UTILITY OF SERUM LYSOSOMAL ENZYME ASSAY IN THE DETECTION OF CEREBRAL SPHINGOLIPIDOSES IN PATIENTS WITH PROGRESSIVE NEUROLOGIC DYSFUNCTION

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ABSTRACT

The cerebral sphingolipidoses which form part of a larger group of lysosomal disorders can be detected and conclusively confirmed by the demonstration of the relevant enzyme deficiency in easily available tissue samples like serum. We have assayed acid β -galactosidase, β -hexosaminidase and its isozymes hexosaminidase A and B, and arylsulfatase A in the serum of patients with progressive cerebral dysfunction and detected 18 patients with enzyme defects, thereby confirming the diagnosis of a specific type of cerebral lipidosis in these patients. The assay of serum lysosomal enzymes was of immense diagnostic use as it obviated the need for highly invasive techniques like a brain biopsy.

KEY WORDS: Cerebral sphingolipidoses serum, acid β -galactosidase, β -hexosaminidase, arylsulphatase A.

INTRODUCTION

The cerebral sphingolipidoses are a group of rare inherited storage disorders resulting from a deficient activity of lysosomal hydrolases required for the metabolism of sphingolipids. The defect may result from the lack of a specific lysosomal enzyme, or a protein cofactor, or a membrane transport protein or the faulty processing of a lysosomal enzyme. The diagnosis of a cerebral lipidosis is often suspected on clinical grounds. The patient is usually an infant or child with a history of mental regression after a period of normal early development. Involvement of the brain gray matter presenting with seizures and abnormalities of cognition, vision, and hearing and pyramidal signs is characteristic of ganglioside storage disorders. Signs of the brain white matter disease like loss of motor skill, spasticity, or ataxia are typical of metachromatic leukodystrophy. However, confirmation of a clinical diagnosis requires the demonstration of the enzyme defect. The activity of the enzymes can be measured in easily obtained tissue samples such as

leukocytes, serum or cultured skin fibroblasts (1). The present paper deals with our experience in demonstrating the relevant lysosomal enzyme deficiency in the serum of patients with cerebral lipidoses presenting with symptoms of progressive neurologic dysfunction in order to conclusively establish the diagnosis.

MATERIALS AND METHODS

All patients from infant to adult, who had unexplained developmental delay, suppression of previously learned skills, loss of fine motor control, or ataxia associated with seizures, startle myoclonus, coarse facies, organomegaly, skeletal deformities, speech, hearing or visual impairment, were included in the study. 6-8 ml clotted blood was collected from these patients and serum separated immediately. A short clinical history was recorded at the time of sample collection and the enzymes to be assayed decided according to the clinical findings (Table 1). Clotted blood was also collected from 102 clinically normal control subjects with no history of any neurologic disorder.

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Table 1.

Disorder	Onset	Enzyme defect	Clinical features
GM1 gangliosidosis			
Infantile	at birth	Acid β -galactosidase	Seizures, rapid neurologic decline, bony abnormalities. Ataxia, strabismus, loss of speech, mental motor deterioration. Progressive dysarthria spasticity, ataxia.
Juvenile	6-20 mths	"	
Adult	teens	"	
GM2 gangliosidosis			
Tay-Sachs	3-6 mths	Hexosaminidase A	Mental-motor regression startle myoclonus, blindness, seizures, cherry red, maculae.
Sandhoff	3-6 mths	Hexosaminidase A and B	As above, bony abnormalities.
Late-onset	>6 yrs	Hexosaminidase A	Ataxia, muscle weakness tremors
Metachromatic leukodystrophy			
Late-infantile	1-3 yrs	Arylsulfatase A	Psychomotor regression ataxia, visual, speech impairment.
Juvenile	4-16 yrs	"	Ataxia, blindness, loss of speech, peripheral neuropathy, dementia.
Adult	>17 yrs	"	Ataxia, peripheral neuropathy, dementia.

The enzymes estimated were total β -hexosaminidase, hexosaminidase isoenzymes, hexosaminidase A and B, acid β -galactosidase and arylsulfatase A. Serum total β -hexosaminidase was assayed by the fluorimetric method of O'Brien et al (2) using 4-methylumbelliferyl N-acetyl β -D-glucosaminide as the substrate. The enzyme activity was expressed as nanomoles of 4-methylumbelliferone released by 1 ml of serum per hour at 37°C. Hexosaminidase isoenzymes were estimated by the heat inactivation method. Serum was incubated at 60°C for 3 minutes to inactivate the heat labile hexosaminidase A, and the heat

stable hexosaminidase B was assayed by the above method. The activity of hexosaminidase A was calculated by subtracting the hexosaminidase B activity from the total activity. Acid β -galactosidase was estimated by fluorimetric method using 4-methylumbelliferyl β -galactoside as the substrate (3). The measurement of serum arylsulfatase A was done using p-nitrocatechol sulfate essentially as described by Baum et al (4,5). The arylsulfatase A activity was expressed as nanomoles of p-nitrocatechol released/ml serum/4 hours at 37°C. The conditions employed for the assay of the individual enzymes are enumerated in table 2.

Table 2. The important assay conditions for the lysosomal enzymes.

Assay conditions	β -hexosaminidase	β -galactosidase	Arylsulfatase A
Method	Fluorimetric	Fluorimetric	Spectrophotometric
Substrate	4MU- β -GlcNAc	4MU- β -gal	p-nitrocatechol sulfate
Concentration	1.0 mM	0.5 mM	20 mM
Buffer	0.04 M citrate-phosphate pH 4.4	0.1 M acetate 0.1 M NaCl pH 4.0	1.0 M acetate 1.7 M NaCl+0.5mM Na pyrophosphate pH 4.9
Assay composition	50 uL of 1:10 diluted serum + 100 uL substrate in buffer	100 uL of 1:10 diluted serum + 100 uL substrate in buffer	0.2 ml serum + 0.4 ml substrate in buffer
Incubation time (37°C)	1 hr	1 hr	4 hrs
Stopping solution	0.17 M glycine-carbonate buffer, pH 9.5	0.17M glycine-carbonate buffer, pH 9.5	2mM EDTA in 2.5 N NaOH
Wavelength	Excitation 365nm Emmission 450nm	Excitation 365nm Emmission 450nm	515 nm
4MU- β -GlcNAc..	4-methylumbelliferyl N-acetyl β -D-glucosaminide		
4MU- β -Gal ..	4-methylumbelliferyl β -galactoside		

RESULTS

Seven cases of GM2 gangliosidosis with abnormalities in the serum total hexosaminidase or hexosaminidase isoenzymes were detected among the 567 patients tested. This includes 3 cases of Tay-Sachs disease with severely reduced hexosaminidase A activity, 3 cases of Sandhoff disease with almost absent total hexosaminidase activity and one case of late-onset GM2 gangliosidosis with decreased hexosaminidase A (Figure 1). Eleven cases of metachromatic leukodystrophy (MLD) with reduced serum arylsulfatase A were also detected in the 352 patients screened. Based on the age of onset, 3 patients had the late-infantile form (onset < 3 years), 6 patients had the juvenile form (4-16 years), and two had the adult form of MLD (>17 years) (Figure 2). Although a deficiency of arylsulfatase A was found in all patients with MLD, it was significantly greater in the late-infantile form

when compared to the adult form. A deficient activity of acid β -galactosidase was not detected in the patients screened.

The important clinical features of the patients with GM2 gangliosidosis and metachromatic leukodystrophy is shown in table 3. Tay-Sachs disease presented with psychomotor regression, startle myoclonus, macular cherry red spots and blindness due to optic atrophy. In addition to these features, patients with Sandhoff disease had skeletal abnormalities and hepatosplenomegaly. The brain CT scan showed cerebral and brain stem atrophy in all the cases of Tay-Sachs disease and in two cases of Sandhoff disease. EEG recordings of all these cases revealed slowing of the background activity to theta and delta range with myoclonic startle response to external stimuli. The single patient with the late-onset GM2 gangliosidosis presented with ataxia, dysarthria and dystonia.

Figure 1 **Legend** : Serum hexosaminidase activity in GM2 gangliosidoses

Total: Total hexosaminidase

Hex A: Hexosaminidase A

Hex B: Hexosaminidase B

Figure 2 **Legend** : Serum arylsulfatase A activity in metachromatic leukodystrophy

Table 3. Some important clinical features in the cerebral lipidoses detected.

Clinical finding	GM2 Tay-Sachs disease	gangliosidoses Sandhoff disease	Late-onset GM2	Late Infantile	MLD Juvenile	Adult
	n=3	n=3	n=1	n=3	n=6	n=2
Psychomotor regression	3	3	-	3	2	-
Ataxia	-	-	1	3	6	2
Seizures	2	2	-	1	1	1
Startle myoclonus	3	3	-	-	-	-
Visual impairment	3	3	-	2	2	1
Speech impairment	3	3	1	3	6	2
Deafness	-	-	-	1	-	1
Muscle weakness	-	-	1	-	-	-
Tremors	-	-	1	1	2	-
Macrocephaly	-	1	-	-	-	-
Gait disturbances	-	-	1	3	6	2
Peripheral neuropathy	-	-	-	3	6	2
Skeletal abnormalities	-	2	1	-	-	-
Organomegaly	-	2	-	-	-	-
Cherry-red maculae	3	3	-	-	-	-
Psychosis	-	-	-	-	-	2
Dementia	-	-	-	-	-	2

All MLD patients presented with ataxia, speech and visual impairment and gait disturbances. In addition, patients with the late-infantile form presented with psychomotor regression and the adult type with psychotic features and dementia. Cranial CT scans showed white matter hypodensity in all cases of MLD. A decrease in the motor nerve conduction velocity was noted in all these cases. Abnormal metachromatic inclusions were seen in the sural nerve biopsy specimens in late-infantile and juvenile forms.

DISCUSSION

The cerebral sphingolipidoses which form part of the larger group of lysosomal disorders can be conclusively diagnosed by specific enzyme assays in the serum, leukocytes or cultured skin fibroblasts. Defects of ganglioside degradation can be divided into 2 groups, the GM1 gangliosidoses and the GM2

gangliosidoses. The GM1 gangliosidoses which is due to a deficiency of acid β -galactosidase include patients with acute infantile onset, rapid neurologic decline, and severe bony abnormalities at one end of spectrum and, normal intelligence and survival to adulthood at the other end. This inherited disorder can be diagnosed by demonstrating a deficiency of acid β -galactosidase in the serum or leukocytes using the artificial methylumbelliferyl substrate.

The GM2 gangliosidoses include the infantile forms, Tay-Sachs and Sandhoff disease and their variants and the late-onset GM2 gangliosidoses. These inborn errors of GM2 ganglioside metabolism cause GM2 ganglioside to accumulate within the lysosomes of the nerve cells. Infants with Tay-Sachs disease develop psychomotor regression, blindness, seizures, startle myoclonus and macrocephaly and die within a few years. The

diagnosis is established by demonstration of a deficiency of the β -hexosaminidase isoenzyme, hexosaminidase A, in the serum, leukocytes or cultured fibroblasts using 4-methylumbelliferyl N-acetyl- β -glucosaminide as the substrate. since hexosaminidase B is more heatstable than hexosaminidase A, the latter can be demonstrated as the proportion of the heat-labile β -hexosaminidase after a controlled heat inactivation prior to enzyme assay.

Sandhoff disease results from a combined deficiency of hexosaminidase A and B in all tissues and is accompanied by added storage of hexosamine-containing neutral glycolipids. It is clinically very similar to Tay-Sachs disease. The disease can be diagnosed by demonstrating deficiency of total β -hexosaminidase (both A and B forms) in the serum. The patients with late onset GM2 gangliosidoses present with signs of cerebellar and anterior horn cell involvement, muscle weakness, ataxia and tremors. The deficiency of hexosaminidase A in late-onset GM2 gangliosidoses varies from partial to almost complete. The residual activity of hexosaminidase A can be as high as 30-40% in the serum.

Metachromatic leukodystrophy (MLD), an inherited disorder of myelin metabolism resulting from a deficient cerebroside sulfatase (arylsulfatase A) activity is characterised by accumulation of galactosyl sulfatide in white matter of the central nervous system and in the peripheral nerves. The earliest signs in the various forms of this disease are gait disturbances and ataxia. It can be diagnosed by demonstrating a severe deficiency of arylsulfatase

A activity in the serum. Assay of arylsulfatase A is usually carried out using p-nitrocatechol sulfate as substrate. Since arylsulfatase B also releases sulfate from this substrate, conditions of the assay are adjusted to minimize arylsulfatase B activity. The procedure of Percy and Brady is carried out in pH 4.9, rather than pH 6, which favour arylsulfatase B activity, and in the presence of chloride and pyrophosphate, which inhibit arylsulfatase B more than arylsulfatase A. Using these conditions arylsulfatase B contributes only 2% of activity.

Using the above methods, patients with undiagnosed neurodegenerative disease were screened for a few lysosomal disorders which resulted in confirming the diagnosis in a few patients. In our screening of 567 patients we have conclusively diagnosed 18 patients with an overall diagnostic rate of about 3.17%. Although this percentage in no way reflects the incidence of these disorders in the population studied, it does show that patients with cerebral sphingolipidoses can be correctly diagnosed without the need for highly invasive techniques.

In conclusion, assay of serum lysosomal enzymes offers an unique opportunity for detection of cerebral lipidoses in patients with signs of progressive neurologic dysfunction. serum is easily accessible and offers an excellent source of information from living patients that does not require brain biopsy with its associated morbidity. With a little effort, these methods can be performed as a routine diagnostic procedure in any well equipped biochemistry laboratory.

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