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CLINICAL INVESTIGATION

Prenatal Diagnosis of β-Thalassemia in the Antalya Province

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Abstract: Beta-thalassemia and Sickle cell anemia are serious health problems in the Antalya Province of Turkey as well as World Wide. We aimed to summarize data obtained from the prenatal diagnosis for beta-thalassemia and sickle cell anemia of 103 fetuses. All samples were cytogenetically and molecular genetically examined during a period of 4 years. Molecular testing using RDBH and direct DNA sequencing was performed by using amplified DNA from chorionic villi samples at 9-14 weeks of gestation, while mutations in the parents were determined in advance. Cytogenetic analysis was carried out from a primary culture set up. Of the 103 fetuses, 26 were affected; 25 had beta-thalassemia major, and one had HbS/ beta-thalassemia. All of the affected fetuses were theraupatically aborted with the written permission of their parents. VNTR analysis was used to eliminate maternal contamination. IVSI-110 (G-A) was reported as the most frequent allele(50.4%) in all fetuses. Heterozygosity for IVSI-110 was the most prevalent combination. Of the 26 affected fetuses, one was found to have a 46,XY/47,XY,+22 mosaic karyotype. On the other hand, one fetus with normal genotype for beta-globin gene was cytogenetically found to have a 45,X karyotype. As a result, we suggest that for accurate genetic counselling the CV and other samples obtained for molecular prenatal testing of the beta-thalassemia should also be studied to identify possible chromosomal abnormalities.

Key Words: beta-globin gene, beta-thalassemia, sickle cell anemia, prenatal diagnosis, Antalya

Introduction

Beta-thalassemia is the most common hereditary disease and is characterized by reduced synthesis or absence of the beta-globin chain of hemoglobin (1-3). Beta-thalassemia creates a serious health problem in Turkey, especially in the Antalya region (4-6). The frequency of β-thalassemia carriers in the Antalya Province is 10.2% due to the high rate of consanguinity (4), while the frequency of β-thalassemia throughout Turkey was reported to be 2 % (7). The heterogeneity of β-thalassemia is associated with more than 34 different mutations in Turkey (8). The prenatal diagnosis is performed with several molecular methods by using chorionic villi (CV) sampling, amniotic fluid, and cord blood (8). Due to the high consanguinity rate (35.17%) (9) and high frequency of β-globin gene mutation carriers, a prenatal diagnosis program was needed and we set up a laboratory in 2000 to screen the globin gene mutations in Antalya. In this study, we aimed to analyse the CV samples for both mutation screening of the beta-globin gene and chromosomal abnormalities, using the same sample divided in two parts for each case, in 103 fetuses.

Materials and Methods

The cases diagnosed as carriers by Hb electrophoresis were referred to our laboratory from the Department of Perinatology for mutational analysis of β-globin gene. CV samples were obtained by transabdominal processing at 9-14 weeks of gestation. Maternal parts were eliminated under the dissection microscope. The CV sample was divided into two parts; one of these was used for screening β-globin gene mutations and the other was used for tissue culture to analyse the chromosomal abnormalities by standard methods.

The reverse dot blot hybridization (RDBH) kit (22 mutations for Beta-globin Strip Assay, VienLab) and dideoxy termination chain of DNA sequencing were used to determine the mutations of the β-globin gene (10). When the mutation found in the fetus was the same mutation as her/his mother, a variable number of tandem repeat (VNTR) markers (ApoB, MCT, IgJH, and D4S95) were used to avoid maternal contamination (11).

Results

Molecular analysis of 206 chromosomes revealed 14 different mutations in the β-globin gene including a very rare mutation, Codon 22 (A-C), which is detected by sequence analysis of the amplified β-globin gene. Results are listed in table 1. IVSI-110 was the most frequently observed β-thalassemia mutation (50.4%), followed by IVSI-6 (9.7%), IVSII-1 (7.7%), IVSII-745 (7.7%), IVSI-1 (5.7%), and Codon 5 (4.7%). Codon 22 (A-C) mutation was firstly identified in a fetus at risk and his mother who was a carrier. Heterozygosity for IVSI-110 appears as the most common genotype, with a percentage of 27.1 among all heterozygous genotypes associated with beta-thalassemia traits. HbS constituted 2.7% of all mutations detected in a total of 103 fetuses. Homozygosity for IVSI-110, Codon 39, IVSI-1, and IVSI-6 mutations (Table 2), and compound heterozygosity in various combinations for IVSI-110, IVSII-745, IVSI-6, IVSI-1, IVSII-1, IVSI-5, Codon 5, Codon 8, Codon 22, and HbS mutations were detected in 25 fetuses who were later aborted with the written permission of the families. Fifty-eight fetuses were found to be heterozygous for the above mentioned mutations, and the remaining fetuses were found to have the normal genotype for β-globin gene. Two fetuses were found to have abnormal karyotypes, namely 45,X with normal genotype and 46,XY/ 47,XY,+22 with IVSI-110/IVSII-745 genotype (Table 3). Karyotypes were confirmed by amniosynthesis, and were aborted with written permission from their families.

Table 1. Frequencies of beta-globin gene mutations detected prenatally.

Type of Mutation	Chromosome	
	n	%
IVSI-110 (G-A)	52	50.4
IVSI-6 (T-C)	10	9.7
IVSII-1 (G-A)	8	7.7
IVSII-745 (G-A)	8	7.7
IVSI-1 (G-A)	6	5.7
Cod5 (-CT)	5	4.7
-30 (T-A)	3	2.7
HbS	3	2.7
Cod8 (-AA)	2	1.7
Cod39 (C-T)	2	1.7
IVSII-848 (C-A)	1	0.9
IVSI-5 (G-T)	1	0.9
Cod44 (-C)	1	0.9
Cod22 (A-C)	1	0.9
Total:	103	100.0

Table 2. Frequencies and percentage of homozygous genotypes.

Type of Mutation	Patients	
	n	%
IVSI-110/IVSI-110	10	76.9
Cod39/Cod39	1	7.6
IVSI-1/IVSI-1	1	7.6
IVSI-6/IVSI-6	1	7.6
Total:	13	100.0

* 13 of 26 affected fetuses had compound heterozygous mutations for beta globin gene.

Table 3. Abnormal karyotypes in CV samples of 103 fetuses analysed.

Karyotype	Genotype for Beta-globin Gene
45, X*	Normal/Normal
46,XY[11]/47,XY,+22[39]	IVSI-110/IVSII-745

* Karyotype was confirmed with amniocentesis to eliminate the placental mosaicism.

Discussion

Hemoglobinopathies are a major public health problem, causing both severe socio-economic and psychologic debilitation in the population due to symptomatic care and hospitalization. Therefore, since 1999, we have been performing the prenatal diagnosis of β-thalassemia, which is seen frequently in the Antalya Province of the country in our molecular genetic laboratories. In recent years, the number of prenatal diagnoses have been increased by conferences and seminars given by experts to the public. As a result, 103 CV samples for prenatal diagnosis were analysed at 9-14 weeks. All of the CV samples could evaluated both for beta-globin gene mutations and possible chromosomal abnormalities. There was no miscarriage after CV

sampling. All 26 affected fetuses were aborted according to the decision of the parents. The results of the molecular prenatal diagnosis were available within 4+1 days after sampling. To eliminate the risk of maternal contamination, VNTR analysis using ApoB, MCT, IgJH, and D4S95 alleles was performed, when the genotype of the fetus was found to be identical to that of the mother. There has been no misdiagnosis in our laboratory to date.

Our results show similarities with those of other groups that study the prenatal diagnosis of β-thalassemia and sickle cell anemia (8,12). We performed cytogenetic analysis, which was not carried out in other studies, regarding molecular analysis of β-globin gene in Turkey, in addition to molecular analysis on the CV samples. The abnormal karyotypes detected from CV samples revealed that cytogenetic analysis could also be useful in detecting genetic defects in fetuses, because a postnatal case who coinherited Down syndrome and compound heterozygosity for IVSI-110 and IVSII-745 mutations was reported for the first time (13). Cytogenetic analysis along with mutation screening requires only one invasive procedure.

In conclusion, our data is valuable in that it includes the mutation screening of patients for prenatal diagnosis in Antalya and nearby towns and villages, one of the regions with the highest frequency of beta-thalassemia mutations in Turkey. Also, we aim to record all the carriers and patients for β-thalassemia using a program via Thalassemia Unit, Faculty of Medicine, Akdeniz University, in Antalya, in addition to discovering novel and rare mutations in the rich genetic pool of our region, in order to reduce the frequency of consanguineous marriages and hemoglobinopathies, and to educate the population and inform the physicians in our region.

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