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Co-inheritance of α-and β-thalassemia: challenges in prenatal diagnosis of thalassemia

Zahra Kiani Moghaddam[1] BSc, Narges Bayat[1] BSc, Atefeh Valaei[1] MSc, Alireza Kordafshari[1] BSc, Behnaz Zarbakhsh[2] DVM, Sirous Zeinali[1,2] PhD, Morteza Karimipoor[1,2] MD, PhD

1. National Reference Center for Thalassemia and Hemoglobinopathies, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran.
2. Molecular Medicine Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

Corresponding Author: *Morteza Karimipoor MD, PhD, Molecular Medicine Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran Email address: mortezakarimi@pasteur.ac.ir, Alternative email: mortezakarimi@yahoo.com*

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Abstract

Background: The double heterozygous state of α/β thalassemia may alter the hematological indices and modify the phonotype. In addition, definite characterization of co-inheritance of α- and β-thalassemia heterozygous carriers may change the process of genetic counseling.

Materials and Methods: An Iranian couple with low hematological indices was analyzed for α-globin gene deletions using multiplex gap PCR method and β-globin gene mutations by ARMS-PCR method and DNA sequencing.

Results: The -20.5kb α-globin gene deletion was found in both individuals, and the IVSI-110(G>A) mutation in β-globin gene in male partner. The β -globin gene sequence was normal in female partner. Therefore, the couple was informed about the risk of having fetuses with hemoglobin Bart's hydrops fetalis.

Conclusion: The co-inheritance of α/β thalassemia should be considered in genetic counseling of families screened for β-thalassemia major prevention.

Keywords: α-thalassemia, β-thalassemia, Polymerase Chain Reaction, Mutation.

Introduction

The thalassemias are a group of disorders resulting from absent or reduced production of globin chains in hemoglobin tetramer.[1] α- and β-thalassemia are caused by different types of mutations in α- and β-globin genes located at short arms of chromosomes 16 and 11, respectively. The thalassemia is prevalent in the Mediterranean region, China, Southeast Asia, and West Africa.[2] Alpha thalassemia has four manifestations that correlate with the number of defective genes: silent carrier, alpha-thalassemia trait, HbH disease, and hemoglobin Bart's hydrops fetalis syndrome. These manifestations occur because of the inheritance of molecular defects affecting one (-α/ α α), two (-α/- α or --/ α α), three (--/-α), or four of the alpha-globin genes, respectively. The most widely occurring single α-globin gene defect is a 3.7kb deletion.[3] According to Hadavi et al.[4], the prevalence of –α3.7 kb deletion is around 30% in the Iranian α-thalassemia carriers. The prevalence of β-thalassemia carreiers in Iran is around 4-5% with higher frequency (~10%) in northern and southern coasts, leading to establishment of a national program for the prevention of β-thalassemia major. This program has been implemented at premarital stage since 1997.[5] The rate of α –thalassemia carriers is not well characterized. However, the rate of %33 has been reported in some areas.[6] Therefore the co-inheritence of α- and β-thalassemia is not uncommon and is a challenge for the screening program. This co-inheritence may modify the CBC and HbA2 parameters in β-thalassemia carriers; therefore a definite characterization of couples at risk of β-thalassemia is critical. The characteristic presenting hematological features of the α-thalassemia are low mean corpuscular volume (MCV), low mean corpuscular hemoglobin (MCH) and the normal value of hemoglobin A2 (Hb A2<3.5). In carriers of β-thalassemia low hematological indices are accompanied by higher levels of HbA2 (HbA2 ≥3.5). The severity of β-thalassemia depends in part on the type of β-thalassemia allels

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inherited by the patient and also the co-inheritence of α –thalassemia. Clinically double heterozygous patients for β- and α-thalassemia are usually diagnosed as typical β-thalassemia carriers and alpha-thalassemia is usually ignored.

Here we report the co-inheritence of α- and β-globin gene defects in a couple and the impact on genetic counseling of the family.

Materials and Methods

A young couple with reduced hematological indices from Theran, the Capital city of Iran, was referred to our laboratory from a primary health care (PHC) center for further characterization of thalassemia. A written informed consent was obtained from both individuals to report their case. The hematological parameters of the couple and the parents are shown in table 1.

Fresh blood was collected in heparin and hematological indices were repeatedly measured using SYSMEX KX-21(KOBE, JAPAN), and the HbA2 was checked using column chromatography. For DNA extraction, peripheral blood was collected into tubes containing EDTA and genomic DNA was extracted by the salting out method.[7]

Multiplex Gap PCR was used to detect the common α- globin gene deletions[8] (-α3.7, -α4.2. --MED, and –α-20.5) in both individuals. PCR was carried out using10pmol of each primes, 0.2mmol/L of each dNTP, 2.5 units of smarTaq DNA polymerase (CinnaGen, Iran), and 200-500 ng of genomic DNA. Amplified reaction was carried out with the following conditions: 95 °C 4 min, 95 °C 1 min, 60 °C 1 min,72 °C 2 min, repeated 35 cycles and a final extension at 72 °C for 5 minutes. The PCR products were run on %1 agarose gel and the bands were visualized by UV irradiation after staining with ethidium bromide. For characterization of mutations in β-globin gene a multiplex amplification refractory mutation system PCR (ARMS -PCR)[9] method was used to detect the common mutations in Iranian population.[10] The PCR condition and the primers used in ARMS-PCR method are from a published report.[11] The whole β-globin gene was analyzed by direct DNA sequencing of two PCR amplified fragments. The first fragment (766 bp) was amplified by Seq1F primer:
(5'-CTGAGGGTTTGAAGTCCAACTCC-3') (nt 61956 - 61978)
and Seq1R (5'-CTGTACCCTGTTACTTCTCCCCTTC-3') (nt 62697-62721).
The second fragment (578bp) was amplified by Seq3F (5'ATGTATCATGCCTCTTTGCACC-3') (nt 63226-63247) and Seq3R (5'-GCACTGACCTCCCACATTCC-3') (nt 63784- 63803).
The reference for nucleotide numbers are GenBank accession number: U01317. The DNA sequencing was performed using an ABI 3730 XL Genetic Analyzer (MacroGen, South Korea), by the primers used for PCR amplification.

Results

An unrelated couple at risk of being carrier of β-thalassemia was referred to our laboratory at Molecular Medicine Department, Pasteur Institute of Iran for molecular characterization and genetic counseling. At first, the hematological parameters and family studies of both parental lines showed the female to be a probable α-thalassemia trait and her partner to be β-thalassemia trait. Molecular analysis of common α-globin gene deletions was performed for both individuals and the data revealed that both are carriers of a -20.5 kb deletion with $\alpha\alpha/\alpha^{-20.5}$ genotype. Family study depicted that both have inherited the α-thalassemia determinant from maternal line (figure 1). On the other hand, the molecular analysis of β-globin gene revealed the IVSI-110 (G>A) mutation in heterozygous

Table 1. the hematological values of the couple and their parents

Subject	RBC	Hb	MCV	MCH	MCHC	HbA	HbA2	HbF
I-1	6.40	12.4	66.9	19.4	29	94.6	4.2	1.2
I-2	5.50	10.8	68.2	19.6	28.8	97.5	2.0	0.5
II-1	6.8	15.7	74.0	23.1	23.1	95.5	4.0	0.5
I-3	5.4	18.1	96.2	33.2	34.4	97.2	2.3	0.5
I-4	6.29	12.6	65.2	10.0	30.6	97.0	2.5	0.5
II-2	6.3	12.9	66.9	20.3	30.4	97.5	2.2	0.3

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form for the husband and his paternal line. No common mutation in β-globin gene was found for the wife and her β-globin gene sequence was normal. Thus according to familial hematological studies and molecular analyses of α- and β-globin genes, we concluded that the female is a carrier for α-thalassemia with αα/$\alpha^{-20.5}$ genotype and her husband is a compound heterozygous of α/β thalassemia with IVSI-110/-20.5 genotype. In this situation the main risk for the couple is pregnancies with % 25 risks of hemoglobin Bart's hydrops fetalis.

Discussion

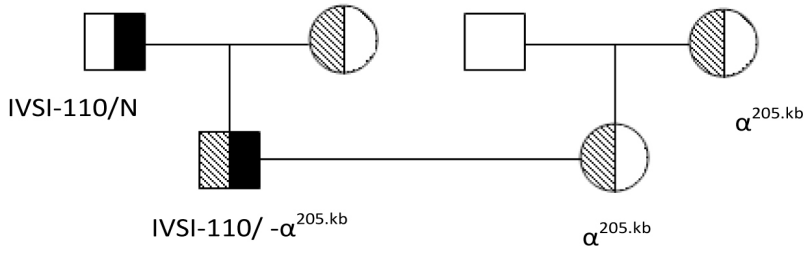
Rapid and correct diagnosis as well as genetic counseling for different hemoglobinpathies is critical among populations where organized prevention programs are implemented. Many problems arise from the heterogeneity and the combination of different hemoglobinopathies among population. The hemoglobinopathy carriers are usually asymptomatic but their offspring have a risk to be symptomatic with variable clinical severities and dependence to blood transfusion.[12] Usually cases with microcytic hypochromic anemia with normal HbA2, who do not respond to iron supplement therapies, are considered carriers of α-thalassemia (normal HbA2 β-thalassemia in population where the δ-thalassemia alleles are present should not be missed). In many cases the presence of α-globin defect is masked by the coexistence of β-globin gene mutation. In this situation the HbA2 may be lowered by α-thalassemia allele, but still is in higher than normal range.

In this study we reported a young couple sent to us to define the risk of having children with β-thalassemia major. As mentioned, the hematologic and genetic studies of the family showed one of the partners to be an α-thalassemia carrier (αα/-$^{\alpha 20.5}$) and the other to be an α-thalassemia carrier (αα/$^{-\alpha 20.5}$) combined with β-thalassemia. As was depicted in table1for II-1 proband the α-thalassemia allele has been masked by the coexistent β-thalassemia allele and the hematological parameters are in favor of β-thalassemia carrier. In this case it was reasonable for the laboratory to conclude the investigation. But the family's hematological studies and the modified CBC parameters of the husband made us to analyze the α-globin gene deletions. For this family the risk of having children with β-thalassemia major was changed to a 25% risk of having fetuses with hemoglobin Bart's hydrops fetalis. Hemoglobin Bart's hydrops fetalis is common in south east Asia[13,14] , where several α° defects like --FIL ,--SEA, THAI are common. However, based on some studies performed on carriers of α-thalassemia among Iranian population, the frequency of one allele defect or two trans alleles are much more common than α° alleles. Hence, it seems that the risk of couples having fetuses with hemoglobin Bart's hydrops fetalis is not high in our population.

Conclusion

The coinheritance of α- and β-thalassemia is not uncommon in Iranian population, mainly in southern provinces. Therefore, it is highly recommended that health care professionals including hematologists, genetic counselors and medical geneticist pay enough attention to the combination of different forms of thalassemia in cases referred to them by national thalassemia prevention program.

Fig1: pedigree analysis and genotype of each individuals of the family



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