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Lysosomal Storage Disorders

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This study was conducted to assess the variability of clinical expression of Lysosomal storage disorders (LSDs) and the selection of specific enzyme investigation to reach the differential diagnosis. Initially 150 children in the age range of 15 days to 13 years were screened for common metabolic disorder and based on screening results, clinical signs and symptoms, 30 children (4 mo-12 yr) of these were selected for the leukocyte enzyme study. Of these 21 were confirmed to have LSDs. The most common disorder was GM2-gangliosidosis (47.61%, 10/21) followed by mucopolysaccharidosis (33.33%; 7/21). All showed variable phenotypic expression. Metachromatic leukodystrophy (MLD) was observed in 9.5% (2/21) of children with arylsulphatase A enzyme deficiency, while two children had shown pseudodeficiency of arylsulphatase A. One case each of galactosialidosis and GM1-gangliosidosis were observed. We conclude that children with developmental delay, seizures, dysmorphic features and organomegaly, with or without positive urinary screening for common metabolic disorders, need to be investigated further for LSDs. Variability of clinical expression is commonly observed in LSDs which require further confirmation by specific leukocyte enzyme study.

Key words: *Lysosomal storage disorders (LSDs), Lysosomal enzyme, Metachromatic leukodystrophy (MLD), GM2-gangliosidosis.*

Lysosomal storage disorders (LSDs) are a group of genetic diseases characterized by an inherited defect in the functional expression of any of the lysosomal enzyme(1). The resultant accumulation of substrates of lysosomal enzymes inside the cell as well as in the blood stream causes a loss of function in one or several crucial areas of the body and the clinical features are very much dependent upon the rate and magnitude of accumulation of the undegraded substances. Therefore, a wide spectrum of phenotypic expression is observed in children with LSDs(2). These disorders are progressive and most affected

individuals appear normal at birth, but subsequently develop severe morbidity and die after months or years. As a result, many of the LSDs are missed or incorrectly diagnosed. Some of the disorders are fatal in childhood or appear during adolescence(3).

The clinical consequences of the enzyme defect can vary, but commonly this may result in developmental defects, mental and physical disability, defects in the immune systems, skeletal abnormality, ophthalmic abnormality, *etc*.(4).

Paucity of Indian studies(5) makes reporting of even small series of data worthwhile. Despite the lack of precise biochemical assay, a clinician should have a high index of suspicion leading to early diagnosis. This would facilitate genetic counseling and possible enzyme replacement therapy (ERT).

Subjects & Methods

The present study involves initial investigation of 150 children in the age range of 15 days to 13 years with suspected metabolic disorder due to most common clinical symptoms of milestone regression, seizures and organomegaly. All were screened for the most common metabolic disorder by urine tests as described elsewhere(6). Based on screening results and clinical observations, thirty children were selected for leukocyte enzyme study. Selection of four children with suspected MLD was based on positive CT/ MRI findings of leukoencephalopathy.

Thirteen different lysosomal enzyme studies were carried out in leukocytes isolated from peripheral blood collected in a heparinized vacutainer. Enzyme activity was measured using the synthetic substrate 4-methylumbelliferrone (4MU) - fluorogenic substrate and p-nitrocatechol sulfate (NCS) - spectrophotometric substrate obtained from Sigma Chemicals(3). For hexosaminidase A (Hex-A), the heat inactivation method was used(7). The enzyme activity was expressed as nmol/h/mg of protein.

Results

Of 150 children, urine screening detected seven children with positive Azure test (spot test) indicative for mucopolysaccharidosis (MPS). Six children with positive reducing substance were also selected for LSD's study due to specific clinical signs and symptoms. Rest of the cases showed negative findings.

The common clinical signs observed in all LSDs were regression in milestone (78%) followed by dysmorphic features (75%), organomegaly (60%), macrocephaly (43%), hypotonia (52.17%) and seizures in 21% of the cases. Other signs were more specific to the specific LSDs. *Table I* provides detailed clinical description of these 21 cases.

Table II demonstrates, reduced activity of Hex-A and *b*-Hexosaminidase T (Hex-T) in 10 of 21. Of these, four children showed positive reducing substances in urinary screening. Depending on the variable concentration of the enzyme activity they were diagnosed as B-variant, Bl-variant and Sandhoff 'O' variant.

The deficient enzyme activity of arylsulfatase B was observed in MPS VI and B galactosidase activity was found to be almost absent in MPS IV (*Table III*). Of four children suspected for MLD, two had shown very low activity of arylsulphatase A (ARSA) confirming MLD, while two children had moderately reduced activity of ARSA (43.10 and 40.0 nmol/h/mg protein respectively) with only positive finding of leukoencephalopathy in MRI. In the absence of other clinical symptoms and signs they were diagnosed as

TABLE I–*Clinical Symptoms and Signs in Children with Suspected Metabolic Disorder.*

Clinical features	Disease					
	GM1 Gangliosidosis (1)	GM2 Gangliosidosis (10)	MPS VI (4)	MPS IV (3)	MLD (2)	Galactosialidosis (1)
Global developmental Delay/Milestone regression	✓	✓(10)	✓(4)		✓(2)	✓
Age of onset	4 month	5mo to 5 y	8 mo to 10 y	3 to 12 y	4.5 to 6 y	4 y
Facial dysmorphism	✓	✓(4)	✓(4)	✓(3)	–	✓
Seizures	✓	✓(4)	–	–	–	–
Macrocephaly	✓	✓(3)	✓(4)	–	✓(2)	–
Organomegaly	–	✓(6)	✓(4)	✓(3)	–	✓
Hypotonia	✓	✓(10)	✓(1)	–	–	–
Cherry red spot	–	✓(3)	–	–	–	–
Photophobia	–	–	–	–	–	✓
Vomiting	–	✓(1)	✓(2)	–	–	–
Cerebral atrophy	–	✓(1)	–	–	–	–
Leukoencephalopathy	–	–	–	–	✓(2)	–
Skeletal dysplasia	–	–	–	✓(3)	–	–
Kyphosis	–	–	✓(4)	✓(3)	–	–
Genu valgus	–	–	–	✓(3)	–	–
Mental retardation	–	✓(2)	–	–	–	–
Neural abnormalities	–	✓(1)	–	–	–	–
Adenoid hyperplasia	–	–	–	✓(3)	–	–
Additional findings	No vision in right eye, left eye small	Optical atrophy (1)	Short trunk dwarfism-(4)	Short trunk dwarfism (4) Interior beaking of lumbar spine and restriction of joint movement (1)	Limb deformity and gait distrur-bance (1) Depressed nasal bridge and curved fingers (1)	White spot on sclera

Values in parentheses are the number of cases and ✓ and - sign represent the presence and absence of character respectively.

TABLE II–*Related Enzyme Activities in Children with Lysosomal Storage Disorders.*

Case No.	Enzyme	Assay values nmol/hr/mg protein	Diagnosis
		GM_2–gangliosidosis	
1.	B-Hexosaminidase T B-Hexosaminidase A	91.42 50	GM_2–gangliosidosis
2.	B-Hexosaminidase T B-Hexosaminidase A	320 Undetectable	B variant
3.	B-Hexosaminidase T B-Hexosaminidase A	362.64 5.7	B variant
4.	B-Hexosaminidase T B-Hexosaminidase A	333.33 2.35	B variant
5.	B-Hexosaminidase T B-Hexosaminidase A	186 24	Tay Sachs Variant B1
6.	B-Hexosaminidase T B-Hexosaminidase A	840.0 31.25	Tay Sachs Variant B1
7.	B-Hexosaminidase T B-Hexosaminidase A	872.18 26.32	Tay Sachs Variant B1
8.	B-Hexosaminidase T B-Hexosaminidase A	3.33 5	Sandhoff Variant O
9.	B-Hexosaminidase T B-Hexosaminidase A	283.68 5	Sandhoff Variant O
10.	B-Hexosaminidase T B-Hexosaminidase A	201.43 5.9	Sandhoff Variant O
		Mucopolysaccharidosis	
11.	Arylsulphatase B	4.0	Maroteaux-Lamy (MPS-VI)
12.	Arylsulphatase B	11.11	Maroteaux-Lamy (MPS-VI)
13.	Arylsulphatase B	13.73	Maroteaux-Lamy (MPS-VI)
14.	Arylsulphatase B	Undetectable	Maroteaux-Lamy (MPS-VI)
15.	B-Galactosidase	Undetectable	Morquio-B Syndrome
16.	B-Galactosidase	Undetectable	Morquio-B Syndrome
17.	B-Galactosidase	2.27	Morquio-B Syndrome
		Metachromatic Leucodystrophy	
18.	Arylsulphatase A	3.125	MLD
19.	Arylsulphatase A	Undetectable	MLD
		GM_1-gangliosidosis	
20.	B-Galactosidase	6.66	GM_1-gangliosidosis
		Galactosialidosis	
21	B-Galactosidase	16.92	Galactosialidosis

* Normal range: Beta-Hexosaminidase T:801 ± 190 nmol/hr/mg protein, Beta-Hexosaminidase A: 55–72%, Arylsulphatase A: 71 ± 11.4 nmol/hr/mg protein, Arylsulphatase B: 121 ± 107 nmol/hr/mg protein, Beta-Galactosidase: 97.9 ± 19.8 in nmol/hr/mg protein.

Key Messages

- Variability of clinical expression is commonly observed in lysosomal storage disorders.
- Specific enzyme selection may be difficult. It is advisable to do a group of lysosomal enzymes in absence of very specific clinical signs and symptoms.

pseudo deficiency for ARSA. One child with GMI-gangliosidosis had reduced activity of *b*-galactosidase while one with moderately low activity of *b*-galactosidase was diagnosed as probable galactosialidosis. Though in latter case confirmative enzyme activity of neuraminidase was not carried out. Both these cases had positive reducing substance on urine screening.

Discussion

Although individually rare, lysosomal storage disorders constitute a significant burden on society and an important health problem. High prevalence of 8 per 100,000 births has been reported in the population of British Columbia (20% of confirmed metabolic disorders(6). Estimate of such prevalence is not possible in present study due to small number of cases. Nonetheless it is important to observe that the most common LSDs observed were GM_2-gangliosidosis in 47.61% (10/21) of cases and MPS in 33.33% (7/21), with variable clinical expression. Similar observation was made by Beek(2). He observed a broad phenotypic spectrum in MPS-I (Hurler-Scheie disease), Gaucher disease, several forms of GM_2-gangliosidosis and the different manifestations of Beta-galactosidase deficiency (GM_1-gangliosidosis and Morquio disease type B). It is not yet clearly understood why the same enzyme defect can manifest variable clinical expression. However, different mutation in the same gene could explain the different phenotypic expression in individuals with an identical enzyme deficiency(2). The residual activity of the affected enzyme and the turnover rate of their substrate also determine the severity of the lysosomal storage disorder(8). It is also likely that a specific activator protein modulates specific enzyme activity, as has been observed in Tay-Sachs due to hexosaminidase enzyme activity(9).

Two children with MLD had very low to undetectable activity of ARSA that depends very much on the type of mutation in the respective gene(l 0). None of our patients were investigated for mutations in the ASA gene. Moderately low activity of ARSA in two children in absence of specific clinical signs except leukoencephalopathy were considered to have pseudodeficiency of ARSA. Alternatively, they could be heterozygous for ASA deficiency. Barth *et al.*(11) have shown mutations in a healthy population responsible for pseudo deficiency of ARSA GM_1-gangliosidosis in a child aged 4 months was found to have about 12% of the normal activity of beta-galactosidase. Sopelsa *et al.* (12) made similar observation in such cases. Similarly, low beta-galactosidase activity with normal activities of other leucocyte enzyme was observed in a 4 year child and based on clinical observations he was diagnosed with galactosialidosis, although further confirmation by neuraminidase assay was not available in our center.

We observe from this study that milestone regression, mental handicap, dysmorphic

features, seizures, abnormal fundus findings and organomegaly are the clinical features leading to the suspicion for LSDs. Variability in clinical expression always need further confirmation by specific lysosomal enzyme. Urine metabolic screening has very little role except for MPS. While the presence of reducing substance seems to be by chance and likely to be due to high carbohydrate diet in those children. In children with suspected MLD, a high index of suspicion coupled with clinical observation and an imaging technique such as CT scan or MRI provide clue for further confirmation. Once the confirmed diagnosed is made in the proband, the prenatal diagnosis in subsequent pregnancies provide great relief to the parent (13,14).

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