Phenotypic expression of primary hyperoxaluria: Comparative features of types I and II

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Phenotypic expression of primary hyperoxaluria: Comparative features of types I and II.

Background. The primary hyperoxalurias are autosomal recessive disorders resulting from deficiency of hepatic alanine:glyoxylate aminotransferase (PHI) or d-glycerate dehydrogenase/glyoxylate reductase (PHII). Marked hyperoxaluria results in urolithiasis, renal failure, and systemic oxalosis. A direct comparison of PHI and PHII has not previously been available.

Methods. Twelve patients with PHI and eight patients with PHII underwent similar laboratory evaluation, clinical management, and follow-up. Diagnosis of PHI and PHII was made by hepatic enzyme analysis (N = 11), increased urinary excretion of glycolate or glycerate (N = 7), or complete pyridoxine responsiveness (N = 2). Six PHI and five PHII patients had measurements of calcium oxalate crystalluria, urine supersaturation, and urine inhibition of calcium oxalate crystal formation.

Results. PHI and PHII did not differ in age at the onset of symptoms, initial serum creatinine, or plasma oxalate concentrations. Urine oxalate excretion rates were higher in PHI (2.19 ± 0.61 mmol/1.73 m²/24 hours) than PHII (1.61 ± 0.43, P = 0.04). Urine osmolality, calcium, citrate, and magnesium concentrations were lower in PHI than PHII (P = 0.001, P = 0.019, P = 0.0002, P = 0.03, respectively). Crystalluria scores and calcium oxalate inhibitory activity of the urine did not differ between PHI and PHII. Calcium oxalate supersaturation in the urine was less in PHI (7.3 ± 1.9) compared with PHII (14.0 ± 3.3, P = 0.002). During follow-up of 10.3 ± 9.6 years in PHI and 18.1 ± 5.6 years in PHII, stone-forming activity and stone procedures were more frequent in PHI than PHII (P < 0.01 and P = 0.01, respectively). Four of 12 PHI compared with 0 of 8 PHII patients progressed to end-stage renal disease (P = 0.03).

Conclusion. The severity of disease expression is greater in type I primary hyperoxaluria than in type II. The difference may be due to greater oxalate excretion and lower concentrations of urine citrate and magnesium in patients with PHI compared with PHII.

Key words: autosomal recessive disorder, hepatic alanine:glyoxylate aminotransferase, d-glycerate dehydrogenase/glyoxylate reductase, plasma oxalate, crystalluria, calcium oxalate, stone forming activity.

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The primary hyperoxalurias are inborn errors of metabolism that result from specific hepatic enzyme deficiencies [1]. In type I primary hyperoxaluria (PHI), deficiency and/or mistargeting of hepatic alanine:glyoxylate aminotransferase results in metabolic overproduction of oxalate and glycolate [2]. In type II primary hyperoxaluria (PHII), deficiency of d-glycerate dehydrogenase/glyoxylate reductase results in metabolic overproduction of oxalate and glycerate [3]. In both PHI and PHII, the excess oxalate is excreted in the urine but is of low solubility and precipitates as a calcium salt. High urinary oxalate concentrations result in urolithiasis. Over time, renal parenchymal damage caused by calcium oxalate deposition leads to renal insufficiency, hyperoxalemia, and systemic deposition of calcium oxalate. End-stage renal failure, metabolic bone disease, erythropoietin refractory anemia, skin ulcers, digital gangrene, cardiac arrhythmias, and cardiomyopathy reflect systemic oxalate deposition. Survival is poor with maintenance dialysis and in patients with extensive tissue oxalosis. Treatment strategies include pharmacologic doses of pyridoxine for patients with PHI responsive to this cofactor of alanine:glyoxylate aminotransferase and the use of agents such as orthophosphate and citrate, which reduce calcium oxalate crystallization in the urine and in the renal parenchyma [4–6]. For patients with end-stage renal disease, kidney transplantation or combined kidney and liver transplantation has been successful [7, 8].

Type I primary hyperoxaluria (McKusick 259900) and PHII (McKusick 260000) are rare, autosomal recessive disorders. Recent studies suggest a prevalence of PHI in France of 1.05/million population with an incidence rate of 0.12 per million per year [9]. As of 1997 [10, 11], only 24 patients with PHII had been described in the world literature, 8 of whom were identified during evaluation of an extensive Canadian pedigree [12]. Seventeen of the 24 patients had urolithiasis, and 1 had nephrocalcinosis [13]. Normal renal function was noted in seven of the patients, reduced renal function in one, and end-stage renal disease in three [11, 14–17]. Renal function of the remaining 13 patients was not reported. Only 10
of the 24 patients had follow-up information available [11, 13, 15, 16]. It has been suggested that patients with PHII may have milder disease expression when compared with PHI [11, 18], although not all authors agree [19]. Both PHI and PHII are characterized by marked hyperoxaluria. Reasons for the apparent difference in clinical outcome have not been elucidated.

At the Mayo Clinic, we have identified 12 patients with PHI and 8 patients with PHII who underwent similar diagnostic evaluation and consistent medical management with similar duration of follow-up. Information from these two groups permits understanding of the differences and of the similarities in the clinical and in the laboratory expression of these subtypes of primary hyperoxaluria. This report provides the first direct data comparing patients with PHI and PHII.

METHODS

Medical records of all patients with primary hyperoxaluria seen at the Mayo Clinic from 1966 to 1995 were reviewed by the authors. Of the 28 patients with a definitive diagnosis of PHI or PHII, 8 patients who presented with end-stage renal disease are not included in this report. All eight of these excluded patients had PHI by hepatic enzyme analysis. Twelve PHI patients and seven PHII patients presented for initial evaluation with good [23]. The normal reference range for glycolate was 14
hepatic enzyme analysis. Twelve PHI patients and seven
report. All eight of these excluded patients had PHI by tween children and adults [22]. Urine glycolate and
glycerate concentrations and normal glycolate. 2 mL aliquot of each voiding was filtered immediately
during the night. Their usual diet was not changed. Six-
teen patients were instructed to drink eight ounces (a glass)
of water every hour while awake and when up to void
distilled water to remove traces of soluble salts and was
air dried for crystal determination. Ten percent of each
voiding was placed under mineral oil for measure-
ment with similar duration of follow-up. Information from these two groups permits understanding of the differences and of the similarities in the clinical and in the laboratory expression of these subtypes of primary hyperoxaluria. This report provides the first direct data comparing patients with PHI and PHII.

METHODS

Medical records of all patients with primary hyperoxaluria seen at the Mayo Clinic from 1966 to 1995 were reviewed by the authors. Of the 28 patients with a definitive diagnosis of PHI or PHII, 8 patients who presented with end-stage renal disease are not included in this report. All eight of these excluded patients had PHI by hepatic enzyme analysis. Twelve PHI patients and seven PHII patients presented for initial evaluation with good renal function, defined as a creatinine clearance of to 114
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renal function, defined as a creatinine clearance of to 114
the method of Olthuis et al [20] [normal reference rangecomparing patients with PHI and PHII.

From 1980 to 1988, urinary oxalate was measured by
the method of Olthuis et al [20] [normal reference range
range 10 to 40.5 mg (0.11 to 0.46 mmol)/day] [21]. Urine samples were collected for 24 hours into contain-
ers with concentrated hydrochloric acid to prevent ox-
lute crystallization and to prevent ascorbate conversion
to oxalate. All urine oxalate values are expressed as
mmol/1.73 m²/24 hours to provide valid comparisons be-
tween children and adults [22]. Urine glycolate and
l-glyceric acid were measured by gas chromatography
[23]. The normal reference range for glycolate was 14
to 114 μg/mg creatinine (21 to 168 μmol/mmol creati-
nine). The normal reference range for l-glyceric acid
was 22 to 185 μg/mg creatinine (24 to 198 μmol/mmol
creatinine). Enzyme studies of liver tissue were per-
formed in the laboratories of Drs. C. Danpure and G.
Rumsby [19, 24].

Eleven patients (6 with PHI and 5 with PHII) had
measurements of calcium oxalate crystalluria, calcium oxalate supersaturation in urine, and inhibition of cal-
cium oxalate crystal formation in urine. These studies
were obtained when the patients were not receiving med-
ications. After obtaining informed consent, patients were
admitted to the General Clinical Research Center for
two days while on their normal fluid intake and normal
diet. Reference values were obtained in 16 normal sub-
jects [7 women and 9 men; median age 31 years (range
17 to 39 years)] who were unrelated to the patients. Two
consecutive 24-hour urine collections were obtained.
Each voiding was collected in a beaker kept at 37°C.
The volume and pH of each sample were measured. A
2 mL aliquot of each voiding was filtered immediately
by vacuum through a 0.22 μm Nucleopore filter (25 mm
diameter). The filter was then rinsed with four drops of
distilled water to remove traces of soluble salts and was
air dried for crystal determination. Ten percent of each
urine voiding was placed under mineral oil for measure-
ment of carbon dioxide, and 10% was stored with addi-
tion of 6N hydrochloric acid and the remainder in a pool.
Each of these aliquots was stored at 4°C until completion of the 24-hour collection. Measurements were then made immediately, or the fractions were frozen. These 24-hour pooled collections were analyzed for the major ionic species (Na, K, Ca, Mg, NH₄, citrate, sulfur, phosphorus, carbon dioxide, chloride, oxalate, and uric acid). Osmolality was analyzed by freeze point osmometry; sodium and potassium by flame emission spectrophotometry; creatinine and phosphorus by Auto Analyzer; chloride by coulometric titration; uric acid enzymatically with uricase; calcium and magnesium by atomic absorption; sulfate by a modification of the gravimetric method [25]; oxalate by the method of Olthuis et al [20]; citrate by the method of Natelson, Pincus, and Lugovoy [26]; ammonium ion by the Berthelot technique; and carbon dioxide content by microgasometer. Crystalluria was assessed by the method of Werness using petrographic microscopy and scanning electron microscopy with energy dispersive x-ray spectroscopy [27]. Twenty-four-hour pooled collections were analyzed with a seeded crystal growth system to determine whether formation of calcium oxalate crystals was inhibited [28]. In this system, one inhibitor unit was defined arbitrarily as the concentration of inhibitor required for 50% reduction in the rate of crystal growth (k/k – kₐ = 2) under standard conditions. Ionic strength, free ion activity, and supersaturation with calcium oxide were estimated using EQUIL 2 [29]. The results from the two 24-hour collections were averaged.

Statistical analysis was by t-test for continuous variables and by chi square for nominal variables. Spearman correlation coefficients were used to compare the rates of decline in renal function. Kaplan–Meier survival percentages were used to compare the numbers of patients progressing to end-stage renal disease during follow-up.

**RESULTS**

Of the PHI patients, six were male, and six were female. Among the PHII patients, one was male, and seven were female. The 12 patients with PHI were from 10 unrelated families, and the 8 patients with PHII were from 4 unrelated families. Initial clinical and laboratory characteristics are displayed in Table 1. No differences were found between PHI and PHII with regard to age at onset of symptoms, age at diagnosis, or initial serum creatinine. Twenty-four-hour creatinine clearance was 13.7 ± 0.9 mL/min/1.73 m² in PHI and 17.9 ± 1.2 mL/min/1.73 m² in PHII (P = 0.04; Fig. 1). Urine oxalate concentrations did not differ between groups (P = 0.41). Mean urine volume was higher in PHI than PHII, although the difference did not reach statistical significance. The urinary concentrations of citrate, calcium, and magnesium were lower in patients with PHI compared with those with PHII (P = 0.0002, P = 0.019, and P = 0.03, respectively). Urinary concentrations of pyrophosphate, while lower in PH patients than normal subjects, did not differ between PHI and PHII. Calcium oxalate supersaturation of 7.3 ± 1.9 in PHI and 14.0 ± 3.3 in PHII was higher than the 2.5 ± 0.6 observed in control subjects (PHI vs. control P = 0.04, PHII vs. control P = <0.001). Calcium oxalate supersaturation was greater in PHII than PHI (P = 0.002; Table 2). Crystal scores were greater in PHI (2.7 ± 1.4) and PHII (2.4 ± 0.5) compared with control subjects (P = 0.001, PHI vs. control P = 0.0001, but did not differ between the PH subtypes. Calcium oxalate inhibition was significantly reduced in both PHI at 23.8 ± 13.7 and PHII at 29.8 ± 5.2 compared with control subjects 97 ± 36 (PHI vs. control P = 0.0001, PHII vs. control P = 0.0002), but not different between subtypes.

In the PHI and PHII groups combined, the 24-hour urine oxalate excretion rate correlated inversely with the initial creatinine clearance (P = 0.01). Urine citrate

<table>
<thead>
<tr>
<th>Table 1. Clinical and laboratory characteristics</th>
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<tbody>
<tr>
<td>Type I</td>
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<tr>
<td>--------</td>
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<tr>
<td>Age onset symptoms years</td>
</tr>
<tr>
<td>Age at diagnosis years</td>
</tr>
<tr>
<td>Serum creatinine baseline mg/dL</td>
</tr>
<tr>
<td>C₄, baseline mL/min/1.73 m²</td>
</tr>
<tr>
<td>Urine oxalate mmol/1.73 m²/24 h</td>
</tr>
<tr>
<td>Plasma oxalate μmol/L</td>
</tr>
<tr>
<td>Fractional excretion oxalate</td>
</tr>
<tr>
<td>Urine glycerate μg/mg creatinine</td>
</tr>
<tr>
<td>Urine glycerate μg/mg creatinine</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

*Normal urine oxalate <0.46 mmol/24 hours

*Normal plasma oxalate 0.5 to 3.0 μmol/L.

*Normal FEoxalate 0.47 ± 0.3

*Normal urinary glycerate ≤114

*Normal urinary glycerate ≤185

Fig. 1. Urine oxalate in patients with primary hyperoxaluria types I (PHI; O) and II (PHII; ●), P = 0.04. The shaded area denotes a normal range of <0.46 mmol (40.5 mg).
excretion, expressed as mg/g creatinine was 236 ± 130 in PHI and 787 ± 432 in PHII (P = 0.002) and also correlated with initial renal clearance (P = 0.02).

During mean follow-up of 10.3 years in PHI and 18.1 years in PHII (Table 3), stone-forming activity was found in 37% of evaluable visits in PHI and in 7% of evaluable visits in PHII (P < 0.01). Surgical intervention for urolithiasis was required more often in PHI than PHII (P = 0.016). Four of the 12 patients with PHI developed end-stage renal disease during the course of follow-up. One PHII patient presented initially with renal failure but maintained a renal allograft iohexol clearance of 83 mL/min/1.73 m² at 20 years following renal transplantation despite persistent hyperoxaluria on treatment with neutral phosphate. Of the eight patients with PHI and eight patients with PHII who had preserved renal function at last follow-up, renal clearance was similar with mean values of 95 and 110 mL/min/1.73 m², respectively (P = 0.44).

**DISCUSSION**

Patients with PHI and PHII have increased urinary calcium oxalate supersaturation, increased calcium oxalate crystalluria, and decreased urine inhibition of calcium oxalate crystal formation when compared with normal subjects, and they demonstrate a life-long propensity for urolithiasis. Our data confirm that patients with PHII have a more favorable long-term clinical course than those with PHI. This difference was evident with regard to metabolic stone-forming activity, number of surgical interventions required for management of urolithiasis, and preservation of renal function. These differences occurred despite a common management approach for all patients with primary hyperoxaluria included in this report. Although PHII appears less severe, the potential for end-stage renal disease and systemic oxalate deposition remains. This possibility was reflected by our patient who presented with end-stage renal disease as well as by patients reported in the literature [14–16, 30].

The explanation for more active stone formation and greater renal parenchymal damage in PHI compared with PHII is suggested by the greater oxalate excretion rates observed in PHI as well as by differences in urine composition. Oxalate excretion rates were inversely correlated with renal clearance at initial evaluation (P = 0.01). In cell culture systems and in animal models, hyperoxaluria causes injury to renal tubular epithelial cells, inducing proliferation and cell disruption [31–33]. The interaction of calcium oxalate monohydrate crystals and renal epithelial cells results in induction of genes encoding plasminogen activator inhibitor and platelet-derived growth factor (abstract; Hammes et al, J Am Soc Nephrol 5:864A, 1994), suggesting a pathophysiologic mechanism for renal interstitial fibrosis in hyperoxaluric states. Calcium oxalate crystals also can be demonstrated in renal interstitium [31, 33]. In humans, interstitial inflammation and interstitial fibrosis are reported in hyperoxaluria [34–37].

**Table 2. Urine findings**

<table>
<thead>
<tr>
<th></th>
<th>Type I</th>
<th>Type II</th>
<th>Normal subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>6</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>pH</td>
<td>5.6 ± 0.3</td>
<td>5.8 ± 0.5</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>Volume mL/24 h</td>
<td>2193 ± 181</td>
<td>1651 ± 601</td>
<td>1925 ± 519</td>
</tr>
<tr>
<td>U_ox mOsm/kg</td>
<td>302 ± 98</td>
<td>499 ± 58</td>
<td>560 ± 212</td>
</tr>
<tr>
<td>Ionic strength</td>
<td>0.080 ± 0.013</td>
<td>0.155 ± 0.021</td>
<td>0.150 ± 0.045</td>
</tr>
<tr>
<td>U_ca mg/dL</td>
<td>8.3 ± 5.8</td>
<td>10.8 ± 6.8</td>
<td>2.1 ± 1.0</td>
</tr>
<tr>
<td>U_Ox mg/dL</td>
<td>2.8 ± 1.7</td>
<td>7.0 ± 5.2</td>
<td>8.8 ± 3.6</td>
</tr>
<tr>
<td>U_Oxmg/dL</td>
<td>8.0 ± 4.4</td>
<td>47.7 ± 24.8</td>
<td>38.4 ± 19.2</td>
</tr>
<tr>
<td>U_BAL mg/dL</td>
<td>4.4 ± 2.5</td>
<td>7.5 ± 1.6</td>
<td>9.0 ± 3.6</td>
</tr>
<tr>
<td>CaOx SS</td>
<td>0.05 ± 0.03</td>
<td>0.17 ± 0.16</td>
<td>0.42 ± 0.17</td>
</tr>
<tr>
<td>Crystal score</td>
<td>2.7 ± 1.4</td>
<td>2.3 ± 0.5</td>
<td>0.6 ± 0.7</td>
</tr>
<tr>
<td>CaOx inhibition</td>
<td>23.8 ± 13.7</td>
<td>29.8 ± 5.2</td>
<td>97 ± 36</td>
</tr>
</tbody>
</table>

*Type I compared with type II

**Table 3. Primary hyperoxaluria outcome**

<table>
<thead>
<tr>
<th></th>
<th>Type I</th>
<th>Type II</th>
<th>Normal subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>12</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Duration follow-up years</td>
<td>10.3 ± 9.6</td>
<td>18.1 ± 5.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Patients with stones</td>
<td>12/12</td>
<td>7/8</td>
<td>0.21</td>
</tr>
<tr>
<td>Stone-forming activity*</td>
<td>0.37 (31/84)</td>
<td>0.07 (7/96)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(proportion of visits)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stone procedures per patient</td>
<td>2.6 ± 1.6</td>
<td>1.0 ± 0.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Change in renal clearance mL/min/1.73 m²/year</td>
<td>-1.65c</td>
<td>-1.04c</td>
<td>0.39</td>
</tr>
<tr>
<td>ESRD during follow-up</td>
<td>4/12</td>
<td>0/8</td>
<td>0.03</td>
</tr>
<tr>
<td>Renal clearance mL/min/1.73 m²</td>
<td>95 ± 39</td>
<td>110 ± 14</td>
<td>0.44</td>
</tr>
</tbody>
</table>

*Stone-forming activity is defined as an increase in number of stones or size of stones demonstrated by renal imaging studies when compared with the previous visit.

**Median**
When compared with PHII, the PHI group had lower concentrations of urinary citrate and magnesium. Both citrate and magnesium form complexes in urine, citrate with calcium and magnesium with oxalate, reducing the amount of oxalate and calcium available for precipitation [28]. Citrate also inhibits calcium oxalate crystal formation [28]. Whether the low concentrations of these inhibitors in PHI result from metabolic differences is not clear from these studies. However, our observations in other PHI patients of persistently low urine citrate concentrations during periods of continued high urinary oxalate excretion following successful hepatic transplantation (unpublished data) suggest that the hypocitric aciduria is not a result of the hepatic enzyme abnormality in PHI. Adsorption onto crystal surfaces as the cause of hypocitric aciduria is supported by our previous observation that calcium oxalate inhibitory activity in the urine of PH patients returns to normal when crystalluria decreases or disappears with neutral phosphorus treatment [4].

Reduced urine citrate concentrations and reduced citrate excretion could reflect renal tubulointerstitial injury resulting from high urine oxalate concentrations or calcium oxalate crystals. Damage to renal tubular epithelium and interstitial inflammation have been reported in hyperoxaluric patients [34, 38, 39]. The observed correlation between urine citrate excretion and renal clearance ($P = 0.02$) is consistent with this hypothesis.

Despite the clinical observation of more active metabolic stone formation in PHI as compared with PHII, other laboratory parameters that might be predictive of such activity, the calcium oxalate crystal score and calcium oxalate inhibitor activity, when measured directly, did not display differences between PHI and PHII. The discrepancy between the clinical observations and these laboratory findings may reflect limitations in the predictive value of these in vitro studies or, due to the dynamic nature of crystal formation, limitation in use of 24-hour urine collections for calcium oxalate inhibition and supersaturation measurement. The higher urinary concentration of calcium and the lower urine volume in patients with PHII as compared with PHI may account for the unexpectedly higher calcium oxalate supersaturation in PHII.

The lower urine osmolality observed in PHI compared with PHII is of interest. Renal structural findings did not provide a satisfactory explanation. Nephrocalcinosis was not demonstrated in any of the patients in either group. Among patients with PHI, one had diffuse calcification within calices bilaterally, and two had solitary kidneys following previous nephrectomies for stone disease. Among patients with PHII, one had a large stone that formed a cast of the renal pelvis and calyces, and two patients had solitary kidneys following nephrectomies for stone disease. Interstitial calcium oxalate crystal deposition, not visible on imaging studies, could potentially compromise renal concentrating capacity. Water deprivation studies were not performed in the patients and would be needed to address this observation further. It is also possible that PHI patients, who had more frequent symptomatic stone episodes, were more conscientious regarding maintenance of a large oral fluid intake.

CONCLUSION

Clinical characteristics such as age at onset of symptoms and age of diagnosis are not helpful in differentiating PHI from PHII. Furthermore, while there are differences with regard to urine oxalate excretion rates and other urinary parameters, the overlap is such that these values are not sufficient in an individual patient to differentiate between PHI and PHII. Urinary glycolate and glycerate values and hepatic enzyme analysis are necessary for accurate diagnosis. Advances in genetic testing may soon permit DNA analysis as an alternative [40, 41]. The finding of eight patients with PHII from four unrelated families among our patients with primary hyperoxaluria suggests that PHII may not be as rare as previously thought. Our observations confirm that PHII follows a more benign long-term clinical course than PHI. Differences in metabolic production rates of oxalate and in urinary chemical composition may account, in part, for this observation. Other factors remain to be evaluated.

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