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Characterization of IVS-110,IVS-6 and Codon 39 beta-thalassemia mutations using amplification refractory mutation system (ARMS) technique in Bisha, Saudia Arabia

KEYWORDS: Beta-thalassemia- ARMS – Molecular- mutations-primers-pecific amplifications

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ABSTRACT *The current study aim to characterized the thalassemia mutations in Bisha,Saudia rabia using amplification refractory mutation system(ARMS) in detection of the IVSI-110, IVSI-6 and codon 39 mutations. The study comprised of 50 cases from King Abdullah hospital, 20 females and 30 males and the age between 3 -25 years. The result revealed that IVS110 were7 cases (14%) homozygous and 15 cases (30%) heterozygous, IVSI-6 were 6 cases(12%) homozygous and 16 cases(32%) heterozygous , finally the codon 39 mutations were 2 cases(4%) homozygous and 12 cases(24%) heterozygous. The conclusion is the high incidence of mutations were in IVSI-110 were 7% and the low incidence were codon 39 for beta thalassemia in Bisha, Saudia Arabia by using ARMS technique .*

Introduction

β-Thalassemia is a common autosomal recessive disorder among the hereditary diseases worldwide. The β-thalassemias refer to that group of inherited hemoglobin disorders which are characterized by a reduced synthesis (β^+-thalassemia) or absence (β^0thalassemia) of β-globin chain production which causes anemia (23,24). It is mostly caused by point mutations, a small deletions or insertions within the β-globin gene which is located as a cluster on the short arm of chromosome 11 (17). More than 200 different mutations of β-globin genes have been identified (11,20). It is most prevalent around the Mediterranean Sea, that is, countries like Greece, Italy, Turkey, and North African, Middle East and South East Asian countries. It is also seen in Iran; the gene frequency of β–thalassemia in Iran is high and alters significantly from area to area, but around the Caspian Sea and Persian Gulf, more than 10% have the highest rate (18). Since the Iranian population is a mixture of different ethnic groups, it is necessary to determine the frequency and distribution of mutations in the different parts of the country. It has been estimated that about 1.5% of the global population (80 to 90 million people) are carriers of beta-thalassemia, with about 60,000 symptomatic individuals born annually, the great majority in the developing world. The total annual incidence of symptomatic individuals is estimated at 1 in 100,000 throughout the world and 1 in 10,000 people in the European Union. However, accurate data on carrier rates in many populations are lacking, particularly in areas of the world known or expected to be heavily affected (22). Clinical presentation of thalassemia major occurs between 6 and 24 months. Affected infants fail to thrive and become progressively pale. Feeding problems, diarrhea, irritability, recurrent bouts of fever, and progressive enlargement of the abdomen caused by spleen and liver enlargement may occur. In some developing countries, where due to the lack of resources patients are untreated or poorly transfused, the clinical picture of thalassemia major is characterized by growth retardation, pallor, jaundice, poor musculature, genu valgum, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoiesis, and skeletal changes resulting from expansion of the bone marrow. Skeletal changes include deformities in the long bones of the legs and typical craniofacial changes(21). More than 200 mutations have been so far reported; the large majority are point mutations in functionally important regions of the beta globin gene(10). The ARMS/PCR products were electrophoresed on 2% Agars gel. They were stained with etidum bromid. To Screen PCR products on acryl amid gel, RCR reaction mixture(15).

The aim of the current study is to perform a mutation spectrum analysis of beta thalassemia in Bisha area, Saudia Arabia

Materials and methods

The study was carried out on 50 patient (20 females and 30 males) age was between 3 and 25 years from pediatric department, King Abd Allah Hospital, Bisha,Saudia Arabia. Specimens were taken on the sterile EDTA anticoagulant tubes by pediatrician in the hospital. The samples were taken between October 2012 and February 2013.The study was approved by the ministry of health ethics in Saudia Arabia, the research is supported by the project no.153/2012 from King Khaid university, Saudia Arabia. The aliquot of blood containing EDTA was used for measurement of red blood cell indices (ABX Diagnostics, Montpellier, France), alkaline hemoglobin electrophoresis (6), measurement of HbA2 by elution (5).

Extraction of genomic DNA

Extraction of genomic DNA was done using Qiagen GmbH, Germany (QiaAmpDNA blood Midi/Maxi) column kit, the DNA extraction procedure was done according to Qiagen protocol.

Amplification The multiplex ARMS was carried out in a 25 ul reaction containing 1x PCR buffer, 3.0mMMgCl2, 2.5 U Hotstart Taq polymerase (Qiagen GmbH,Germany), 200uM dNTPs, 200 pmol of each primer and 250ng of template DNA. After incubation at 95 C for 15 min, followed by 35 cycles of denaturation at 94C for 1 min, annealing at 65 C for 1 min and 72 C for 1 min, with the final extension at 72 C for 7 min. The PCR products were analyzed using 2% agarose gel containing ethidium bromide and were visualized by ultraviolet illumination. Table(3) show oligonucleotide primers used in ARMS techniques (16) and Figure(1) show agarose gel electrophoresis of IVSI-110 heterozygous mutations base pair(bp) of the studied cases.

Statistaical analysis

Statistical analysis was done using spss microsoft program version 12, and student t-test independent.

Result

In this study, all patients were genotyped for the same markers using PCR-based technique (ARMS). The patient's blood indices and hemoglobin are shown in Table(1) which indicated that correlation between IVSI-110 heterozygous and homozygous in hemoglobin are significant $p \leq 0.001$, also IVSI-6

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heterozygous and homozygous and codon 39 heterozygous and homozygous hemoglobin are significant p≤ 0.001 but on the other side the red blood cells count for IVSI-110 heterozygous and homozygous not significant, IVSI-6 heterozygous and homozygous not significant and finally codon 39 heterozygous and homozygous not significant. Table(2) show the most common mutations were IVSI-110 (G→A) 7 cases (14%) and the lowest mutations were codon 39 (C→T) 2 cases only (4%). The IVSI-110 mutations were 7 cases homozygous (14%), heterozygous were 15 cases (30%) and normal cases were 28 (56%). IVSI-6 mutations were 6(12%) homozygous cases, 16 (32%) heterozygous cases and 28(56%) normal cases were discovered. Codon 39 mutations revealed 2(4%) cases were homozygous, 12 (24%) cases were heterozygous and 36(72%) cases were normal.

Discussion

Thalassemia syndromes are common in Saudi Arabia, the Beta-Thalassemia genes occur with variable frequency in different regions of Saudi Arabia and both B+ and Bo thalassemia have been reported. Alpha-Thalassemia is also highly prevalent here and the interaction with the sickle cell gene is commonly observed (2). The current study in Bisha,Saudia Arabia revealed 3 mutations from beta thalassemia the first is IVSI-110, the second is IVSI-6 and the third is codon 39.The IVSI-110 study discovered 7 cases (14%) homozygous and 15 cases heterozygous and 28 cases(56%) normal from the total study 50 specimens from King Abdullah hospital in Bisha,Saudia Arabia. But Ibraheim etal.,2001 recoded 31.1% IVSI-110 mutations in Egypt, Shams El-Din etal.,1998 study was 39.2% IVSI-110 mutations in Egypt.This denoting the high frequency in Egypt than Saudia Arabia, Basak et al., 1992 revealed 41% IVSI-110 mutations in Turkey, Kollia etal.,1992 recorded 43.1% IVSI-110 in Greece. Shehab etal.,1987 reported 46.6 IVSI-110 mutations in Lebanon and El-Hazmi etal., 1995 reported 40.0% in Saudia Arabia, all these frequencies may be due to different genes in area where the geographical distribution is different. Our study for IVSI-6 revealed 6 cases (12%) homozygous mutations, 16 cases (32%) heterozygous and 28 cases(56%) normal specimens. Ahmad A. et al.,2006 discovered that frequency of IVSI-6 is 17.5% , Ibraheim et al.,2001 reported that IVSI-6 frequency was 14.2% in Egypt, this is similar to our study result. Basak et al.,1992 revealed that IVSI-6 mutations were 13.3 % in Turkey, also DiMarzo et al., 1988 reported 28.9 % of IVS-I mutations in Sicily. The current study demonstrated that codon 39 mutations were 2 cases(4%) homozygous, 12 cases (24%) heterozygous and 36 cases(72%) were normal, by comparison this result by the result of Ibraheim et al., 2001 which the codon 39 mutations revealed 4 % frequency and 2-3% of codon 39 mutations were discovered with Novelletto et al., 1990 and Hussein etal.,1993 we can see similarities in spite of different geographical origin of studied cases.

(Table 1) IVSI-110,IVSI-6 and Codon 39 comparison with blood indices of the patients

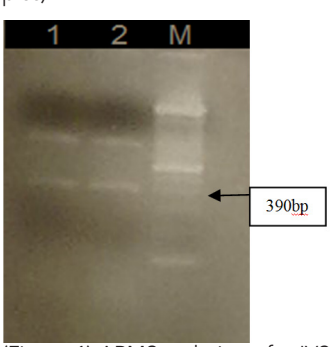
Blood indices	Types of mutations					
	heterozygous IVSI-110(n=15)	homozygous IVSI-110(n=7)	heterozygous IVSI-6(n=16)	homozygous IVSI-6(n=6)	heterozygous Codon39(n=12)	homozygous Codon 39(n=2)
Hemoglobin(g/dl)	9.6±0.6*	5.8±0.7*	10.9±0.8	5.9±0.5*	11.4±0.6	6.4±0.6*
Red blood cells(x10^{12}/L)	5.0±0.70	4.4±0.60	5.1±0.81	4.10±0.60	5.6±0.72	4.0±0.73
MCV(fL)	64.2±3.6	60.0±5.2	64.0±4.9	60.1±4.6	65.0±5.2	60.0±4.6
MCH(pg)	19.0± 1.5	16.9±1.4	20.3±1.7	16.8±1.5	21.2±1.5	15.8±1.8
HemoglobinA(%)	94.3±1.7	84.6± 4.6	94.8±0.6	83.2±0.5	95.2±0.7	83.3±0.9
Hemoglobin A_2(%)	5.1±0.6	5.8±0.7	5.3±0.5	5.9±0.9	5.0±0.6	6.0±0.5

*p≤0.05 significant

(Table 2) Beta thalassemia frequency mutations by ARMS techniques

Mutations	ARMS method		
	Heterozygous cases N(%)	Homozygous Cases N (%)	Normal cases N(%)
IVSI-110	15(30)	7 (14)*	28 (56)
IVSI-6	16(32)	6 (12)*	28 (56)
Codon39	12(24)	2 (4)*	36 (72)

*P value ≤ 0.05 is significant (student t-test independent samples)



(Figure 1) ARMS technique for IVSI-110 mutations, (1 and 2) are the heterozygous cases, IVSI-110 normal at 630bp and IVSI-110 mutant at 390bp, (M) DNA 100 bp ladder.

Table (3) oligonucleotide primers used in ARMS technique

IVSI-nucleotide 6-N	5'-TCTCCTTAAACCTGTCTTGTAACCTTCATA-'3
IVSI-nucleotide 6-M	5' -TCTCCTTAAACCTGTCTTGTAACCTTCATG-3'
IVSI-nucleotide 110-N	5 '-ACCAGCAGCCTAAGGGTGGGAAAATAGTCC-3'
IVSI-nucleotide 6-110 M	5 '-ACCAGCAGCCTAAGGGTGGGAAAATAGTCT-3'
Codon 39-N	5 '-CAGATCCCCAAAGGACTCAAAGAACCTGTG-3'
Codon 39-M	5'-CAGATCCCCAAACGACTCAAAGAACCTGTA-3

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