Evidence of oxidative stress in peroxisomal disorders

Hala Tabie El-Bassyouni[1], MSc, MD, *Soheir A Abdel Maksoud*[2], MSc, MD, *Fadia A Salem*[4], MSc, MD, *Reem Badr El-Deen*[4], MSc, MD, *Hisham Abdel Aziz*[3], MSc, MD, *Manal Michel Thomas*[1], BSc, MSc

INTRODUCTION Peroxisomal disorders are subdivided into peroxisome biogenesis disorders (PBDs) and single peroxisomal enzyme deficiency. Many peroxisomal diseases exhibit excessive oxidative stress, leading to neurological alterations and dysfunction. Peroxisomes use oxygen in oxidative reactions that generate hydrogen peroxide. This study aimed to investigate various oxidative stress parameters in patients suffering from peroxisomal disorders.

METHODS A total of 20 patients with peroxisomal disorders, aged six months to 13 years (mean age 5.9 ± 3.2 years), were compared to 14 healthy controls. All individuals were subjected to full history-taking, including a three-generation pedigree analysis concerning parental consanguinity and similarly affected members in the family, with meticulous clinical examination to detect any malformation or anomaly. Estimation of very-long-chain fatty acids and phytanic acid was done to verify the diagnosis. Brain magnetic resonance imaging, electroencephalogram, visual evoked potential, auditory potential and plain radiography were conducted to assess the pathological condition of the patients. Oxidative stress parameters, including nitric oxide (NO), malondialdehyde (MDA) and superoxide dismutase (SOD), were estimated in both the patients and controls.

RESULTS Significant increases in both MDA and NO were found in patients with PBDs. It was also demonstrated that SOD was significantly lower in patients with PDB than the controls.

CONCLUSION This study sheds more light on the link between oxidative stress and peroxisomal disorders, as oxidative stress may be a hallmark of peroxisomal disorders. Consequently, one of the useful neuronal rescue strategies could be treatment with antioxidant agents in addition to other lines of treatments.

Keywords: oxidative stress, peroxisomal disorders, reactive oxygen species
Singapore Med J 2012; 53(9): 608–614

INTRODUCTION

Oxidative stress is the imbalance between free radical production and the antioxidant cascade. Oxidative stress occurs when the reactive oxygen species (ROS) generated in a system exceeds its ability to eliminate them. The imbalance can result from a lack of antioxidant capacity caused by a disturbance in their production or distribution, or by an overabundance of ROS. If not regulated properly, the excess ROS could damage cellular organelles and enzymes, cause lipid peroxidation and contribute to disease generation via the activation of gene regulatory proteins such as nuclear factor (NF) kappa B.[1] Since ROS induce apoptosis in healthy cells, they could have harmful effects when produced in excessive amount. Oxidative stress may be a hallmark of several neurodegenerative disorders, including peroxisomal disorders.[2]

Peroxisomal disorders are a group of genetically heterogeneous metabolic diseases that share the dysfunction of peroxisomes, which are cellular organelles that play an integral part in the metabolic pathway. Peroxisomal disorders are divided into two large categories: (1) peroxisome biogenesis disorders (PBDs), which represent a clinical continuum, with Zellweger syndrome (ZS) having the most severe phenotype and neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease having progressively milder phenotypes; and (2) single peroxisomal (enzyme) protein deficiency, e.g. adult-onset adrenoleukodystrophy.[2,3] Peroxisomal disorders participate in important cellular functions, such as the beta oxidation of very-long-chain fatty acids (VLCFAs), the production of plasmalogen and the synthesis of bile acid. These organelles are responsible for the metabolism of myelin lipids (i.e. plasmalogens, cholesterol and VLCFAs) as well as the detoxification of ROS.[4] Peroxisomal disorders have been associated with oxidative stress, as oxygen radicals are both produced and scavenged in peroxisomes.[3] Peroxisomes use oxygen in oxidative reactions that generate hydrogen peroxide; they also contain catalase, which induces oxidative stress.[5] Many peroxisomal diseases exhibit excessive oxidative stress, leading to neurological alterations and dysfunction, since it affects synaptic plasticity and dendritic morphology, and induces neurotoxic damage through the generation of free radicals.[6,7]

The prime targets of ROS attack are the polyunsaturated fatty acids in the membrane lipids causing lipid peroxidation (LP), which may lead to disorganisation of cell structure and function. LP occurs by a radical chain reaction, i.e. once started, it spreads rapidly and affects a large number of lipid molecules.[8] Furthermore, decomposition of peroxidised lipids yields a wide variety of end products, including malondialdehyde (MDA), which is used as a marker of free radical-mediated reactions.[9] LP can damage membrane proteins as well as lipids. Free radicals

[1]Department of Clinical Genetics, [2]Department of Clinical and Chemical Pathology, [3]Department of Medical Biochemistry, National Research Centre, [4]Department of Paediatrics, Abul Resh Hospital, Cairo, Egypt
Correspondence: Dr Hala Tabie El-Bassyouni, Professor, Division of Human Genetics and Genome Research, National Research Centre, El-Tahreer Street, Dokki, Cairo, Egypt. halabassyouni@yahoo.com

can also act as second messengers and activate factors or genes involved in the development of various pathologies.[10]

Oxidative stress has been shown to cause severe perturbation of the peroxisome compartment with evidence of peroxisome proliferation and induction of PEX genes, suggesting their involvement in cellular rescue from ROS.[11] This increased oxidative stress, induced probably by defective peroxisomal antioxidant mechanisms combined with accumulation of lipid intermediates of the peroxisomal β-oxidation system, could contribute significantly to the pathogenesis of multiple organ dysfunctions.[12,13] Oxidative stress biomarkers include superoxide dismutase (SOD), catalase, LP and nitrite concentration.[14] Nutritional supplementation with Vitamin E is effective in enhancing the levels of glutathione, activities of SOD and catalase, and decreasing LP. It scavenges free radicals generated in the tissues and reduces oxidative stress.[7] This study aimed to examine the role of oxidative stress in peroxisomal disorders by investigating the various oxidative stress parameters in patients suffering from such disorders.

METHODS

This study was conducted at the paediatric outpatient clinic, Faculty of Medicine, Cairo University and the outpatient clinic of Clinical Genetics Department, National Research Centre, Egypt, during the years 2004–2006. A total of 20 patients (15 male, 5 female) with peroxisomal disorders, who complained of delayed milestones, learning disabilities, convulsions, limb weakness, inability to walk and skeletal problems, were included in the study. The mean age of the patients was 5.95 ± 3.24 years (range 6 months to 13 years). They were compared to 14 age- and gender-matched healthy controls.

All patients were subjected to thorough history-taking, including family history and a three-generation pedigree analysis of parental consanguinity and any similarly affected family members. Full clinical examination was done to detect any malformation or anomaly that usually co-exists with peroxisomal disorders. Full neurological and developmental examination, including both motor and intellectual skills, was conducted using portage scale. Patients were also subjected to brain magnetic resonance (MR) imaging, visual evoked potential, fundus examination, auditory brain response and hearing tests.

Quantification of plasma VLCFAs (C26:0, C26:0/C22:0 and C24:0/C22:0) and phytanic acid was done using gas chromatography-mass spectrometry, based on the method of Takemoto et al.[15] Estimation of oxidative stress parameters, including MDA as an indicator of LP, nitrites and nitrates as indicators of endogenous nitric oxide (NO) formation and SOD as an antioxidant, was done. MDA was estimated using the method described by Fukunaga et al.[16] Nitrites and nitrates were determined by estimating NO using Griess reaction (Bioxytech® Nitric Oxide Non-Enzymatic Assay, Oxis International Inc, Portland, OR, USA) according to the method of Tsukahara et al.[17] SOD was measured using an enzyme-linked immunosorbent assay kit.[18] Data was analysed using the Statistical Package for the Social Sciences version 7.5 for windows (SPSS, Chicago, IL, USA). Simple *t*-test, correlation and analysis of variance were performed according to the method described by Hirsh and Riegl.[19]

RESULTS

In this study, NALD was the commonest disorder among the patients (70%), followed by X-linked adrenoleukodystrophy (XALD) (10%), rhizomelic chondrodysplasia punctata (RCDP) (10%), ZS (5%) and adult Refsum disease (5%). Positive parental consanguinity was noted in 15 of our patients (75%), while 13 (65%) patients had other similarly affected family members. Clinical findings of the patients are illustrated in Tables I and II. The phenotypic pictures of some patients are shown in Figs. 1 & 2. The ZS patient had more severe clinical affection and biochemical derangements when compared with NALD, RCDP, XALD and adult Refsum disease patients. Brain MR imaging in NALD patients (Figs. 3 & 4) revealed white matter demyelination around the occipital horns, brain atrophy and cerebellar atrophy. The levels of C26:0, C26:0/C22:0 and C24:0/C22:0 ratios and phytanic acid levels were elevated in the PBD group (ZS, NALD and RCDP), with the ZS patient being the most severely affected (Table III, Fig. 5).

The single peroxisomal enzyme deficiency group showed elevated levels of VLCFAs as compared to the controls, with the highest phytanic acid level noted in patients with Refsum disease (Fig. 5). It was found that MDA and NO levels were higher in the ZS patient than in the controls, whereas the SOD level was lower in this patient than the controls (Table IV). Similarly, the MDA and NO levels were higher and the SOD level lower in single peroxisomal enzyme deficiency patients as compared to the controls (Table V). The results of oxidative stress parameters in NALD patients revealed higher MDA levels than in the controls ($p = 0.091$). Their NO level was also significantly higher than that in the control group ($p = 0.022$), and the SOD level was significantly lower than that in the control group ($p = 0.007$). In NALD patients, higher levels of C26:0 were associated with lower levels of SOD (Fig. 6), while higher levels of MDA correlated with higher levels of C26:0/C22:0 (Fig. 7). In the PBD group, higher levels of MDA correlated with higher levels of C26:0/C22:0 (Fig. 8); this is due to higher LP in these patients as compared to the controls. MDA and NO levels in RCDP patients were higher than those in the controls, while the SOD level was lower. XALD patients had higher MDA and NO levels but lower SOD levels than the controls.

DISCUSSION

According to the classification of Wanders and Waterham,[20] peroxisomal disorder subjects were divided into two groups: (1) the PBD group (n = 17), which included ZS, NALD and RCDP patients; and (2) the single peroxisomal enzyme deficiency group (n = 3), which included XALD and adult Refsum disease patients.

Table I. Clinical findings in patients with peroxisomal biogenesis disorders.

Case No.	Age (yrs)/ gender	Cons	FH	Clinical features	Abdominal examination	CNS examination	Seizures	MR imaging finding	Diagnosis/ Inherit
1	3/M	+ve	+ve	Dolicocephaly, depressed nasal bridge, long philtrum, visual affection	Inguinal hernia	Hypotonia	Seizures	Cortical brain atrophy	ZS/AR
2	1/F	–ve	+ve	Dolicocephaly, broad forehead with bossing, face haemangioma, hypertelorism, depressed nasal bridge, low-set ears, long philtrum, short neck, bilateral simian crease, bilateral flat feet, wide space between big toe & 2nd toe.	Umbilical hernia, hepato-megaly	-	-	White matter demyelination	NALD/AR
3	3/M	–ve	+ve	Visual affection	-	Hypotonia & hyporeflexia	-	White matter demyelination	NALD/AR
4	5/F	+ve	–ve	Prominent forehead, depressed nasal bridge, long philtrum, epicanthal folds, nystagmus	-	Hypotonia & hyporeflexia	-	Cerebral cortical atrophy	NALD/AR
5	7/M	–ve	+ve	Broad forehead, low-set ears and bilateral simian crease.	-	-	-	White matter demyelination	NALD/AR
6	5/M	+ve	–ve	Forehead bossing, beaked nose	-	-	-	White matter demyelination	NALD/AR
7	5/M	–ve	+ve	Broad forehead, low-set ears and bilateral simian crease.	-	-	-	White matter demyelination	NALD/AR
8	4/M	–ve	+ve	-	-	-	-	White matter demyelination	NALD/AR
9	5/M	+ve	–ve	Narrow forehead, epicanthal folds, long philtrum, nystagmus	-	Hypotonia & hyporeflexia	-	White matter demyelination	NALD/AR
10	5/M	+ve	+ve	Dolicocephaly, depressed nasal bridge, long philtrum, clinodactyly of the 4th finger, nystagmus & squint	-	Right hand hypotonia	-	White matter demyelination	NALD/AR
11	3/F	+ve	–ve	Prominent forehead, long philtrum, nystagmus	-	Hypotonia & hyporeflexia	-	Diffuse cerebellar atrophy	NALD/AR
12	3/F	+ve	–ve	Epicanthal folds, depressed nasal bridge	-	Hypotonia & hyporeflexia	Seizures	White matter demyelination	NALD/AR
13	5/M	+ve	+ve	-	-	Hypotonia & hyporeflexia	Seizures	White matter demyelination	NALD/AR
14	7/F	+ve	+ve	-	-	Hypotonia & hyporeflexia	Seizures	White matter demyelination	NALD/AR
15	9/M	+ve	+ve	Depressed nasal bridge, corneal opacity	-	Hypotonia & hyporeflexia	-	Diffuse brain atrophy	NALD/AR
16	1/M	+ve	–ve	Disproportionate short stature, brachycephaly, depressed nasal bridge, upturned nostrils, low-set ears and simian crease, nystagmus, limited extension of knees & elbows and arthrogryposis affecting knees & elbows.	-	-	-	Brain atrophy	RCDP/AR
17	4/M	+ve	–ve	Disproportionate short stature, depressed nasal bridge, long philtrum, low-set ears, limited extension of knees & elbows and arthrogryposis affecting knees & elbows	-	-	-	White matter demyelination	RCDP/AR

M: male; F: female; Cons: consanguinity; FH: family history; CNS: central nervous system; MR: magnetic resonance; Inherit: inheritance; +ve: positive; –ve: negative; ZS: Zellweger syndrome; NALD: neonatal adrenoleukodystrophy; RCDP: rhizomelic chondrodysplasia punctata; AR: autosomal recessive

Table II. Clinical findings in patients with single enzyme deficiency.

Case No.	Age (yrs)/ gender	Cons	FH	Clinical features	Abdominal examination	CNS examination	MR imaging finding	Diagnosis/ Inherit
18	10/M	+ve	+ve	Broad forehead, depressed nasal bridge, epicanthal folds, squint	-	-	White matter demyelination	XALD (adult)/ XLR
19	12/M	+ve	+ve	Broad forehead, long philtrum, low-set ears	Hepatomegaly	Hypotonia	White matter demyelination	XALD (adult)/ XLR
20	12/M	+ve	+ve	Bulging forehead, depressed nasal bridge, visual affection	-	-	White matter changes in Rt. occipital area and mild cerebellar atrophic changes.	Refsum disease (adult)/AR

M: male; cons: consanguinity; FH: family history; +ve: positive; CNS: central nervous system; MR: magnetic resonance; XALD: X-linked adrenoleukodystrophy; AR: autosomal recessive; XLR: X-linked recessive

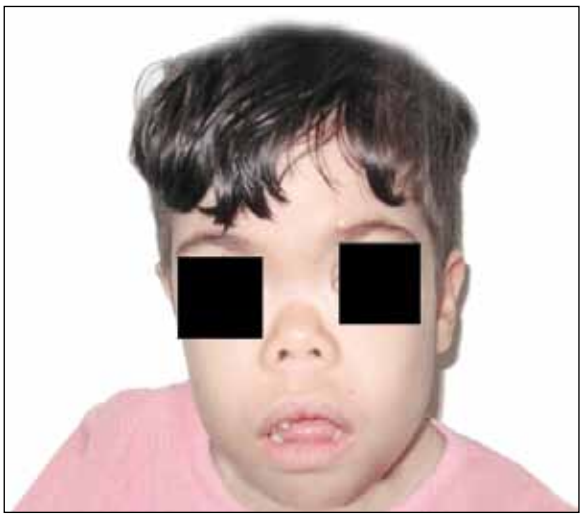

Fig. 1 Photograph of an NALD patient (Case 4) shows broad forehead, hypertelorism, depressed nasal bridge, epicanthal folds, long philtrum and low-set ears.

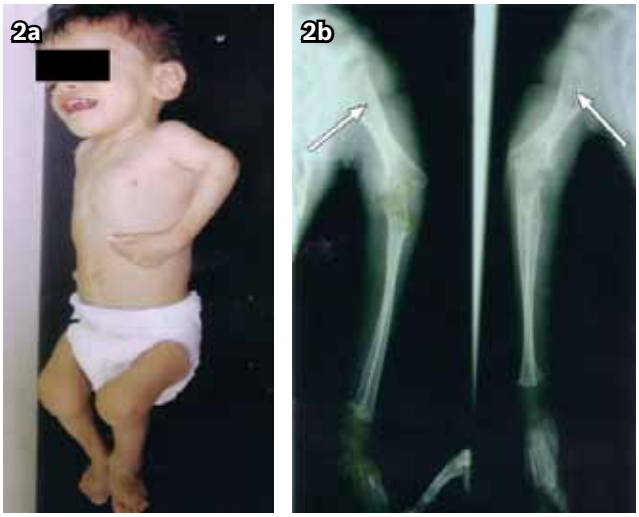

Fig. 2 Photograph of an RCDP patient (Case 16) shows broad forehead, depressed nasal bridge, long philtrum, low-set ears and hypertelorism, hypotonia, shortening of the humerus and femur (rhizomelia), contracture deformities at the elbow, wrist and knees. (b) Plain radiograph of the upper arm shows shortening of the humerus (rhizomelia) and contracture deformities. Punctate calcification is demarcated by arrows.

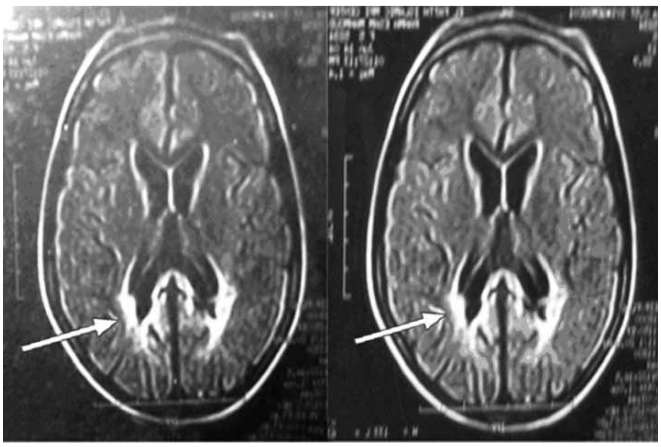

Fig. 3 Axial T2-W images show white matter demyelination around the occipital horn of the lateral ventricle in an NALD patient (Case 10). Demyelination sites are demarcated by arrows.

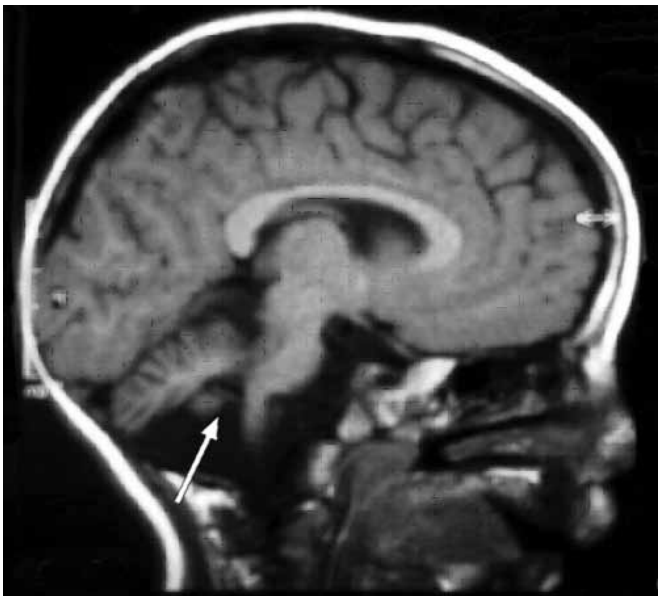

Fig. 4 MR image of the mid-sagittal section of the brain shows severe vermal atrophy in an NALD patient (Case 11).

In our study, NALD was the commonest disorder among the reported patients. This finding coincides with the results of EL-Bassyouni et al.[21] On the contrary, Giros et al[22] found that XALD was the commonest disorder (76%) among the 116 patients included in their study, followed by ZS (11.2%) and NALD (4%). Positive parental consanguinity was noted in 15 of our patients (75%), while 13 (65%) patients had other similarly affected family members. These findings suggest autosomal recessive inheritance and agree with the results of Walter et al and Grayer.[23,24] The ZS patient in our study had more severe clinical

Table III. Comparative study of VLCFAs and phytanic acid levels between NALD patients and controls.

Variable	Mean level ± SD; range	T-value	p-value
C26 (µg/ml)		−7.19	≤ 0.01
NALD	2.93 ± 1.07; 1.21–4.69		
Control	0.67 ± 0.29; 0.15–1.12		
C26/C22		4.65	≤ 0.01
NALD	2.37 ± 1.56; 0.89–5.75		
Control	0.30 ± 0.29; 0.02–0.89		
C24/C22		−4.73	≤ 0.01
NALD	2.87 ± 1.07; 0.29–6.35		
Control	0.40 ± 0.39; 0.02–1.02		
Phytanic acid (µg/ml)		−2.04	0.06
NALD	4.46 ± 3.18; 0.31–11.56		
Control	2.07 ± 2.19; 0.02–8.50		

NALD: neonatal adrenoleukodystrophy; SD: standard deviation; VLCFAs: very-long-chain fatty acids

Table IV. Oxidative stress parameters in PBD group and controls.

Variable	Mean level ± SD; range
MDA (nmol/ml)	
NALD*	2.86 ± 0.83; 1.8–4.20
RCDP†	3.70 ± 0.14; 3.60–3.80
ZS (mean)‡	4.62
Control§	2.14 ± 1.00; 0.90–4.00
NO (U/L)	
NALD	77.18 ± 11.56; 54.00–99.90
RCDP	88.85 ± 3.61; 86.30–91.40
ZS (mean)	95.73
Control	66.50 ± 10.27; 51.00–89.30
SOD (U/gHb)	
NALD	926.43 ± 149.15; 770.00–1200.00
RCDP	905.50 ± 133.64; 811.00–1000.00
ZS (mean)	812.00
Control	1070.20 ± 126.50; 790.00–1236.00

*n = 14; †n = 2; ‡n = 1; §n = 14
SD: standard deviation; PBDs: peroxisome biogenesis disorders; MDA: malondialdehyde; NO: nitric oxide; SOD: superoxide dismutase; NALD: neonatal adrenoleukodystrophy; RCDP: rhizomelic chondrodysplasia punctata; ZS: Zellweger syndrome

Table V. Oxidative stress parameters in single enzyme deficiency group and controls.

Variable	Mean level ± SD; range
MDA (nmol/ml)	
XALD*	3.10 ± 0.00; 3.10
Refsum disease† (mean)	2.40
Control‡	2.14 ± 1.00; 0.90–4.00
NO (U/L)	
XALD	84.60 ± 2.26; 83.00–86.20
Refsum disease	77.30
Control	66.50 ± 10.27; 51.00–89.30
SOD (U/gHb)	
XALD	859.50 ± 132.23; 766.00–953.00
Refsum disease	911.00
Control	1070.20 ± 126.50; 790.00–1236.00

SD: standard deviation; XALD: X-linked adrenoleukodystrophy; MDA: malondialdehyde; NO: nitric oxide, SOD: superoxide dismutase

affection and biochemical derangements compared with the other peroxisomal disease patients. Likewise, Mandel and Korman[25] have emphasised that the severity of the clinical phenotype correlates with the degree of biochemical dysfunction.

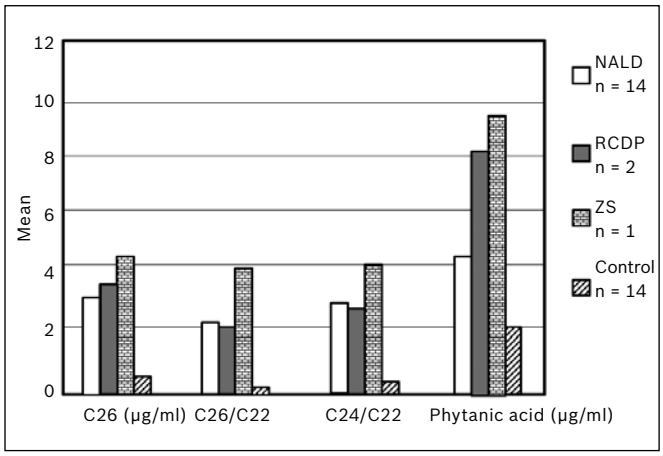

Fig. 5 Graph shows VLCFA and phytanic acid levels in the PBD group and controls.

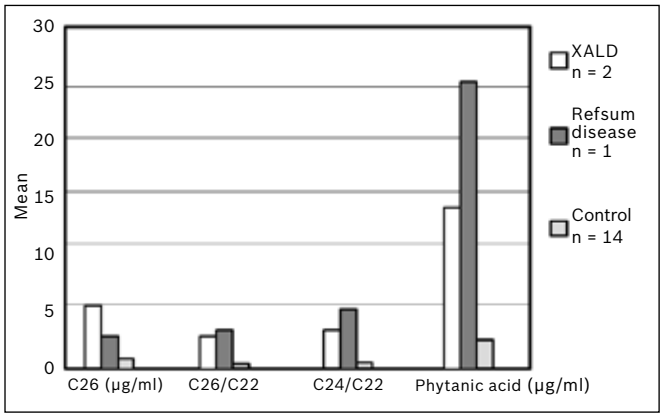

Fig. 6 Graph shows VLCFA and phytanic acid levels in the single enzyme deficiency group and controls.

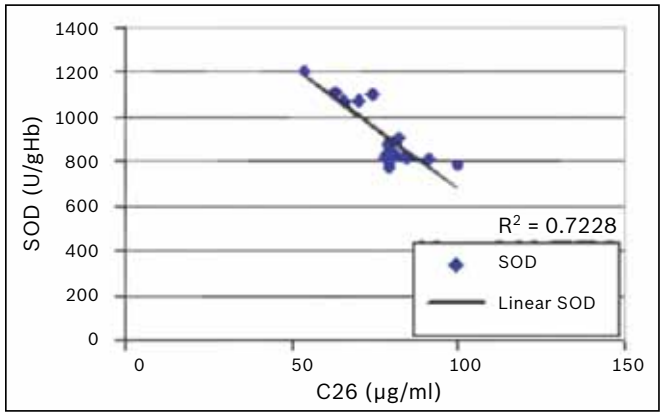

Fig. 7 Graph shows the correlation between C26 and SOD in NALD patients.

Similar to the results of Cheon et al,[26] the brain MR images of our NALD patients showed white matter demyelination around the occipital horns, brain atrophy and cerebellar atrophy. VLCFAs are exclusively oxidised in peroxisomes and their levels are significantly increased in the tissue of patients with peroxisomal disorders. Studies have shown that the analysis of plasma VLCFAs is a very reliable test with unequivocal results for diagnosis of peroxisomal disorders. The increased plasma levels of VLCFAs, especially C26:0 and C26:0/C22:0 ratio is pathognomonic for PBD and adult adrenoleukodystrophy (ALD).[15] In our study, the MDA and NO levels were higher in the ZS patient than in the

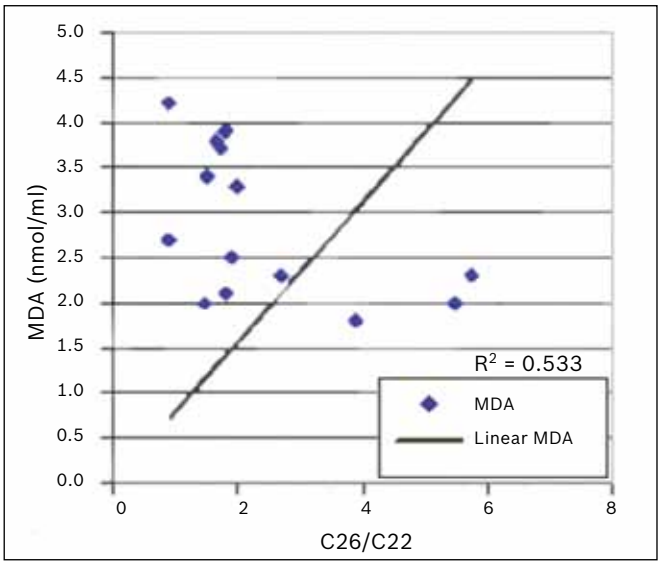

Fig. 8 Graph shows the correlation between C26/22 and MDA in NALD patients.

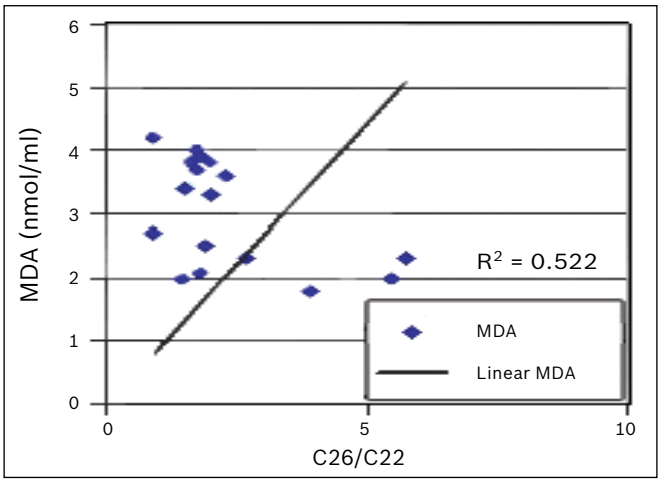

Fig. 9 Graph shows the correlation between C26/22 and MDA in the PBD group.

controls, whereas the SOD level was lower in this patient than in the controls. These results are similar to those of Surdacki et al,[27] who stated that NO production was highly elevated in infant s diagnosed with ZS. The oxidative stress parameters in the NALD and RCDP patients showed that their MDA level was higher than that in the controls. Their NO level was also significantly higher than that in the control group, although their SOD level was significantly lower. This is in agreement with the findings of Dhaunsi et al.[28] The XALD patients had higher MDA and NO levels than the controls, but their SOD level was lower. Powers et al[29] also reported high levels of MDA in the brains of XALD patients, denoting oxidative stress and oxidative damage, as well as lower levels of SOD. Vargas et al[6] reported a significant increase in plasma MDA and a significant decrease of total antioxidant reactivity measurement and alterations of SOD activity.

Khan et al and Biase et al[30,31] reported that the accumulation of VLCFAs paralleled the decrease in peroxisomal beta-oxidation activity, and that the 12- to 14-fold increase in the production of NO suggests the role of NO toxicity in the neuropathological changes associated with abnormal VLCFA metabolism in XALD patients. In adult Refsum disease patients, the levels of oxidative stress parameters, including MDA and NO, were higher than those in the controls while the SOD level was lower. This coincides with the findings of Dhaunsi et al, and Schonfeld and Reiser.[28,32] PBD patients had higher MDA levels than the control group, but the difference was not statistically significant ($p = 0.091$). Leipnitz et al[33] elucidated increased MDA levels in peroxisomal disorders, reflecting an increase of LP. NO levels were significantly higher in these patients than in the control group ($p = 0.022$). SOD levels in PBD patients were significantly lower than those in the control group ($p = 0.007$). These results coincided with the findings of Dhaunsi et al,[28] who stated that increased levels of VLCFAs cause a significant increase in LP. In contrast, Ferdinandusse et al[34] measured MDA levels and found no evidence of increased oxidative stress in patients with PBD. The MDA and NO levels were higher and the SOD level lower in single peroxisomal enzyme deficiency patients as compared to the controls (Table V). This finding coincides with the results of Ferdinandusse et al,[34] who found increased levels of MDA and oxidative DNA damage, and decreased levels of the lipophilic antioxidants alpha-tocopherol and coenzyme Q10, which proved a state of increased oxidative stress in patients with single peroxisomal enzyme deficiency.

In conclusion, our patients showed elevated MDA and NO levels and decreased SOD levels; this finding emphasises oxidative stress in peroxisomal disorders and suggests that oxidative stress plays a role in their pathogenesis. Our results also suggest that early administration of antioxidants in addition to docosahexaenoic acid should be considered as a potential form of therapy for patients with peroxisomal disorder, as this may help to ameliorate symptoms and improve the general health of patients.

REFERENCES

1. Cross CE, Halliwell B, Borish ET, et al. Oxygen radicals and human disease. Ann Intern Med 1987; 107:526-45.
2. Leipnitz G, Amaral AU, Fernandes CG, et al. Pristanic acid promotes oxidative stress in brain cortex of young rats: a possible pathophysiological mechanism for brain damage in peroxisomal disorders. Brain Res 2011; 1382:259-65.
3. Weller S, Gould SJ, Valle D. Peroxisome biogenesis disorders. Ann. Rev. Genom. Hum Genet 2003; 4:165-211.
4. Krysko O, Bottelbergs A, Van Veldhoven P, Baes M. Combined deficiency of peroxisomal beta-oxidation and ether lipid synthesis in mice causes only minor cortical neuronal migration defects but severe hypotonia. Mol Genet Metab 2010; 100:71-6.
5. Kawada Y, Khan M, Sharma AK, et al. Inhibition of peroxisomal function due to oxidative imbalance induced by mistargeting of catalase to cytoplasm is restored by vitamin E treatment in skin fibroblasts from Zellweger-like patients. Mol Genet Metab 2004; 83:297-305.
6. Vargas CR, Wajner M, Sirtori LR, et al. Evidence that oxidative stress is increased in patients with X-linked adrenoleukodystrophy. Biochem Biophys Acta 2004; 1688:26-32.
7. Zaidi SM, Banu N. Antioxidant potential of vitamins A, E, and C in modulating oxidative stress in rat brain. Clin Chem Acta 2004; 340:229-33.
8. Meagher EA, FitzGerald GA. Indices of lipid peroxidation in vivo: strengths and limitations. Free Radic Biol Med 2000; 28:1745-50.
9. Pryor WA. Detection of lipid peroxidation products. Free radic Biol Med 1987; 3:317-64.
10. Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, Oxidative Damage and Oxygen Deprivation Stress: A Review. Ann Bot 2003; 91:179-94.
11. Lopez-Huertas E, Charlton WL, Johnson B, Graham IA, Baker A. Stress induces peroxisome biogenesis genes. EMBO J 2000; 19:6770-7.

12. Baumgart EI, Vanhorebeek M, Grabenbauer MB, et al. Mitochondrial alterations caused by defective peroxisomal biogenesis in a mouse model for Zellweger syndrome (PEX5 knockout mouse). Am J Pathol 2001; 159:1477-94.
13. Schrader M, Fahimi HD. Mammalian peroxisomes and reactive oxygen species. Histochem. Cell Biol 2004; 122:383-93.
14. Ferdinandusse S, Denis S, Mooyer PAW, et al. Clinical and biochemical spectrum of D-bifunctional protein deficiency. Ann Neurol 2005; 59:92-104.
15. Takemoto Y, Suzuki Y, Horibe R, et al. Gas chromatography/mass spectrometry analysis of very long chain fatty acids, docosahexaenoic acid, phytanic acid and plasmalogen for the screening of peroxisomal disorders. Brain Dev 2003; 25:481-7.
16. Fukunaga K, Takama K, Suzuki T. High-performance liquid chromatographic determination of plasma malonaldialdehyde level without a solvent extraction procedure. Anal Biochem 1995; 230:20-3.
17. Tsukahara H, Jiang MZ, Ohta N, et al. Oxidative stress in neonates: Evaluation using specific biomarkers. Life Sci 2004; 75:933-8.
18. Beckman JS and Koppenol WH. Nitric oxide, superoxide and peroxynitrite: the good, the bad and ugly. Am J Physiol 1996; 271: C1424-37.
19. Hirsh R, Riegl R. Studying a study and testing a test, how to read the medical literature. 2nd ed. Boston: Little, Brown and Company, 1989.
20. Wanders RJ, Waterham HR. Peroxisomal disorders I: biochemistry and genetics of peroxisome biogenesis disorders. Clin Genet 2005; 67:107-33.
21. El-Bassyouni HT, Abdel Mawgoud SA, Khalipha AG. Peroxisomal disorders in Egyptian children: estimation of very long chain fatty acids by GC/MS. Med J Cairo Univ 2003; 71:391-401.
22. Giros M, Ruiz M, Ribes A, Pampols T. The diagnosis of peroxisomal disorders in Spain during the period 1987-1997. Rev Neurol 1999; 28:S40-4.
23. Walter C, Gootjes J, Mooijer PA, et al. Disorders of peroxisome biogenesis due to mutations in PEX1: phenotypes and PEX1 protein levels. Am J Hum Genet 2001; 69:35-48.
24. Grayer J. Recognition of Zellweger syndrome in infancy. Adv Neonatal Care 2005; 5:5-13.
25. Mandel H, Korman SH. Phenotypic variability (heterogeneity) of peroxisomal disorders. Adv Exp Med Biol 2003; 544:9-30.
26. Cheon JE, Kim IO, Hwang YS, et al. Leukodystrophy in children: a pictorial review of MR imaging features. Radiographics 2002; 22:461-76.
27. Surdacki A, Tsikas D, Mayatepek E, Frolich JC. Elevated urinary excretion of nitric oxide metabolites in young infants with Zellweger syndrome. Clin Chim Acta 2003; 334:111-5.
28. Dhaunsi GS, Kaur J, Alsaeid K, Turner RB, Bitar MS. Very long chain fatty acids activate NADPH oxidase in human dermal fibroblasts. Cell Biochem Function 2005; 23:65-8.
29. Powers JM, Pei Z, Heinzer AK, et al. Adreno-leukodystrophy: oxidative stress of mice and men. J Neuropathol Exp Neurol 2005; 64:1067-79.
30. Khan M, Pahan K, Singh AK, Singh I. Cytokine-induced accumulation of very long-chain fatty acids in rat C6 glial cells: implication for X-adrenoleukodystrophy. J Neurochem 1998; 71:78-87.
31. Biase DA, Benedetto DR, Fiorentini C, et al. Free radical release in C6 glial cells enriched in hexacosanoic acid: Implication for X-linked adrenoleukodystrophy pathogenesis. Neurochem Int 2004; 44:215-21.
32. Schonfeld P, Reiser G. Rotenone-like action of the branched-chain phytanic acid induces oxidative stress in mitochondria. J Biol Chem 2006; 281:7136-42.
33. Leipnitz G, Amaral AU, Fernandes CG, et al. Pristanic acid promotes oxidative stress in brain cortex of young rats: a possible pathophysiological mechanism for brain damage in peroxisomal disorders. Brain Res 2011; 1382:259-65.
34. Ferdinandusse S, Finckh B, de Hingh YC, et al. Evidence for increased oxidative stress in peroxisomal D-bifunctional protein deficiency. Mol Genet Metab 2003; 79:281-7.