

Case Report

Peroxisomal Disorders: Short Review with Four Case Reports

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INTRODUCTION

Peroxisomes are subcellular organelles that are present in all human cells except the mature erythrocytes. They were first observed in mice kidney's cells in 1954 and were named as such by De Duve^[1] in 1966. Although much remains to be elucidated, it is known that these organelles carry out a large variety of metabolic functions including detoxification of H₂O₂, beta oxidation of some fatty acids, i.e., very long chain fatty acids (VLCFA), di- and tri-hydroxycholestanic acid, pristanic acid, alpha oxidation of branched chain fatty acids (phytanic acid), early steps in synthesis of phospholipids, plasmalogens and cholesterol and pipecolic acid degradation^[2].

Normal peroxisomal assembly and import of proteins from cytosol into peroxisome depend on peroxisome biogenesis factors or peroxins (with corresponding genes PEX)^[3]. Most peroxins are localized in the peroxisomal membrane. The selection of proteins destined for transport from cytosol into the peroxisome depends on peroxins Pex5 and Pex7. They act as receptors for specific sequences on proteins called peroxisomal targeting signals (PTS-1 and PTS-2). Pex5 (receptor for PTS-1) binds to its protein ligand in the cytosol and carries it to the peroxisome. During this process it interacts with Pex1 which belongs to ATP-ases. Pex7 is receptor for PTS-2, carried by enzymes necessary in plasmalogen synthetis and phytanic acid oxidation^[3].

Peroxisomal disorders were clinically recognized over half a century ago. In 1923 Siemerling and Creutzfeldt^[4] reported a 7-year old boy with bronzed skin, progressive behavioral abnormalities and spasticity of the lower limbs. Postmortem examination disclosed atrophy of the adrenal cortex and diffuse cerebral sclerosis, currently known as adrenoleukodystrophy. The knowledge about peroxisomes and number of affected patients extensively increased and the group currently includes more than 20 diseases,

most manifesting as neurological diseases of infancy and childhood^[5]. In spite of the accumulated knowledge, the group of peroxisomal disorders still remains a challenging field for basic scientists and clinicians. We report four patients with peroxisomal disorders recently diagnosed in Kuwait.

CASE REPORTS

Case 1: A baby girl was born to consanguineous parents after an uneventful pregnancy at full term by Cesarean section, BW 3450g, Apgar score 4 and 8. She had dysmorphic features (wide nasal bridge, large fontanel, webbed short neck, micrognathia) and was profoundly hypotonic with very poor spontaneous movements and absent deep tendon reflexes. At several hours of age, the patient developed recurrent convulsions, which were controlled by Phenobarbitone. CT head was reported normal. On the 3rd day after birth, she developed respiratory failure due to hypoventilation and was connected to the ventilator.

Progressive hepatomegaly with abnormal liver function tests was observed at the age of 1 week but abdominal US revealed normal liver echogenicity. A cortical cyst of the right kidney was detected at the same time. Patellar X-ray was normal. The ophthalmologist reported lens opacities.

Neurophysiological testing revealed decreased nerve conduction velocities (NCV rt median motor 18 m/s, norm 22-42 m/s, rt peroneal 11 m/s, norm 23-53 m/s). Peroxisomal screen showed a significant increase of VLCFA (C26 5.52 umol/L, norm 0.15-0.91), increased ratios C24/C22 (1.91, norm up to 0.96) and C26/C22 (0.409, norm up to 0.022) with normal phytanic (1.58 umol/L, norm up to 15 umol/L) and pristanic acid (0.34 umol/L, norm up to 2.00 umol/L). Other metabolic tests were normal. The diagnosis of Zellweger-like syndrome was made. It was not possible to carry out further specification. Lorenzo's oil - a mixture

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of short chain fatty acids reported to be of value in these disorders - was started without clinical effect. The patient remained on the ventilatory support no psychomotor development was observed and she died at five months of age.

Case 2: A baby boy, born to far related parents as a first child after uneventful pregnancy, presented during the neonatal period with mild dysmorphic features, nystagmus, abdominal distension and diarrhea. He was found to have malabsorptive disorder, but no specific diagnosis was settled. CT head was normal. His parents noticed progressive generalized hypotonia and a delay in psychomotor milestones. On his first visit in the neurology OPD at seven months of age, the examination revealed somatic growth parameters below the 3rd percentile (HC 43cm, Wt 4.75kg, Ht 61cm), dysmorphic features (frontal bossing, large anterior fontanelle), sun set eye phenomenon, rotatory nystagmus, macular degeneration and optic atrophy. The child showed marked generalized hypotonia with brisk deep tendon reflexes. Neurophysiological tests showed low normal NCV (rt median motor 46 m/s, norm 41-62 m/s, rt peroneal 48 m/s, norm 44-74 m/s). BERA confirmed deafness. MRI brain showed deformed skull with narrow base, prominent basal cisterns and mild changes of periventricular white matter.

Hematological and biochemical tests were normal apart from peroxisomal screen showing very high values of phytanic (219 $\mu\text{mol/L}$), pristanic acid (46.47 $\mu\text{mol/L}$) and VLCFA (C26 4.95 $\mu\text{mol/L}$), increased ratios C24/C22 (1.8) and C26/C22 (0.510) diagnosing infantile Refsum disease (IRS). The patient was lastly seen at the age of 18 months when he showed severe failure to thrive (Wt 5.4 kg), microcephaly (HC 45 cm) and global developmental delay. He died several months later.

Case 3: A five-year-old boy was doing well until the age of three years when he developed Addison's disease. At the age of 4 1/2 years, his gait became abnormal and his speech deteriorated. CT head and MRI spine were normal. Peroxisomal screen showed increased values of VLCFA (C26 12.658 $\mu\text{mol/L}$) with increased ratios C24/C22 (1.82) and C26/C22 (0.065), phytanic (1.14 $\mu\text{mol/L}$) and pristanic acid (traces) were normal. Diagnosis of X-linked adrenoleukodystrophy (XALD) was settled. Two months later the child suddenly developed drowsiness progressing to coma and generalized convulsions necessitating admission to the ICU. No electrolyte disturbance was detected, blood glucose and blood pressure were normal excluding Addisonian crisis.

CT brain showed bilateral hypodense areas with small calcifications in the white matter near occipital horns, however, MRI showed extensive changes (Fig. 1). After four days, he was extubated successfully but abnormal neurological findings were more pronounced than before. Lorenzo's oil was started.

In the next several months, he deteriorated quickly, became blind, severely spastic and vegetative. MRI brain showed further progression of the disease.

Case 4: A male child of consanguineous healthy parents, born at term after uneventful pregnancy, had normal developmental history. He had 3 siblings (2 healthy sisters and 11 years old brother with unsteady gait and cutaneous hyperpigmentation). He presented at the age of 5 1/2 years with progressive deterioration of school performance, behavioral changes, unsteady gait, loss of bladder and bowel control. On examination, he had hyperpigmented skin and mucous membranes, wide based ataxic gait, impaired coordination and intention tremor.

CT head showed bilateral symmetrical low attenuation of white matter involving the peritrigonal region extending peripherally in temporal and parieto-occipital white matter, splenium of corpus callosum and centrum semiovale. Enhancement at leading edges of the lesion indicated active demyelinating disease. MRI brain showed low density on T1 and hyperintensity on T2 images involving white matter of the occipital, temporal and parietal lobes symmetrically, splenium of corpus callosum and posterior limbs of cerebellum.

Peroxisomal screen showed raised levels of VLCFA (C26 3.659 $\mu\text{mol/L}$), raised ratios C24/22 (1.48) and C26/22 (0.065), normal phytanic (10.07 $\mu\text{mol/L}$) and pristanic acid (2.01 $\mu\text{mol/L}$). He had very high level of ACTH (1012 ng/L, norm 9-52 ng/L) and impaired cortisol response to ACTH. Consequently, a diagnosis of XALD was settled. The patient was started on appropriate hormonal substitution and Lorenzo's oil. He was deteriorating quickly and he stopped follow up soon. (Parents refused to investigate patient's elder brother who might suffer from milder form of XALD).

DISCUSSION

With the awareness of possibility of peroxisomal disorders and availability of screening tests, it seems that these are not rare diseases and incidence might be higher than reported (1/100,000 for XALD and 1/40,000 for whole group of peroxisomal disorders)^[6].



Fig. 1: MRI brain (post contrast T1 study): extensive abnormal enhancement is seen in both parietal and occipital regions adjacent to the trigones of the ventricles, extending to the posterior limbs of the internal capsules.

All human peroxisomal disorders are genetically determined, most of them are autosomal recessive diseases. Currently, they are classified in two major groups^[7] but many unresolved questions still exist and modifications might follow.

The first group comprise disorders of peroxisomal biogenesis (PBD) in which the organelle is not formed normally due to PEX abnormalities and multiple peroxisomal enzymes are deficient. Histochemical staining of the patient's tissues show variable (usually severe) reduction in the number of peroxisomes, while the remaining organelles are often enlarged and misshapen. Biochemical tests might demonstrate elevated plasma VLCFA, phytanic and pristanic acid, deficient erythrocytes plasmalogen lipids and accumulation of abnormal bile acid intermediates. Unfortunately many patients share similar biochemical abnormalities but biochemical tests lack the power to discriminate one clinical form of PBD from other.

At least 19 PEX genes are required for normal peroxisomal assembly. Clinically, the diseases of this group present with 4 phenotypic syndromes, however it is becoming increasingly recognized that there is rather a clinical continuum^[8]. The most severe form is the cerebrohepatorenal or Zellweger syndrome (ZS) characterized by abnormal morphogenesis, severe neurological dysfunction, regressive changes, hepato-digestive involvement and early death. Less severe form is an autosomal recessive neonatal adrenoleukodystrophy (NALD) where there is mild or absent craniofacial dysmorphism and pronounced demyelination. The mildest form is the infantile Refsum disease (IRD)

with pronounced hepatodigestive, visual and hearing impairment and relatively slow neurological regression. The fourth phenotype presents as rhizomelic chondrodysplasia punctata (RCDP) and is associated with PEX7 alternations^[9]. With the growing number of reported cases, however, it is emerging that the clinical picture is even more variable^[5]. Saudubray et al.^[10] reported a patient with PBD presenting as pseudo Charcot Marie Tooth disease. On the basis of complementation and biochemical studies, 12 disorders have been identified in PBD group till now and mutations of 10 genes have been found^[7]. Most frequent are the mutations of PEX1 gene which typically present variably as ZS-NALD-IRD spectrum^[11].

In the second group of peroxisomal disorders are the diseases caused by a single peroxisomal enzyme defect^[5]. This group includes XALD, classic Refsum disease, mevalonic aciduria, hyperoxaluria type 1 and acatalasemia as well as diseases clinically mimicking ZS-NALD-IRD spectrum or RCDP^[12-14].

Obviously there is no strict genotype-phenotype correlation: different genotypes might result in the same phenotype and mutations of the same gene present with different phenotypes^[7]. The reasons might be different. First, clinical manifestations of each PEX disorder vary according to the nature of the mutation^[15]. In general, those mutations that do not significantly affect function are associated with milder phenotypes and vice versa. Another reason for clinical variations might be tissue mosaicism - variable expression of the gene defect in different tissues or within the same tissue^[4].

Clinical findings of our first patient were typical (dysmorphic features, hepatomegaly, renal involvement, cataract, severe hypotonia and other abnormal neurological findings) and suggested early evaluation in the direction of PBD^[8]. As only VLCFA were increased in peroxisomal screen, suggesting abnormal beta oxidation due to a single enzyme defect, a diagnosis of Zellweger-like syndrome was settled. CT brain was normal, and MRI brain was not performed for technical reasons. Moser et al.^[8] reported neuronal migration defects in 67% and demyelination in 20% of their patients with ZS. Nerve conduction velocities were decreased in our case. Peroxisomal disorders frequently affect peripheral nervous system mostly in the form of sensorineural hearing loss (almost all ZS and NALD patients are deaf) and peripheral neuropathy^[16]. Axonal and demyelinating changes with onion bulb formation are reported^[17-18]. Rather typical for PBD is ocular involvement in the form of cataract (80% of ZS and 45% of NALD) and retinopathy (71% of ZS and 82% of NALD patients).

In the beginning, the patient with IRD presented with nystagmus and diarrhea, otherwise the child was considered normal. Developmental delay, failure to thrive and dysmorphic features were observed at 7 months of age. MRI brain showed dyscranic skull with narrow base, prominent basal cisterns and cortical fissures and slightly increased signal in white matter. Neuropathological studies of patients with IRD^[19] disclosed reduction of axons and myelin in corpus callosum, periventricular white matter, corticospinal tracts and optic nerves, but there was no clear evidence of active demyelination. According to Moser,^[8] retinopathy was found in all his patients with IRD and hearing impairment in 93%. Our patient had nystagmus, retinopathy, optic atrophy and deafness, confirmed by BEPA. The course of the disease was rather rapidly progressive as the child died before the age of three. The average age of death in Moser's group was 6.4 years. It might be stressed that the level of phytanic acid was very high in our case possibly reflecting rather severe functional defect with negative impact on the clinical picture and course^[20].

The first patient with XALD presented with rapidly progressive neurological deterioration. His CT scan on admission to ICU demonstrated small calcifications in the white matter near the occipital lobes. MRI brain performed soon afterwards showed extensive white matter changes in the same area which are typical for XALD^[6]. Calcifications are unusual finding in peroxisomal disorders but are rather frequent finding in some rapidly progressive leukodystrophies such as Krabbe's disease. In the last several months, parents observed patient's rapid mental regression, gait deterioration, and darkening of the skin in spite of appropriate substitutional hormonal treatment. The second patient had similar clinical course.

XALD, the most frequent peroxisomal disorder^[6], is caused by a deficiency of the protein ALDP^[21, 22]. It is a peroxisomal membrane half-transporter and probably interacts with other related half-transporter peroxins forming a functional transporter for enzymes involved in beta oxidation of VLCFA. The clinical presentation of XALD varies strikingly, even in the same kindred^[6, 17]. This is attributed not only to very different types of genetic abnormalities (often named as "private mutations")^[23] and X linked inheritance, but also to other factors which are not clear yet. Rapidly progressive cerebral forms of XALD are associated with an extensive inflammatory response in the regions of affected white matter^[24, 25]. Such process is absent or present only to a low degree in milder form of the disease known as adrenomyeloneuropathy (AMN)^[22]. It seems that inflammatory response involves T and B cells,

macrophages and cytokines^[24]. An autosomal modifier gene^[26] is thought to modulate intensity of inflammatory reaction. XALD is one of the rare neurodegenerative diseases in which there are treatment perspectives^[25].

A bone marrow transplant is recommended in the early stages of cerebral forms^[26, 27]. There have been studies^[28] which reported beneficial effects of such management on further deterioration. Normalization of VLCFA is recommended before the procedure^[27]. Martinez^[29] reported some clinical improvement in several patients with PBD after docosahexaenoic acid (DHA) therapy which decreased values of VLCFA in blood. DHA is a major constituent of membrane phospholipids in the central nervous system and photoreceptors and is deficient in PBD patients. Moser reported clinically insignificant results^[30], but his group included more severely affected patients. Lorenzo's oil, combined with a reduced dietary fat intake decreases levels of saturated VLCFA in plasma^[31].

It has also been reported^[32, 33] that 4-phenylbutyric acid (4-PBA) upregulated one of the peroxin genes and stimulated the formation of new peroxisomes in human cell culture. 4-PBA is currently used for treating patients with urea cycle disorders and appears safe for long-term use in human^[34]. Hopefully, gene therapy will be a successful therapeutic option in the future.

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