

ZELLWEGER (CEREBRO-HEPATO-RENAL) SYNDROME: FIRST BRAZILIAN CASE WITH CONFIRMED PEROXISOMAL DEFECT

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ABSTRACT

Zellweger syndrome (ZS) is a recessive disorder caused by a generalized failure in the peroxisomal metabolism. It is an illustrative example of an inherited metabolic disease that is usually noticed in the neonatal period due to its typical dysmorphic features. Though suspicion of ZS is frequently raised in neonatal units, diagnosis is seldom confirmed as it should be based on sophisticated biochemical procedures. In this paper we describe a case of ZS that, to the best of our knowledge, is the first Brazilian report where the peroxisomal defect was biochemically confirmed. We stress the importance of the biochemical confirmation of ZS, as it enables not only a better delineation of the ZS phenotype, but mainly because it allows the prevention of further cases through genetic counseling and prenatal diagnosis.

INTRODUCTION

The Zellweger syndrome (ZS) is an autosomal recessive disorder (McKusick 21410) originally described by Bowen *et al.* (1964) as a "familial syndrome of multiple

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congenital defects". ZS patients are characterized mainly by the presence of typical craniofacial dysmorphia (high forehead, large fontanels, shallow orbital bridges, low/broad nasal bridge, epicanthus, high arched palate, external ear deformities, micrognathia) and neurological abnormalities (severe hypotonia, abnormal Moro reflex, hypo- or areflexia, epileptic seizures and psychomotor retardation). The picture is usually completed by defects in other systems and tissues, like the eye (optic disk pallor, cataract, cloudy cornea), the liver (hepatomegaly, fibrosis, cholestasis) and the kidney (cysts). Comprehensive reviews of this entity, also known as "cerebro-hepato-renal syndrome", were recently published (Kaiser and Kramar, 1988; Singh *et al.*, 1988; Wanders *et al.*, 1988; Wilson *et al.*, 1988).

A clear suspicion that ZS was not just an additional MCA/MR (multiple congenital anomalies/mental retardation) syndrome was advanced once it was observed that affected patients do not have peroxisomes (Goldfisher *et al.*, 1973). That discovery called attention to this subcellular organelle, which is now recognized as having an essential role in human cellular metabolism (Table I). Further work on peroxisomal biochemistry enabled Borst (1983) to associate ZS with impaired synthesis of plasmalogens, and Singh *et al.* (1984) to demonstrate that affected patients have a defective oxidation of very-long-chain fatty acids. Many other biochemical abnormalities were already detected in ZS patients all related to peroxisomal dysfunction (Wanders *et al.*, 1988).

Table I - Peroxisomal functions in mammalian cells (modified from Lazarow, 1987).

H₂O₂ metabolism (including many oxidases and catalase)

Ether-phospholipid biosynthesis (plasmalogens)

Beta-oxidation of fatty acids and other compounds (very-long-chain fatty acids, polyunsaturated fatty acids, prostaglandins, bile acids, xenobiotics)

Glyoxylate metabolism.

Polyamine metabolism.

Cholesterol and dolichol synthesis.

Phytanic acid and pipercolic acid degradation.

In addition to ZS, a large number of diseases are now recognized to be caused by defects in one or more peroxisomal functions (see Table II).

Table II - Classification of peroxisomal disorders (according to Wanders *et al.*, 1988).

Disorder	Enzyme defect
<i>Generalized peroxisomal dysfunction</i>	
Zellweger syndrome	Generalized
Neonatal adrenoleukodystrophy	Generalized
Infantile Refsum disease	Generalized
Hiperpipecolic acidemia	Generalized
<i>Multiple peroxisomal dysfunction</i>	
Rhizomelic chondrodysplasia punctata	DHAP-AT alkyl DHAP synthase phytanic acid oxidase
Combined peroxisomal beta-oxidation enzyme deficiency (Zellweger-like syndrome)	Peroxisomal beta-oxidation enzymes
<i>Isolated peroxisomal dysfunction</i>	
Adrenoleukodystrophy	Peroxisomal very-long-chain fatty acyl-CoA synthetase
Thiolase deficiency (pseudo-Zellweger syndrome)	Peroxisomal thiolase
Acyl-CoA oxidase deficiency (pseudo-neonatal ALD)	Acyl-CoA oxidase
Classical Refsum disease	Phytanic acid oxidase
Hyperoxaluria type I	Alanine:glyoxylate aminotransferase
Acatalasemia	Catalase

In this paper we describe a case of ZS that, to the best of our knowledge, is the first Brazilian report for which the peroxisomal defect has been biochemically confirmed.

CASE REPORT

Clinical findings

J.S., white, female, was the first child of a young and healthy couple. The pregnancy was unremarkable, except for a mild vaginal bleeding in the first trimester. The child was born by vaginal delivery at the expected time. Her weight was 2,650 g and her length 47 cm. In the first examination it was noticed wide fontanelles, low nasal bridge, upturned palpebral fissures, epicanthus, low-set ears, redundant folding of the neck, limitation of extension of knees and elbows, simian crease, camptodactily

and hip dislocation were noticed. She presented seizures in the neonatal period, which were treated with phenobarbital. At that time cerebral ecography and EEG were normal. A hypothesis of chromosome anomaly was raised, but discarded after a normal result for chromosome analysis with G-banding.

When examined again at the age of 2 months, physical and neuromotor retardation were apparent as was global hypotonia. At that time a transient episode of jaundice was noticed, together with high serum levels of blood urea nitrogen and creatinine. An IV urography and a renal ecography were performed, with normal results. An ophthalmologic examination was also normal. Standard screening for metabolic diseases (for amino acid, carbohydrate and some lysosomal diseases) was also performed with normal results.

ZS was then postulated, and a skin biopsy was collected in order to perform the biochemical studies. A treatment based on medium-chain triglycerides was introduced, and a slight improvement in weight gain and a decrease in bilirubin levels was subsequently noticed (but not documented).

At the age of 4 months her weight was 3,200 g, and she had no reactions to visual and auditive stimuli. Nystagmus was noticed and microcephaly was obvious. The fontanelles were still very wide. a CT scan disclosed a diffuse cerebral atrophy. Some of the patient's characteristics may be observed in Figures 1 and 2.

Development was marked by severe failure to thrive and lack of neuromotor development. Seizures and broncopneumonia episodes became very frequent, leading to death at the age of 18 months.

Biochemical investigation

The purpose of the biochemical investigation was to check the activity of acyl-CoA: dihydroxyacetone phosphate acyl-transferase (DHAP-AT, E.C. 2.3.1.42), a peroxisomal enzyme that is involved in the first step in the plasmalogen biosynthetic pathway (Hajra *et al.*, 1979).

Fibroblasts were grown from a skin biopsy on Ham F-10 culture medium supplemented with glutamine, antibiotics and fetal calf serum. When confluent, the cells from early passages were collected with trypsinization, and the cell pellet kept at -20°C until the analyses were performed.

The DHAP-AT assay was performed essentially as described by Schutgens *et al.* (1984). The fibroblasts were suspended in 5 mM Tris-HCl (pH 7.5) buffer, containing 50 mM NaCl, and the enzyme liberated by sonication (3 x 15 sec) plus 3 cycles of freeze-thawing. Homogenates (20-40 μg protein, assayed by Lowry's method) were incubated at 37°C for 2 h with 0.6 mM L-(U- ^{14}C) dihydroxyacetone phosphate (10 μCi) (previously prepared from L-(U- ^{14}C) glycerol-3-phosphate) in the presence of 8 mM NaF, 8 mM MgCl_2 , 0.4 mg bovine serum albumin and 0.15 mM palmitoyl-CoA in a final volume of 0.12 ml. The product was recovered through an extraction step

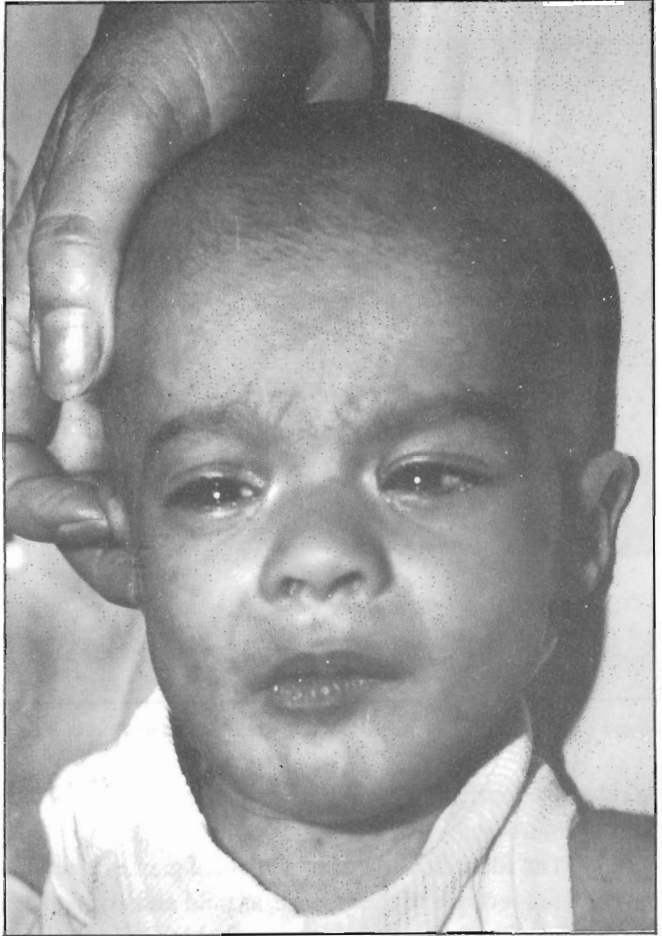


Figure 1 - Facial appearance of the patient J.S. at the age of 14 months, displaying a high forehead, low nasal bridge and epicanthus.

and dried on filter paper, which was subsequently washed four times in trichloroacetic acid solutions of decreasing concentrations, in order to remove any traces of the labeled substrate. The paper was then dried and placed in a scintillation vial with Aquasol for radioactivity measurement.

The enzyme activity in the patient's fibroblasts was $0.242 \text{ mM}/2 \text{ h}/\text{mg}$ protein, clearly below the normal range (2.098 to $4.472 \text{ mM}/2 \text{ h}/\text{mg}$ protein) and indicative of severe DHAP-AT deficiency.

DISCUSSION

ZS is a remarkable example of an inborn error of metabolism with noticeable dysmorphic features already present in the neonatal period. These findings may



Figure 2 - At the age of 14 months, failure to thrive and neuromotor delay were evident.

be related to early defects in neuronal migration due to failure of peroxisomal functions (Powers *et al.*, 1985).

The identification of biochemical markers for ZS allowed the expansion of the clinical spectrum of the disease, as mild and atypical cases that previously did not fit the clinical criteria are now recognizable (Wilson *et al.*, 1986; Barth *et al.*, 1987). Despite massive investigation in the field of peroxisomal disorders, the basic defect of ZS is still unknown. A defect in the ability to import proteins into peroxisomes has been suggested by the finding that ZS cells have empty membrane structures called "peroxisomal ghosts" (Santos *et al.*, 1988).

The diagnosis of ZS is frequently suspected in neonatal units, but the final diagnosis is seldom confirmed as it depends on the performance of sophisticated procedures in order to check peroxisomal functions, such as the assay of DHAP-AT levels or of very-long-chain fatty acids in the plasma (Brown *et al.*, 1982). With this report we aim to stress to the pediatrician and to the clinical geneticist the importance of making efforts to obtain a biochemical confirmation of ZS, mainly because it allows the family to benefit from genetic counseling and prenatal diagnosis, which can be performed either with amniotic fluid or with chorionic villi (Moser *et al.*, 1984; Schutgens *et al.*, 1985).

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RESUMO

A síndrome de Zellweger (ZS) é uma doença recessiva causada por uma deficiência generalizada das funções peroxissômicas, sendo um exemplo ilustrativo de uma doença metabólica herdada que usualmente chama a atenção já no período neonatal pelo seu quadro dismórfico típico. Embora a suspeita de ZS seja freqüentemente levantada nas unidades neonatais, o diagnóstico é raramente confirmado uma vez que ele exige procedimentos bioquímicos sofisticados. Neste artigo é descrito um paciente com ZS que, tanto quanto estejamos informados, é o primeiro caso brasileiro no qual o defeito peroxissômico foi confirmado bioquimicamente. Os autores ressaltam a importância da confirmação bioquímica do diagnóstico de ZS, uma vez que ela permite não apenas um melhor delineamento do fenótipo da síndrome mas principalmente porque permite a prevenção de casos adicionais através do aconselhamento genético e do diagnóstico pré-natal.

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