# IDENTIFICATION OF β-GLOBIN MUTATIONS WHICH PRODUCED β-THALASSEMIA BY ARMS-PCR ASSAY AND DIRECT SEQUENCING

Gaza F. Salih * and Hersh Abdul Hamakarim *

Submitted: 13/9/2015; Accepted: 14/11/2016; Published: 1/12/2016

## ABSTRACT

### *Background*

Thalassemia is the most common recessive single gene disease in humans which is caused by inheritance of an affected allele from both parents resulting in impaired production of the globin chain.

### *Objectives*

This study was established in order to; 1. Identify β-globin mutations, which produced β-thalassemia by ARMS-PCR assay and direct sequencing and identifying the spectrum of mutations causing β-thal in the KRG. Also to investigate the usefulness of the PCR-ARMS technique followed by DNA sequencing as diagnostic tools that could be applied for carrier detection and prenatal diagnosis; 2. Establishment and present a feasible protocol for molecular diagnosis of β-thalassemia in KRG region

### *Methods*

Screening for β- thalassemia mutations using PCR-ARMS for frequent mutations in the KRG population followed by DNA sequencing of the unknown alleles could be useful for the implementation of a strategy for carrier detection and preimplantation genetic diagnosis in high risk families.

### *Results*

A total of thirty β-thalassemia patients including 16 males (53.33%) and 14 females (46.66%) were examined by PCR assay using specific primers for each of mutations. The results indicate that these mutations detected in this study were also detected in surrounding countries which occurred with varying frequency.

### *Conclusion*

These results are in line with studies in other parts of the world which have shown that gene flow due to population migration is common. Rapid, accurate genotyping methodologies for specific, causative mutations of the β-globin gene are needed for pre- and postnatal screening and diagnosis of this disease in different ethnic populations.

**Keywords:** *β-globin, β-thalassemia, ARMS-PCR.*

---

* Department of Biology, Sciences, University of Sulaimani.
Correspondence: gazasalih@home.se

https://doi.org/10.17656/jsmc.10096

## INTRODUCTION

Thalassemia is the most common recessive single gene disease in humans which is caused by inheritance of an affected allele from both parents, and the fundamental abnormality in thalassemia is impaired production of the globin chain [1, 2], so thalassemias are named by reference to the affected globin chain: α-thalassemia involves the α-chain, β-thalassemia the β-chain [3, 4].

The β-thalassemias are among the most common autosomal recessive disorders, and it is failure of β-globin chain synthesis resulting from different mutations in the β-globin gene. These alterations give rise to significant changes in the level of gene transcription that lead to absent (β0) or markedly diminished (β+) amounts of β-globin gene mRNA [5, 6]. The mutations in the β-globin gene are scattered throughout the length of the gene. At present, more than 200 different mutations resulting in a β-thalassemia have been reported in different parts of the world, over 90% of which are gene substitutions, insertions or deletions involving only one or several nucleotides within the β-gene cluster [7].

Mutations causing thalassemia can affect any step in the pathway of globin gene expression: transcription, processing of the mRNA precursor, translation, and posttranslational metabolism of the β-globin polypeptide chain. The most common forms arise from mutations that derange splicing of the mRNA precursor or prematurely terminate translation of the mRNA [8]. The strategy for identifying, β-thalassaemia mutations in most diagnostic laboratories depends on knowing the spectrum of common and rare mutations in the ethnic group of the individual being screened, so information provided on the distribution and the frequency of beta thalassemia alleles is useful to establish a program for carrier screening, genetic counseling, prenatal diagnosis, and for physicians to establish specific therapeutic approaches for patients with β-thalassemia major [9]. For the purpose of prevention and control of the clinically severe thalassemia diseases molecular diagnosis and genetic counseling are most useful and informative [10, 11]. The study aimed at identifying the spectrum of mutations causing in the KRG, Iraq. We also wanted to investigate the usefulness of the PCR-ARMS technique followed by DNA sequencing as diagnostic tools that could be applied for carrier detection and prenatal diagnosis [12].

## MATERIALS AND METHODS

Venous blood samples were collected prior to transfusion in ethylenediaminetetraacetic acid (EDTA) containing tubes from thirty patients of random age with transfusion-dependent β-thalassemia major that referred to the department of Hiwa hospital in Sulaimania province, KRG, Iraq.

DNA was isolated from white blood cells using salting out method [13]. Amplification refractory mutation system (ARMS) technique was used for molecular detection of mutations in 30 samples and sequence analysis was done for two samples. Polymerase chain reaction (PCR-ARMS) primers were used for detect 8 beta thalassemia mutations. Sequences of allele-specific oligonucleotide primers (mutant) used are listed in table1.

**Table 1. Primer sequences for the ARMS-PCR.**

| Primer | Nucleotide sequence (5"→"3) | Position | Product size bp |
|---|---|---|---|
| **A F** | ACCTCACCCTGTGGAGCCAC | 62028-62047 | Sq* |
| **IVSI-I R** | TTAAACCTGTCTTGTAACCTTGATACCGAT | 62308_62279 | 280 |
| **IVSII-I R** | AAGAAAACATCAAGGGTCCCATAGACTGAT | 62661_62632 | 633 |
| **IVSI-110 R** | ACCAGCAGCCTAAGGGTGGGAAAATAGAGT | 62417_62388 | 389 |
| **Codon 44 R** | CAGCATCAGGAGTGGACAGATCCCCAATGA | 62478_62450 | 450 |
| **IVSI-5 R** | CTCCTTAAACCTGTCTTGTAACCTTGTTAG | 62312_62283 | 284 |
| **Codon 39 R** | CAGATCCCCAAAGGACTCAAAGAACCTGTA | 62463_62434 | 435 |
| **C R** | CCCCTTCCTATGACATGAACTTAA | 62703_62680 | Sq* |
| **-87 F** | CACTTAGACCTCACCCTGTGGAGCCACACA | 62021_62050 | 682 |
| **Codon 5 F** | TCAAACAGACACCATGGTGCACCTGAGTCG | 62174_62203 | 529 |

*** (Sq) direct Sequencing**

After optimizing PCR assay, according to [9], the ARMS analysis was performed in a reaction mixture of 50 µl contained 4 µl of genomic DNA, 1 µl 10 mM dNTP mix, 2 µl of each primer (foreword and reverse), 5 µl 10X PCR buffer, 1.5 µl 50 mM $MgCl_2$, 0.25 µl Taq DNA polymerase and 36.5 µl distilled water (total volume 50 µl). The thermocycler was programmed to incubate the sample for initial denaturation at 95°C for 5 minutes followed by 35 cycles, consisting of denaturation at 95°C for 1 minute, variable annealing temperatures (ranged between 55°C to 58°C) depending on each mutations) for 45 second, elongation at 72°C for 1.30 minutes with final elongation at 72°C for 7 minutes and lastly hold at 4 °C.

### *Direct sequencing*

To identify the other β-thalassemia alleles in the study, the beta globins of the two samples were amplified by two sets of primers (Set I and Set II), table 2 [14], were sequenced in IBL laboratory in Vienna.

Set I represents the primer pair that amplified exon I and exon II region and produced the fragments which is of amplified sizes of 760 base pairs (bp). Set II represents the primer pair that amplified exon III region and produced the fragments which is of amplified sizes of 690 bp [15].

The reaction was carried out on a 50µl reaction mixture containing 5 µl of genomic DNA, 1 µl of each primer (foreword and reverse), 25µl of green master mix, and 18 µl distilled water.

The thermal cycling of set I consists of heating the reaction at 95°C for 5 min and 40 cycles of denaturation at 94°C for 45 second, primer annealing at 58 °C for 30 second, DNA extension reaction at 72°C for 2 minute, and with final elongation at 72°C for 10 minutes. The thermal cycling of set II was performed like the set III condition except that the primer annealing

**Table 2. The sequences of primers used for the direct DNA sequencing .**

| Primer | Nucleotide sequence (5"→"3) | Position | Product size bp |
|---|---|---|---|
| **S1 F** | AGAAGAGCCAAGGACAGGTACG | 61991-61990 | 760 bp |
| **S2 R** | TGCAATCATTCGTCTGTTTCCC | 62730-62751 | |
| **S3 F** | TCCCTAATCTCTTTCTTTCAGG | 63190-63211 | 660 bp |
| **S4 R** | TTTTCCCAAGGTTTGAACTAGC | 63829-63849 | |

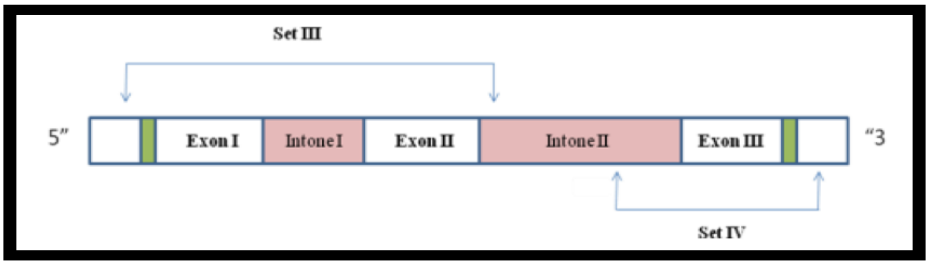



**Figure 1: Schematic diagram showing the β -globin genes and two fragments amplified for the direct DNA sequencing (Mirasenaa *et al*., 2007).**

## RESULTS

Over a period of 2 months 30 blood samples were randomly collected from transfusion dependent β-thalassemia patients, including (16) males (53.33%) and (14) females (46.66%), attending Hiwa hospital, in Sulaimania province. The patient samples were examined by PCR assay using 8 primers, each of them specific for a known mutation. The percentage prevalence of the IVSII-1 mutation was the predominant one (73.33%), figure 2, while the percentage prevalence of the others were: IVSI-1 (56.66%), figure 3, codon 44 (33.33%), figure 4, codon 39 (30%), figure 5, -87 (26.66%), figure 6, IVSI-110 (26.66%), figure 7, codon 5 (20%), figure8, and IVSI-5 (10%), figure 9.

To identify the other β-thalassemia mutations in the study, partial DNA sequencing was done for the β-globin gene (HBB) of two samples. They were amplified by two sets of primers, table 2. The resulting segments (two segments of each sample) were aligned with normal HBB sequence by balstn on the NCBI web site, and it showed four mutant sits, which are codon 2 (C→T), codon8/9 (+G), IVSI-6 (T→C) are related to beta thalassemia while the fourth one which is codon 85 (T→C) mutation produce an unstable Hb and cause hemolytic anemia.

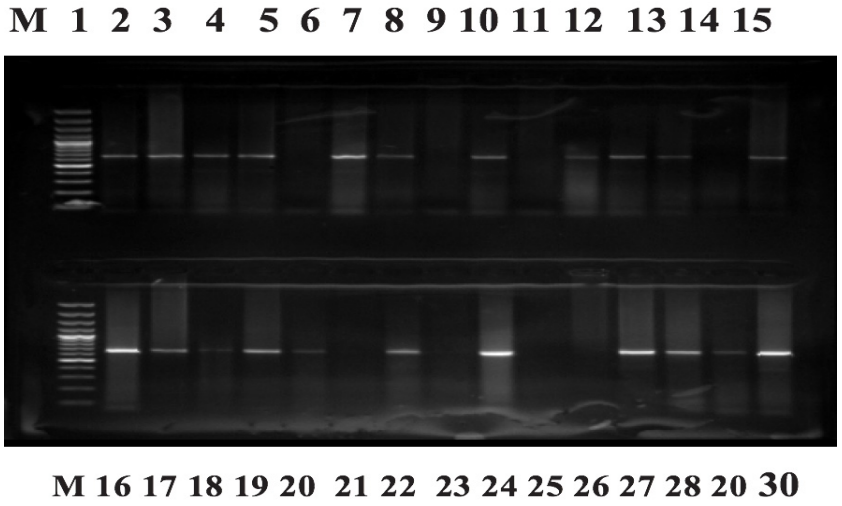



**Figure. 2 Amplification of (633bp) β-globin gene indicate detection of IVSII-1 (G→A) mutation using ARMS-PCR Lanes (m): 100 bp ladder Lanes (1, 2, 3, 4, 5, 7, 8, 10, 11, 12, 13, 14, 16, 17, 20, 22, 24, 25, 26, 27, 28 and 29): positive samples. Lanes (6, 9, 15, 18, 19, 21, 23 and 30): negative samples**

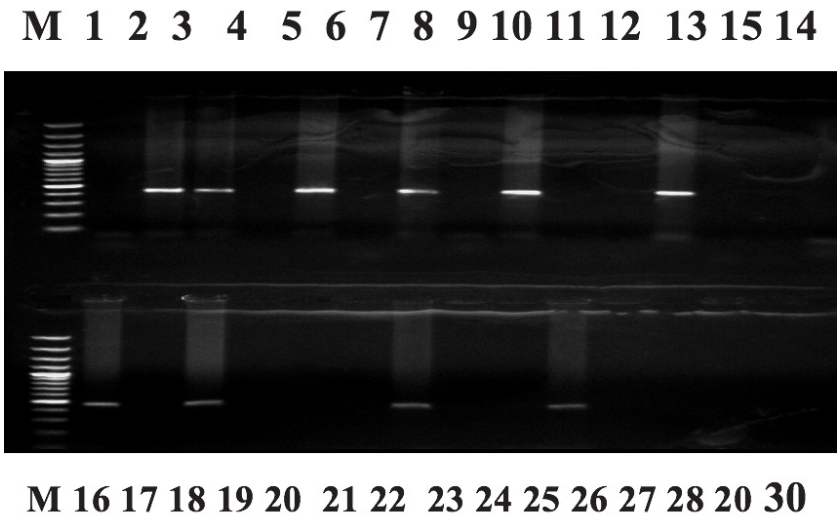



**Figure 3. Amplification of (280bp) β-globin gene indicate the detection of IVSI-1 (G→A) mutation using ARMS-PCR Lanes (m): 100 bp ladder. Lanes (1, 2, 3, 5, 6, 8, 9, 11, 15, 16, 17, 18, 19, 21, 25, 26 and 28): positive samples. Lanes (4, 7, 10, 12, 13, 14, 20, 22, 23, 24, 27, 29 and 30): negative samples.**

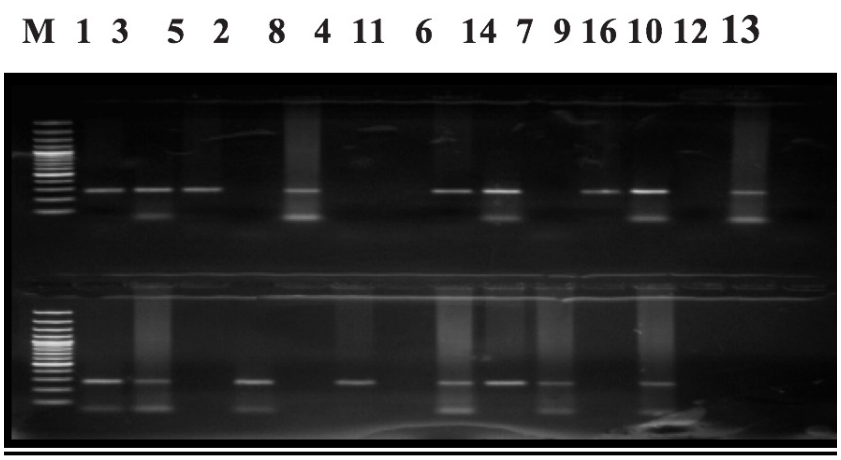



**Figure 4. Amplification of (450bp) β-globin gene indicate the detection of Codon 44 (-C) mutation using ARMS-PCR Lanes (m): 100 bp ladder. Lane (3, 5, 8, 11, 14, 16, 22, 24, 25 and 30): positive samples. Lane (1, 2, 4, 6, 7, 9, 10, 12, 13, 15, 17, 18, 19, 20, 21, 23, 26, 27, 28 and 29): negative samples.**

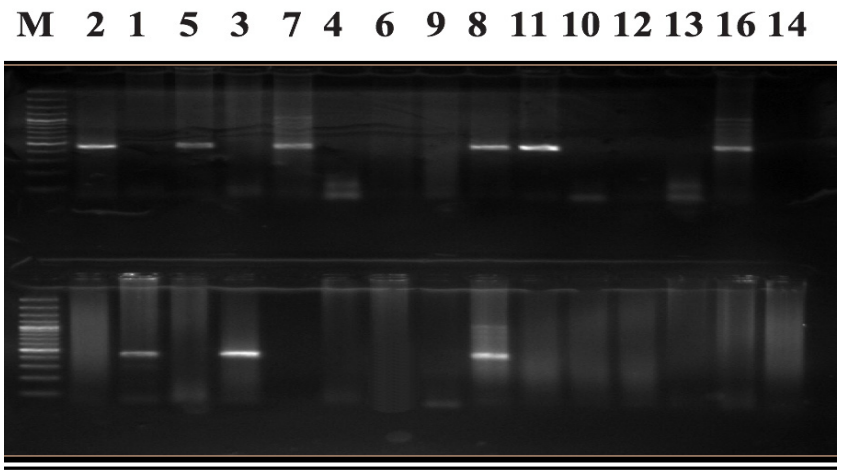



**Figure 5. Amplification of (435bp) β-globin gene indicate the detection of Codon 39 (C→T) mutation using ARMS-PCR Lanes (m): 100 bp ladder. Lanes (2, 5, 7, 8, 11, 16, 21, 28 and 30): positive samples.Lanes (1, 3, 4, 6, 9, 10, 12, 13, 14, 15, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27and 29): negative samples.**

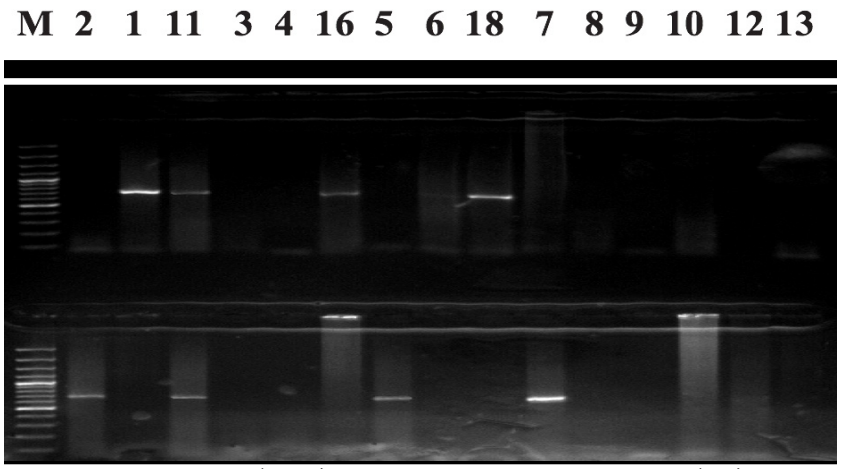
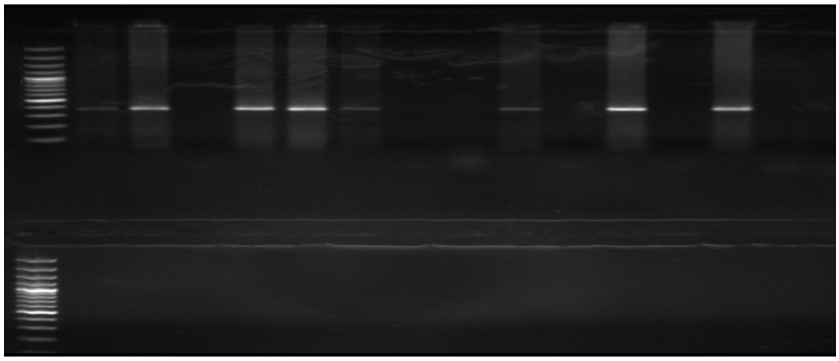



**Figure 6. Amplification of (682bp) β-globin gene indicate detection of -87 (C→T) mutation using ARMS-PCR Lane (m): 100 bp ladder Lane (1, 11, 16, 18, 23, 25, 29 and 30): positive samples. Lane (2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 17, 19, 20, 21, 22, 24, 26, 27 and 28): negative samples.**

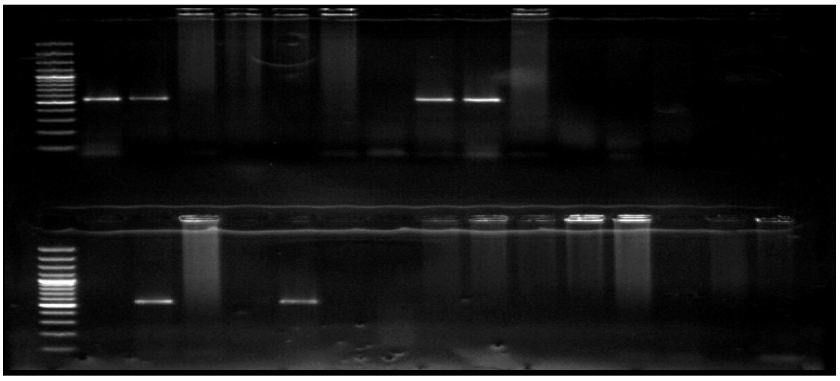
M 5 7 1 12 15 16 2 3 19 4 26 6 30 8 9



M 15 23 17 18 29 19 20 21 22 24 25 26 27 28 30

**Figure 7. Amplification of (682bp) β-globin gene indicate detection of -87 (C→T) mutation using ARMS-PCR Lane (m): 100 bp ladder Lane (1, 11, 16, 18, 23, 25, 29 and 30): positive samples. Lane (2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 17, 19, 20, 21, 22, 24, 26, 27 and 28): negative samples.**

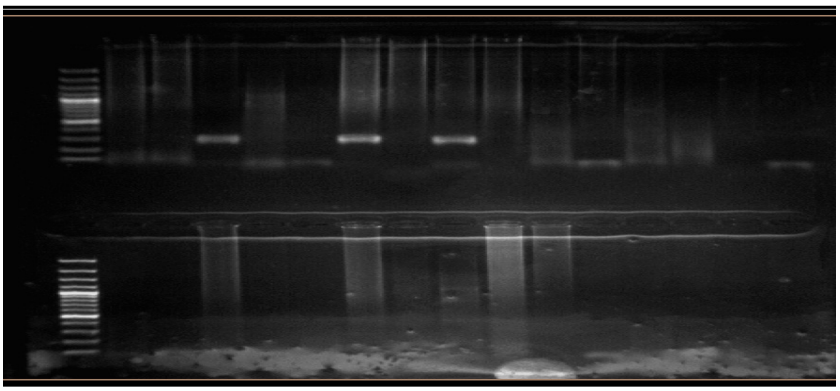
M 4 10 1 2 3 5 6 12 16 7 8 9 11 13 14



M 15 16 17 18 20 21 22 23 24 25 26 27 28 29

**Figure 8. Amplification of (533bp) β-globin gene indicate the detection of Codon 5 (-CT) mutation using ARMS-PCR Lanes (m): 100 bp ladder Lanes (4, 10, 12, 16, 23 and 29): positive samples Lanes (1, 2, 3, 5, 6, 7, 8, 9, 11, 13, 14, 15, 17, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28 and 30): negative samples ladder.**

M 1 2 6 3 4 13 5 19 7 8 9 10 11 12 14



M 10 11 13 14 17 18 20 21 22 23 24 25 27 28 29

**Figure 9. Amplification of (284bp) β-globin gene indicate the detection of IVSI-5 (G→C) mutation using ARMS-PCR Lanes (1 and 17): 100 bp ladder Lanes (4, 7 and 9): positive samples.Lanes (2, 3, 5, 6, 8, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32): negative samples.**

## DISCUSSION

The molecular characterisation of β-thalassemia mutations can be considered as a stepping-stone in identifying the spectrum of mutations in a population. The results of this study will show data on the spectrum of β-thalassemia mutations among β-thalassemia patients in Sulaimani, although a larger number of patients would provide more accurate representation of the spectrum of mutations. The characterisation of β-thalassemia mutations in this region will help to bolster the establishment of a rapid and effective prenatal diagnosis programme, or genetic counselling in this ethnic group in future as one of the effective ways to control the prevalence of β-thalassemia cases.

### *Mutations Analysis by ARMS-PCR*

In this study PCR method based on allele specific priming known as the amplification refractory mutation system (ARMS), was employed for the detection of eight β-thalassemia mutations for 30 patients and direct sequencing for 2 of them, to investigate the prevalence of β-thalassemia mutations in Sulaimani province, in the north of Iraq, which was the first study done for molecular detection and identification of β-thalassemia mutation. In the present study among 30 confirmed patients (male and female), all of the mutations (8 mutations) appeared with different ratio. Table (3) shows the frequencies of various mutations in patients with β-thalassemia major.

Therefore comparing the results of this study with the spectrum of β-thalassemia mutations that has been previously reported among other populations in the other countries illustrated that IVSII-1 (G→A) this type of mutation, which occurs due to base substitution at position 1 in intron II and prevents normal splicing results in absolute absence of β-globin ($\beta^0$)[16] was diagnosed as the most common mutation in Sulaimani (73.33%).

**Table 3. Frequencies of various mutations in patients with β-thalassemia.**

| Mutation | Detected* | Frequency |
|---|---|---|
| TVS II-1 | 22 | 77.33% |
| TVSI-1 | 17 | 56.66% |
| Codon 44 | 10 | 33.33% |
| Codon 39 | 9 | 30% |
| -87 | 8 | 26.66% |
| TVSI-110 | 8 | 26-66% |
| Codon 5 | 6 | 20% |
| TVSI-5 | 3 | 10% |
| Codon 2 | 1 | 3.33% |
| TVSI-6 | 1 | 3.33% |
| Codon 8/9 | 1 | 3.33% |
| Codon 85 | 1 | 3.33% |

Other studies have already reported this mutation with different frequency rates in other countries. For example, in Iran, among 23 β-thalassemia, it forms the high frequency (around 48 % [17]. In Kuwait, it is the most common mutation of about (29%)[18]. In Jordan, it forms the most frequent mutation of about (15%), among 135 unrelated occasionally and periodically transfusion dependent β-thalassemia patients[19]. In Turkey, it forms the third most frequent mutation of about 8%[20], and in the Eastern province of Saudi Arabia, among 69 persons, it is the most frequently encountered with a frequency of 27.5%[21].

IVSI-1 (G→A) this type of mutation occurs due to base substitution at position 1 in intron I, and prevents normal splicing result in absolute absence of β-globin ($\beta^0$)[22]. It is the second common mutation in Sulaimani (56.66%). This can also be found in other countries. For example, in Lebanon, it is the second most frequent β-thalassemia mutation, with an incidence of 15%[23]. In Jordan, it forms the fourth most frequent mutation around 10%[19]. In the Eastern province of Saudi Arabia, among 69 persons, it forms the fourth frequent mutation of about 5.8%[21]. In Palestine, it forms the fourth frequent mutation around (9%) in 148 patients[24]. In Iran, among 23 persons, it forms the seventh β-thalassemia mutation around (4.59%)[17].

The third mutation detected in this study was codon 44 (33.33%), which occurs due to frame shift mutation (deletion C) at position 42 in exon II, which change reading frame results in absolute absence of β-globin ($\beta^0$)[24]. This type was also detected in Saudi Arabia, among 69 persons, it formed eighth frequent β-thalassemia mutation 1.8%[21]. In Turkey, among 108 persons, it forms the eighth frequent β-thalassemia mutation around 3.6%[25]. In Lebanon, it forms the ninth frequent β-thalassemia mutation of around (1.5%) among 260 patients [23]. It forms up to 10% in Oman, while in Tunisia; it forms around (4%)[18] (Zahed, 2001), and in Qazvin province in Iran in 100 patients it forms the five most common mutation of around 6.1% [9].

The fourth mutation detected was codon 39 (30%) which occurs due to base substitution (C→T) at position 26 in exon II, and it forms stop codon, and causes absolute absence of β-globin ($\beta^0$)[22]. This type was also found in other countries. For example, in Bahrain, it forms the second frequent 26% in 70 patients [26]. In Saudi Arabia, it forms the third frequent β-thalassemia 20.3% in 69 patients[21]. In Turkey, it forms the tenth frequent 1.7% in 103 fetuses[32], and in Jordan, it forms the seventh frequent β-thalassemia 4.6% in 135 patients[19].

-87 is the fifth mutation in this study of about (26.66%), which occurs due to base substitution (C→T) at position -87 promoter region and interferes with transcription and causes mild ($\beta^+$)[27]. This type again was also present in other counties with different frequency. For example, in Syria, the frequency of this mutation was around 2%, while in Lebanon, it was 1.2%. In Jordan, it was 2%[18], and in Egypt it occured with (3.2%) frequency among 95 patients[29].

IVSI-110 the sixth mutation which was detected (26.66%) in this study, occurs due to base substitution (G→A) in cryptic splice site at position 110 in intron I, reduces β-globin production and it forms sever ($\beta^+$) phenotype[16] (Higgs *et al*., 2012). In other countries, in Antalya province of Turkey, for example, it was reported as the most frequent allele (50.4%) in 103 fetus [32]. In Jordan, it forms the high frequency of β-thalassemia mutation of 25% in 135 patients[19]. In Lebanon, it forms the high frequency of β-thalassemia mutation of 34.2% in 260 patients [23]. In Palestine, it forms the second common mutation (17.6%) among 148 patients [24], and in Southern Iran, it forms the third frequent β-thalassemia mutation of around (8.38%)[17].

Codon 5 form the sixth mutation (20%) in this study, which occurs due to deletion (-CT) at position 17 in exon I, and leads to $\beta^0$, which changes reading frame (frameshift) results in absolute absence of β-globin ($\beta^0$) [27]. It is also detected in Lebanon, form sixth frequency of β-thalassemia mutation 5% in 260 patients [23]. In Antalya province of Turkey, it forms the sixth frequency of β-thalassemia 4.7% in 103 fetus ([32]. In Jordan, it forms eighth frequency of β-thalassemia 3.8% in 135 patients [19]. In Saudi Arabia, it forms the seventh frequency of β-thalassemia 1.5% in 69 patients[21]. And in Iran, it forms the eighth frequency of β-thalassemia 4.4% n 38 patients[17].

IVSI-5 (G→C), was the last mutation which was detected in this study. It was detected only in 3 patients out of 30 (10%). It occurs due to base substitution in consensus sequences at position 5 in intron I and reduces β-globin production and forms sever ($\beta^+$) phenotype[23]. It also appeared in the UAE, as the most common mutation (55%) and Oman (62%) It is also quite frequent in neighboring Kuwait and Saudi Arabia (17%–19%)[18] (Zahed, 2001), in India, it forms the high frequency of β-thalassemia mutation around 56.8%[28]. And in Isfhan province of Iran, it forms 16.3% among 114 patients [29].

### *Mutation Detected by Sequencing*

The PCR product (which represent HBB gene) of two samples (No.20 and No.27) amplified by two sets of primers, were sequenced in IBL laboratory in Vienna for showing the sequence of HBB gene and detect other mutation if present. The four different types of mutations were detected by direct sequencing, three of them in sample No.20 and the other one in sample No.27, are: Codon 8/9 (+G), it is a frame-shift mutation which occurs due to insertion of (G) between codon 8 and codon 9 at the position 28 of exon I. It prevents β-globin synthesis and forms sever ($\beta^0$) phenotype [30]. It was detected in sample (No.27) by direct sequencing. It also was detected in other countries. For example, in Lebanon, it occurred with the frequency of 0.2% [23]. In Iran, it forms 13.51% among 185 patients [17]. In Pakistan, it was detected as the high frequent β-thalassemia mutation around (38.59%)[31].

In Syria, Saudi Arabia, Kuwait, Bahrain and UAE occurred with frequency of 1.4%, 2.5%, 1.3%, 1.5% and 7%, respectively [18].

IVSI-6 (T→C), is the base substitution mutation, which occurs at the position 6 of first intervening sequence and forms mild β-thalassemia which lead to reduce β- globin synthesis and forms moderate ($\beta^+$) phenotype[23]. It was detected in sample (No.20) by direct sequencing. This mutation was reported in other countries. For example, in Palestine, it forms the most frequent mutation (28.7%) among 148 patients[23]. In Turkey, it was reported as the second frequent allele (9.7%) in 103 fetus [32], in Lebanon, it forms the third common mutation 14.4% among 260 persons[23]. In Jordan, Israel, Kuwait, Gaza, and UAE, the percentage of distribution was 6.6%, 14.7%, 7.3%, 7.5% and 3.5%, respectively[18. Other mutations, which were detected by sequencing, are: Base substitution (C→T) which occurs at position 9 (codon 2) in exon I of sample (No.20), it forms silent mutation, which does not change amino acid (Histidine CAC→CAT). According to [31], silent mutation in β-globin leads to extremely mild β-thalassemia ($\beta^{++}$). This type of mutation was also detected in other part of the world. For example, in references with accession numbers {AY128650.1, L26478.1, EF450778.1, EU760913.1, EU760937.1}. The silent β-thalassemia causes only a minimal deficit of β-globin production and does not produce a detectable haematological phenotype when present in a single copy, the only abnormality is a very mild imbalance of globin synthesis and leads to extremely mild β-

thalassemia ($\beta^{++}$)[16, 33].

Another mutation was detected by sequencing is base substitution (T→C) which occurs at position 165 (codon 85) in exon II of sample (No.20). It forms missense mutation, which changes amino acid (Phenyl-alanine TTT→ Serine TCT) in the β-globin chain. This type of mutation forms an unstable hemoglobin variant, which is called (Hb Buenos Aires) also known as (Bryn Mawr) resulting in congenital hemolytic anemia [34]. The above results indicate that these mutations detected in this study were also detected in surrounding countries, which occurred with varying frequency. These results are in line with studies in other parts of the world, which have shown that gene flow due to population migration is common

## REFERENCE

1. Borgna-pignatti C, Galanello R. Thalassemias and related disorders: quantitative disorders of hemoglobin synthesis. In: Greer JP, Editor. Wintrobe's Clinical Hematology. 12th ed. USA: Lippincott Williams & Wilkins; 2008.

2. Tubman VN, Fung EB, Vogiatzi M, Thompson AA, Rogers ZR, Neufeld EJ, et al. Guidelines for the Standard Monitoring of Patients With Thalassemia: Report of the Thalassemia Longitudinal Cohort. Journal of pediatric hematology/oncology. 2015; 37(3):e162-9.

3. Langlois S, Ford JC, Chitayat D, Desilets VA, Farrell SA, Geraghty M, et al. Carrier screening for thalassemia and hemoglobinopathies in Canada. Journal of obstetrics and gynaecology Canada : JOGC = Journal d'obstetrique et gynecologie du Canada : JOGC. 2008; 30(10):950-71.

4. Long J, Ye X, Lao K, Pang W, Weng X, Fu K, et al. Detection of three common alpha-thalassemia in non-deletion types and six common thalassemia in deletion types by QF-PCR. Clinical biochemistry. 2013; 46(18):1860-4.

5. Huang SW, Liu XM, Li GF, Su L, Wu X, Wang RL. Spectrum of beta-thalassemia mutations in Guizhou Province, PR China, including first observation of codon 121 (GAA>TAA) in Chinese population. Clinical biochemistry. 2013; 46(18):1865-8.

6. Rao S, Saxena R, Deka D, Kabra M. Use of HbA estimation by CE-HPLC for prenatal diagnosis of beta-thalassemia; experience from a tertiary care centre in north India: a brief report. Hematology. 2009; 14(2):122-4.

7. Bahadır A, Öztürk O, Atalay A, Atalay EO. Beta globin gene cluster haplotypes of the beta thalassemia mutations observed in the Denizli province of Turkey. Turkish Journal of Hematology. 2009; 26(3): 129-137.

8. Fauci AS, Kasper DL, Longo DL, Braunwald E, Hauser SL, Jameson J, et al. Harrison principles of internal medicine. 17th ed. United States: The McGraw-Hill Companies; 2008.

9. Sarookhani MR, Ahmadi MH, Amirizadeh N. Molecular spectrum of beta globin mutations in transfusion-dependent patients with thalassemia in Qazvin Province, Iran. Iranian Journal of Medical Science. 2009; 34(1): 17-24.

10. Boonyawat B, Monsereenusorn C, Traivaree C. Molecular analysis of beta-globin gene mutations among Thai beta-thalassemia children: results from a single center study. The application of clinical genetics. 2014; 7:253-8.

11. Villegas A, Ropero P, Gonzalez FA, Anguita E, Espinos D. The thalassemia syndromes: molecular characterization in the Spanish population. Hemoglobin. 2001; 25(3):273-83.

12. Abuzenadah AM, Hussein IM, Damanhouri GA, FM AS, Gari MA, Chaudhary AG, et al. Molecular basis of beta-thalassemia in the western province of Saudi Arabia: identification of rare beta-thalassemia mutations. Hemoglobin. 2011; 35(4):346-57.

13. Epplen, JE, Lubjuhn T. DNA profiling and DNA fingerprinting. (1999). Berlin: Birhkhauser Verlag; 1999. P. 252.

14. Mirasena S, Shimbhu D, Sanguansermsri M, Sanguansermsri T. The Spectrum of β-thalassemia Mutations in Phitsanulok Province: Development of Multiplex ARMS for Mutation Detection. Naresuan University Journal. 2007; 15(1): 43-53.

15. Sirichotiyakul S, Saetung R, Sanguansermsri T. Analysis of beta-thalassemia mutations in northern Thailand using an automated fluorescence DNA sequencing technique. Hemoglobin. 2003; 27(2):89-95.

16. Higgs DR, Engel DE, Stamatoyannopoulas G. Thalassemia. Lancet. 2012; 379: 373-383.

17. Rahimi Z, Muniz A, Parsian A. Detection of responsible mutations for beta thalassemia in the Kermanshah Province of Iran using PCR-based techniques. Molecular biology reports. 2010; 37(1):149-54.

18. Zahed L. The Spectrum of beta-Thalassemia Mutations in the Arab Populations. Journal of biomedicine & biotechnology. 2001; 1(3):129-32.

19. Sadiq MF, Eigel A, Horst J. Spectrum of beta-thalassemia in Jordan: identification of two novel mutations. American journal of hematology. 2001; 68(1):16-22.

20. Ghazi O. Tadmouri and A. Nazlõ Bas°ak. Advances in Hemoglobinopathies Research B-Thalassemia In Turkey: A Review of The Clinical, Epidemiological, Molecular, and Evolutionary Aspects. Hemoglobin. 2001; 25(2), 227-239.

21. Al-Ali AK, Al-Ateeq S, Imamwerdi BW, Al-Sowayan S, Al-Madan M, Al-Muhanna F, et al. Molecular bases of beta-thalassemia in the Eastern Province of Saudi Arabia. Journal of biomedicine & biotechnology. 2005; 2005(4):322-5.

22. Agouti I, Badens C, Abouyoub A, Levy N, Bennani M. Molecular basis of beta-thalassemia in Morocco: possible origins of the molecular heterogeneity. Genetic testing. 2008; 12(4):563-8.

23. Makhoul NJ, Wells RS, Kaspar H, Shbaklo H, Taher A, Chakar N, et al. Genetic heterogeneity of Beta thalassemia in Lebanon reflects historic and recent population migration. Annals of human genetics. 2005; 69(Pt 1):55-66.

24. Galanello R, Eleftheriou A, Trager-Synodinos J, Old J, Petrou M, Angastiniotis M. Prevention of Thalassaemias and Other Haemoglobin Disorders. Thalassaemias international federation. 2003; 1.

25. Pehlivan S, Okan V, Guler E, Yilmaz M, Sever T, Dikensoy E, Kilincarslan C. et al. Molecular basis of β - thalassemia mutations in an urban area of Gaziantep Turkey. Hematologica. 2008; 93(1): 337.

26. Jassim N, Al-Arrayed S, Al-Mukharraq H, Merghoub T, Krishnamoorthy RSpectrum of β-thalassemia Mutation in Bahrain. Bahrain Medical Bulletin. 2000; 22(1)

27. Ngo DA, Steinberg MH. Genomic approaches to identifying targets for treating beta hemoglobinopathies. BMC medical genomics. 2015; 8:44.

28. Gajra B, Chakrabarti S, Sengupta B, De M, Mukherjee S, Talukder G. Prevention of β -Thalassemia Major and E β Thalassemia by Prenatal Diagnosis in Eastern India. International journal of human genetics. 2003; 3(4): 225-235.

29. Derakhshandeh-Peykar P, Hourfar H, Heidari M, Kheirollahi M, Miryounesi M.The Spectrum of β -thalassemia Mutations in Isfahan Province of Iran. Iranian Journal of Public Health . 2008; 37(2):106-111.

30. Weatherall DJ. Disorders of Globin Synthesis: The Thalassemias. In: Lichtman MA, Kipps K, Kaushansk E, Seligsohn U, Prchal JT, Editors. Williams hematology. 7th ed. United states: McGrow Hill; 2006.

31. Baig SM, Azhar A, Hassan H, Baig JM, Kiyani A, Hameed U, et al. Spectrum of beta-thalassemia mutations in various regions of Punjab and Islamabad, Pakistan: establishment of prenatal diagnosis. Haematologica. 2006; 91(3):ELT02.

32. Keser I, Manguolu E, Kayisli Og, Kurt F, Mendilcioglu I, Simsik M, et al. Prenatal Diagnosis of β-Thalassemia in the Antalya Province: clinical investigation. Turkish journal of medical sciences. 2005; 35(2005):251-253.

33. Olivieri NF. The β-Thalassemias: review article. The new England journal of medicine. 1999; 341: 99-109.

34. Larson PJ, Friedman DF, Reilly MP, Kattamis AC, Asakura T, Fortina P, et al. The presurgical management with erythrocytapheresis of a patient with a high-oxygen-affinity, unstable Hb variant (Hb Bryn Mawr). Transfusion. 1997; 37(7):703-7.