Original Article

Prenatal Diagnosis of β-thalassemia in Twin Pregnancies in Iran

Zahra Kainimoghaddam BSc[1], Atefeh Valaei MSc[1], Fatemeh Bayat BSc[1], Maryam Taghavi Basmanj BSc[1], Fatemeh Navabmoghaddam BSc[1], Marjanalsadat Mortezazadeh BSc[1], Ladan Teimoori-Toolabi MD PhD[1], Setareh Ahmadi BSc[1], Shaghayegh Sadegh BSc[1], Alireza Kordafshari BSc[1], Morteza Karimipoor MD PhD [1], Sirous Zeinali PhD•[1,2]

Abstract:

Objective: Prenatal diagnosis of β-thalassemia carrier couples has helped to prevent bearing affected children. Among 177 couples referred to our laboratory for prenatal diagnosis, 14 mothers had twin pregnancies.

Methods: By using direct and indirect methods, we determined their mutations and linkage analysis using polymorphic markers (restriction fragment length polymorphism [RFLP]).

Results: It was shown that in five families both fetuses were heterozygote carriers. In another five families, one fetus was normal and the other one was carrier. In two families, one fetus was affected and the other one was heterozygous carrier; in one case one fetus was affected and the other one was homozygote normal. In the last family both fetuses were homozygote normal. If all fetuses were fraternal then one would expect to see seven homozygote normal and the same number affected, and 14 carriers.

Conclusion: Our results indicated that at least in cases where both fetuses had identical genotypes, then they may be identical twins. Molecular testing indeed showed that in three cases the twins were identical.

Another point is that in three cases, one of the twin fetuses was affected and the other one was either normal or heterozygote in which only the affected fetuses were aborted by the specialist.

Keywords: β-thalassemia, prenatal diagnosis, RFLP, twins

Cite the article as: Kainimoghaddam Z, Valaei A, Bayat F, Taghavi Basmanj M, Navabmoghaddam F, Mortezazadeh M, Teimoori-Toolabi L, Ahmadi S, Sadegh S, Kordafshari A, Karimipoor M, Zeinali S. Prenatal Diagnosis of β-thalassemia in Twin Pregnancies in Iran. *Arch Iran Med*. 2013; **16(10):** 573 – 575.

Introduction

The β-thalassemia syndrome is an inherited hemoglobin disorder characterized by reduced production of β-globin chain. The severe forms of β-thalassemia produce marked anemia starting a few months after birth and survival relies on regular blood transfusion and the lifelong use of drugs to prevent iron accumulation.

It is estimated that 3% of the world's population are carrier of β-thalassemia trait.[1] This disease is a serious public-health problem, particularly in Iran and other parts of Middle-East and Asia[2] where the frequency of β-thalassemia is higher than other parts of the world. More than 25000 affected patients have been reported to live in Iran.[3]

Identification of β-thalassemia carriers is essential in order to reduce the risk of bearing a child affected with severe anemia. For this reason, accurate diagnosis of carriers and proper genetic counseling is highly required. β-thalassemia carriers can initially be identified by measuring the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin (MCH) combined with HbA_2 level. Premarital National Screening Program for Thalassemia Prevention was implemented in1997.[4] Prenatal diagnosis (PND) of β-thalassemia was established in Iran as early as 1994.

PND for genetic disorders causing problems to the affected child, like β-thalassemia, DMD, etc., was legalized by the Iranian Parliament in 2005. Introduction of PND, in Iran, has lowered the birth rate of children affected with this disease dramatically.[5] PND has its complications including identification of molecular defects, coinheritance of α- and β-thalassemia, sample mix-up, etc. Twin pregnancy is another problem since the fetal position may be confused or fraternal twins may have dissimilar genotypes, etc. In the present study, PND was performed for 14 couples with twin pregnancies among 177 families who had been at risk for β-thalassemia.

Authors' affiliations: [1]Molecular Medicine Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran. [2]Kawsar Human Genetics Research Center, Tehran, Iran.
•Corresponding author and reprints: Sirous Zeinali PhD, 69, Pasteur Ave, Tehran-13164, Iran. Tel: +98216953311-20; ext 2473,
E-mail: zeinalipasteur@yahoo.com.
Accepted for publication: 14 August 2013

Patients and Methods

Fourteen twin pregnancies which were at risk of bearing fetuses affected with β-thalassemia were admitted to our PND center. The carrier status of β-thalassemia, in these couples, was assessed by hematologic indices (Table 1). Blood samples were collected in EDTA-containing tubes and DNAs were extracted by using salting out method.[6] Chorionic villi sampling (CVS) was obtained at the age of 10–12 weeks of gestation by the specialist. Chorionic villi (CV) were cleaned from blood clots and possible maternal decidua under microscope. DNA was extracted from CV using DNA Tissue Extraction, DNA Isolation Kit (Roche, Germany). β-globin gene mutations were analyzed using ARMSPCR,[7] in parallel with RFLP/PCR analysis. Haplotype detection was performed by analyzing at least four restriction sites in the β-globin gene cluster mainly *HincII*/3′ψβ, *AvaII*/β, *HinfI*/β, and *RsaI*/β. For RFLP analysis, parental DNAs were tested and linkages were obtained using their CBC and HbA_2 results and if needed, parental mutations were obtained as above. Maternal contamination of fetal DNA was ruled out by using PCR analysis of several VNTRs.[8] These markers include apolipoprotein β-gene (APOB),[9] phenyl-

Table1. Hematologic data of the studied families.

Sex	RBC	Hb	MCV	MCH	HbA_2
Male	6.60 ± 0.44	13.66 ± 1.19	65.62 ± 2.76	20.28 ± 1.21	4.76 ± 0.87
Female	5.37 ± 0.62	10.82 ± 1.94	67.04 ± 3.20	20.10 ± 1.62	5 ± 0.57

Table2. Demographic and molecular data of the studied families.

Gender	Originbyprovince	Consanguinity	Mutation	Number of children	Number of pregnancies
1 F M	Mazandaran	No	IVSII-1	0	First
2 F M	Gilan	Yes	IVSII-1	0	Second
3 F M	Mazandaran	No	IVSII-1 IVSI-110	0	First
4 F M	Khozestan	No	IVSII-1	1/ minor	Third
5 F M	Markazi	No	IVSI-110	0	First
6 F M	Ardebil	Yes	Codon8/9	1 / major	Second
7 F M	Esfehan	No	Codon8/9	1/ major	Third
8 F M	Sistan and Balouchestan	Yes	IVSI-5	1 / major	Second
9 F M	Eastern Azarbayejan	Yes	IVSI-110	1 / major	Second
10 F M	Mazandaran	No	IVSII-1 IVSI-5	0	First
11 F M	Semnan Mazandaran	No	Codon 5 IVSII-1	0	First
12 F M	Hamadan	Yes	Codon 39	0	First
13 F M	Hormozgan	No	-88 IVSI-25	0	First
14 F M	Tehran	Yes	IVSI-5 IVSI-5	0	First

ketonuria (PKU), and DIS80 abbreviated as (PKU, APOB, and DIS80).

Results

Among β-thalassemia carrier couples who had been referred to our center for PND, 14 cases were twins. Eight of these couples were not relatives and the remaining were cousins. Family information is summarized in Table 2. Their relevant hematologic parameters are also included in Table 1. Polymorphic markers on β-globin gene cluster,[10] were used routinely along with mutation analysis, to increase the accuracy of PND. In all cases, ARMS and RFLP results confirmed each other. When the results of mutation and RFLP marker in the fetus and mother were similar, VNTR markers were used to rule out maternal contamination.[11] In all CV samples, fetal positions were marked on the tubes as right, left, anterior, posterior, upper, or lower. Fetal positions were carefully noticed and written down in reports.

Our results showed that in five families both fetuses were heterozygote carrier, while in five others one of the twins was normal and other one was heterozygote. In two families, one fetus was affected with β-thalassemia and the other one was heterozygous, while in the other family one fetus was affected and the other one was heterozygous; in the last family both fetuses were homozygote normal. No maternal contamination was observed. No fetal loss was seen either. The affected fetuses were aborted after receiving permission from the Iranian Legal Medicine Organization by the specialist.

Discussion

PND is the best way for preventing birth of children affected with β-thalassemia. In Iran, premarital screening has become in effect since 1997. The program involves genetic counseling to inform individuals or couples at risk of carrying β-globin gene mutation.

Twin pregnancy is at higher risk of bearing a child with β-thalassemia since in each pregnancy the risk usually doubles. The problem becomes severe when one fetus is normal (homozygote or heterozygote) and the other one affected. Therefore, PND must be performed more accurately than singleton pregnancies. The positions of fetuses must be determined accurately by specialist and should be dealt with throughout the molecular tests. Marking the position of each fetus, in a twin pregnancy, is the most important part of the PND when fetal sampling is carried out, especially when one of them may be affected.

All of the fetuses were clearly genotyped and affected fetuses were aborted, while normal ones were unharmed (pregnancies were continued).

Conclusion

It is suggested to study more cases to see if the probability of 25%–50%–25% (major-minor-normal) is applicable in larger population of those fraternal twins or not. Also, it should be noted that for abortion of the affected fetus (affected with major thalassemia), more precise diagnosis and more caution in abortion should be applied.

Acknowledgment

This study was supported by Pasteur Institute of Iran. We would like to thank Dr. Akhlaghpour for chorionic samplings, and Dr. Behdani and other colleagues at the Molecular Medicine Department for their help and assistance.

References

1. Kazazian HH Jr., Boehm CD. Molecular basis and prenatal diagnosis of beta-thalassemia. *Blood*. 1988; **72:** 1107 – 1116.
2. Rahimi Z, Muniz A, Akramipour R, Tofieghzadeh F, Mozafari H, Vaisi-Rayquani A, et al. Haplotype analysis of beta-thalassemia patients in Western Iran. *Blood Cells Mol Dis*. 2009; **42:** 140 – 143.
3. Najmabadi H, Karimi-Nejad R, Sahebjam S, Pourfarzad F, Teimourian S, Sahebjam F, Amirizadeh N, Karimi-Nejad MH. The beta-thalassemia mutation spectrum in the Iranian population. *Hemoglobin*. 2001; **25:** 285 – 296.
4. Fallah MS, Samavat A, Zeinali S. Iranian National Program for the Prevention of Thalassemia and Prenatal Diagnosis: mandatory premarital screening and legal medical abortion. *Prenat Diagn*. 2009; **29:** 1285 – 1286.
5. 15 June. 2005, autoCAD, 2005.
6. Miller SA, Dykes DD, Polesky AF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988; **16:** 1215.
7. Old JM,Varawalla NY, Weatherall DJ. Rapid detection and diagnosis of β-thalassemia: Studies in India and Cyprior populations in the UK. *The Lancet*. 1990; **336:** 834 – 837.
8. Knott TJ, Wallis SC, Pease RJ, Powll LM, Scott J. A hypervariable region 3' to the human apolipoprotein B gene. *Nucleic Acids Res*. 1986; **14:** 9215 – 9216.
9. Jenner K, Sidoli A, Ball M, Rodriguez JR, Paqani F,Giudici G, et al. Characterization of genetic markers in the 3' end of the Apo B gene and their use in family and population studies. *Atherosclerosis*. 1988; **69:** 39 – 49.
10. Attila, G, Yalin S, Tuli A, Yalin Er, Aksoy K. Prenatal diagnosis of sickle-cell anemia in twin pregnancies and identification by VNTRs. *Clin Chim Acta*. 2004; **350:** 137 – 142.
11. Azizi Z, Delmaghani S, Zeinali M, Moghaddam Z, Zeinali S. The value of ARMS/PCR and RFLP/PCR in prenatal diagnostic accuracy of β-thalassemia. *Iran J Med Sci*. 2001; **26:** 133 – 136.