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Genetic diversity of beta-thalassemia mutations in Pakistani population

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Genetic Diversity of 3-thalassemia Mutations in Pakistani Population

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Abstract

Background: B-thalassemia is one of the most common inherited single gene disorder in Pakistan. It is characterized by reduced or absent B-globin gene expression resulting in abnormal maturation and survival of red blood cells. Due to high prevalence of this disease in the local population, it has become important for the health care providers to encourage people to utilize laboratory facilities for carrier and prenatal genetic testing. **Objective:** To study the frequency of 13-thalassemia mutations in. Pakistani population.
Setting: A tertiary care teaching hospital.
Methods: Blood samples of 72 couples and chorionic villus (CV) biopsy specimen collected at the Aga Khan University Hospital, Karachi were tested by Amplified Refractory Mutation Systems (ARMS) for the 12 most common mutations in the B-globin gene.
Results: Out of 72 chorionic villus biopsy specimen analyzed, 17 (23%) had mutations in both alleles of the B-globin gene. Homozygosity was identified in 6 C\\ samples, whereas 11 CV specimens were diagnosed as double heterozygous. Almost 60% of the CV biopsies showed mutations in one allele and were diagnosed as carriers. IVSI-5 (G-C) was the most common mutation identified in this study. It was found in 53% of the subjects and was represented equally in all the ethnic groups except Pathans. Several regional and ethnic differences were observed in the distribution of common mutations, for example in Pathan families Fr 8-9 (+G) mutation was most prevalent. In addition, variation in the distribution of mutations was also observed between the Northern and the Southern regions.
Conclusion: This study indicates that in Pakistan, the five most common mutations are IVS1-5 (C-C), IVS1-1 (G-T), Fr 41-42 (-TTCT) Fr 8-9 (+G) and deletion 619 hp. An important factor contributing to high incidence of thalassemia is the unawareness among people about the available diagnostic facilities for the prenatal diagnosis in Pakistan. Strict implementation of collective measures including carrier identification, genetic counseling and prenatal diagnosis are required for preventing B-thalassemia in Pakistan (JPMA 50:293, 2000).

Introduction

B-thalassernia is an autosomal recessive disorder characterized by reduced or absent 3-globin gene expression resulting in abnormal maturation and survival of red blood cells. B-thalassemia is primarily caused by mutations on chromosome 11 that affects beta globin product[1]. To date more than 140 different mutations of 3-globin gene have been identified but approximately 20 different mutations account for 80% of the mutations worldwide[2]. This disease is frequently encountered in regions of Mediterranean basin, Africa, South East Asia and in Indian Sub-Continent[3]. It has now been realized that the incidence of thalassemia major can be reduced by taking effective measures such as identification of carriers, genetic counseling and prenatal diagnosis. Rapid advances in prenatal diagnosis of birth defects have opened a new era in preventive medicine and now it is routinely used to identify fetus affected with thalassernia major[4,5].
B-thalassemia is the most common inherited single gene disorder in Pakistan and studies on its frequency have demonstrated 5% carrier rate[6]. According to unofficial estimates over 4000 thalassemic children are born in Pakistan each year and the available health care facilities are inadequate to deal

with the number of sick children. Due to high prevalence of this disease in Pakistan it is important to raise awareness and encourage people to get tested for trait and use the option of prenatal diagnosis in couples at risk of having a thalassernia major offspring.
This report details the findings of B-thalassernia mutations in families who have opted for prenatal diagnosis at the Aga Khan University Hospital, Karachi.

Subjects and Methods

Subject Selection

In this study prenatal diagnosis of married couples who were at the risk of giving birth to thalassemic offspring was camed out using ARMS-PCR. A total of 72 families approached the Aga Khan University Hospital between the period January 1994 to December 1998 for prenatal diagnosis of B-thalassemia. After providing necessary counseling, blood samples and CVs biopsy were obtained under ultrasound guidance. The gestational age of the mothers ranged between 9-12 weeks. Both parents of each family were previously diagnosed for thalassemia trait and had at least one thalassernia major child. Inter family marriages between first cousins were reported in 57% families.
All the subjects included in this study had a history of thalassemia major in the family. Most of them were either referred from hematology clinics of Aga Khan Hospital or from local transfusion centers. After obtaining consent from the couple, 10-mi blood sample was collected from each parent in EDTA anticoagulant containing tubes and chronic villus (CV) biopsy sample from mother in RPM1-1640 medium. Peripheral blood mononuclear cells were separated using Ficoll/Hypaque density gradient technique and stored at -20°C until utilized for DNA extraction. Under a dissecting microscope maternal tissue was removed from the CV biopsy with the help of sterilized forceps and fetal tissue was transferred to fresh RPM1-1640 medium that was later used for isolation of DNA.
DNA extraction from Chorionic Villus Biopsy and mononuclear blood cells
Extraction of DNA from blood cells and biopsy specimen was performed according to the method of Varawalla[3]. Briefly, mononuclear cell pellet or minced biopsy was homogenized in 400ml of Tris EDTA buffer pH 8.0 containing l0ul of 10mg/nil proteinase K and 10% SDS. The homogenate was incubated overnight at 37°C in a water bath. Next day after 15 minutes of incubation at 65°C, CTAB/NaCI solution was added and vortexed until contents became milky. Subsequent to the addition of chioroform/isoamyl alcohol phases were separated by spinning the mixture at 12000g for 15 minutes. Following the addition of isopropanol. DNA was pelleted by centrifugation. After a final wash with 70% ethanol the pellet was reconstituted in Tris EDTA pH 8.0 buffer.
Amplified Refractory Mutation System (ARMS) for f3-thalassemia mutation detection
The B-thalassemia mutations were characterized by PCR method based on ARMS using the method and conditions described by Varawall et al[3-7]. A typical ARMS assay comprised of two PCRs, each conducted using the same substrate DNA. For the identification of a specific mutation, two primers, one complementary to the normal DNA sequence and the other to the mutant DNA were mixed with the target DNA in separate reaction tubes. The amplified products were separated in an agarose gel by electrophoresis, visualised by ethidium bromide staining and exposure to UV light. The presence of specific product of a particular size denoted a positive result. An internal control was included in each PCR reaction to monitor the presence of PCR inhibitors in the specimen and pipetting errors.

Results

Out of 72 CV samples analyzed, 17 (23%) had mutations in both alleles of B-globin gene. Homozygous mutations were identified in 6 CV samples, while 11 CV specimens were diagnosed as double heterozygous. Almost 60% of CV examined demonstrated mutation in one allele and identified as thalassemia minor. Nine (11%) out of 77 CV samples were negative for thalassemia mutations. Out of 72 families included in the study, 61% couples were related to each other and only 38% were unrelated.

IVSI-5(G-C) was the most common mutation identified; it was observed in 53% of the subjects and was represented equally in all the ethnic groups except Pathans. The other two common mutations Fr 8-9(+G) and del 619 showed similar distribution, both were observed in 1 5% of the individuals tested. Several regional and ethnic differences were observed in the distribution of common mutations. For example in Northern Pakistan, Fr 8-9(+G) was identified as the most prevalent mutation, whereas in Southern regions IVSI-5(G-C) was the predominant mutation. In Southern Pakistan, Sindhis, Baluchis and Mohajirs constituted the major ethnic groups. This study showed that 40% Sindhis, 50% Baluchis and 53% Mohajirs (immigrants from India) screened positive for IVS1-5(GC). In addition to IVSI-5(G-C), prevalence of del 619 was also high in Southern region especially in Sindhi and Mohajir population (Table).

Table. Distribution of β-thalassemia mutations in Pakistan according to ethnic background.

Mutation	Punjabi		Pathan		Sindhi		Baluchi		Mohajir	
	n	%	n	%	n	%	n	%	n	%
IVS1-5(G-C)	12	46	-	-	22	39	2	50	25	53
Fr 8-9(+G)	5	19	6	75	9	16	2	50	7	15
Del 619	-	-	-	-	11	22	-	-	7	15
Fr 41-42(-TTCT)	4	15	-	-	3	5	-	-	1	2
IVS1-1(G-T)	2	8	-	-	7	12	-	-	6	13
Codon 15	2	8	-	-	4	7	-	-	1	2
Codon 30	1	4	-	-	-	-	-	-	-	-
Codon 5	-	-	2	25	1	2	-	-	-	-
All	26	100	8	100	57	100	4	100	47	100

Mutations in Codon 5, Codon 15 and Codon 30 were identified at a lower frequency in all the major ethnic groups. When compared among different ethnic groups. Codon 5 mutation was found at a higher level in Pathans.

Discussion

The genetic diversity of b-thalassemia mutations was investigated in Pakistani families who were at high risk of giving birth to B-thalassemic offspring. Several of the mutations common to the region of Indian Sub-Continent were analyzed in this study. Out of 12 mutations tested, eight have accounted for 100% of the total mutations identified. The most common mutations which encompassed about 95% of the total mutations were IVS15(G-C), IVS1-1(G-T), Fr 8-9(+G) Fr 4l-42(-TTCT) and del 619. These observations are in accordance with several published studies carried out in this region. In the Sub-Continent, mostly sixteen different B-globin mutations have been identified and out of those five accounted for 93% of 3-thalassemia alleles[9]. For instance Varawalla et al reported similar 3-thalassemia types in Asian Indian population. In addition, they have also described two rare mutations at IVS-2 and Codon 30[10]. Similarly, we have observed mutation at codon 30 only in one subject.

It is well documented in literature that each population has its own major 3-thalassemia types and because of this unique distribution of B-thalassemia alleles its prenatal diagnosis is greatly facilitated[11]. Our study also demonstrated that the prevalence of different mutations among several ethnic groups was not uniform. For example IVI-5(G-C) was most common in Punjabi. Baluchi, Sindhi and Mohajir families whereas it was not detected in any of 8 Pathan families tested, in Pathan families, Fr 8-9(+G)

was the most prevalent mutation when compared with other ethnic groups. Regional variation in the spectrum of mutations was also observed in population of Northern and Southern areas of Pakistan. In Southern regions the most prevalent mutations were IVS 1-5 and del 619, whereas in Northern Pakistan Fr 8-9(+G) and Codon 5 were dominant. Most of the couples we have screened for 13-thalassemia showed identical mutations that may be mainly due to local marriage customs and traditionally high frequency of marriages among close relatives.

An important factor contributing to high incidence of B-thalassemia in Pakistan is the fact that majority of people are not aware of the available diagnostic facilities, especially pre-natal diagnosis, for the prevention of thalassemia. This is evident from our report, which demonstrated that 98% of all the families that have approached for prenatal diagnosis of B-thalassemia carried a thalassemia major child. In addition, routine screening for B-thalassemia carriers and genetic counseling for couples at risk is not a common practice in Pakistan, but if provided adequately these measures would be beneficial to the thalassemia prevention programmes. Another aspect that made prenatal diagnosis more acceptable is the introduction of chorionic villus sampling. CV sample can be obtained in the first trimester of pregnancy, therefore in case of homozygous fetus the interruption of pregnancy would be physically and emotionally more acceptable then in the later stages of pregnancy[8-13]. Similar approaches have significantly contributed in controlling B-thalassemia in several other countries where such measures have now become the mainstay of the management of thalassemia[14]. In conclusion, we suggest that strict implentation of collective measures including carrier identification, genetic counseling and prenatal diagnosis are required for preventing B-thalassemia in Pakistan.

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