RESEARCH PAPER

Lysosomal Storage Disorders in Indian Children with Neuroregression Attending a Genetic Center

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Objective: To study the etiology of neuroregression in children having deficiency of the lysosomal enzymes.

Design: Review of medical records.

Setting: Specialized Genetic Center.

Participants: 432 children aged 3 mo-18 y having regression in a learned skill, selected from 1453 patients referred for diagnostic workup of various Lysosomal storage disorders (LSDs).

Methods: Plasma chitotriosidase, quantitative and qualitative glycosaminoglycans, and mucolipidosis-II/II screening followed by confirmatory enzyme study using specific substrate was carried out; Niemann-Pick disease Type-C was studied by fillipin stain method on skin fibroblasts.

Results: Total 309 children (71.5%) were diagnosed with different lysosomal storage disorders as the underlying cause of neuroregression. Plasma chitotriosidase was raised in 82 of 135; 64 (78%) of these had various LSDs. 69 out of 90 cases showed high excretion of glycoaminoglycans, and 67 (97.1%) of these were confirmed to have enzyme deficiency for various mucoplysaccharide disorders. While 3/90 children with positive I-cell screening had confirmed mucolipidosis-II/III disease. Among all, glycolipid storage disorders were the most common (50.2%) followed by mucopolysaccharidosis (MPS) (21.7%) and sulphatide degradation defect (17.5%). Neuronal ceroid lipofucinosis-1 & 2 (7.4%), mucolipidosis-II/III (1%), Sialic acid storage disorder (1%), Niemann-Pick disease type-C (1%) and Fucosidosis (0.3%) were observed with less frequency. Most common phenotypes in all subjects were cherry red spot (18.5%), hepatosplenomegaly (17.9%), coarse facies (15%), seizures (13.1%) and skeletal abnormalities (12.14%).

Conclusions: Lysosomal storage disorders are considered to be one of the common causes in children with regression in learned skill, dysmorphic features and cherry red spot. Among these, glycolipid storage disorders are the most common, followed by mucopolysaccharidosis.

Keywords: *Developmental delay, Glycolipid storage disorders, Metabolic disorders, Mucopolysaccharidosis (MPS).*

Neuro-regression in childhood could either be genetic with neurometabolic origin or non-genetic causes such as infections and toxins [1]. It has been observed that more than two-third of the diagnosed cases of progressive neurological decline are due to metabolic disorders [2]. Approximately 4.5% of the cases have mitochondrial disease [3] and several are found to have basic metabolic abnormalities like vitamin B12 deficiency [4] and thyroid disorders [5].

Lysosomal storage disorders (LSDs) are the heritable group of nearly 40 heterogeneous disorders occurring due to genetic defect in one or more specific lysosomal enzymes, activator protein or membrane protein resulting in deficient enzyme activity [6-8]. There is very little information available regarding the role of LSDs in neuroregression, except for few studies demonstrating neurological deterioration as the most commonly occurring pathophysiology of lysosomal storage disorders in around one-third of the cases [9,10].

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Though, individually these disorders are rare (incidence 1:1,00,000), collectively they occur with the frequency of approximately 1:7000-8000 live births [11-14]. Availability of prenatal diagnostic facilities [15,16], newborn screening [12,17] and the possibilities of early therapeutic approaches [18,19] has increased awareness among medical fraternity for different LSDs [20-23]. Therefore, we studied the frequency of various LSDs as the cause of neuroregression in children from India.

METHODS

This work presents the data on 432 children aged 3 months to 18 years referred to our institute between February 1997 and May 2014, and selected from the cohort of 1453 patients referred for various LSDs. Many of these children were also included in our previous report on burden of LSDs in India [23]. They presented with regression in learned skill with/without cherry-red spot, hepatomegaly/ hepatosplenomegaly, coarse facies, seizures, skeletal abnormality, visual impairment and spasticity. Patients with neuroimaging findings such as leukodystrophy, cerebral and/or cerebellar atrophy, white and gray matter involvement were also included in the study. After obtaining an Institutional ethics committee approval, an informed written consent was obtained from the parents or the guardian while enrolling for the previous study.

10-15 mL of random and/or morning void urine samples were collected for screening, and confirmatory enzymes study was carried on 6 mL peripheral blood collected in sodium heparin and/or EDTA vaccutainers. A screening algorithm was used for plasma chitotriosidase (ChT) [21] in 135 cases with hepatomegaly or hepatosplenomegaly and neuroregression. Urine glycosaminoglycans (GAG) screening [24] and mucolipidosis-II/III (ML-II/III) [22] were carried out in cases with coarse facial features and neuroregression (***Fig.* 1**). Confirmatory enzymes studies were carried out from leucocytes and/or plasma using synthetic fluorogenic 4-methylumbelliferrone (4-MU) or p-nitrocatechol sulphate (PNCS) substrates and enzyme activity was expressed as nmol/hr/mg of protein, and for β-Galactocerebrosidase as nmol/17hr/mg protein [25]. The enzyme activity was carried out from plasma in case of Sanfillippo type-B (MPS-IIIB) and ML-II/III, and was expressed in term of nmol/hr/ml plasma [22,25]. For Niemann-Pick disease-C (NPD-C), skin fibroblasts were cultured in lipid-deficient medium followed by fillipin stain to confirm the presence of punctate granules [26,27].

RESULTS

Out of 432 cases with the aforementioned clinical signs and symptoms, 123 (28.5%) were found to be normal for lysosomal enzyme activity and the rest were found to be affected with different LSDs (***Web Table* I)**. Consanguinity among parents was seen in 98 (22.7%) cases. The age of presentation for diagnosis of storage disorder was 7 months to 7 years whereas late juvenile

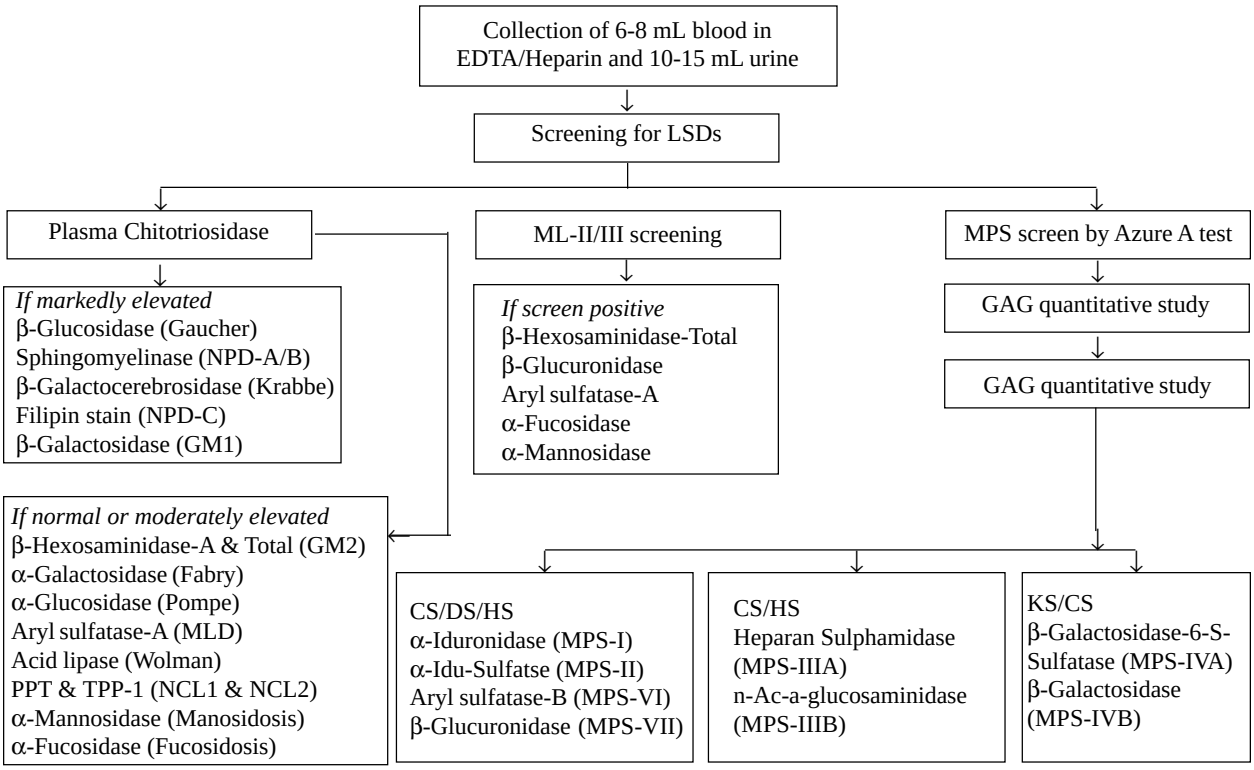

FIG. 1 *Screening approach for various lysosomal storage disorders.*

TABLE I CLINICAL FEATURES OF CHILDREN WITH LYSOSOMAL STORAGE DISORDERS WITH NEUROREGRESSION (*N*=309)

Clinical Phenotype	*No.* (%)(*N=309*)
Cherry red spot	58 (18.8)
Hepatosplenomegaly	56 (18.1)
Coarse features	47 (15.2)
Seizures	41 (13.3)
Skeletal abnormality	38 (12.3%)
Cerebral and/or cerebellar atrophy	23 (7.4)
Psychosis	20 (6.5)
Leukodystrophy	17 (5.5)
Myoclonic jerks	13 (4.2)
Visual Impairment	13 (4.2)
Spasticity	9 (2.9)
White matter disease	8 (2.6)
Grey matter disease	1 (0.3)

presentation was seen in two cases of Niemann-Pick disease-B (NPD-B), and Krabbe disease and Fucosidosis in one each. Adult onset presentation was also seen in four cases of Sanfillipo type A/B (MPS-IIIA/B) and two cases of Neuronal ceroid lipofucinosis type 2 (NCL2) and Metachromatic leucodystrophy (MLD).

Plasma chitotriosidase (ChT) screening of 135 cases revealed raised ChT (106.9-30,000 nmol/hr/mL of plasma) in 82 (60.7%) children. Enzyme study from leucocyte and/or plasma was carried out for various LSDs and 78% (64/82) cases with raised ChT were found to have Gaucher, Niemann Pick disease type A or B (NPD-A/B), Krabbe, GM1 gangliosidosis and Sandhoff disease, whereas 22% (12/53) with normal ChT were affected with LSDs like Tay-Sachs, Neuronal ceroid lipofucinosis type 1 and type 2 (NCL1 and NCL2), Metachromatic leukodystrophy (MLD), NPD A/B and various mucopolysaccharide (MPS) disorders. Urine screening for glycoaminoglycans (GAG quantitative and qualitative study) and plasma screening for ML-II/III was carried out in 90 cases and 69 were screen positive for urine GAG excretion suggesting presence of MPS disorders with excretion of excess dermatan sulphate (DS) and moderate heparan sulphate (HS) to mild chondroitin sulphate (CS) in 40 (57.9%) and excess HS with mild CS in 27 (39.1%) cases. Further confirmation by enzyme study was carried out in all patients. Moderate CS with mild HS in 2 (2.9%) patients were found to be affected with ML-II/III and GM1 gangliosidosis one each. In I-cell screen, 87 cases were found to be normal, while 3 patients were screen-positive; enzyme activity in plasma further confirmed ML-II/III in all 3 screen-positive patients (***Web Table* 1**).

The most commonly diagnosed LSDs were in the group of glycolipid storage disorders (50.16%) with GM2 gangliosidosis (23%), GM1 gangliosidosis (13.9%), Niemann Pick disease (12%) and Gaucher disease (1.3%). (***Web Table* 1**).

The common clinical phenotype observed among patients with neuroregression affected with various LSDs were the presence of cherry red spot (18.5%), hepatosplenomegaly (17.9%), and coarse facies (15%) (***Table* I**).

DISCUSSION

This is the largest data-set from India demonstrating neuroregression in 29.9% of patients suspected with storage disorders, 71.5% of which had different types of LSDs, at a specialized genetics center. The high occurrence of LSDs could be due to selection bias as all referred cases were from the pediatric neurologists or pediatrician or geneticist and it is highly likely that other causes of neuroregression have been ruled out before suspecting for storage disorders. Our study findings are in concordance with the Northern Indian group demonstrating presence of LSDs in 69.2% of children having neuroregression with highest frequency of mucopolysaccharidosis followed by glycolipid degradation defects [10]. In a UK-based study where 40.4% of children with progressive intellectual and neurological deterioration (PIND) had LSDs with the highest frequency (31%) of NCL1 and NCL2 [9], relatively large numbers of PIND cases were due to high rate of consanguinity [9]. This is in accordance with our observation of 80% NCL (1 and 2) cases from the region having 72% consanguinity.

The high proportion of glycolipid degradation defects in this study, which is much higher than the previous study from India [10], could be due to either the presence of founder mutation for Tay-sachs disease in Gujarat [28] or referral bias due to lack of investigative facility at other places in the region. Mucopolysaccharidosis (MPS) was found to be the second most common LSD with highest frequency of Sanfillipo disease (MPS-IIIA and IIIB). This is in contrast to the reports of high number of MPS-I and -II in the literature [10,23] and is likely to be due to overlapping phenotypic features and limited diagnostic facility for these investigations in most of the centers in the country. The third largest group of patients with neuroregression were found with defects in sulphatide degradation (17.5%) associated with MLD and Krabbe disease. This is almost similar to what had been found by our group in an earlier study [23], while Verma, *et al*. [10] have shown the presence of MLD in nearly 22% of cases with neuroregression.

WHAT IS ALREADY KNOWN?

- Neuroregression is one of the common observations in children with lysosomal storage disorders.

WHAT THIS STUDY ADDS?

- Screening by using biomarkers like Plasma ChT, urine GAG and ML-II/III from plasma can provide the first line diagnosis in children with suspected lysosomal storage disorders.

Major limitation of the present study is a referral bias of children with neuroregression where previous workup for the cause has been ruled out in the setting of lack of wider availability of diagnostic facility at most of the places in the country.

To conclude, screening method for storage disorders like mucolipidosis type II/III, MPS disorders and Gaucher/NPD-A/B have a high predictive value for the confirmative diagnosis saving the unnecessary cost of enzyme study. Additionally LSDs should be considered to be one of the common causes of neuroregression in children with regression in learned skill, dysmorphic features and cherry red spot.

Contributors: JS: study design, standardization of technical procedure, preparation of manuscript and guarantor; MM: processing the sample, analysis of data and preparation of manuscript; RB: analysis of data and preparation of the manuscript; FS,HS,CD,MK: critical evaluation of manuscript and patient management. All the authors read and approved the manuscript.

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