

Diagnosis in Lysosomal Disorders

How to Cite this Article: Shakiba M. Diagnosis in Lysosomal Disorders. Iran J Child Neurol Autumn 2012; 6:4 (suppl. 1):15-16.

Marjan SHAKIBA MD'

Lysosomal disorders are a heterogeneous group of Over 40 inherited disorders that individually are rare but as a group have an incidence of 1 in 10000 live births. LDs are caused by enzyme, enzyme activator, membrane transporter, or membrane protein defects that result in accumulation of complex macromolecules normally degraded in lysosomes .LDs classified to Mucopolysaccharidoses, disorders of lysosomal enzyme localization (Mucopolipidoses), sphingolipidoses, sialic acid disorders, Oligosaccharidoses, Neuronal ceroid lipofuscinoses and a number of disease that are more difficult to classify in to specific category such as Wolman disease and cystinosis [Ox]ostosis, pompe disease Danon disease.

Biochemical and genetic diagnosis of LSDs should be performed in specialized laboratories. Various clinical samples can be used for analysis, such as blood, urine, amniotic fluid, skin fibroblasts and tissue biopsies.

Measurement of the accumulated substrate is often the first approach when an LSD is suspected or in screening programmes. Specific secondary changes may also occur in cells associated with a lysosomal deficiency. Such changes may result in the modification of other proteins or cellular components that may be useful as markers of specific diseases, or for following disease progression. Identification of the molecular basis of a disorder may enable the use of more specific testing, such as the assessment of lysosomal enzymes and □

The tests performed for diagnosis can be divided into the six categories outlined below:

Urinary oligosaccharides Urinary oligosaccharide screening is performed by high-performance thin-layer chromatography (HPTLC) .This method, however, has severe limitations. Metabolite quantification is not possible, and the identification of metabolites is assumed on the basis of migration rates. Definitive identification of specific metabolites is therefore not possible. This screening technique can provide an initial indication of a possible diagnosis, although further investigations are needed to confirm the diagnosis. abnormal HPTLC profile are:

Urinary glycosaminoglycans Mucopolysaccharidoses (MPS) may be suspected from measurements of urinary glycosaminoglycans (GAGs).This method assesses the amount of hexuronic acid contained in the extracted GAGs and can give false-negative results when the accumulated GAG is keratin sulphate, which contains galactose instead of hexuronic acid; in this case the diagnosis of Morquio disease may be missed.The second pitfall when

1.Associate Professor of Pediatric Endocrinology and Metabolism, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding Author:
Shakiba M. MD
Mofid Children Hospital, Shariati Ave,
Tehran, Iran
Email:shakibamarjan@yahoo.com

quantifying GAGs is linked to the progressive decrease of urinary GAG levels that occurs with age. In the case of MPS, the normal age-related decline in urinary GAGs should be taken into account when diagnosis is suspected in older individuals. One-dimensional electrophoresis is used to separate the main classes of GAGs: chondroitin sulphate, keratan sulphate, dermatan sulphate and heparan sulphate. In the normal physiological situation, only chondroitin sulphate is detectable, except in the urine of newborns, where a band corresponding to heparan sulphate may be present. Although urinary GAG levels may be affected by nutrient levels and drugs, the detection of keratan sulphate, dermatan sulphate and heparan sulphate may suggest the possibility of an MPS.

Global tests Recognition of the high combined prevalence of LSDs and the increasing availability of specific therapies have encouraged the search for reliable biomarkers that can be used to identify LSDs in newborns or in high-risk populations. It has been suggested that LAMP-1, LAMP-2, saposins and GM2-ganglioside are elevated in almost all LSDs.

Assessment of specific substrates The development of tandem mass spectrometry for the identification and quantification of lysosomal substrates and metabolites has been a significant advance in the diagnosis of LSDs. In almost all cases, glycosphingolipids and oligosaccharides analysed by this method have been shown to differ significantly in controls and affected patients: 12 diseases were identified in 47 patients, with only two cases not presenting with an elevation of the corresponding substrate

Assessment of lysosomal enzyme activities

Lysosomal enzyme activities are usually determined by a fluorometric assay in cultured fibroblasts, leukocytes or sera, using a 4-methylumbelliferyl-containing fluorescent substrate. The activity of another lysosomal enzyme should also be assayed as a control for cell integrity.

LSDs can be associated with either low or undetectable enzyme activity. In some diseases, a correlation has been found between the level of residual enzyme activity and phenotypic severity

It is noteworthy that, in some cases, lysosomal enzyme activity in vitro can be low in samples taken from individuals who do not have an LSD, a condition termed pseudodeficiency. Such pseudodeficiency has been reported for β -galactocerebrosidase, β -glucuronidase, β -glucosidase, β -hexosaminidase A and arylsulphatase A, and is linked to specific mutations

Indirect biomarkers Indirect biomarkers may be useful for the identification of LSDs and for monitoring the effects of treatment. For example, increased plasma levels of two molecules, chitotriosidase and CC 18/pulmonary and activation-regulated chemokine (PARC), have been reported in patients with Gaucher disease.

Molecular genetics

LSDs are monogenic diseases, the majority of which have an autosomal recessive mode of inheritance. To date, X-linked inheritance has been observed in Hunter disease, Fabry disease and Danon disease. Identification of the mutations responsible for the LSDs has facilitated understanding of the pathophysiology of these diseases. It also enables prenatal and postnatal testing and allows the provision of genetic counselling.

Keywords: Lysosomal storage disease; Urinary oligosaccharides; Urinary glycosaminoglycans; Enzyme activities