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Mutation in thalassemia syndrome and clinical manifestation

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Abstract

Introduction: Thalassemia intermedia is a term used to define a group of patients with β thalassemia in whom the clinical severity of the disease is somewhere between the mild symptoms of the β thalassemia trait and the severe manifestations of β thalassemia major. Thalassemia intermedia shows considerable heterogeneity in phenotype and molecular basis.
Objectives: The aim of this study was to identify the common mutations of beta globin gene and the relationship between genotypes and phenotypes in thalassemia intermedia patients in Mazandaran province, in the north of Iran.
Patients and Methods: Fifty unrelated thalassemia intermedia patients, based on clinical and hematological characteristics including age of diagnosis, age of first blood transfusion, history of blood transfusion, mean corpuscular volume (MCV), mean cell hemoglobin (MCH), hemoglobin values, and liver and spleen status were selected. DNA of peripheral blood was extracted and common mutations in beta globin gene were analyzed by reverse dot blot (RDB) method.
Results: Our study showed that 30 patients (60%) had blood transfusion. There was no obvious hepatomegaly in any of the subjects, however 40 patients (80%) showed splenomegaly among which 34 cases (68%) underwent splenectomy. Mutations analysis indicated that HBB:c.315+1G>A [IVS II-1 (G>A)] mutation was the dominant mutation and has been widely associated with the phenotypic manifestations of thalassemia intermedia patients.
Conclusion: It is important to comprehend the molecular basis of thalassemia intermedia and the association between genotype and phenotype in different ethnic groups. Therefore a careful evaluation of genetic, molecular, hematological and clinical aspects is necessary to differentiate thalassemia intermedia in patients at presentation.

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Introduction

Beta thalassemia is one of the most common hereditary autosomal disorders in the world with a high prevalence among Mediterranean populations, characterized by defects in beta-globin gene due to reduced (β^+) or lack (β^0) of β-globin chain production (1-3). Until now more than 200 mutations have been reported in the gene coding for β-globin (2, 4) with more than 60 of them having been identified in Iranian patients (5-10). The carrier frequency of β-thalassemia in Iranian populations is 4%-8%, however in the regions in the north and south of Iran, near the Caspian sea and the Persian gulf, respectively, the prevalence of carriers is higher and about 10% (4,11). The phenotypes of β-thalassemia are classified into three groups including minor, intermedia and major. Thalassemia intermedia is a term used to define a group of patients in whom the clinical severity of the disease is somewhere between the mild symptoms of the β thalassemia trait and the severe manifestations of β thalassemia major. The diagnosis is a clinical entity, that is based on satisfactory maintenance of hemoglobin level of at least 6-10 g/dL at the time of diagnosis without the need for regular blood transfusion (12-14). Patients with thalassemia intermedia may show specific symptoms including cholelithiasis, hepatosplenomegaly, cardiac disease, leg ulcers, pulmonary hypertension, thrombophilia, iron overload, infertility and pregnancy complications, endocrine diseases and bone abnormalities in addition to thalassemia major complications (12, 14). The severity and clinical heterogeneity

Key point

The present study indicated that β-thalassemia intermedia patients in the north of Iran had high levels of heterogeneity in both phenotypes and genotypes.

of thalassemia intermedia depend on the molecular and genetic determinants (15). There are many complications in the diagnosis and treatment of thalassemia intermedia patients. Many patients who were thalassemia intermedia have been considered as thalassemia major, therefore detection of molecular details of thalassemia intermedia can provide a better diagnosis and treatment of patients.

Objectives

This study aimed to investigate the β-thalassemia intermedia mutations profile and the relation between genotypes and phenotypes in patients from the north of Iran.

Patients and Methods

Study design

This study included 50 unrelated thalassemia intermedia patients based on clinical and hematological characteristics including the age of diagnosis, age of first blood transfusion, history of blood transfusion, mean corpuscular volume (MCV), mean cell hemoglobin (MCH), hemoglobin values and liver, and spleen status. All patients were from the north of Iran, Mazandaran province, and were referred from thalassemia and genetic disease research center of Amirkola.

Molecular analysis

Blood samples were collected from 50 patients and DNA was extracted by salting out procedure. Polymerase chain reaction (PCR) was performed using 5´biotinilated forward (GTACGGCTGTCATCACTTAGACCTCA) and reverse (TCATTCGTCTGTTTCCCATT) primers. PCR reaction mixture contained 250 mM dNTPs, 200 nM each forward and reverse primers (Bioneer, Korea), 2 mM MgCl2 and 1.5 unit Taq DNA polymerase (Roche, Germany). Thermocycling program included initial denaturation at 95˚C for 3 minutes, followed by 38 cycles of denaturation at 95˚C for 30 seconds, annealing at 54˚C for 30 seconds, extension at 72˚C for 30 seconds and a final extension at 72˚C for 4 minutes. PCR products were confirmed after electrophoresis on 1.5% agarose LE gel under UV transilluminator. Beta-globin mutations were investigated using reverse dot blot (RDB) method (4), the molecular and clinical data were analyzed using SPSS version 23 software.

Ethics issues

All procedures conducted in studies involving human participants were as per the ethical standards of the Babol University of Medical Sciences Committee (ethics code# IR.MUBABOL.HRI.REC.1395.61) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent obtained from all individual participants included in the study was written. This research was part of the pediatric residency thesis of Leila Gharehdaghly (# 528). Written informed consent was obtained from patients or their parents

Data analysis

The numerical data were expressed with mean ± standard deviation, while categorical data were indicated with frequency and percentage. All statistical analysis was conducted with SPSS 16 (SPSS© Inc., Chicago, IL, USA).

Results

Hematological characteristics of 50 patients are shown in Table 1. The mean age of patients was 33.7±11.45 years (range; 14 to 59 years). The mean age of the patients was 8.44 ± 8 years at the time of diagnosis with the minimum and maximum ages of one and 38 years, respectively. Clinical investigation showed that 30 patients (60%) had a blood transfusion and the mean age of the first blood transfusion was 10.63 ± 9.03 years. The mean intervals between blood transfusion requirements in patients were 1.13 ± 0.72 years. There was no obvious hepatomegaly in any of the subjects. Forty patients (80%) showed splenomegaly among which 34 (68%) underwent splenectomy. The lowest and highest age of splenectomy was 7 and 45 years respectively. Other clinical symptoms like echocardiographic and face changes were reported in 24% and 90% of patients, respectively.

In this study, common mutations in Iran and Mazandaran province were investigated in the beta-globin gene while among them, HBB:c.315+1G>A [IVS II-1 (G>A)]mutation had the highest prevalence. The frequencies of mutations and genotypes are shown in Tables 2 and 3, respectively. Additionally, the relation between genotypes and some important phenotypes were indicated in Table 4.

Mutations analysis indicated that IVS II-1 (G>A) mutation was the dominant mutation and has been widely associated with the phenotypic manifestations of thalassemia intermedia patients.

Discussion

Thalassemia intermedia is an example of a disease with a clinical and genetic heterogeneity that can result from the nature of mutations and other modifier factors such as alpha globin gene mutations, modifier genes and polymorphisms within several genes inside and

Table 1. Hematological characteristic of thalassemia intermedia patients

Hematological characteristics	Range	Mean ± SD
Hemoglobin (g/dL)	8-11.5	9.82 ± 0.9
Hb A1 (%)	0-60	24.8 ± 19.91
Hb A2 (%)	1.1-7	3.58 ± 3.3
Hb F (%)	0.8-98.8	71.43 ± 21.34
MCV (fl)	61-79	71.67 ± 3.99
MCH (pg/cell)	17-25.2	21.31 ± 1.92
Ferritin (ng/mL)	222-2310	614.32 ± 342.9

Hb: hemoglobin, MCV; mean corpuscular volume, MCH; mean cell hemoglobin.

Table 2. Frequency of beta globin mutations obtained from thalassemia intermedia patients

Mutations	No. (%)
IVS II-1 (G>A)	71
C22 (G>T)	4
C8 (-AA)	2
C30 (G>C)	6
+22 (G>T)	1
C26 (G>A)	2
-28 (T>C)	6
C39 (G>T)	1
C6 (T>A)	1
Unknown	6

Table 3. Frequency of genotypes of thalassemia intermedia patients

Types of genotypes	Number of patients	No. (%)
IVS II-1 (G>A) / IVS II-1 (G>A)	31	62
IVS II-1 (G>A) / C30 (G>C)	6	12
IVS II-1 (G>A) / C22 (G>T)	1	2
IVS II-1 (G>A) / C39 (G>T)	1	2
IVS II-1 (G>A) / +22 (G>T)	1	2
C22 (G>T) / C6 (T>A)	1	2
-28 (T>C) / -28 (T>C)	3	6
C22 (G>T) / C22 (G>T)	1	2
C8 (-AA) / C8 (-AA)	1	2
Hb E: C26 (G>A) / IVS II-1 (G>A)	1	2
Unknown	3	6
Total	50	100

outside beta-globin gene family like BCL11A, XmnI, and HBS1L-MYB (13). Because of the variety of phenotypic manifestations, genotypic heterogeneity and the severity of clinical symptoms, appropriate detection and treatment of thalassemia intermedia patients remains a serious challenge. One of the aims of this study was to describe the profiles of beta globin mutations in thalassemia intermedia patients in Mazandaran, a northern province of Iran. In this study, IVS II-1 (G>A) mutation was the dominant mutation and has been widely associated with the phenotypic manifestations of thalassemia intermedia patients. Sixty percent of patients requiring blood transfusion, were homozygote for IVS II-1 (G>A) mutation and the second genotype was IVS II-1 (G>A)/ C30 (G>C) with the frequency of 12%. Among 40 patients with splenomegaly, 23 patients (57.5%) were homozygote for IVS II-1 (G>A) and 6 (15%) had IVS II-1 (G>A)/C30 (G>C) genotype, and among 34 patients who needed splenectomy, 19 patients (55.9%) were homozygote for IVS II-1 (G>A) mutation and 6 (17.6%) were IVS II-1 (G>A)/C30 (G>C). It should be noted that the need for blood transfusion after splenectomy was resolved in all patients. IVS II-1 (G>A) is a β° mutation and is common in Mediterranean regions (16). In agreement with previous studies on the mutation spectrum of the beta-globin gene in Iranian populations and thalassemia intermedia patients, IVS II-1 (G>A) was considered as the most frequent beta-globin mutation among the patients investigated in this study (1, 2, 4, 6, 17-22). This is contradicted by the finding in Kuwait (23), Iraq (24) and some other ethnic groups (25, 26). It is therefore important to comprehend the molecular basis of thalassemia intermedia and the correlation between genotype and phenotype in different ethnic groups. The first important step in determining the association between genotypes and phenotypes is to provide an appropriate definition of each phenotype and factors affecting it. As the severity and frequency of complications in thalassemia intermedia patients are more variable, a careful evaluation of molecular, hematological and clinical aspects is necessary to differentiate thalassemia intermedia in patients at presentation, to predict the severity of clinical symptoms and to prevent from early transfusion (27). Therefore conduction a genetic counseling and prenatal diagnosis especially in high prevalence areas is necessary. The present study indicated that thalassemia intermedia patients in the north of Iran had high levels of heterogeneity in both phenotypes and genotypes, however alpha globin gene mutations, other probable genetic factors and modifier genes (28)should be investigated to a better description of genotype-phenotype association in thalassemia intermedia patients.

Conclusion

It is important to comprehend the molecular basis of thalassemia intermedia and its association between genotype and phenotype in different ethnic groups. Therefore a careful evaluation of genetic, molecular, hematological and clinical aspects is necessary to differentiate thalassemia intermedia in patients at presentation.

Table 4. Relation between genotypes and some important phenotypes in thalassemia intermedia

	Blood transfusion (n = 30)	Splenomegaly (n = 40)	Splenectomy (n = 34)	Highest ferritin level (n = 1)
Genotypes	IVS II-1 (G>A)/ IVS II-1 (G>A) (20)	IVS II-1 (G>A)/ IVS II-1 (G>A) (31)	IVS II-1 (G>A)/ IVS II-1 (G>A) (26)	IVS II-1 (G>A)/ C39 (G>T)
	IVS II-1 (G>A)/ C30 (G>C) (9)	IVS II-1 (G>A)/ C30 (G>C) (9)	IVS II-1 (G>A)/ C30 (G>C) (8)	
	-28 (T>C)/ -28 (T>C) (1)			

Limitations of the study

The limitations of the study were small sample size and cross-sectional design.

Authors' contribution

AT as a corresponding author, prepared patients sample and interpreted the patients' data. LGD, performed genetic examination. MB analyzed and interpreted the patients data and was a major contributor in writing the manuscript. All authors have read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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