Lysosomal Storage Disorders: An Underdiagnosed Metabolic Disorder

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Abstract

Lysosomal storage disorders (LSDs) are a group of 70 different metabolic diseases with a likely incidence of 1:5000 to 1:8000. They present with heterogeneous overlapping phenotypes mainly involving regression in learned skill, organomegaly, skeletal dysplasia, mental retardation, cherry red spot and progressive visual loss. Main cause of these disorders is due to the accumulation of cellular debris, undigested protein, fat, carbohydrates, complex lipids due to defect in the gene that regulates the respective enzyme synthesis required for the degradation of the above material. Early diagnosis can help for the initiation of enzyme replacement therapy (ERT) or Bone marrow transplantation (BMT) for a few of theses while prenatal diagnosis holds promise for the prevention of LSDs in future generations. Gene therapy for many of these diseases are evolving in near future as some of them are in clinical trials

Keywords: Lysosomes, Lysosomal storage disorders, metabolic disorders, Enzyme replacement therapy, Neuroregression, hepatosplenomegaly, skeletal dysplasia

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Introduction

Lysosomal storage disorders (LSDs) are a group of over 70 diseases that are characterized by lysosomal dysfunction, in the form of deficiency of lysosomal enzymes, causing accumulation of complex substrates in the lysosomes. These disorders are individually rare but collectively affect 1 in 5,000 live births[1]. Amongst these, the most common LSD are lipid storage disorders followed by Mucopolysaccharidosis (MPS) as a group. Among all LSDs, Gaucher disease is the most common treatable lipid storage disorder[2,3]. With 26 million births occurring in India annually, the extrapolated burden of LSD in India is nearly 3,700 to 17,000 affected babies born every year[2]. A large multicentric study carried out at FRIGE-Institute of Human Genetics has shown that nearly 39% of children with progressive neuroregression, skeletal dysplasia, hepatosplenomegaly, coarse facial features, regression in learned skill, and presence of cherry red have an underlying cause of LSDS[3]. In short considering an overall presence of lysosomes in every cell, it affects almost every part of the body that includes mainly face, brain, liver, spleen, skin, bone, eyes, heart, muscles and kidney (Fig 1). Likewise, a retrospective study reported in 1994, conducted at premier Institutes in Delhi and

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LSD: Multi-organ involvement

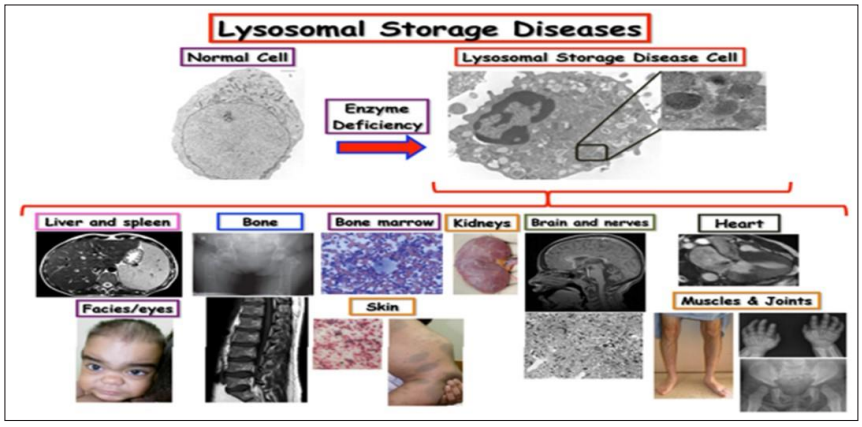

Figure 1
Source: Dr Jayesh Sheth, Personal communication

Mumbai and recently at FRIGE, reported LSDs to be the most common metabolic cause of mental retardation at these centres[4,5]. Although not completely representative of the true burden, these figures indicate that LSDs are common in India. Considering the large population of India, the absolute number of cases must be larger. Most of these diseases are inherited as autosomal recessive manner, except Hunter disease, Fabry disease and Danon disease which depict X-linked inheritance. LSDs typically present in infancy and childhood, although fetal and adult-onset forms also occur[6] that have been recently reported for Gaucher diseases by Sheth *et al* [7]. Several LSDs can be treated with approved, disease-specific therapies that are mostly based on enzyme replacement. In spite of advances in therapies and increased diagnostic ability with next generation DNA sequencing technology, LSDs are underdiagnosed in India. In this article, we plan to review the spectrum of LSDs, current status of their diagnosis, therapies and their future perspective in India.

Pathophysiology

Most LSDs have a progressive neurodegenerative clinical course, although symptoms in other organ systems are frequent. The lysosome is the key cellular organelle for catabolism of macromolecules, clearing of cellular debris, recycling etc. Defects that impair any of these functions cause the accumulation or storage of undigested or partially digested macromolecules like protein, fat, carbohydrates, nucleic acid, complex lipids and cellular debris in the lysosomes or impair the transport of molecules, which can result in cellular damage and different phenotypes depending on the nature and site of the storage material accumulation. More than two-thirds of lysosomal diseases affect the brain, with neurons appearing particularly vulnerable to lysosomal compromise. In a study by Sheth and his group it was shown that nearly 71% of children with neuroregression, dysmorphism and cherry red spots have an underlying cause of LSDs[5]. While failure of lysosomal function characteristically leads to lysosomal storage, new studies argue that lysosomal diseases may also be appropriately viewed as 'states of deficiency' rather than simply storage*.

Need For Early Diagnosis

A recent study from KEM Hospital, Mumbai outlines the diagnostic journey of 119 cases of LSD from the onset of symptoms up to the final diagnosis[2] (Figure 2). This study concluded that the delay in the diagnosis is due to delayed suspicion of LSD by the primary physician and the subsequent delayed referral to a tertiary Genetic Centre, rather than the diagnostic work-up thereafter at the tertiary center [2].

In some centres, the average time taken for the diagnosis of LSDs, following suspicion ranges from 2 months to 6 years and sometimes, the diagnosis is offered as late as in adulthood. (Sheth *et al*-Personal communication) This was witnessed recently in one of

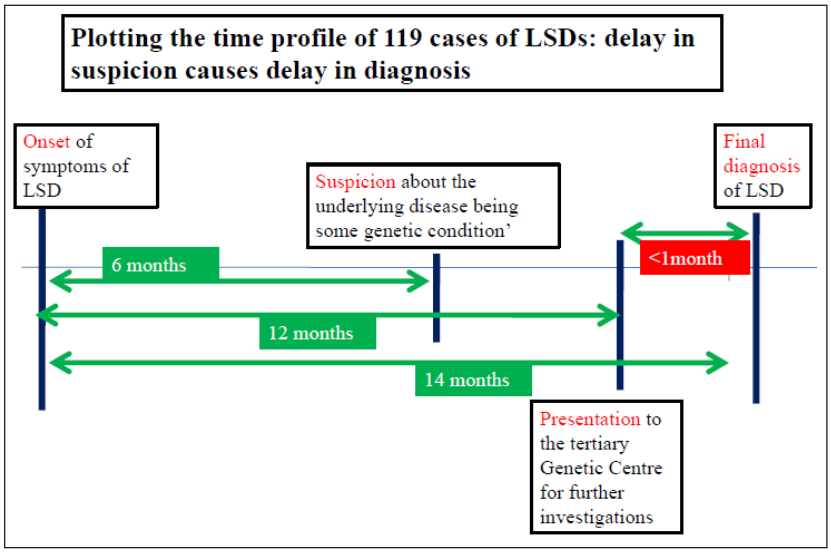

Figure 2: Median time intervals from onset of symptoms to diagnosis 119 patients with LSD diagnosed at KEM Hospital, Mumbai

Source: Information extracted from- Agarwal S, et al. The face of lysosomal storage disorders in India: a need for early diagnosis. Indian J Pediatr 2015;82:525-29.

the cases of Hunter disease diagnosed at 23 years at FRIGE, followed by successful initiation of enzyme replacement therapy.

Early diagnosis is important in the case of LSDs for the following reasons:

1. **Prenatal diagnosis:** Barring a few exceptions, most LSDs are inherited in an autosomal recessive manner. Thus, there is an innate 25% chance of recurrence of the same disease in every subsequent pregnancy. Given the morbidity and mortality associated with LSD, the financial and emotional burden of raising an affected child, difficulties in accessing treatment options for the select disorders; 'prevention' of recurrence is best 'cure'. Lysosomal enzyme study is the gold standard for the index case diagnosis and can be used for the prenatal diagnosis[8]. However, due to lack of experienced centers across the country for enzyme based prenatal diagnosis, and also the overlapping activity of enzymes in normal and carrier fetus, molecular (genetic) diagnosis, is the cornerstone to prevent the recurrence of particular LSD in the subsequent pregnancy. In the study at KEM hospital, nearly one-fifth of the 119 patients had another affected sibling with LSD, highlighting the impact of delayed diagnosis[2].
2. **Timely institution of treatment when available:** LSDs are multisystemic, chronic and progressive disorders. There are a few amongst those having definitive treatment options available[9]. However, timely institution of the treatment modalities is important for optimal response to therapy. Timely treatment, when available, is known to significantly reduce the complications associated with the specific LSDs and improve the quality as well as the longevity of life[10]. Most of the new treatment options, including enzyme replacement therapy (ERT), substrate reduction therapy (SRT) are now available even in India on a charitable, research trial or a state-sponsored basis[10]. Many major tertiary Institutes in New Delhi, Lucknow, Mumbai, Kochi, Chennai, Ahmedabad, Bengaluru and Hyderabad have the facilities to administer these sophisticated and life-saving drugs[10].
3. **Limiting the complications by planned and specific surveillance:** Even in cases where treatment is not available, it is often rewarding to diagnose the LSD correctly. Diagnosing an LSD in time, can help to mitigate the various complications associated, by timely and targeted surveillance of the systems affected and also its prevention through prenatal diagnosis as has been reported by Sheth *et al* for several LSDs[8]. For example, MPS4A is associated with risk of atlantoaxial instability. Recognizing this disorder early can help counsel the family about the precautions to be taken for cervical stability and remind the physician to proactively look for signs of cord compression at regular clinical intervals (lower limb paresis, hyperreflexia, bowel and bladder incontinence, neck pain). Another example includes

Common MIMICS of Treatable LSD:

Table 1: Common mimics of treatable LSD[4]

Treatable LSD	Clinical feature prone to be mistaken for another disorder	Misdiagnosis
Gaucher disease	Anemia, pancytopenia, splenohepatomegaly	Thalassemia, Hemat-oncological malignancy
MPS 1/2/6/7 Alpha mannosidosis	Joint stiffness	Rheumatological disorders (juvenile arthritis)
	Recurrent respiratory tract infections, otitis media	Common ENT disorders, immunodeficiency
MPS 4A	Short stature, wrist widening, other skeletal features	Rickets
Pompe disease	Muscle weakness, respiratory difficulties, cardiomyopathy, macroglossia	Acute flaccid paralysis (poliomyelitis), myocarditis, hypothyroidism
Fabry's disease	Recurrent abdominal pain	Appendicitis, renal colic
	Cerebrovascular stroke	Non-genetic, essential/ primary causes, underlying hypertension, diabetes mellitus
	Cardiac ischemia, hypertension	
	End stage renal disease	
	Acroparesthesia	Growing pains, rheumatological disease, arthritis, neuropsychological disease
	Angiokeratomas	Petechiae
Wolman disease	Hepatosplenomegaly	Thalassemia, Hemat-oncological malignancy
Neuronal ceroid lipofuscinosis	Intellectual disability, seizures, progressive vision loss	Cerebral palsy

Hurdles to Diagnose LSD Early:

Table 2: Hurdles to early diagnosis of LSD[4]

Physician related	Patient related	Healthcare system related
Lack of awareness	Lack of awareness of the symptoms and/ or denial	Lack of easily accessible tertiary centres to diagnose LSD in time
Delayed suspicion, delayed referral to tertiary centre	Lack of follow-up	Lack of universal medical insurance to cover the sophisticated and expensive diagnostic and treatment options
Wrong labeling/ misdiagnosis	Delay in seeking medical attention even after being guided rightly	Lack of medical and laboratory expertise in this field
Ordering the wrong tests/ incomplete tests even after suspecting correctly	Lack of incentive to diagnosis, since many LSD may not necessarily have a definitive treatment	Lack of inclusion of awareness of these disorders in mainstream CMEs and academic programs

early detection of valvular heart disease in children with MPS and instituting infective endocarditis prophylaxis when necessary, early on, so as to prevent the complication later[11].

The responsibility of the primary physician to diagnose these cases early; thereby maximizing the benefits to the family increases in the light of this information.

Clinical Features

LSDs are genetically and clinically heterogeneous disorders. Some of the common LSDs have already been described in table no. 1. They frequently present as pediatric neurodegenerative diseases that are often accompanied by hepatosplenomegaly. LSDs can also manifest as skeletal deformities called as dysostosis multiplex. Patients present with a continuum of disease severity that loosely correlates with the type of mutation and residual activity of the mutant protein. They are generally classified on the basis of the type of disorder and the age of onset of the clinical signs as congenital or infantile with usually severe phenotypes and late-infantile, juvenile and adult types with usually milder phenotypes. Table 3 describes various clinical features in various LSDs[1]. Even many pregnancies with recurrent non-immune hydrops fetalis have been found to have an underlying cause of LSDs with most common one as Sly syndrome, Hurler syndrome, Mucolipidosis, Gaucher disease etc[12].

Treatment Modalities in LSDS: [3]

Supportive

Since LSDs have multisystemic involvement, it is necessary to have a multidisciplinary approach with

Organ Involved	Phenotypic features	Examples of LSDs
Central nervous system and peripheral nervous system	Developmental delay, neuro-regression, Ataxia, Dystonia, epilepsy, peripheral neuropathy, macrocephaly, oculomotor apraxia, tremors, action myoclonus, acro-paresthesias	Mucopolysaccharidosis (MPS), Mucolipidosis, sphingolipidososes, oligosaccharidosis, metachromatic leukodystrophy, neuronal ceroid lipofuscinosis (NCL), Fabry disease etc.
Gastrointestinal system	Hepatosplenomegaly, cholestatic jaundice, diarrhea, malabsorption	Gaucher disease, Niemann pick disease, mucopolysaccharidosis, Wolman disease, etc.
Cardiovascular system	Cardiomyopathy, stroke, aortic valve abnormalities	Fabry disease, Pompe disease, Danon disease etc.
Renal system	Renal tubular Fanconi syndrome, renal failure, nephropathy	Cystinosis, Fabry disease etc.
Respiratory system	Interstitial lung disease	Niemann Pick Disease
Ophthalmic findings	Corneal opacities, cherry red spot, progressive visual loss, corneal verticillate, supranuclear gaze palsy,	Mucopolysaccharidosis, NPD, GM1 gangliosidosis, oligosaccharidosis
Congenital	Hydrops fetalis	Gaucher disease, sialidosis II, sialic acid storage disease etc.
Musculoskeletal system	Dysostosis multiplex including metaphyseal widening at large joints, bullet shaped metacarpals, kyphoscoliosis, central or inferior beaking of vertebral bodies, squared iliac wings, hour glass deformity, skeletal dysplasia, polyarticular arthritis	Mucoplysaccharidosis, mucolipidoses etc.

Table 3

specialists being available for all kinds of presentations and complications. Most of the LSDs require physiotherapy which should be available.

Haematopoetic stem cell transplantation

HSCT have shown improvement in clinical features if done before CNS involvement in Hurler syndrome or type I MPS. But with availability of Enzyme replacement therapy (ERT) which is a curative treatment, it is preferred over HSCT.

Enzyme Replacement Therapy (ERT)

The goal of ERT, is to replace the deficient enzymes by infusions of recombinant enzymes. Using a specific receptor, the intravenously applied enzymes can be taken up by cells and transported to the lysosomes where they act. The therapeutic enzymes may be produced from genetically modified cells from different organisms such as animals, humans, plants, or eggs. ERT has shown to reduce organomegaly (liver, spleen, heart), and to improve some organ function (e.g. heart function in Fabry disease or liver function in acid lipase deficiency). Patients exhibit a broad variation in clinical manifestations, and severely affected patients with irreversible organ damage may not respond to ERT if it is initiated after damage has been established. Table 4 gives an overview of drugs approved for lysosomal storage diseases[13].

Substrate Reduction

The aim is to slow the production of substrate, to a level that can be effectively cleared by mutated enzyme. Miglustat and eliglustat are approved molecules for Gaucher disease. Patients who have mild to moderate symptoms, who are not candidates for ERT, can be given miglustat.

Chaperon Therapy

Newly synthesized enzymes undergo appropriate folding with the help of special molecules like heat shock protein, calnexin, Bip etc. These molecules are called as chaperones. In many of the lysosomal storage diseases, because of missense mutation, these enzymes are misfolded and hence undergo degradation and can't function properly. If chaperones are provided, some catalytic activity of enzymes is regained and symptoms reduce. This is the basis of 'chaperone therapy'. As an example, the imino sugar migalastat (1-deoxygalactonojirimycin) is available for patients with Fabry disease.

Some of the advanced treatment modalities in pipeline for future are gene therapy, CRISPR/Cas 9 (clustered regularly interspaced short palindromic repeats/caspase 9) and STOP-CODON Read-Through all of which are going to work at the DNA level to achieve cure over these genetic diseases.

Role of Laboratory diagnosis in LSDs diagnosis

The main challenges faced by the treating physician for the clinical diagnosis of LSDs, is the dilemma about the choice of the test, due to overlapping clinical phenotype. This necessitates the need for three tier test right from screening to the confirmative enzymes study followed by genetic mutation study. At FRIGE, a special screening technique has been developed to diagnose children with suspected eight different mucopolysaccharide disorders (MPS 1 to MPS VII) by urine study for glycoaminoglycans (GAG) excretion quantitation and electrophoresis study to identify the GAG excretion pattern[14], mucolipidosis screening by indigenously developed and patented technique called as I cell screening for type II and II[15] and plasma chitotriosidase study for Gaucher and Niemann Pick a and Niemann Pick B disease[16] (Figure 3).

Based on urine GAG study MPS I, II, VI and VII can be suspected in those with excess of Dermatan sulfate (DS) excretion with mild Heparan sulfate (HS) and Chondrotin sulfate (CS). While in those with excess of HS MPS IIIA, IIIB, IIIC and IIID can be suspected and those with excess of Keratan sulfate are likely to have Morquio-a or Morquio-B (MPS IVA and IVB). This approach was found to be 100% sensitive and 68% specific for the screening of various MPS disorders in our study of 128 cases [14].

While our study of I cell screening is found to be 100% sensitive and specific for the screening of ML II and ML III[15] as has been

Disease	Approved drug
Gaucher Disease	Imiglucerase (Cerezyme), Velaglucerase (Vpriv), taliglucerase (Elelyso), Miglustat, (Zavesca) Eliglustat (cerdelga)
Fabry Disease	Agalsidase beta (Fabrazyme),Agalsidase alfa (Raplagal) , Migalastat (Galafold)
MPS I (Hurler, Hurler -Scheie, Scheie)	Laronidase (Aldurazyme), HIRMab
MPS II (Hunter Disease)	Idursulfase (Elaprase)
MPS IV A (Morquio A)	Elosulfase (Vimzim)
MPS VI (Maroteoux Lamy)	Galsufase (Naglazyme)
Pompe Disease	Alglucosidase alfa (Myozyme)

Table 4

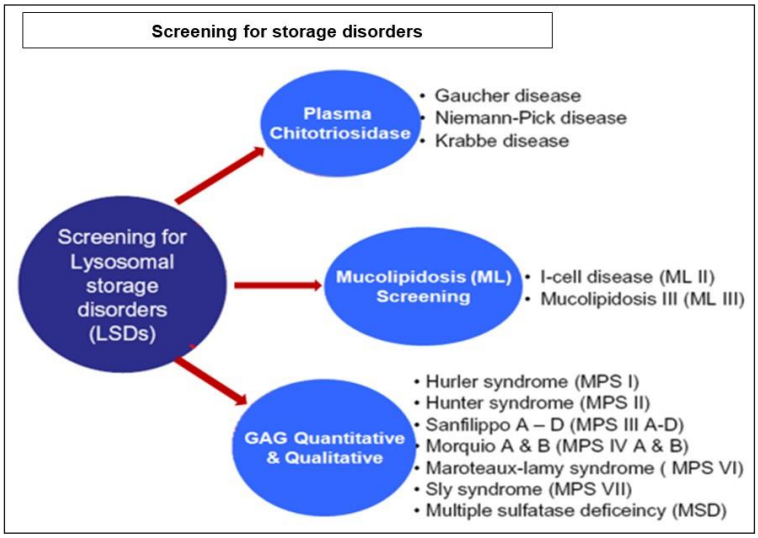

Figure 3

Source: Dr Jayesh Sheth, Personal communication

reported earlier by our group.

Those with hepatomegaly or hepatosplenomegaly, plasma chitotriosidase is found to be very useful biomarker for the diagnosis of Gaucher and NPD disease[16] where chitotriosidase is nearly 100-fold elevated in almost 85% of cases.

Thus, in our experience, the above screening strategy minimizes the burden of enzyme study from the leucocytes; as this provides a specific clue to the confirmative diagnosis and minimizes the cost of enzymes study [16].

Molecular Diagnosis of LSDs

Once an index case is confirmed with biochemical enzyme investigation, further study of the proband and parents is required to be carried out by Sanger sequencing of the coding region of the gene. This is mainly required to identify carrier status of other family members and for prenatal diagnosis. Nonetheless, with recent development of Next Generation sequencing technique (NGS), all LSD related genes can be sequenced together at one go. Though considering the high cost of NGS, there are newer techniques known as small molecule Molecular inversion probe (smMIP) that seem to be very promising and are likely to reduce the cost of molecular diagnosis of various LSDs.

Role of Support Group

LSDs are a group of rare diseases with limited option of therapeutic approach and patients once diagnosed need a social, financial and medical support. In India three support group play an active role in bringing awareness about the diseases. They are mainly LSDSS support group (www.lsdss.org), Organization for rare disease India (ordi.in) and Indian organization for rare diseases (www.i-ord.org). The main function of support group is to help patients with rare diseases to facilitate diagnosis and treatment. They are also advocating with the Government to form a uniform policy on rare diseases like, provide free treatment to these patients, orphan drug research policy so more and more pharmaceutical companies are encouraged to develop indigenous drug for these diseases.

What lies ahead in the future for LSDs

Though ERT is available for a wide range of LSDs, the biggest challenge is the exorbitant cost and absence of Govt or insurance company support for the treatment cost. Another problem is the Development of antibodies in certain disorders mainly in Pompe disease as was seen in Indian patients. There are plenty of challenges in LSDs treatment. The first critical step has been achieved by targeting lysosomes but developing an enzyme that reaches brain and bones, and prevents and improves all LSD related manifestations, will cause dramatic change in the outcome. For this, two main approaches that are promising and are being explored include bone marrow transplantation at an early age of onset and secondly development of gene therapy to overcome blood-brain barrier, and these are already in phase 2 or phase 3 clinical trials[13].

Studies using combinations of HSCT and ERT have also shown promising results. Here ERT was initiated prior to transplantation and continued until full engraftment that has demonstrated this combination does not disturb engraftment or increase morbidity.14

Conclusion

Nearly 40 per cent of children with regression of learned skill, coarse facial features, dysostosis multiplex, hepatosplenomegaly, neuroregression and visual loss are likely to have an underlying cause of lysosomal storage disorders. One should for these red flags pointing towards an underlying LSD, in practice. Confirmed early diagnosis of an index case can help to prevent the recurrence by prenatal diagnosis and initiation of an early treatment in some of the treatable LSDs.

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