The Turkish Journal of Pediatrics 36: 215-221 1994

A STUDY ON ENZYME ACTIVITIES OF SOME SPHINGOLIPIDOSES*

*H. Asuman Özkara MD**, M. Çevik Arıkan PhD***, Meral Topçu MD****
*Serap Emre PhD***, Yavuz Renda MD*****

SUMMARY: Özkara HA, Çevik Arıkan M, Topçu M, Emre S, Renda Y. (Department of Biochemistry, Hacettepe University Faculty of Medicine, Ankara, Turkey). A study on enzyme activities of some sphingolipidoses. Turk J Pediatr 1994; 36: 215-221.

Enzyme activities were determined in fibroblast cell cultures of eight patients suspected of having a type of sphingolipidosis. The patients were 0 to 4 years of age; four were female and four were male. Thirteen age-matched controls were also included in the study.

In one of the cases, hexosaminidase A activity was found to be 0% (43-82%), while in two other cases β-galactosidase activity was found to be 5 nmol/h/mg protein (100-1035 nmol/h/mg protein) and arylsulfatase activity was found to be 12 nmol/h/mg protein (106-990 nmol/h/mg protein), respectively. Two more enzymes, α-galactosidase (11-39 nmol/h/mg protein) and cerebroside β-galactosidase (3.7-6.9 nmol/h/mg protein), were also evaluated but were found to be in the normal ranges in these patients. Therefore, these patients were considered to have Tay-Sachs disease, GM1 gangliosidosis and metachromatic leukodystrophy, respectively.

The remaining five patients were normal in respect to the five enzyme activities determined.

For the prenatal diagnosis of metachromatic leukodystrophy, arylsulfatase A activity was determined in one amniotic cell culture. The activity found in this case was lower than normal (34 nmol/h/mg protein versus 387 nmol/h/mg protein found in three control amniotic cell cultures). *Key words: sphingolipidoses, lysosomal enzymes, prenatal diagnosis, postnatal diagnosis.*

The sphingolipidoses are defined as disorders caused by genetic defects in catabolism of sphingosine-containing lipids. Underlying genetic defects are mutations in lysosomal hydrolases or activator proteins[1]. Major sphingolipidoses are Farber disease, Niemann-Pick disease, Krabbe disease, metachromatic leukodystrophy, Gaucher disease, Fabry disease, Tay-Sachs disease, GM1 gangliosidosis and Sandhoff disease (Table I)[1].

* From the Department of Biochemistry, Hacettepe University Faculty of Medicine, Ankara.
** Instructor in Biochemistry, Hacettepe University Faculty of Medicine.
*** Assistant Professor of Medical Biology, Hacettepe University Faculty of Medicine.
**** Associate Professor of Pediatrics and Pediatric Neurologist, Hacettepe University Faculty of Medicine.
***** Professor of Pediatrics and Pediatric Neurologist, Hacettepe University Faculty of Medicine.

Table I: Major Sphingolipidoses

Disease	Storage Material	Deficient Enzyme
1. Farber	Ceramide	Acid ceramidase
2. Niemann-Pick	Sphingomyeline	Sphingomyelinase
3. Krabbe	Galactosylceramide, Galactosylsphingosine	Cerebroside β-galactosidase
4. Metachromatic leukodystrophy	Sulphatide	Arylsulphatase A
5. Gaucher	Glucosylceramide, Glycosylsphingosine	Glucosylceramidase
6. Fabry	Trihexosylceramide	α-galactosidase A
7. GM1 gangliosidoses	GM1 ganglioside, Glycoproteins	β-galactosidase
8. Tay-Sachs	GM2 ganglioside	β-hexosaminidase A
9. Sandhoff	GM2 ganglioside, Asialoganglioside GM2	β-hexosaminidase A and B

The disorders are generally classified according to the age of onset as infantile, late infantile, juvenile and adult cases. Infantile patients have virtually no detectable enzyme activity[2]. Patients affected by the sphingolipidoses can be diagnosed definitively by appropriate enzyme assays, and heterozygous carriers can be identified in most instances with reasonable reliability in serum, urine, leukocytes and fibroblasts. Affected fetuses can be diagnosed during pregnancy either with cultured amniotic fluid cells or with biopsied chorionic villi[3-9].

In Turkey, the incidence of consanguineous marriages is relatively high (21.2%)[10]. Therefore, we may expect a high incidence of autosomal recessive disorders in the Turkish populatilon. The aim of this study was to diagnose sphingolipidoses either prenatally or postnatally by related enzyme activity determinations.

Material and Methods

The patients were from Hacettepe University Children's Hospital Pediatric Neurology Unit and were suspected of having a type of sphingolipidosis. The study group comprised four female and four male patients between the ages of 0 and 4 years. Enzyme activity was determined in only one amniotic fluid cell culture for metachromatic leukodystrophy. Thirteen age-matched controls (skin fibroblast

culture) and three amniotic fluid cell culture controls were also included in the study.

Until the time of enzyme activity determination, cells from tissue cultures were frozen at −50°C and investigated within one month.

Case Reports

Case 1

E.Y. was a 10-month-old boy admitted to Hacettepe Children's Hospital with complaints of hypotonicity, motor and mental deterioration which started at five months of age. He was the second child of nonconsanguineous parents. On physical examination his height was 75 cm, weight was 12 kg and head circumference was 45 cm. He had prominent hypotonicity and hyperacusia. On eyeground examination cherry-red spots were seen bilaterally. There was no organomegaly. Deep tendon reflexes were hypoactive bilaterally. On laboratory examination, hexosaminidase A (Hex. A) activity was found to be 0% in cultured skin fibroblasts. The patient was diagnosed with Tay-Sachs disease.

Case 2

İ.M. was a 16-month-old boy followed up at Hacettepe Children's Hospital with motor and mental deterioration, recurrent pulmonary infections and convulsions. He was the first child of nonconsanguineous parents. Signs of deterioration first appeared at six months of age. On physical examination his height was 72 cm, weight was 9 kg and head circumference was 45 cm. He had coarse facial features and was unable to support his head. The liver was palpable six cm below the right costal margin. On eyeground examination, the optic discs were pale bilaterally. On laboratory examination, urine mucopolysaccharides were found to be less than 3 mg/dl (normal: <3 mg/dl), bone marrow aspiration was normal: X-rays of his extremities and skull displayed changes compatible with mucopolysaccharidosis, while computerized brain tomography displayed a leukodystrophic appearance. In the postmortem skin fibroblast cell cultures, β-galactosidase activity was found to have 4.78 nmol/h/mg protein. The patient was diagnosed with GM1 gangliosidosis.

Case 3

S.T. was a stillbirth from a 32-week pregnancy at risk for metachromatic leukodystrophy. The family also had a child with metachromatic leukodystrophy who had died at three years of age. The mother did not come for amniotic fluid examination during the early months of pregnancy. The patient was born spontaneously at 32 weeks gestation. Arylsulphatase A activity was found to be

severely deficient (12 nmoles/h/mg protein) in postmortem fibroblast cell cultures, and the patient was diagnosed with metachromatic leukodystrophy.

Skin Fibroblast Culture Procedure

After skin disinfection and local anesthesia, a skin sample of 3 mm by 3 mm by 1 mm deep was taken from the medial aspect of the forearm. The skin fragment was then transferred to a sterile bottle containing Ham's F10 culture medium with antibiotic-antimycotic solution (10.000 µ penicillin/ml, 10 µg streptomycine/ml, 2.5 µg amphotericin B/ml).

The sample was then divided into three to five pieces and transferred to one or two 25 cm^2 culture flasks which had previously been moistened with 2-3 ml of culture media. To facilitate adherence of the skin fragments, the flasks were inverted and incubated at 37°C in an environment consisting of 5 percent CO_2 and 95 percent air. After two to three hours, the flasks were turned right-side-up and supplemented with 5 ml of fresh growth medium (Ham's F10, Sigma, USA) containing 20 percent fetal calf serum (Seromed, Germany), 100 U/ml penicillin and 100 µg/ml streptomycine (Flow, England). When confluency was attained, the cells were trypsinized with trypsin-EDTA (0.05%), 0.02%, Sigma, USA). This subcultivation (p1 passage) was continued until sufficient cells were available, and samples were kept at −50°C until the time of investigation.

Amniotic Fluid Cell Culture Procedure

A sample of 10-20 ml of amniotic fluid was removed by transabdominal puncture at 15-16 weeks gestation. The amniotic fluid and cells were separated by centrifugation, and the cells were placed in a culture medium (Ham's F12) (Seromed, Germany) supplemented with 20 percent fetal calf serum. Penicillin (100 U/ml), streptomycine (100 µg/ml) and Hepes (100 u/ml) were added. The primary cultures of all the amniotic cells underwent one passage before the enzyme analysis.

Enzyme Activity Determination

Galactosylceramide sulphate, p-nitrocatecole sulphate, trinitrophenylaminolauryl-galactocerebroside, 4-methyl-umbelliferyl ∝-galactoside, 4-methylumbelliferyl β-galactoside, 4-methylumbelliferyl N-Acetyl β-glucosaminide and p-nitrocatecole were purchased from Sigma, USA; 4-methyl-umbelliferone, bovine serum albumin were purchased from Serva, Germany.

Fibroblast and amnion fluid cell cultures were homogenized with freezing and thawing three times before protein determination. The protein concentration was measured using the method of Lowry et al[11] with bovine serum albumin as standard.

Hexosaminidase A, β-galactosidase, arylsulphatase A and α-galactosidase were assayed using 5-15 µg, 20-50 µg, 100-400 µg and 50-200 µg of cellular proteins, respectively, according to Suzuki[1]. Cerebroside β-galactosidase was assayed using 100-400 µg of cellular protein according to Besley and Gatt[12]. All the assays were performed in duplicate.

Enzyme activities were expressed as nanomoles of substrate hydrolysed per milligram of protein per hour.

Results

Normal values for enzyme activities are given in Table II. In one patient, Hex A was totally inactive (0%). In another patient, β-galactosidase activity was found to be significantly low (4 nmol/h/mg protein). Arylsulphatase A activity was found to be lower than normal controls (12 nmol/h/mg protein) in a third patient. In another case, arylsulphatase A activity was determined as 33 nmol/h/mg of protein in cultured amniotic cells, which led to a prenatal diagnosis of metachromatic leukodystrophy.

Table II: Enzyme Activity Levels in Control Skin Fibroblasts and Amniotic Cell Cultures

	Skin Fibroblast Culture		Amnion Cell Culture	
Enzyme	Sample (n)	Mean±SD	Sample (n)	Mean±SD
Hexosaminidase A (%)	14	63.65±12.63 (43–82.6)*	4	76.68±5.24 (74.38±86.2)
β-galactosidase (nmol/h/mg protein)	14	417.65±224.54 (100-1035)	3	838.9±625.7 (235.8-1485)
α-galactosidase (nmol/h/mg protein)	9	24.89±9.71 (11.1±39.2)	–	–
Arylsulphatase A (nmol/h/mg protein)	10	442.7±348.37 (106-990)	1	387.16
Cerebroside β-galactosidase (nmol/h/mg protein)	7	5.3±1.33 (3.69±6.87)	–	–

* Numbers in parentheses indicate lowest and highest values.

Discussion

Sphingolipidoses, which are a group of metabolic and inherited diseases causing mental retardation, can be diagnosed prenatally by measuring the activities of

some specific enzymes in amniotic cells[1]. The correct diagnosis of affected siblings is an important prerequisite for prenatal testing of the risk groups[13].

The normal values of the enzyme activities reported here correlated closely with the results of the following reports. O'Brien et al[14] reported Hex A levels of 62-79 percent and 50-60 percent as normal values for amniotic cell cultures and fibroblast cell cultures, respectively. In two other studies, normal levels were reported as 62-80 percent for amniotic cells[15] and 46-47 percent for fibroblasts[16]. Our patient with undetectable Hex A activity had a clinical diagnosis of Tay-Sachs disease. The activity of arylsulphatase A increases during fetal development and postnatal life, a fact which must be taken into consideration in the evaluation of the test results[5]. Similar studies revealed normal levels of 146-760 nmol/h/mg protein[17], and 181-576 nmol/h/mg protein[15] for amniotic cells, and 263-981 nmol/h/mg protein[18] for skin fibroblasts. Our patient with 4 nmol/h/mg protein β-galactosidase activity had nonspecific clinical signs, and the diagnosis was established as GM1 gangliosidosis only after enzyme activity determination. α-galactosidase activity was 35-90 nmol/h/mg protein and 25-60 nmol/h/mg protein and 25-60 nmol/h/mg protein for normal fibroblast and amniotic cell cultures, respectively[7]. Normal cerebroside β-galactosidase activity was reported to be 2.2-3.6 nmol/h/mg protein for amniotic cells in another study[8].

Although we had no patients with the diagnosis of Fabry's or Krabbe's disease, we tried to establish normal levels of α-galactosidase and cerebroside β-galactosidase enzyme levels.

In conclusion, our study is a preliminary attempt to establish a prenatal and postnatal diagnostic set of assays for evaluating groups at risk for sphingolipidoses in our country.

Acknowledgements

This study was supported in part by the Turkish Pediatric Neurology Society.

REFERENCES

1. Suzuki K. Enzymatic diagnosis of sphingolipidoses. In: Ginsburg V (ed). Methods in Enzymology Vol.: 138. Florida: Academic Press; 1987: 727-63.
2. Sandhoff K, Conzelmann E, Neufeld EF, Kaback MM, Suzuki K. The GM2 gangliosidoses. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). The Metabolic Basis of Inherited Disease (6th ed). New York: McGraw-Hill; 1989: 1807-39.
3. O'Brien JS. The gangliosidoses. In: Stanbury JB, Wyngaarden JB, Fredrickson DS (eds). The Metabolic Basis of Inherited Diseases, (5th ed). New York: McGraw-Hill; 1983: 945-66.
4. Benson PF, Fensom AH. Genetic Biochemical Disorders. Oxford: Oxford University Press; 1985: 81-149.

5. Kolodny EH. Metachromatic leukodystrophy and multiple sulphatase deficiency: sulphatide lipidosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds), The Metabolic Basis of Inherited Disease (6th ed). New York: McGraw-Hill; 1989: 1721-50.
6. Hatton CE, Cooper A, Sardharwalla IB. Detection of Fabry's disease carriers by enzyme assay of hair roots. J Inherited Metab Dis 1989; 12: 369-71.
7. Kleijer WJ, Hussaarts-Odijik LM, Sachs Es, Jahoda MGJ, Niermeijer MF. Prenatal diagnosis of Fabry's disease by direct analysis of chorionic villi. Prenat Diag 1987; 7: 283-7.
8. Giles L, Cooper A, Fowler B, Sardharwalla IB, Donnai P. Krabbe's disease: first trimester diagnosis confirmed on cultured amniotic fluid cells and fetal tissues. Prenat Diag 1987; 7: 329-32.
9. Harzer K, Schuster I. Prenatal enzymatic diagnosis of Krabbe disease (globoid-cell leukodystrophy) using chorionic villi. Pitfalls in the use of uncultured villi. Hum Genet 1989; 84: 83-5.
10. Başaran N, Şaylı BS, Başaran A, Solak M, Artan S, Stevenson JD. Consanguineous marriages in the Turkish population. Clin Genet 1988; 34: 339-41.
11. Lowry OH, Rosebrough NJ, Farr L, Randell RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193: 265-75.
12. Besley GTN, Gatt S. Spectrophotometric and fluorimetric assays of galactocerebrosidase activity, their use in the diagnosis of Krabbe's disease. Clin Chim Acta 1981; 110: 19-26.
13. Winchester B. Prenatal diagnosis of enzyme defects. Arch Dis Child 1990; 65: 59-67.
14. O'Brien JS, Okada S, Fillerup DL, et al. Tay-Sachs disease: prenatal diagnosis. Science 1971; 172: 61-4.
15. Gatti R, Lombardo C, Filocamo M, Borrone C, Porro E Comparative study of 15 lysosomal enzymes in chorionic villi and cultured amniotic fluid cells. Prenat Diag 1985; 5: 329-36.
16. Hechtman P, Khoo K, Isaacs C. A new form of residual hexosaminidase activity in infantile Tay Sachs disease fibroblasts. Clin Genet 1983; 24: 206-215.
17. Sanguinetti N, Marsh J, Jackson M, Fensom AH, Warren RC, Rodeck CH. The aryl-sulphatases of chorionic villi: potential problems in the first-trimester diagnosis of metachromatic leucodystrophy and Maroteaux-Lamy disease. Clin Genet 1986; 30: 302-8.
18. Suzuki Y, Nakamura N, Fukuoka K, Shimada Y, Uono M. β-galactosidase deficiency in juvenile and adult patients. Hum Genet 1977; 36: 219-29.