RESEARCH PAPER

Spectrum of Lysosomal Storage Disorders at a Medical Genetics Center in Northern India

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Background: There is limited literature available on the phenotypic and mutation spectrum of Indian patients with Lysosomal storage disorders (LSD).

Objective: To elucidate the clinical, biochemical and mutation spectrum and to study the management options in Indian patients with lysosomal storage disorders.

Design: Descriptive study.

Subjects and Methods: All patients with lysosomal storage disorders diagnosed in the Medical Genetics department of a tertiary care institute in North India over a three year period from January 2008 to December 2010.

Results: Out of the total of 93 patients clinically suspected to have LSDs, 68 (mean age at presentation 4.5 years) were confirmed to have LSDs based on the laboratory/neuroimaging findings and documentation of deficient enzymatic activity in the peripheral blood (leucocytes or plasma) and/or skin fibroblasts. The commonest clinical features at presentation were growth retardation (failure to thrive 47.2% and short stature 17.6%), hepatosplenomegaly (41.2%) and neuroregression (33.8%). A history of consanguinity was present in 32.4% of the families. Prenatal diagnosis was done in a total of 6 affected families; two pregnancies were found to be affected (one each with Gaucher disease and Tay Sachs disease) and in both cases the parents opted for termination of pregnancy. Of the remaining four pregnancies which were found to be unaffected and therefore continued, three were confirmed to be normal on post-natal follow up. Enzyme replacement therapy (ERT) is being given for a total of 8 LSD patients and all of them are showing a gradual amelioration of their symptoms and an improvement in the quality of life.

Conclusions: Lysosomal storage disorders constitute an important group of genetic metabolic disorders for many of which therapeutic options are now available.

Key words: *Lysosomal storage disorders, India, Clinical features, Management, Mutation analysis.*

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Lysosomal storage disorders (LSDs) are a group of inborn errors of metabolism (IEM) characterized by the intra-lysosomal accumulation of complex macro-molecules. LSDs usually occur due to deficiencies of these lysosomal enzymes but can also result from defects in key lysosomal membrane proteins, proteins involved in lysosomal enzyme trafficking or lysosomal enzyme activator proteins [1]. Almost fifty different LSDs are known at present and although each disorder is rare, as a group LSDs have a frequency of around 1 in 5000 live births worldwide [2]. There are only a few studies available regarding the clinical features and mutation spectrum, use of enzyme therapy and antenatal diagnosis in Indian patients with LSDs [3-6]. This study aims to address this gap in knowledge.

METHODS

The study was a descriptive study done over a 3-year period from January 2008 to December 2010, in the Medical Genetics department of a tertiary care, referral hospital in North India. All patients diagnosed to have lysosomal storage disorders on the basis of their clinical features and laboratory findings and confirmed through enzyme analysis were included in the study. The relevant clinical, biochemical, imaging and molecular genetic data and the management/ intervention details were collected for each patient. Clinical observations noted included a complete medical history of the patient, a detailed family history, a three generation pedigree, and a full physical examination comprising general as well as systemic examination. The details of the baseline laboratory investigations and enzyme analysis results were also noted. Enzyme assay was done in the peripheral blood sample (leucocytes or plasma) and in addition, in two patients with Gaucher disease and one patient with Pompe disease, enzyme assay was repeated in skin fibroblasts for confirmation because of equivocal results

in the blood assay. Enzyme analysis was done from standard diagnostic laboratories, as per recommendations [7]. Mutation reports, where available, were recorded (blood samples for molecular genetic studies for the different LSDs were sent to different national and international research groups, who used the whole gene sequencing technique for identifying the mutations). The collected data was statistically analyzed.

RESULTS

Ninety three patients were suspected to have LSDs on the basis of their clinical features during the 3-year study period; out of these, 68 (76.4% males) patients were confirmed to have different types of LSDs, seven were lost to follow up and did not undergo the necessary enzyme assays for confirmation of the clinical diagnosis and in the remaining 18, the enzyme assay results were normal. The age at presentation of the LSD patients varied from 5 months to 26 years (with the exception of one MPS I case which presented as non-immune hydrops fetalis at 26 weeks gestation), with an average age of 4.5 years. Consanguinity was present in 22 families (32.4%). Thirty patients (44.1%) had history of one or more siblings with similar clinical features, but except in four cases, the diagnosis had not previously been established in the similarly affected siblings.

The diagnosis made and the enzyme activity levels in patients with LSDs are shown in ***Table* I**. Most of the patients had enzyme activity levels between 0 to less than 10% of the normal reference range of the respective enzyme. The pathogenic genetic mutation could be identified in only 7 families. Hurler (type I MPS) and Hunter (type II MPS) syndromes accounted for 78.2% of the MPS group. One case of MPS I was incidentally diagnosed through fetal autopsy; this fetus had died *in-utero* at 26 weeks gestation due to non-immune hydrops fetalis and MPS I enzyme (β-iduronidase) assay done in cord blood as a part of the work-up for non-immune fetal hydrops revealed very low levels of the enzyme (0.2 nmol/ h/ mg; reference range 22-56 nmol/h/ mg) against normal values of the control enzyme (β-galactosidase 110 nmol/h/mg; reference range 70-324 nmol/h/mg), thereby leading to the diagnosis. Out of the ten cases of Gaucher disease, eight had the type I non-neuronopathic form, one had the type II acute neuronopathic form and one had the type III sub-acute neuronopathic form.

TABLE I PATIENTS WITH THE DIFFERENT TYPES OF LSDS (*N*=68)

Type of LSD	*No.*	*Blood enzyme levels (range) (nmol/ h/ mg)*	*Mutations identified in the causative gene*
MPS I	9	0.4-5.2	–
MPS II	9	0.4-4.9†	–
MPS IVA	4	0.6-1.6	–
MPS VI	1	0.6	–
Gaucher disease	10	0.38-3 (Skin fibroblast: 10 - 21)	*GBA* gene: S237F/R496C; S356F/ S356F; L444P/L444P
Metachromatic leukodystrophy	10	1.5-18 nmol/ 17h/ mg	–
GM1 gangliosidosis*	6	0.64 - 4.2	–
Tay-Sachs disease	4	0.5 - 1.3 †	*HEXA* gene: 1277_ 1278insTATC/ 1277_1278insTATC
Pompe disease	3	2.4 - 5.6 (Skin fibroblast: 3)	*GAA* gene:D489N/ R600H; E655del/ D489N
Sandhoff disease	3	54-149 †	–
Niemann-Pick disease	3	0.57-1.2 nmol/ 17h/ mg	–
Fabry disease	2	0.1- 0.02	*GLA* gene: W236X
Mucolipidosis III	2	Hexosaminidase A+B: ~ 20,000 - 28,000 †	Iduronate sulphate sulphatase:
		560-730†	–
Neuronal ceroid lipofuscinosis	1	10	–
Wolman disease	1	0.0	–

†*Enzyme assay done in plasma; value in nmol/ h/ mL; * Lysosomal sialidase assay was not done in any of the six cases with low beta galactosidase activity. Hence, galactosialidosis could not be definitively ruled out.*

The most common reasons for referral were visceromegaly (particularly hepatosplenomegaly, 41.2%) and neuroregression (33.8%). The common findings were growth retardation (failure to thrive 47.2%; short stature 17.6%), dysostosis multiplex (41.1%), joint contractures (36.7%) and coarse facies (36.7%). Extensive Mongolian spots over the trunk, back and extremities found in many children with the infantile onset GM1 gangliosidosis were noted in four of our six patients with this condition. Apart from neuroregression, other neurological features noted were hypotonia (8.8 %) and progressive ataxia and dysarthria (5.9%). Of the two male sibs with Fabry disease, the elder sib presented with acute episodes of severe pain and paraesthesias in the extremities and the other had recurrent diarrhea; the elder sib had received several courses of non-steroidal anti-inflammatory drugs and due to the non-specificity of his symptoms, had even been thought to have a psychiatric disorder. The child with Wolman disease presented with a history of recurrent episodes of loose stools starting from around 4 to 5 months of age with severe failure to thrive and the diagnosis was suspected after his abdominal ultrasonography revealed bilateral homogenous calcification of the adrenal glands.

Prenatal diagnosis was done for 6 families (2 of Pompe disease and 1 each of Gaucher disease, MPS I, MLD and Tay Sachs disease) after appropriate genetic counseling and with the informed consent of the couples. The method used for prenatal diagnosis was enzyme assay in the chorionic villus sample (CVS), taken at around 11-12 weeks of gestation (enzyme assay was done directly in the obtained CVS sample without any prior culturing). In addition, targeted mutation analysis in the CVS DNA, based on the mutations identified in the proband, was done in 3 families (2 with Pompe disease and 1 with Tay-Sachs disease) for further confirmation. The results of the prenatal diagnostic tests are mentioned in ***Table* II**. Two pregnancies were found to be affected (one with Gaucher disease and one with Tay-Sachs disease) and in both cases the parents opted for termination of pregnancy. The remaining four pregnancies that were found to be unaffected were continued; three were confirmed to be normal on postnatal follow up and 1 was lost to follow up.

Enzyme replacement therapy (ERT) was initiated for 8 patients, four with type I Gaucher disease, 3 with MPS type I H/S (Hurler – Schie) and 1 with Fabry disease. All of them showed a gradual amelioration of their symptoms. The four patients with non-neuronopathic Gaucher disease on ERT showed improvement of their growth and hematological parameters and reduction of liver and spleen size. The three patients with MPS I H/S on ERT showed a reduction of liver and spleen size, improvement in growth parameters and 6-minute walk test, and a mild improvement in their joint mobility. There has been no change noted in the corneal opacity, facial coarseness or dysostosis. The patient with Fabry disease had a significant reduction of limb pain and paraesthesias and improvement in height and weight after initiation of ERT. He had hypersensitivity reactions during three successive infusions in the third year of his ERT, which probably occurred in response to some allergen present in one particular batch of recombinant enzyme.

TABLE II RESULTS OF THE PRENATAL DIAGNOSTIC TESTS IN SIX FAMILIES WITH LYSOSOMAL STORAGE DISORDERS

Type of LSD in proband (enzyme assay done)	*CVS enzyme valuein nmol/h/mg (Normal reference range of laboratory)*	*Targeted mutation analysis*	*Interpretation (affected/ not affected)*
Gaucher disease (β-glucosidase)	0 (250-782)	Not done	Affected
Metachromatic leukodystrophy (Arylsulphatase A)*	31 nmol/ 17 h/ mg (25 – 80 nmol/17 h/mg)	Not done	Not affected
Mucopolysaccharidosis I (α–iduronidase)$	187 (110-226)	Not done	Not affected
Pompe disease (family 1) (α-glucosidase)#	Lab I: 26 (101-305) Lab II: 60 (140-280)	No mutations in *GAA* gene	Not affected
Pompe disease (family 2) (α-glucosidase)•	Lab I: 13.3 (132-525) Lab II: 56 (140-280)	Heterozygous carrier of 1 mutation (c.1962_1964 delAGA) in *GAA* gene	Not affected
Tay Sachs disease (Hexosaminidase A)	108 (1560-3100)	Homozygous for 1277_1278 insTATC mutation in *HEXA* gene	Affected

***** *Blood leucocyte arylsulphatase A assay at 5 months after birth: 78 nmol/ h/ mg (reference range 67 – 396 nmol/ 17 hr/ mg);* **$** *Lost to follow up;* **#** *Blood leucocyte α-glucosidase assay at 3 months after birth: 100 nmol/ h/ mg (reference range 86 – 296 nmol/ h/ mg);* **•** *Child normal on clinical evaluation at 10 months of age.*

DISCUSSION

The age of presentation and clinical manifestations of LSDs depend on the substrates accumulated, the rate and the magnitude of their intracellular accumulation, the percentage of residual functional enzyme and presence of alternative functional pathways [8,9]. In the present study, we detected one case of mucopolysaccharidosis type I in the antenatal period in a fetus with non-immune fetal hydrops.

The most common causes of morbidity and mortality in LSDs are due to neurological, visceral, cardiovascular and skeletal involvement, which were observed in our patients also [3]. Apart from the mucopolysaccharidoses, the skeletal changes of dysostosis multiplex are also seen in GM1 gangliosidosis and various oligosaccharidoses [10]. The present study had five patients of oligosaccharidoses and GM1 gangliosidosis who presented with an MPS-like picture and were differentiated based on the absence of urinary glycosaminoglycans.

In Pompe disease, alpha-glucosidase enzyme assay in leucocytes may give falsely normal results due to the presence of isoenzymes such as maltase-glucoamylase in the blood, as happened in one of our cases with Pompe disease; therefore, conventionally, enzyme assay in skin fibroblasts or in the muscle tissue is considered to be more reliable. However, modifications in the blood leucocyte alpha-glucosidase assay protocol have now made enzyme assay in blood samples reliable [11].

Non-specific symptoms and lack of definite physical findings often lead to misdiagnosis of Fabry disease cases as gout or other rheumatological disorders or malingering, as happened in our patient. The diagnosis must be kept in mind in any patient with suggestive features. This is especially important as effective enzyme replacement therapy (ERT) is now available for Fabry disease and early and timely institution of ERT can prevent/ ameliorate the renal and cardiovascular complications of the disease [12].

A complete ophthalmological evaluation including slit lamp and fundus examination and electroretinogram studies, where required, can provide important clues for the diagnosis of lysosomal storage disorders [13]. Bilateral fundal cherry red spots were detected in all of the four Tay-Sachs cases, 2 out of the three Sandhoff cases and in 2 of the three GM1 gangliosidosis patients who underwent a fundus examination.

Accurate diagnosis of the type of LSD is imperative not only for appropriate management of the affected child, but also for prenatal diagnosis for future pregnancies in the family. Prenatal diagnosis is conventionally being done through enzyme assay in the chorionic villus sample or cultured amniocytes [14,15]. However, enzyme assay results can be erroneous at times due to problems related to technical expertise, sample transportation and maternal contamination. If the causative pathogenic mutations are identified in the proband or in the carrier parents, targeted mutation analysis in the fetal DNA can also help determine if the fetus is affected. Combining molecular genetic testing with enzyme assay has been found to significantly increase the reliability of the prenatal diagnostic procedure [16]. The main limitations with molecular genetic testing are the limited availability of centers for such testing and the cost. In the present study, there were two families with Pompe disease, where targeted mutation analysis in the chorionic villus sample DNA had to be done for prenatal diagnosis, as the enzyme assay results were equivocal and inconclusive. Targeted mutation analysis was also done for confirmation of the enzyme assay results for prenatal diagnosis of Tay-Sachs disease in one family.

Mutations were identified in 7 families with LSDs. Of the three families with Gaucher disease in whom the mutations were identified, only 1 family had one of the common mutations in the GBA gene (L444P/ L444P) reported in Ashkenazi Jewish and other Caucasian populations [17]. The mutation found in the HEXA gene in our Tay-Sachs disease patient (+TATC1278) is the same mutation that has been reported to be found in around 70% of carriers of Tay-Sachs disease in the Ashkenazi Jewish population [18].

ERT is currently available for six LSDs (Gaucher disease, Pompe disease, Fabry disease and mucopolysaccharidoses types I, II and VI) [19]. Regular intravenous infusions of the recombinant enzyme have been demonstrated to be safe and effective in reversing the features resulting from hematologic and visceral involvement, in reducing bone pain and the frequency of bone crises, and in significantly improving the quality of life in Gaucher disease [20]. ERT for MPS disorders (types I, II and VI) has been shown to produce significant improvement in pulmonary function and the six-minute walk test performance, reduce urinary glycosamino-glycan excretion, reduce the liver and spleen volume, improve growth and joint mobility and decrease sleep apnea [21]. Similar improvement has been noted in our Gaucher and MPS I H/S patients on ERT. However, the currently available forms of ERT do not have any effect on the neurological features of the LSDs, as they cannot cross the blood-brain barrier [19].

WHAT IS ALREADY KNOWN?

- Lysosomal storage disorders, are fairly common genetic disorders which present with multi-organ involvement including neuro-degeneration and visceromegaly.

WHAT THIS STUDY ADDS?

- Considering the possibility of LSDs in patients with the relevant clinical and laboratory findings and confirming the diagnosis through appropriate enzyme assays helps in the appropriate management of these patients and in providing correct genetic counseling and prenatal diagnosis for their families.

Though exact prevalence studies are not available for the Indian population, lysosomal storage disorders as a group are not uncommon. The present study gives us an insight into the clinical and biochemical spectrum of Indian patients with LSDs. There is a need to increase awareness about these disorders in the medical community to ensure accurate diagnosis and appropriate management of the affected patients as well as appropriate genetic counseling and prenatal diagnosis for their families.

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Contributors: PKV collected the data and helped in the drafting of the paper, PR analysed the data and drafted the manuscript, ABD helped in performing and interpreting the tests and SRP conceived and designed the study and revised the manuscript for important intellectual content. The final manuscript was approved by all authors.

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