oxalate excretion, under fasting conditions, was normal in group 3 and high in group 4 (> 18.9 μmol/h).

Of the 115 calcium-stone-forming patients evaluated in this study 30% were classified as hyperoxaluric. Both the 24-h urinary excretion of oxalate (0.59 ± 0.03 mmol) and plasma oxalate (6.39 ± 0.7 μmol/l) in this group were significantly higher than observed in the remaining patients (0.31 ± 0.13 mmol/24 h and 4.04 ± 0.54 μmol/l, $n=80$, urine and plasma respectively) and healthy controls (0.22 ± 0.02 mmol/24 h and 2.55 ± 0.11 μmol/l, $n=15$, urine and plasma respectively). It is noteworthy that no significant differences were found in dietary oxalate intake between/among the healthy, non-stone-forming controls and any of the four patient groups examined.

A breakdown of the major risk factors for stone formation identified in the 24-h urine collections of the four subgroups is presented in Table 1. Low urinary volume was found in the majority of patients (72%) and occurred usually in combination with one or more additional risk factors. Low urinary volume was the only risk factor found in 7% of cases and more than one risk factor was identified in 91% of the 115 patients evaluated. The next most common risk factors determined, in decreasing order of frequency, were hypocitraturia (35%), fasting and absorptive hypercalciuria (24% and 19% respectively), and hyperuricosuria (9%).

A comparison of the concentration of urinary oxalate before and after the ingestion of the calcium load by the stone-formers is presented in Table 2. Oxalate excretion following the calcium load was not significantly different from oxalate excretion under fasting conditions in any subgroup of stone-formers or in the control group

Table 2. Comparisons of urinary oxalate excretion (μmol/l) before and after calcium (1 g) ingestion

Group	*n*	Before	After
1	64	56.6 ± 1.1	45.6 ± 18.9
2	16	70.0 ± 32.2	73.3 ± 98.8
3	22	61.1 ± 13.3	38.9 ± 36.6
4	13	137.8 ± 34.4[a]	144.4 ± 43.3[a]
All	115	73.3 ± 10.0	64.4 ± 8.9

Values are means ± SE
[a] Significantly different from the corresponding value in groups 1, 2, 3, and the control group

(73.3 ± 15.0 to 85.5 ± 16.3 μmol/l, $n = 15$, before and after, respectively). A significantly higher concentration of urinary oxalate was evident in group 4, both before and after the calcium load, when comparisons were made among the groups. Similar results were obtained when all of the data were computed on a temporal basis, i.e. as excretion in micromoles per hour (not shown). In addition, when the 115 patients were grouped on the basis of calcium excretion, the results did not change. Fifty-seven percent of the patients were normocalciuric on the basis of the calcium load study and mean urinary oxalate excretion was 10.98 ± 1.55 and 11.22 ± 2.11 μmol/h, $n = 65$, before and after the standard 1 g calcium load, respectively. The group of 50 patients (43%) with fasting and/or absorptive hypercalciuria had a mean urinary oxalate excretion of 13.55 ± 1.89 μmol/h, under fasting conditions, which decreased to 9.55 ± 1.11 μmol/h after the calcium load. As in the previous study [8], significant increases in urinary excretion of calcium and magnesium and a decrease in phosphate excretion were confirmed (not shown) after the oral calcium load.

A profile of urinary oxalate excretion parameters and plasma oxalate for each group is presented in Table 3. There was no difference between the patients in group 1 and the control group in any aspect of oxalate status that was examined. A significantly higher mean urinary oxalate excretion (on a random diet) was seen in groups 2, 3, and 4 when these were compared with group 1 and the non-stone-forming control group (0.22 ± 0.02 mmol/24 h). Under fasting conditions urinary oxalate excretion is significantly increased in group 4 but is within normal limits in groups 2 and 3. While urinary oxalate excretion (expressed on a temporal basis) under fasting conditions and on a random diet compared well in groups 1 and 4, a significant reduction in urinary oxalate in groups 2 and 3 was noted under fasting conditions. Plasma oxalate levels were also significantly higher in groups 2, 3, and 4 when these were compared with group 1 and the controls (2.55 ± 0.11 μmol/l, $n = 15$).

It is evident from Table 3 that renal clearance of oxalate differed widely among the subgroups of stone-formers, and while oxalate clearance was lower in groups 2 and 3 than in the healthy controls (66.2 ± 8.3 ml/min, $n = 15$) there were no significant differences among the groups. Creatinine clearance was not found to be significantly different among the groups when the four patient groups and the group of healthy controls were compared. The mean oxalate/creatinine clearance ratio for the group of healthy normals was found to be 0.41 ± 0.05, indicating net tubular reabsorption of oxalate. It can be seen that the mean oxalate/creatinine clearance ratios for each group were also less than unity and no significant differences were found between these means and that of the control group.

Discussion

A profile of major stone-formation risk factors in this patient series (shown in Table 1) indicates that low urinary volume was frequent in all patients and in each group of patients when the 115 patients were subdivided according to their oxalate excretion patterns. The diagnosis of either absorptive or renal leak calciuria was made in 43% of these patients on the basis of how each individual responded to the oral calcium load, and this consistent with the incidence of hypercalciuria found in other studies [4, 13] and the previous study [8]. Although there are

Table 3. Profile of urinary oxalate excretion, serum oxalate, oxalate clearance, and oxalate/creatinine clearance ratios in the four subgroups of 115 stone-formers

	Group			
	($n = 64$)	($n = 16$)	($n = 22$)	($n = 13$)
24 h urine Ox (mmol/24 h)	0.26 ± 0.01	0.41 ± 0.01[a]	0.57 ± 0.03[a]	0.64 ± 0.07[a]
Fasting urine Ox (μmol/h)	10.55 ± 2.22	8.88 ± 1.00	8.00 ± 1.00	24.55 ± 4.44[b]
Fasting serum Ox (μmol/l)	2.38 ± 0.10	7.54 ± 1.30[a]	6.80 ± 0.80[a]	5.71 ± 1.00[a]
Ox clearance (ml/min)	84.2 ± 16.0	39.2 ± 16.0	42.2 ± 9.0	82.3 ± 19.0
Clearance ratio: Ox/Creat	0.65 ± 0.10	0.33 ± 0.10	0.31 ± 0.06	0.75 ± 0.24
	(64)	(16)	(22)	(13)

Values are means ± SE
Creat, creatinine; *Ox*, oxalate
[a] Significantly different from the corresponding value in group 1 (and the control group)
[b] Significantly different from the corresponding values in all groups

apparent differences in the frequency and concomitance of hypocitraturia, hypercalciuria, and hyperuricosuria among the subgroups, the clinical relevance of such interactions cannot easily be explained in such a complex disease as urolithiasis. For example, the finding of no individuals with hyperuricosuria in group 4 is most probably coincidental. These data confirm, however, that multiple risk factors for stone formation exist in the majority (91% in the present study) of patients with urolithiasis.

The additional findings reported in the earlier study [8] of (1) a higher mean plasma oxalate in stone-formers than in non-stone-formers, and (2) no significant difference in excretion of exalate after a calcium load by patients grouped either on the basis of oxalate excretion (see Table 1) or on the basis of urinary calcium excretion, were confirmed here. These results contrast with the observations of Schwille et al. [16], who reported both lower serum oxalate levels in calcium-stone-formers and an elevation in postprandial oxalate excretion among patients who were grouped according to calcium excretion patterns [16]. Grouping our patients according to the type of calciuria did not change the result, and the reason for this contrast in results is not clear. In another study by Schwille et al. [15] postprandial urinary oxalate excretion increased in patients with absorptive and renal leak calciuria following the ingestion of a calcium load similar to that consumed by our patients. In contrast, stone-formers in the present study exhibiting absorptive and renal leak calciuria had somewhat decreased urinary oxalate excretion after ingesting the calcium load (10.3 ± 1.7 to 9.1 ± 1.1 μmol/h, $n = 22$; 15.0 ± 3.3 to 11.8 ± 1.4 μmol/h, $n = 28$, before and after, respectively). A decrease in urinary oxalate excretion is predicted, and may possibly occur, if excess intraluminal calcium is available to bind with oxalate. A decrease in the absorption of ^{14}C tracer oxalate was reported by Schwille et al. [15] when they determined its excretion over 24 h following the test calcium load; however, these authors could not reconcile their apparently contradictory results.

In an earlier report from this laboratory [8] hyperoxaluria was described in 30% of 80 patients evaluated, which was acknowledged to be a larger number than previously observed. This percentage was confirmed here in a larger series of 115 patients forming calcium-containing stones. Although we can speculate that the majority of the patients formed oxalate stones, we admit that the exact composition of the stones formed by each patient studied here was not known. Given normal renal function (as indicated by creatinine clearance) hyperoxaluria and/or hyperoxalemia can conceivably result from: (1) enhanced endogenous production of oxalate, (2) increased absorption of dietary oxalate, (3) alterations in renal oxalate clearance, or (4) a combination of the above. Previous studies have indicated that the hyperoxaluria observed in stone-formers is of an endogenous origin [15], of a dietary source [10], or renally mediated [16]. The data presented here indicate that the hyperoxaluria and hyperoxalemia in groups 2 and 3 may be explained on the basis of increased absorption of dietary oxalate and decreased renal clearance of oxalate, but enhanced endogenous production of oxalate cannot be definitively excluded. In contrast, the hyperoxaluria and hyperoxalemia in group 4 appears to be due to enhanced endogenous oxalate production alone. That is, when 24-h and 2-h oxalate excretion were compared on a temporal basis within group 4, there was no significant difference. Additional support for this diagnosis is the finding that oxalate clearance is comparable to that observed in group 1, which presumably excludes a renally mediated cause for the hyperoxalemia found in group 4. Renal function, as indicated by creatinine clearance (which was determined for each individual) was not compromised and no significant differences were found in creatinine clearance among the stone-forming patient groups and the non-stone-formers.

A group of patients with characteristics similar to those of group 4 have been described previously and given the diagnosis "mild metabolic hyperoxaluria" [14], the term proposed to describe a type of hyperoxaluria that was effectively reduced by pyridoxine treatment [14]. Although a pyridoxine deficiency was never identified and pyridoxine supplementation was not always a successful treatment, a metabolic basis for the hyperoxaluria was nonetheless concluded. The study presented here provides more solid evidence for the entity of mild metabolic hyperoxaluria than a retrospective assessment of pyridoxine treatment. In addition, since the diagnosis here is based upon measurement of oxalate and not glycolate (which may or may not be elevated in mild metabolic hyperoxaluria), this represents an advance in characterizing the etiology of hyperoxaluria.

The mechanisms of oxalate transport and excretion by the human kidney have not been definitively characterized and require further investigation. Evidence has been provided by in vitro animal investigations that oxalate is freely filtered at the glomerulus and undergoes bidirectional tubular transport [5]. Ultimately, it was shown in animal studies [5] that oxalate clearance exceeded inulin clearance, implicating net tubular oxalate secretion. In studies in humans using a rapid injection (^{14}C-labelled oxalate) intrarenal technique, Osswald and Hautman [11] came to a similar conclusion, which is contrary to our findings and those of Kasidas [10]. In 86% of 115 patients and in all of the controls in this study the oxalate/creatinine clearance ratio was less than unity. Kasidas [10] reported a similar finding in 79 of 94 patients (84%), indicating that net tubular reabsorption of oxalate occurs in most individuals. A ratio of greater than 1 was found in the remaining patients in each study (1.72 ± 0.25 in the present study and 1.42 ± 0.09, $n = 15$ in Kasidas' study [10]) and half of these patients in our study were hyperoxaluric. Assuming that oxalate is freely filtered at the glomerulus, the present results indicate that oxalate is secreted and/or reabsorbed in both hyperoxaluric and normo-oxaluric individuals; what determines the directional movement of oxalate is not known. It is clear, however, that all of the oxalate/creatinine ratios determined for non-stone-formers in this study are less than unity and this can be substantiated by a calculation which incorporates currently accepted normal values for 24-h urine volume, urinary and serum oxalate levels and creatinine clearance. If it is assumed that (1) average 24-h

urine volume of healthy non-stone-formers is 1.5 l [1, 18] (present study), which gives a urine excretion rate of 1.04 ml/min; (2) average oxalate excretion is 0.28 mmol/24 h; and (3) plasma oxalate concentration is 2.5 μmol/l (i.e. average estimate of recently reported values [3, 10] (present study), renal clearance of oxalate can be calculated to be 77 ml/min. If creatinine clearance is a "textbook" 120 ml/min, it follows that the estimated clearance ratio is 0.64. Both the estimate of oxalate clearance and the oxalate/creatinine ratio are comparable to those determined in the present study for both group 1 (Table 3) and the control group.

Unfortunately, there is no current consensus view of the mechanism of oxalate excretion in humans because of the different approaches and species employed in the various studies reported to date. Further investigations are necessary to define more clearly what renal oxalate transport mechanisms are operable under normal and pathophysiological conditions. In addition, the factors affecting unidirectional and/or bidirectional tubular oxalate transport need to be determined.

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