

# The Spectrum of Lysosomal Storage Diseases at Paediatric Genetic Clinic in Maharashtra, India

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**Abstract:** ***Objective:** To study the demographic features, clinical characteristics and diagnostic evaluation, treatment availability for lysosomal storage diseases at Paediatric genetic clinic, Aurangabad. **Design:** Descriptive study. **Methods:** Retrospective analysis of case records of patients attending Paediatric Genetic clinic, for last 15 months i.e. March 2018 to May 2019, was undertaken. Out of 250 patients attending genetic OPD for various genetic Diseases, children with global developmental delay and /or Neuro-regression associated with hepatosplenomegaly were evaluated for various lysosomal storage diseases. The age at first suspicion, diagnostic work up and time required for final diagnosis by enzyme assay and/ or mutation analysis was considered. **Results:** A total 250 patients were referred to Genetic clinic from March 2018 to May 2019. Out of which lysosomal storage Diseases were suspected in 25 patients (10%). Out of which diagnostic work up could be completed in 18 patients (7.2%). Commonest clinical features were growth failure, developmental delay, short stature, and neuro-regression. History of Consanguinity was present in 88 % families. Enzyme replacement therapy is being given to 1 patient of Hurler Disease and he is showing gradual improvement in symptoms. One of the parents opted for prenatal testing and fetus was detected to be carrier for neuronal ceroid lipofuscinosis. **Conclusion:** Lysosomal storage diseases constitute one of the common disease group presenting to paediatric Genetic Clinic. In spite of sufficient awareness and Indian Literature, the diagnosis is delayed. Various enzyme replacement therapies are available for few of the LSDs with raising hopes of treatment in future. Definitive diagnosis is must, so that conditions which are treatable will have hopes of treatment. By definitive molecular and/or enzyme assay diagnosis, we can give opportunity to couples to decide about reproductive choices of future pregnancies.*

**Keywords:** Lysosomal storage disorders, India, Clinical features, Management, Mutation analysis

## 1. Introduction

Lysosomal storage disorders (LSDs) are a group of inborn errors of metabolism (IEM) characterized by the inability of defective lysosomal enzymes to degrade macromolecules or transport them outside the cells causing intra-lysosomal accumulation of complex macromolecules (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). For a larger population in India, the absolute number of cases obviously must be larger. However, the cases diagnosed in India at present represent the tip of an iceberg. Presences of high level of consanguinity in India is obvious in LSDs, and account for about one-third of cases (3). This study aims to throw a light on new hotspot of lysosomal storage diseases in India, being it marathwada. In spite of increased awareness and literature from India, the diagnosis is still delayed and training of paediatricians for diagnosing lysosomal storage diseases needs to be increased.

## 2. Methods

The study was a descriptive study done over a period of 15 months period from March 2018 to May 2019, in the Genetics OPD of a tertiary care, referral hospital in Maharashtra. All patients suspected to have lysosomal storage disorders on the basis of their clinical features and laboratory findings were advised enzyme assay and mutation analysis. The relevant clinical, biochemical, imaging and molecular genetic data and the management/intervention details were collected for each patient. A complete medical history of the patient, a detailed family history, a three-generation pedigree, and a full physical

examination was done. 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). 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 analysed.

## 3. Results

Out of the 250 patients attending genetic OPD, 25 patients were suspected to have LSDs on the basis of their clinical features during the 15 months study period (10%); out of these, 12 patients (48%) were confirmed to have different types of LSDs (based on enzyme assay and /or mutation analysis), 8 were lost to follow up and did not undergo the necessary enzyme assays for confirmation of the clinical diagnosis. 5 patients completed some work up but not enzyme assay or mutation. The age at presentation of the LSD patients varied from 5 months to 11 years with an average age of 4.2 years. Consanguinity was present in 22 families (88%) out of suspected cases. Five patients (20%) had history of one or more siblings with similar clinical features, but in none of the cases, the diagnosis had 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 5 families.

## Patients with different types of LSDs (Table 1)

Table 1

Type of LSD	Electrophoretic pattern on urine for MPS/urine for oligosaccharides	No.	Blood enzyme level(nm/ml/hr)	Mutations identified in the causative gene
MPS I	-	2	0.14-0.15	p.Tyr265Cys, p.Leu490Pro
MPS II	HS+,DS+,	1	Enzyme assay for type I MPS normal	-
MPS III	-HS+++ KS++	6	-	-
Gaucher Disease	-	3+3*	b-glucosidase : 0.45-1.68	p.Leu483Pro p.L444p
GM1 Gangliosidosis	Urine oligosaccharides positive	1	b-galactosidase : 0.4	-
TaySach Disease		1	Beta hexaminidase-1.8(62-310)	-
Mucopolidosis		1	I-cell screen : Positive Arylsulfatase A : 3813.3(very high) a-fucosidase : 5900.6 (very high)	-
Metachromatic leukodystrophy	-	1	Aryl sulfatase A assay-14 (50-150)	-
Neuronal Ceroid lipofuscinosis	-	2	Tripeptidyl Peptidase 1 (TPP-1) : 3.5	CLN6 gene c.556_558delTTC
Pompe Disease	-	1	Lysosomal alfa glucosidase-1.13(5.5-13)	-

Out of the 22 cases described above, 6 patients of suspected type III MPS, age range 5-11 years, had, global developmental delay, attention deficit hyperactivity, mild coarse facies, with mild to moderate hepatosplenomegaly, urine for MPS qualitative assay was asked for the children, but one of the patient underwent and showed significant presence of heparan sulphate. Total 6 patients of Gaucher diseases were enrolled in Genetic OPD, but 2 of the siblings had, diagnosis on bone marrow aspiration by haematologist and one of the girl siblings, aged 15 had already undergone splenectomy. Other three patients of Gaucher Disease were diagnosed by enzyme assay and mutation analysis in Genetic OPD. Age range for diagnosis 1 year to 3 years. One of the patients underwent splenectomy for hypersplenism. Out of the two patients of MPS I diagnosed by enzyme assay and mutation analysis, one patient with Hurler Schieie Phenotype has been started with aldurazyme enzyme replacement therapy under India Charitable Access program of Sanofi Genzyme Company. This child has received therapy for two months and is already showing increased joint mobility and regression of hepatosplenomegaly. 1 patient each of GM 1 ganliosidois and Mucopolidosis II were suspected at the age of 3 and 6 months respectively. The diagnosis was confirmed within 1 month of visit to genetic OPD. One child was referred from cardiologist for hypertrophic cardiomyopathy. On clinical evaluation, child had delayed motor milestones and hypotonia, with increased CPK levels. Enzyme assay for Pompe disease confirmed the diagnosis. Two children with neuro regression without coarse facies or skeletal changes were suspected to be lysosomal storage diseases on the basis of decline in cognitive function and reduced vision. MRI brain of one child showed changes suggestive of metachromatic leukodystrophy and enzyme assay confirmed the diagnosis. MRI brain of other two children showed cerebellar degeneration and fundus examination showed macular degeneration suggestive of late infantile neuronal ceroid lipofuscinosis. Enzyme assay for late infantile NCL confirmed the diagnosis. Mutation analysis in another patent confirmed infantile NCL. Another child with regression of milestones at 8 months with convulsions and hepatosplenomegaly had cherry red spot,

enzyme assay for Tay Sach disease confirmed the diagnosis. Prenatal testing by amniocentesis was done for 1 patient's pregnant mother and Fetus was heterozygous for mutation in CLN6 gene.

#### 4. Discussion

The lysosomal storage group of diseases are one of the commonest groups of inborn errors of metabolism as there are many studies published regarding burden of LSDs in India (3-6). The primary treating paediatrician suspects the diagnosis of lysosomal storage diseases and sends to the Geneticist or neurologist for further work up and identifying exact diagnosis of Lysosomal storage diseases. Hepatosplenomegaly, coarse facial features, developmental delay and skeletal changes and neuro-regression. The age of presentation, severity of clinical presentation, depends on sub type of storage disease, type of mutation, residual enzyme activity and other functional pathways (7). Most common reasons of morbidity were developmental delay, neuro-regression, dysostosis multiplex changes, cardiovascular changes etc. Dysostosis multiplex changes are also seen in oligosachharidosis, (GM1 gangliosidosis in our case). The child with mucopolidosis also had coarse facial features and dysostosis changes.

##### What is already known

Lysosomal storage diseases are not rare in India

##### What this study adds

Genetic work up is delayed till second child is affected, so we need more awareness amongst paediatrician to consider diagnostics in first affected child and in fact any affected child, so that prenatal testing can be done which is primary prevention of genetic diseases.

Ophthalmological evaluation showed corneal clouding in two of the MPS I patients and cherry red spot on retina in GM1 gangliosidosis and Tay Sach disease patient. Clinical features and ophthalmological findings give a clue regarding type of Lysosomal storage diseases. On the basis of these, urine for Mucopolysaccharidosis screening

and urine for oligosaccharidosis screening can be done in step wise manner, to narrow down the diagnosis (8,9). Accurate diagnosis of the type of LSD is imperative for appropriate management of the affected child if enzyme replacement therapy or bone marrow transplant treatment is available for particular type of LSD. ERT is currently available for six LSDs (Gaucher disease, Pompe disease, Fabry disease and mucopolysaccharidoses types I, II and VI) [10]. Accurate diagnosis by enzyme assay and/or mutation analysis is important 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 [11,12]. 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. The main limitations with molecular genetic testing are the limited availability of centres for such testing and the cost. 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 new hotspot of lysosomal storage diseases from region of Marathwada, Maharashtra. 22 of the 25 patients having consanguineously married parents, contributing to high number of cases. Another thing is the mutations identified were, known common mutations in Indian patients. 5 out of 12 patients had one sibling affected who was undiagnosed, pointing towards unawareness of recurrence risk of these diseases amongst doctors and need of exact diagnosis in first child itself. 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 counselling and prenatal diagnosis for their families.

## References

- [1] Lysosomal disorders. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Childs B, Kindler KW, et al, editors. *The Metabolic and Molecular Bases of Inherited Disease*. 8th ed. New York; McGraw-Hill, 2001. p. 3371-877.
- [2] Mickle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. *J Am Med Assoc*. 1999;281:249-54.
- [3] Verma PK, Ranganath P, Dalal AB, Phadke SR. Spectrum of lysosomal storage disorders at a medical genetics center in Northern India. *Indian Pediatr*. 2012;49:799-804.
- [4] Sheth J, Mistri M, Sheth F, Shah R, Bavdekar A, Godbole K, et al. Burden of lysosomal storage disorders in India: experience of 387 affected children from a single diagnostic facility. *JIMD Rep*. 2014;12:51-63.
- [5] Agarwal, S., Lahiri, K., Muranjan, M. et al. The Face of Lysosomal Storage Disorders in India: A Need for Early Diagnosis *Indian J Pediatr* (2015) 82: 525. <https://doi.org/10.1007/s12098-014-1628-8>
- [6] Sheth, Jayesh & Mistri et al. Lysosomal Storage Disorders in Indian Children with Neuroregression Attending a Genetic Center. *Indian Pediatrics*. Dec. 2015 ,52. 1029-1033. 10.1007/s13312-015-0768-x.

- [7] Beck M. Variable clinical presentation in lysosomal storage disorders. *J Inher Metab Dis*. 2001;24:7-51.
- [8] J E Stone, Urine analysis in the diagnosis of mucopolysaccharide disorders, *Ann Clin Biochem* 1998; 35: 207-225
- [9] Casado, M., Ferrer-López, I., Ruiz-Sala, P., et al. (2017). Urine oligosaccharide tests for the diagnosis of oligosaccharidoses. *Reviews in Analytical Chemistry*, 36(3),
- [10] Muro S. New biotechnological and nanomedicine strategies for treatment of lysosomal storage disorders. *Wiley Interdiscip Rev of Nanomed Nanobiotechnol*. 2010;2:189-204.
- [11] Carey WF, Hopwood JJ, Poulos A, Petersons D, Nelson PV, Muller V, et al. Prenatal diagnosis of lysosomal storage diseases. Review of experience in 145 patient referrals over a period of eight years. *Med J Aust*. 1984;140:203-8.
- [12] Sheth, Jayesh & Mistri, Mehul & Sheth, Frenny & Datar, Chaitanya & Godbole, Koumudi & Kamate, Mahesh & Patil, Kamal. (2014). Prenatal Diagnosis of Lysosomal Storage Disorders by Enzymes Study Using Chorionic Villus and Amniotic Fluid. *Journal of Fetal Medicine*. 1. 10.1007/s40556-014-0001-3