GM1-gangliosidosis is a very rare autosomal recessive genetic-metabolic disorder, caused by deficiency of the lysosomal enzyme ganglioside-β-galactosidase that results in accumulation of glycosaminoglycans, oligosaccharides, and especially GM1-ganglioside. Herein, we report the clinical and laboratory findings of two Iranian families with 6 affected cases. In one of the families, four of the affected children died in the childhood period. All of the deceased cases were investigated thoroughly before and the diagnosis of Nieman-Pick's disease was suggested for them; enzymatic analysis had not been carried out for these cases. In the alive probands of the two families, enzyme assays showed that the sphingomyelinase activity was within normal limits, but ganglioside-β-galactosidase activity was deficient in both of them. Enzyme assays of the patients was carried out in the Metabolic Department of the Erasmus University based in the Rotterdam the Netherlands.

Measurement of the activity of the lysosomal enzyme ganglioside-α-galactosidase showed deficiency in both of the probands. Sphingomyelinase and other lysosomal enzyme activity were within normal limits.

Diagnosis in these two families was GM1-gangliosidosis. To date these are the only affected cases confirmed by enzyme assays in Iran.

Key Words: Lipid storage diseases, GM1-gangliosidosis, Ganglioside-β-galactosidase deficiency, Prenatal diagnosis, Genetic counseling

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Introduction

Lysosomal storage diseases (LSDs) including the sphingolipidoses, account for a substantial proportion of neurometabolic disorders. The sphingolipidoses are genetic diseases in which a mutation in a gene responsible for the production of the lysosomal hydrolases or activator proteins blocks sphingolipid degradation, leading to lysosomal accumulation of the enzyme's specific sphingolipid substrate. Since glycosphingolipids are essential components of all cell membranes, the inability to degrade these sub-
stances and their subsequent accumulation result in the physiologic and morphologic alterations and produce characteristic clinical manifestations. There is almost no effective therapy. Although very rare, but in a few isolated ethnic populations, such as Ashkenazi Jews, some of these disorders, like Tay-Sacks and Sandhoff's diseases are very common. The prevalence of some of the LSDs, are reduced by population-based screening programmes (illustrated by the success of the Tay-Sachs initiative)\textsuperscript{11}.

One of these diseases, generalized or GM\textsubscript{1}-gangliosidosis, was recognized and reported by O'Brien in 1965 for the first time.\textsuperscript{2} This disorder is characterized by deficiency of the activity of the enzyme ganglioside-\(\beta\)-galactosidase, resulting in accumulation of glycolipids, keratan sulfate, and especially GM\textsubscript{1}-ganglioside in different tissues.

GM\textsubscript{1}-ganglioside considerably increased, especially in the brain, liver and spleen. In addition, keratan sulfate, a mucopolysaccharide, accumulates in the liver and is excreted in the urine of these patients.\textsuperscript{3} There are four types of \(\beta\)-galactosidase isoenzyme; A\textsubscript{1} (monomeric), A\textsubscript{2} (Dimeric), A\textsubscript{3} (multimeric), and a neutral type. The enzyme ganglioside-\(\beta\)-galactosidase gene is located on the short arm of the chromosome 3 (3p21.33). The complete genomic region has been isolated, mapped, sequenced, and several mutations have been identified.

**GM\textsubscript{1}-gangliosidosis classifications**

Clinically GM\textsubscript{1}-gangliosidosis is classified into three phenotypes:

**Infantile type I:** Symptoms appear during early infantile life with growth deficiency; progressive neurological impairment and death are mostly caused by bronchopneumonia when the children are 3 to 4 years old. Hepatosplenomegaly, bone alterations similar to dysostosis multiplex, coarse facies, cherry red spot on the retina and macrocephaly are frequent findings in the infantile type I disease.

**Juvenile type II:** This type occurs less common than type I, disease. The age of onset is delayed, and the illness progresses slowly, and death occurs usually by the end of the childhood period though some patients may survive through the 4\textsuperscript{th} decade of their life. Initial manifestations may be gait disturbance, spasticity, dystonia and ataxia. The natural history will progress to the loss of language and motor milestones, seizure, and progressive deterioration to a persistent vegetative state. Bony abnormalities may be mild and organomegaly can be absent.\textsuperscript{4}

**The adult type:** The patients start to show progressive cerebellar impairment during adolescence, and evolution is very slow (O'Brien 1983).\textsuperscript{2} There is no specific treatment for either form of GM\textsubscript{1}-gangliosidosis. All forms are inherited by the autosomal recessive pattern.

**Materials and methods**

We studied 153 families with 229 cases, referred with various neurologic symptoms and suspected of having one of the lysosomal storage disorders, and separated 57 families with 94 affected cases, suffering from one of the sphingolipidoses indicated by lysosomal enzyme assay. In two of these families GM\textsubscript{1}-gangliosidosis was detected. The diagnosis was confirmed by urine oligosaccharide chromatography, and blood and skin fibroblast enzyme assays conducted in the Genetics and Metabolic Department of the Erasmus University based in Rotterdam in the Netherlands.

**Family 1:** This family referred for genetic counseling with an affected daughter [S.F]. They lived in a small city in the northern province of Mazandaran. The proband was a product of the mother's 8\textsuperscript{th} pregnancy. Pregnancy and delivery were uneventful. Parents were healthy, first cousins. Mother's obstetrical history was Gravida 10, but they had only 2 live children, one of whom was healthy while the other was affected (Fig. 1).
Birth weight was 3600 grams and there was history of mild jaundice in the first few days of her life. The chief complaint in the proband was severe progressive psychomotor retardation and neurological regression. Convulsions were observed from birth, and recurrent pneumonias were reported since the age of seven months (Fig. 2).

Brain CT scan showed mild generalized cerebral atrophy. Bone marrow aspiration revealed accumulation of abnormal foamy histiocytes with eccentric nuclei. Liver biopsy was suggestive of lipid storage disease, too. The family had four affected children with a similar illnesses before, all of whom showed almost the same neurological deterioration, and succumbed between the ages 4 to 5 years (see the pedigree). The mother was pregnant and naturally worried about her fetus and for the confirmation of the diagnosis in the proband and prenatal screening for the fetus, leukocytes, isolated from the peripheral blood, and skin fibroblasts in a culture media of the proband and also amniotic fluid cells were sent for enzyme assay.

**Family 2:** The second family was not related to the first one, their origin being from the southern province of Shiraz. Parents were healthy first cousins and the proband was a girl and the only child of the family. There was no similar case in the pedigree; in her case although the symptoms began from early infancy, we saw her at the age of two years. The clinical findings were coarse facies, hypotonia, macrocephaly, frontal bossing, gum hypertrophy, joint stiffness, and hepatosplenomegaly (figures 3 and 4). Skeletal examination showed slight abnormalities similar to dysostosis multiplex congenita. Cherry red spots were detected on fundoscopic examination. Urine MPS was negative. According to the clinical and paraclinical findings, one of lipid storage disorders was suspected. Samples were sent for enzyme assay in this case too.

**Results**

The activity of β-galactosidase was grossly deficient (4 nmol/h/mg protein with normal range of 95 to 220) for the first case, and (4 nmol/h/mg protein or nmol/h/ml with the normal range of 100 to 250) for the second family probands, in the peripheral white blood cells and skin fibroblasts (courtesy of van Digglen et al).

Sphingomyelinase, Hexosaminidase A and B, Arylsulphatase A, and β-glucosidase activities were all within normal limits. Diagnosis of “GM1-gangliosidosis” was...
confirmed in these two families. Enzyme analysis of fetal cells in the first family was normal.

**Discussion**

During a period of over 15 years, we saw 57 families with 94 affected cases suspected of one of the sphingolipidoses that were referred for enzyme assay; in two families with 6 affected children the diagnosis was GM1-gangliosidosis. The relative incidence of GM1-gangliosidosis in our patient population is about 4%. In a similar study in Turkey, conducted by Ozkara and Topcu, evaluating 300 patients that were suspected of sphingolipidoses, 11.7% cases with GM1-gangliosidosis were found. Because of geographic, cultural, ethnic, and also consanguineous marriage similarities, the prevalence of this disease might be higher in our population too; this however needs a population based screening program to ascertain the exact rate.

Although symptoms are evident during the first months of life in type I GM1-gangliosidosis, diagnosis would be possible only when obvious physical and neurological deterioration is apparent. There is no ordinary method for screening to identify high risk families and cases. Vacuolated lymphocytes in peripheral blood smears were observed in all of the patients examined for characteristics. Because of the simplicity of the procedure, identification of vacuolated lymphocytes is a useful screening test. Cha-noles NA. et al. reported a new method for screening of different lipid storage disorders on a dry blood spot from newborn screening Guthrie cards stored at room temperature for about 15 months. In an affected symptomatic patient, activity of the enzyme β-galactosidase was less than 10% of that of normal controls.

Accumulation of GM1 ganglioside is due to a deficiency of ganglioside β-galactosidase, which results in an inability to cleave the terminal galactose from a number of compounds, including GM1 ganglioside, galactose-containing oligosaccharides and Keratan Sulfate. Several studies in animal models have shown that the accumulation of GM1-ganglioside may result in a progressive increase in local microglial activation / expansion.
sion and infiltration of inflammatory cells in CNS. These data suggest that inflammation may play an important role in the pathogenesis of the gangliosidoses.\(^\text{11}\)

Examination of urine oligosaccharides by chromatography method can show a characteristic pattern for GM1-gangliosidosis in the patients.\(^\text{7}\) Recently (Ramsay SL. et al.) introduced a method by ElectroSpray Ionization-Tandem Mass Spectrometry (ESI-MS/MS) for semiquantity measurement of urinary oligosaccharides from patients suffering from oligosaccharidurias. Disease specific oligosaccharides were identified for several entities, including GM1- and GM2-gangliosidoses, Sialidosis, Galactosialidosis, I-cell disease, Fucosidosis, Pompe and Gaucher’s disease, and α-Mannosidosis.\(^\text{12}\)

The human gene for ganglioside-β-galactosidase (GLB1) is located on the short arm of chromosome 3 (3p21.33). The gene produces two alternatively spliced transcripts that encode the lysosomal enzyme β-galactosidase(GBP1), and the elastin binding protein(EBP).\(^\text{13}\) Several mutations have been described till now, but many of them were in Japanese patients.\(^\text{4,7,8}\) Caciotti A et al evaluated 9 cases and found 4 new mutations and developed an RT-PCR method for GLB1 gene expression profile in Italy.\(^\text{13}\) Georgiou T et al reported high prevalence of a founder mutation in an isolated small mountainous village in Cyprus. Carrier rate for GM1-gangliosidosis in the village was about 8%.\(^\text{14}\)

The mutations in the infantile and adult forms of GM1-gangliosidosis interfere with the phosphorylation of β-galactosidase precursor. As a result, the precursor is secreted instead of being compartmentalized into the lysosomes and further being degraded. The impaired phosphorylation might be due to conformational change of the precursor molecule.\(^\text{6}\)

In view of the apparent low prevalence of GM1-gangliosidosis in the entire group of inborn metabolic errors, we should consider this entity in the differential diagnosis of neurometabolic disorders as well, because most of them present with similar symptoms and course, enzymatic assay is essential for confirmation of diagnosis in the lipid storage disorders. Determination of ganglioside-β-galactosidase activity in leukocytes and skin fibroblasts, proved to be a precise method both for detecting heterozygotes, and affected individuals. There are several new methods for evaluation like ElectroSpray Ionization-Tandem Mass Spectrometry (ESI-MS/MS), and dry blood spot screening Guthrie cards, that make diagnosis more easier and in the near future these will be available to most metabolic centres. Recent advances in molecular genetics also allow us to arrive at diagnosis by detection of private mutations in affected families.

For most of the sphingolipidoses including GM1-gangliosidosis, there are no efficient cures and treatments. Hence precise diagnosis can enable us to provide informative genetic counseling, perform prenatal diagnosis, and implement prevention measures for such patients.

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