Obstetrics; *Maternal - Fetal Medicine and Perinotology*

Prenatal Diagnosis of Sandhoff Disease by Enzyme Analysis of Chorionic Villus Sample

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OBJECTIVE AND STUDY DESIGN: The prenatal diagnosis of Sandhoff disease (SD) was performed in 14 fetuses of families by the analysis of chorionic villus sample obtained at 11-14 weeks of gestation. The diagnosis was based on the absence or near-absence of total hexosaminidase activity using fluorogenic synthetic substrate 4-methylumbelliferyl beta-D-glucosaminide.
RESULTS: 7 fetuses were found to be affected and the pregnancies were terminated. The remaining seven fetuses were found to be normal. The diagnosis were confirmed after delivery by enzyme analysis of leucocytes isolated from peripheric blood.
CONCLUSION: Chorionic villus sampling (CVS) is a safe and accurate method for the first trimester prenatal diagnosis of various genetical disorders if the amount of the chorionic villi taken is sufficient for enzyme analysis and if it is well separated from maternal blood or decidua. Hexosaminidase (Hex) assay using fluorogenic substrate is useful for specific prenatal diagnosis of SD.

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Key Words: Prenatal diagnosis, Sandhoff disease, Chorionic villus sampling, Hexosaminidase

Introduction

Sandhoff Disease (SD) is an autosomal recessively inherited fatal neurodegenerative disease, resulting from the deficiency of lysosomal enzyme β-hexosaminidase (β-hex). β-hex activity is absent or near-absent in SD. The function of β-hex is to hydrolyze the β-glycosidic linkage between the N-acetylgalactosaminyl residue and the galactose residue of glycosphingolipid GM2 ganglioside. This reaction requires the GM2 activator protein in vivo. In case of enzyme deficiency, GM2 ganglioside and related lipids accumulate in lysosomes of cells, particularly of neurons, and could lead neuronal death.[1]

The minimum incidence of SD in Turkish population is 1/104,838 live births and it is the third frequently seen disease among sphingolipidoses.[2] The importance of prenatal diagnosis for SD originates from the reality that there is no available specific therapy for the disease to date. Prenatal diagnosis of SD has been performed by using different samples such as chorionic villi, cultured amniotic fluid cells and fetal blood samples.[1] Among these, chorionic villus sample is preferred for providing a reliable material for the early prenatal detection of SD.[3] CVS has been used for the prenatal diagnosis of SD since 1992 in Hacettepe University.[4]

We report here the prenatal diagnosis of 14 at-risk fetuses by enzyme analysis using CVS taken by a single surgeon during the 2000-2007 period.

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Material and Methods

Patients

Fourteen at-risk pregnancies from 9 families who had a history of having a child with SD previously were evaluated by enzymatic analysis of CV samples.

CVS Procedure

Chorionic villus sampling procedure was performed transabdominally under ultrasound guidance at 11-14 weeks of gestational age using an 18-gauge needle (90 mm long) after ensuring that the fetus was alive. The villi were collected into MEM solution and any maternal decidual tissue among the villi was seperated under a dissection microscope.

Sample preparation and enzyme analysis

Sample preparation and enzyme analysis were performed as described before.[3,5] All the assays were performed quartet with a normal control sample. Protein concentrations were measured by the method of Lowry[6] and enzyme activities were expressed as nanomoles of substrate hydrolyzed per mg protein per hour.

Results

Total hexominidase activity levels obtained from the CV samples are shown in Table 1. All families had a previous

child with SD. Seven fetuses were diagnosed as having SD and these pregnancies were terminated at different centers. The remaining 7 fetuses were found to be normal and they were born as healty children. After birth their diagnosis were confirmed by enzyme analysis of leuocytes isolated from peripheric blood. Prenatal diagnosis was performed 3 times for 1 family, and 2 times for 3 families.

Table 1: Prenatal diagnosis results of 14 fetuses from 9 families

Case No	Gestational Age (Week-day)	Date of CVS	Total Hex Activity (normal range:600-2675 nmol/mg protein/hr)
1.	11w-5d	2001	81 (Sandhoff Disease)
2.	13w-4d	2001	40 (Sandhoff Disease)
3.	13w-2d	2001	85 (Sandhoff Disease)
4.	12w-3d	2002	626 (Normal)
5.	12w-0d	2002	739 (Normal)
6.	14w-0d	2002	1330 (Normal)
7.	11w-5d	2003	41 (Sandhoff Disease)
8.	12w-5d	2003	971 (Normal)
9.	11w-6d	2003	1054 (Normal)
10.	13w-2d	2004	69 (Sandhoff Disease)
11.	12w-6d	2005	1187 (Normal)
12.	13w-4d	2005	20 (Sandhoff Disease)
13.	14w-0d	2006	3554 (Normal)
14.	11w-6d	2007	45 (Sandhoff Disease)

Discussion

Prenatal diagnosis of inherited metabolic diseases such as SD is important to reduce the delivery of affected child. We diagnosed 7 patients with SD and 7 unaffected fetuses prenatally. Confirmation of the diagnosis in aborted fetuses could not be possible because the pregnancies were terminated in different cities of Anatolia. Although, we did not have the opportunity to confirm the diagnosis in the abortus materials, at least based on the postnatal confirmation of "normal" diagnosis by studying enzyme activity of leuocytes isolated from peripheric blood, we conclude that CVS obtained in the first trimester is a reliable and rapidly obtained material for the prenatal diagnosis of SD at Hacettepe University.

Demonstration of the maturity and activity of the enzyme in a fetal tissue is necessary for prenatal diagnosis. Comparative previous studies revealed that Hex levels in CV samples is sufficient to make the diagnosis.[4,7-9] CVS procedures were performed at 11-14 weeks of gestational age, and the prenatal diagnostic studies for SD were completed within 3 days of obtaining the sample in our laboratory. This rapid workup reduces the stressful waiting period associated with amniocentesis (which is performed at 16 weeks of gestational age) and the following cell culture which requires in additional 2-3 weeks. However, dissection of chorionic villus samples is important to avoid contamination from maternal blood or decidua. Based on mixing experiments with CV samples of normal fetuses and those with Tay-Sachs disease, hex A activity that was observed due to contamination by decidua was as little as 2 %.[10] In our study, because of careful dissection of CV we did not have misdiagnosis. The most serious complications of CVS are fetal damage or loss. The rate of fetal loss decreases after 12th gestational week.[11-13] In our study, 3 of the patients undergoing CVS were below the 12th gestational week. We did not have any fetal loss. Vaginal spotting after CVS is reported in up to one-third of women, but frank bleeding occurs in less than 6 % and is more common after transservical (TC) than transabdominal (TA) CVS.[12,14-16] A subchorionic hematoma is detected by ultrasound following up to 4 % of TC-CVS.[18] All of the CVS procedures in our patients were performed by transabdominal (TA) approach and none of them had vaginal bleeding or subchorionic hematoma. Rare cases of clinically-evident infectious complications including chorioamnionitis (0 to 0.5 %),[14,17] maternal septic shock,[18,20] and maternal intestinal perforation (during TA-CVS) leading to peritonitis have been reported.[14] TC-CVS appears to carry a higher risk of infection than TA-CVS. Of greater concern is the possibility that subclinical intrauterine infection is the cause of a part of the excess fetal losses reported after TC-CVS compared to TA-CVS.[13,21] We did not have any complications such as chorioamnionitis, maternal septic shock or intestinal perforations.

We concluded that TA-CVS is a safe method to determine whether the fetus has SD if Hex enzyme analysis is available

Sandhoff Hastalığının Koryonik Villus Örneğinde Enzim Analizi ile Prenatal Tanısı

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Sandhoff hastalığı riski taşıyan 9 aileye ait 14 gebelikte, 11-14. gebelik haftaları arasında alınan koryonik villus örneğinde (CVS) enzim analizi ile prenatal tanı yapıldı. Tanı, total heksozaminidaz aktivitesinin, fuluorojenik sentetik substrat, 4-methylumbelliferyl beta-D-glucosaminide kullanılarak, tam veya tama yakın yokluğunun saptanması ile konuldu. Yedi fetus hasta bulundu ve gebelik sonlandırıldı. Kalan 7 fetus normal bulundu. Tanı doğumdan sonra enzim analizi ile doğrulandı. Koryonik villus örneği eğer enzim analizi için yeterli miktarda alınır ve maternal kan veya desiduadan ayrılırsa ilk trimester tanısı için güvenli ve doğru bir örnektir. Heksozaminidaz (hex) ölçümünde kullanılan fuluorojenik substrat Sandhoff hastalığının özgül prenatal tanısında kullanışlıdır.

Anahtar kelimeler: Prenatal tanı, Sandhoff hastalığı, Koryonik villus örneklenmesi, Heksozaminidaz

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