Primary hyperoxaluria type 1: clinical, genetic and biochemical studies

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CHAPTER 06  **High incidence of hyperoxaluria in generalized peroxosomal disorders**

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**ABSTRACT**  The Zellweger spectrum disorders (ZSDs) are characterized by a generalized loss of peroxisomal functions caused by deficient peroxisomal assembly. Clinical presentation and survival are heterogeneous. Although most peroxisomal enzymes are unstable in the cytosol of peroxisome-deficient cells of ZSD patients, a few enzymes remain stable among which alanine:glyoxylate aminotransferase (AGT). Its deficiency causes primary hyperoxaluria type 1 (PH1, MIM 259900), an inborn error of glyoxylate metabolism characterized by hyperoxaluria, nephrocalcinosis, and renal insufficiency. Despite the normal level of AGT activity in ZSD patients, hyperoxaluria has been reported in several ZSD patients. We observed the unexpected occurrence of renal stones in a cohort of ZSD patients. This led us to perform a study in this cohort to determine the prevalence of hyperoxaluria in ZSDs and to find clinically relevant clues that correlate with the urinary oxalate load. We reviewed medical charts of 31 Dutch ZSD patients with prolonged survival (more than 1 year). Urinary oxalate excretion was assessed in 23 and glycolate in 22 patients. Hyperoxaluria was present in 19 (83%), and hyperglycolic aciduria in 14 (64%). Pyridoxine treatment in six patients did not reduce the oxalate excretion as in some PH1 patients. Renal involvement with urolithiasis and nephrocalcinosis was present in five of which one developed end-stage renal disease. The presence of hyperoxaluria, potentially leading to severe renal involvement, was statistically significant correlated with the severity of neurological dysfunction. ZSD patients should be screened by urinalysis for hyperoxaluria and renal ultrasound for nephrocalcinosis in order to take timely measures to prevent renal insufficiency.
INTRODUCTION

The Zellweger spectrum disorders (ZSDs) include the severe classic Zellweger syndrome with an early lethal course, and much milder phenotypes with prolonged survival known as neonatal adrenoleukodystrophy and infantile Refsum disease. Most common clinical features are neurodevelopmental impairment, retinopathy, perceptive deafness, and hepatic dysfunction (1). The extent of the neurodevelopmental impairment is highly variable. Some patients reach adolescence and are socially and intellectually educable, underscoring the need for proper medical attendance. Although renal involvement is known from pathological studies (e.g., renal cysts), functional renal impairment is uncommon.

Peroxisome biogenesis is impaired in ZSDs, resulting in a loss of peroxisomal functions, including the deficient β-oxidation of certain fatty acids, notably hexacosanoic acid (C26:0) and pristanic acid as well as the impaired formation of polyunsaturated fatty acids and plasmalogens (1). Survival is best predicted biochemically by the dihydroxyacetonephosphate acyltransferase (DHAPAT) activity and C26:0 β-oxidation activity as measured in fibroblasts (2). The genetic basis is heterogeneous with 12 mutant PEX genes identified so far (3). The most common c.2528 G>A mutation, which leads to an amino acid substitution at position 843 of the PEX1 protein (G843D), is generally associated with a mild phenotype (4-8). At the other end of the clinical spectrum, the c.2097_2098insT (null) mutation gives rise to a truncated PEX1 protein and is associated with the classical Zellweger syndrome phenotype (9,10).

Most peroxisomal enzymes are unstable in the cytosol which explains the functional deficiency of most peroxisomal enzymes in ZSD. A few enzymes, however, remain stable, among which alanine:glyoxylate aminotransferase (AGT; EC 2.6.1.44). This enzyme catalyzes the conversion of glyoxylate to glycine. Mutations in the AGXT gene, occurring in the inherited disorder of glyoxylate metabolism primary hyperoxaluria type 1 (PH1), render the enzyme inactive, either by inactivating AGT directly or by mistargeting of AGT to mitochondria (11). The resulting excess glyoxylate is either oxidized to oxalate or reduced to glycolate and excreted into the urine. The resulting excess oxalate easily precipitates as calcium oxalate leading to the formation of urolithiasis, nephrocalcinosis, and renal insufficiency (12,13). Pyridoxine administration reduces the urinary oxalate load in approximately 30% of the patients (14).

Over the past decade, several ZSD patients have been described with hyperoxaluria (6,15,16). To date, a systematic survey on the occurrence of hyperoxaluria in ZSDs has not been performed. Therefore, we undertook a cohort study in order to delineate the prevalence of hyperoxaluria in patients with ZSDs and to find clinically relevant variables that correlate with the severity of urinary oxalate excretion.
MATERIALS AND METHODS

Patients
Patient data were retrieved from a recently collected cohort of patients diagnosed between 1975 and 2002, comprising 31 Dutch ZSD patients with survival beyond the first year of life (6). Clinical data were reviewed, including data on renal ultrasounds and radiographies. The diagnosis was confirmed by appropriate studies in plasma (very-long-chain fatty acids, bile acid intermediates, pipecolic acid, pristanic acid, and docosahexaenoic acid), erythrocytes (plasmalogen levels), and fibroblasts (de novo plasmalogens biosynthesis, activity of dihydroxyacetonephosphate acyltransferase (DHAPAT), oxidation rates of C26:0, pristanic acid, and phytanic acid, immunoblot analysis of acyl-CoA oxidase and peroxisomal thiolase, and immunofluorescence microscopy analysis of catalase), followed by complementation studies and molecular analysis of the relevant PEX gene in most cases. Neurological development was assessed by using the compound developmental score (CDS) as designed and published previously (6). This score is a measure of the development of statural motor control, hand control, verbal development, and visual development. A maximum of 10 points may be given to patients with appropriate development on all aspects of the score. For patients under the age of four, a general assessment of neurological development was made, as the CDS can only be used for patients over four years of age.

Biochemical and genetic analysis
Urinary oxalate levels were assayed by ion chromatography and urinary glycolate levels by gas chromatography. Urinary levels exceeding 54 mmol oxalate/mol creatinine and 140 mmol glycolate/mol creatinine, respectively, were considered as elevated. Age related reference values were used as determined by Reusz et al. (17). For patients with primary hyperoxaluria the median urinary oxalate level is 185 (range 60-600) mmol/mol creatinine and the median urinary glycolate level is 345 (range 180-1740) mmol/mol creatinine as detected in our laboratory. The DHAPAT activity and C26:0 β-oxidation in fibroblasts and plasma levels of C26:0 were investigated for potential predictive value with respect to neurological development as expressed by the compound developmental score. Mutations in the PEX genes were investigated for their association with neurological development and hyperoxaluria.

Statistical analysis
For analysis, patients were categorized with respect to developmental outcome into a group of poor (CDS<5) and favorable (CDS>5) outcome. The correlation of the different biochemical markers and the neurological development between the two groups was evaluated using the Mann-Whitney U test. Additionally, dichotomous variables were composed regarding presence or absence of hyperoxaluria. The Fischer's Exact Test was used to analyze the association between neurological development and hyperoxaluria. Statistical package used was SPSS 11.5.1 Statistics UK.
RESULTS

Urinary tract symptoms were recorded in five patients and consisted of colic pains, hematuria, and recurrent urinary tract infections (TABLE 06.1). Renal ultrasound was performed in 15 out of the 19 patients with hyperoxaluria. Urolithiasis or nephrocalcinosis were found in five patients. One of them (nr 1) underwent lithotripsy and another (nr 2) underwent surgical removal of kidney stones. In this patient, a huge obstructing renal stone, causing extensive hydronephrosis without signs of colic pains, was found by chance on an abdominal X-ray that was made in order to assess a scoliosis. In retrospect, hematuria had already been present in this patient. Renal function was preserved in all but one patient. This child (nr 1) died at 15 years of age due to the consequences of end-stage renal disease. Four patients (nrs 2, 3 as well as two others) suffered from recurrent episodes of diarrhea. Patient nr 3 also suffered from steatorrhea.

\[\text{Plasma C26:0, reference range: 0.45-1.32 \, \mu M, C26:0/C22:0, reference range: 0.0-0.02.}\]

\[\text{NQ, not quantified.}\]

\[\text{Reference range for oxalate < 54 mmol/mol creatinine}\]

\[\text{Reference range for glycolate < 174 mmol/mol creatinine}\]

\[\text{G843D genotype: (++) homozygous G843D; (+/-) heterozygous G843D.}\]

\[\text{This patient is mutated in the PEX5 gene.}\]
### TABLE 06.1  
*Clinical, biochemical, and genetic characteristics of the five ZSD patients with renal involvement*

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
</tr>
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<tbody>
<tr>
<td>Age at last follow-up (years)</td>
<td>15</td>
<td>11</td>
<td>2.8</td>
<td>1.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Death</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Neonatal period</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>● Poor feeding</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>● Failure to thrive</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Motor milestones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>● Walks</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>● Stands with support</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>● Sits</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Extinguished ERG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Profound hearing impairment</td>
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<td>+</td>
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<tr>
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<td>Feeding problems</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Urinary tract complications</td>
<td></td>
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<td></td>
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<tr>
<td>● Hematuria</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>● Urinary tract infections</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>● Urolithiasis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>● Nephrocalcinosis</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>● Hydronephrosis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>● End-stage renal disease</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Compound development score</td>
<td>3</td>
<td>5</td>
<td>Poor</td>
<td>Poor</td>
<td>2</td>
</tr>
<tr>
<td>Plasma very-long-chain fatty acids^a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>● C26:0</td>
<td>4.73</td>
<td>5.24</td>
<td>NQ^b</td>
<td>5.08</td>
<td>3.96</td>
</tr>
<tr>
<td>● C26:0/C22:0</td>
<td>0.28</td>
<td>0.36</td>
<td>0.19</td>
<td>0.24</td>
<td>0.26</td>
</tr>
<tr>
<td>Oxalate levels mean (range) mmol/mol creatinine^c</td>
<td>100 (90;110)</td>
<td>260 (30;540)</td>
<td>200 (200)</td>
<td>480 (310;740)</td>
<td>275 (230;390)</td>
</tr>
<tr>
<td>Glycolate levels mean (range) mmol/mol creatinine^d</td>
<td>NQ</td>
<td>98 (5;353)</td>
<td>94 (94)</td>
<td>63 (37;134)</td>
<td>26 (15;41)</td>
</tr>
<tr>
<td>Genotype</td>
<td>G843De</td>
<td></td>
<td></td>
<td>++</td>
<td>+/-</td>
</tr>
</tbody>
</table>

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^a Plasma very-long-chain fatty acids:
- C26:0
- C26:0/C22:0

^b Oxalate levels mean (range) mmol/mol creatinine:
- 100 (90;110)
- 260 (30;540)
- 200 (200)
- 480 (310;740)
- 275 (230;390)

^c Glycolate levels mean (range) mmol/mol creatinine:
- NQ
- 98 (5;353)
- 94 (94)
- 63 (37;134)
- 26 (15;41)

^d Genotype:
- G843De
- ++
- +/-
Urinalysis was performed in 23 (74%) of the 31 patients. The remaining eight patients had died. In 19 (83%) of these 23 patients hyperoxaluria was detected at least once, with hyperglycolic aciduria in 12. Six patients had isolated hyperoxaluria and two had isolated hyperglycolic aciduria. Two patients had completely normal oxalate and glycolate levels. Glycolic acid was not measured in one patient with hyperoxaluria. Urinary oxalate and glycolate levels did not change consistently with progression of the disease over time. All 11 patients with a poor neurological development had hyperoxaluria. A representation of oxalate and glycolate measurements as compared to the degree of neurological dysfunction is shown in FIGURE 06.1. A poor neurological development was statistically significant correlated with elevated levels of urinary oxalate (Kendall’s tau-b coefficient = 0.44, p = 0.015). The activity of hepatic AGT was assessed in one patient (nr 2) and was found to be normal. For peroxisomal C26:0 β-oxidation and plasma levels of C26:0 a correlation was found with the degree of neurological development (p = 0.02 and p = 0.01, respectively) but not for DHAPAT activity and neurological development (p = 0.438).

**FIGURE 06.1** Representation of urinary oxalate and glycolate for all ZSD patients, categorized for the degree of neurological dysfunction. Patients with a mild degree of neurological development are shown on the left part of the X-axis and patients with a severe degree of neurological development on the right side. (-----) Upper level of reference values for urinary oxalate. (-----) Upper level of reference values for urinary glycolate. Dots (●) represent urinary oxalate. Diamonds (◆) represent urinary glycolate.
Genetic analysis revealed that the mild c.2528 G>A mutation in the PEX1 gene was present in 13 out of 23 patients: seven in the group with a favorable neurological development and six in the group with a poor neurological development. The other patients had other, less common mutations.

Pyridoxine was administered to six of the 19 ZSD patients with hyperoxaluria but did not result in a decline of oxalate excretion. None of the patients received vitamin C supplementations at the time of oxalate measurements. Detection of hyperoxaluria was irrespective of diarrhea episodes.

**DISCUSSION**

This is the first systematic survey on the prevalence of hyperoxaluria in a cohort of patients with Zellweger spectrum disorders. Our results show that hyperoxaluria is a common finding in ZSD patients. The highest levels of urinary oxalate in the ZSD patients as described in this paper equal the levels found in patients with primary hyperoxaluria type 1. In addition, levels of urinary glycolate are mostly raised albeit to a lesser extent as compared to patients with primary hyperoxaluria. In addition, urinary tract involvement was always accompanied by hyperoxaluria. Hyperoxaluria in patients with ZSD resulted in significant clinical morbidity and even mortality. Lithotripsy was mandatory in one patient and one patient required surgical intervention for stone removal. End-stage renal disease, most probably caused by hyperoxaluria, developed in another patient.

In four patients, intermittent diarrhea was seen in combination with hyperoxaluria. Patients with malabsorption syndromes can display hyperoxaluria due to elevated enteric oxalate uptake (18). We excluded this pathophysiological mechanism in our cohort, as oxalate excretion did not vary with stool pattern. A high intake of vitamin C may also result in a high urinary oxalate excretion (19). However, as none of the patients received vitamin C supplementations during our study, we can exclude this as a confounding factor.

In most of the patients with ZSD and hyperoxaluria, glycolic acid was also raised up to levels seen in PH1. As detected in one of our patients and reported before in others, AGT activity is normal at least when measured in total liver homogenates of ZSD patients (15,20). Apparently, the absence of normal compartmentalization of AGT in peroxisomes as a result of the defective import machinery leads to an impairment in glyoxylate metabolism. In ZSD patients with hyperoxaluria, at least part of the produced glyoxylate was not converted into glycine by AGT but appeared to be converted into oxalate probably by the cytosolic enzyme lactate dehydrogenase (FIGURE 06.2).
Reactions involved in oxalate synthesis in man. In controls, AGT converts glyoxylate into glycine. In ZSD patients, peroxisomes are absent and AGT is localized in the cytosol. AGT, alanine:glyoxylate aminotransferase; DAO, D-amino acid oxidase; GO, glycolate oxidase; GR/HPR, glyoxylate reductase/hydroxypyruvate reductase; LDH, lactate dehydrogenase.

We found a statistically significant positive relation between the severity of the disease, as expressed by the compound developmental score, on the one hand, and the presence of hyperoxaluria on the other hand. Apparently there is a relative preservation of both neurological functions as well as of functional AGT activity in some patients suggesting a common mechanism. This could be due to functional AGT activity outside the peroxisome or some residual AGT import in peroxisomes in mildly affected patients. Other, yet unknown, genetic or environmental factors may influence the functional activity of some of the peroxisomal enzymes when localized in the cytosol. Further studies are necessary to address these hypothetical mechanisms. The identification of C26:0 β-oxidation in fibroblasts and levels of C26:0 in plasma as a prognostic marker for neurological development further supports the hypothesis that a variable degree of cellular dysfunction underlies both the clinical heterogeneity and the heterogeneity with respect to urinary oxalate levels.

The equal presence of the mild c.2528 G>A mutation in both the mild and the severe patient groups shows that the genotype in patients with ZSD cannot predict outcome, either clinically as expressed by the level of neurological dysfunction, or biochemically as expressed by the level of urinary oxalate. The frequencies of the genotypes of the other patients were too low to draw conclusions.
Our results strongly indicate the need for periodic urinalysis in all patients with ZSDs immediately after diagnosis has been made. As collection of a 24-h sample of urine can be difficult in patients with severe developmental delay and poor bladder control, one should be aware of potential sample errors if only small collections of urines are used (21). Therefore, repeated tests may be necessary. If hyperoxaluria is detected, sufficient fluid intake in combination with oral citrate are mandatory in order to try to prevent formation of calcium oxalate and thus of renal stones and nephrocalcinosis. In addition, it is important to search for urolithiasis or nephrocalcinosis in ZSD patients by means of abdominal ultrasound, in order to initiate appropriate therapy as early as possible trying to prevent further renal damage.

REFERENCES


8. Walter C, Gootjes J, Mooijer PA,


