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High incidence of hyperoxaluria in generalized peroxisomal 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 (>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.

Keywords: Zellweger spectrum disorders; Hyperoxaluria; Hyperglycolic aciduria; Urolithiasis; Nephrocalcinosis; End-stage renal disease; Peroxisomal disorders; Alanine:glyoxylate aminotransferase

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*

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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, phytanic 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 $\leqslant$ 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 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.

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 Fig. 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