Archives of Razi Institute, Vol. 77, No. 3 (2022) 976-980

DOI: 10.22092/ARI.2022.357209.1997

Original Article

Genetic Analysis of Alpha-Thalassemia Mutations in Thi-Gar Province, Iraq

Odah Al-Musawi, A. H[1 *], Jumaah Alhussna, A[2], Hussein Jalood, H[2]

1. Collage of education for women, University of Thi-Qar, Nasiriyah, Iraq
2. General Directorate of Education in Thi-Qar Iraq

Received 22 December 2021; Accepted 16 January 2022
Corresponding Author: ali.habeeb2020@utq.edu.iq

Abstract

The prevalence of alpha-thalassemia as a major health problem in the south of Iraq has highlighted the necessity of investigations and screening of patients with thalassemia. The present study aimed to characterize the spectrum of alpha-globin gene mutations in patients who were followed up in a genetic diseases center in Thi-Qar province. A total of 30 subjects were collected from thalassemia patients and 15 cases as the control group. Polymerase chain reaction (PCR) and direct sequencing were performed for functionally regions of the gene (exon 1 and exon 2). The fragment size amplified was 442 bp in the Exon 1 region and 324 bp in the Exon 2 region of α-globin. The molecular analysis of the sequence of PCR products revealed that 13 point mutation within the α-thalassemia gene included deletion and substitution mutation, while the rest of the mutations were in the intron site of the gene. These results indicated that mutations may constitute a risk of developing hemophilia B disease. Molecular mechanisms in the expression of globin genes are used to help manage patients with thalassemia.

Keywords: alpha-thalassemia, Iraq, mutation, PCR

1. Introduction

Hemoglobinopathies are groups of inherited disorders of hemoglobin synthesis, mutation, or deletion of one or more globin genes, resulting in the production of structurally abnormal Hb variants and reduced synthesis rate of structurally normal globin chains (1). Hemoglobin disorders are the most frequent genetic diseases in the world caused by structural changes in the globin protein chains of hemoglobin and thalassemia, which are disorders of globin expression (2). Thalassemia is a genetic disorder that involves mutations in the genes responsible for hemoglobin production in the blood. It has two broad types, alpha and beta-thalassemia, each of which has a different prevalence among certain ethnicities (3).

Alpha thalassemia represents a group of recessively inherited hemoglobin disorders due to deficient or absent synthesis of α-globin genes. It is the most common monogenic disorder affecting 5% of the world population (4). The first reports on thalassemia major and sickle cell disease from Iraq appeared in the 1960s; therefore, these inherited disorders have been recognized as major health problems in this area (5). The inheritance of alpha thalassemia is complex since each individual has four distinct α-globin genes (two genes per chromosome 16).

The deletion of all four α-globin genes leads to a condition known as Hb Barts syndrome (homozygous alpha-thalassemia). The prevalence of these disorders has been reported in Middle Eastern countries (6). The heterozygous of α-thalassemia is often clinically

asymptomatic, and the high frequency of inherited hemoglobin variants has been reported in Sub-Saharan Africa to the Indian subcontinent, as well as East and Southeast Asia (7, 8).

Thalassemia is caused by impaired synthesis of globin chains, altering the production of hemoglobin (Hb). It is a common cause of various degrees of anemia with different clinical signs (9, 10). Sickle cell disorders were mainly observed in the extreme north and south of Iraq, with the highest carrier rates in the south reaching up to 16% in some localities (11). Thalassemia major is one of the major problems in the north and south of Iraq. In the north of Dohuk and Erbil provinces with an area of about 20,000 square kilometers, in the south of T-Qar province, we are witnessing an increase in the prevalence of this disease. Due to the prevalence of Alpha-thalassemia in this region, the present study was carried out at the University of Thi-Qar on Alpha-thalassemia patients, and genetic mutations of this disorder were evaluated.

2. Materials and Methods

2.1. Sampling

The present study was conducted on 30 Iraqi patients (13 males and 17 females) with α -thalassemia disease and 15 healthy subjects as the control group from January to May 2021. The patients were within the age range of 2-28 years and were selected from the center of the genetic diseases in Thi-Qar province, south Iraq, while the healthy cases were selected randomly from the same area. The data were collected by a questionnaire. A venous blood sample (5 ml) was collected from each patient and healthy control by a medical specialist from a genetic diseases center. Each blood sample was collected in EDTA tubes for molecular studies.

2.2. Genomic DNA Isolation and Amplification

Genomic DNA was extracted using AccuPower® gsync DNA mini kit 100 prep (Bioneer, South Korea). The extracted DNA was stored at -20°C until use and then quantified through the measurement of its OD260 by ND-2000 spectrophotometer (Thermo Scientific Inc., USA) and specific primers displayed in table 1. The PCR was performed using AccuPower® PCR, 5 µl of pre-Mix (Bioneer, South Korea), 2.5 µl of 10 pmol/µl of each forward and reverse primers, and 5 µl template DNA extract and the volume was completed to 20 µl with deionized distilled water.

Table 1. Sequences of primers used in this study

Primers	sequences	Annealing	Band size
Exon1 1F	AGACTCAGAGA GAACCCACC	58 C	442 bp
Exon1 1R	GCTCACCTTGAA GTTGACC		
Exon2 1F	GGTCGAGGGGC GAGATGGCG	56 C	324 bp
Exon2 1R	CCACGGGGGTA CGGGTGCAGG		

The PCR Programs for exon 1 were carried out in Veriti™ thermal cycle (Applied Biosystem) using the standard cycle procedure: one cycle of 5 min denaturation at 95°C, then 30 cycles of 30-sec denaturation at 95°C, 30 sec of annealing at 58°C, 30 sec of extension at 72qaqq°C, and 5 min for the final extension at 72°C. The PCR products were then analyzed by sequencing. For exon 2, the annealing temperature was 56°C, and the other steps were performed like exon 1. The electrophoresis was conducted on 2% agarose gel at 1 volt/cm^2 for 75 min and ethidium bromide staining.

2.3. Polymerase Chain Reaction Product Sequencing

The PCR products of 13 samples of the exon 1 gene primer and 17 samples of the exon 2 gene primer were sent to Macrogen (South Korea) for sequencing. Sequence analysis was performed by a direct sequence of the PCR products. The results were compared with sequences in the national center biotechnology information (NCBI) Gene Bank.

3. Results and Discussion

The PCR was used to amplify the target fragment using specific primers. The fragment size amplified

was 442 bp in the Exon 1 region and 324 bp in the Exon 2 region of the *A-globin* gene *(HBA)*, as illustrated in figures 1 and 2.

The analysis of gene sequence results demonstrated transversion and deletion mutation in the Alpha-1-globin gene in alpha-thalassemia patients (Figures 3 and 4). The location and type of mutation in the gene are presented in table 2.

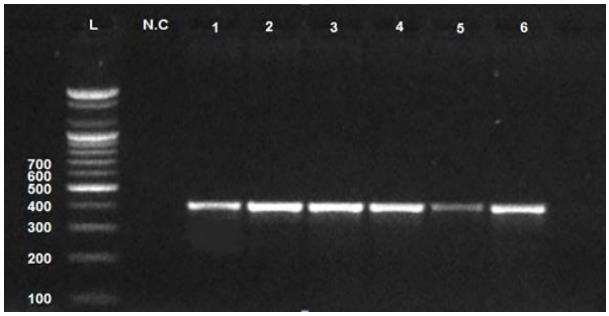

Figure 1. Polymerase chain reaction product of Exon 1 region (442bp) visualized under UV light, DNA ladder=100bp; N=negative control

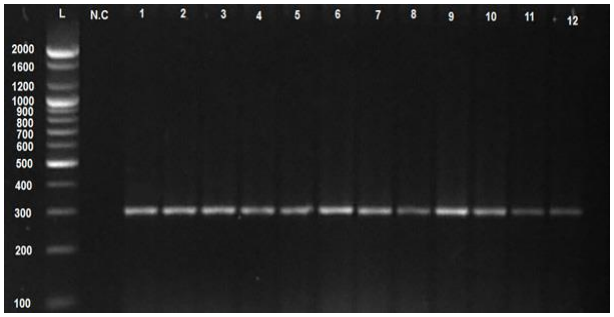

Figure 2. Polymerase chain reaction product of Exon 2 region (324bp) visualized under UV light, DNA ladder=100bp; N=negative control

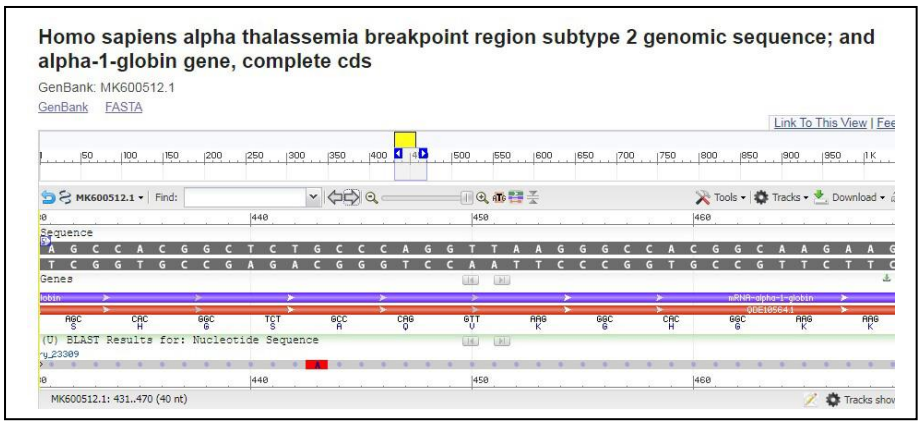

Figure 3. Transversion mutation in Alpha-1- globin gene

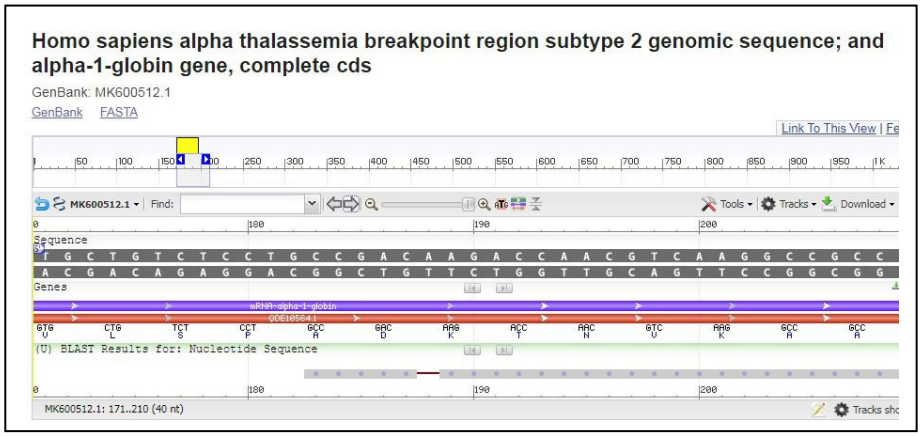

Figure 4. Deletion mutation in Alpha-1- globin gene

Table 2. Location and type of mutation in Alpha-1 globin gene in Alpha Thalassemia patients

S.no	Mutations	Location	Type	Effect	Originality
S1	g.679 G > C	Intronic	Transversion	-	Rs 600512.1 Mk 600512.1
S2	g.188 *del* A	Lysine > Arginine	deletion	Frame shift	Novel
S5	g.188 *del* A	Lysine > Arginine	deletion	Frame shift	Novel
S7	g.188 *del* A	Lysine > Arginine	deletion	Frame shift	Novel
S8	g.188 *del* A	Lysine > Arginine	deletion	Frame shift	Novel
S9	g.494 G > T	Valine > Leucine	transition	Frame shift	Novel
S10	g.502 *del* C	Histidine > Glutamine	deletion	Frame shift	Novel
S14	g.188 *del* A	Lysine > Arginine	deletion	Frame shift	Novel
S20	g.188 *del* A	Lysine > Arginine	deletion	Frame shift	Novel
S20	g.679 G > C	Intronic	Transversion	-	Rs 600512.1 Mk 600512.1
S24	g.502 *del* C	Histidine > Glutamine	deletion	Frame shift	Novel
S25	g.443 G > A	Alanine > Threonine	transition	Frame shift	Novel
S25	g. 509 G > A	Aspartic acid > Asparagine	transition	Frame shift	Novel
S25	g.679 G > C	Intronic	Transversion	-	Rs 600512.1 Mk 600512.1

Alph-thalassemia as one of the most important genetic diseases plays a major role in human life, and in the past years, there has been a marked increase in the number of people infected with this disease. About 1, 500 children are suffering from different types of thalassemia in the southern cities of Iraq, and around 250 causative mutations are characterized in the α-globin gene. Alpha thalassemia diagnosis is very complicated due to the genetic diversity of the HBA gene across different geographical regions of the world (1, 5, 6).

The α-globin gene has three exons and two introns involved in α-thalassemia pathogenesis. The mutations detection of α-thalassemia is absolutely necessary for molecular diagnosis (2). One of the main reasons for this increase is inbreeding (closed marriage), which directly affects the concentration and transmission of defective genes from parents to children. Therefore, the current study aimed to find out the most important mutations that cause thalassemia, especially in southern Iraq. As evidenced by the results of this study, many mutations were observed in the studied samples, some of which had a slight effect since they occurred in the intron.

In the sequence examination obtained, the code AAG that encodes Leucine acid, a deletion occurred in the second base, replaced by the base, and became the code AGA that encodes for arginine acid, as well as the genetic code GUG that encodes for Valine. The first base was replaced and the code was changed to one that codes for Leucine TTG, also the genetic code CAC that encodes for Histidine, in which the third base was deleted, and therefore, replaced by the next base and became the code CAG that codes for glutamine. The GCA that encodes the amino acid alanine has been replaced by the third base and becomes the one that codes for the acid threonine ACC.

Based on the aforementioned results, there have been significant changes in the genetic codes and the translation process, affecting the emergence and spread of the disease. In addition to that, the above mutations were frequent in the majority of samples in the current study. Therefore, it is considered a major indicator of the prevalence of this disease, especially in the study area, and can be used as brands or indications to detect this type of disease. These results pointed out that mutations may constitute a risk of developing thalassemia disease.

The mechanisms that are particularly important in modulating the severity of thalassemia include those that lead to chromosomal rearrangements, gene conversion, copy number variation, telomere cleavage, and homologous recombination (12). Mutations may lead to globin instability due to intermolecular interactions in the Hb tetramer, interactions with other proteins, such as AHSP, or possible post-translational changes (13). Liebhaber and Kan (14) pointed out that non-deleting defects in the alpha 2 gene are more

severe since the gene is encoded two or three times more than the alpha 1 gene.

Given the high cost of treatment and catastrophic problems in families with this disorder, control and prevention programs are necessary in this regard. Researchers need to thoroughly investigate the molecular mechanisms involved in the expression of globin genes to help manage patients with thalassemia. One way is to study molecular markers that are associated with globin gene expression and changes during growth stages. It is also important to enhance awareness, neonatal and prenatal screening programs, and monitoring of patients.

Authors' Contribution

Study concept and design: A. H. O. A.
Acquisition of data: A. J. A.
Analysis and interpretation of data: A. J. A.
Drafting of the manuscript: A. H. O. A.
Critical revision of the manuscript for important intellectual content: H. H. J.
Statistical analysis: A. H. O. A.
Administrative, technical, and material support: A. H. O. A.

Ethics

All participants signed a written consent form and all research steps had the approval of the Ethics Committee of the University of Thi-Qar, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Polus R. Prevalence of hemoglobinopathies among marrying couples in Erbil province of Iraq. 2017;6(2):90-3.
2. Sabath DE. Molecular Diagnosis of Thalassemias and Hemoglobinopathies: An ACLPS Critical Review. Am J Clin Pathol. 2017;148(1):6-15.
3. Nezhad FH, Nezhad KH, Choghakabodi PM, Keikhaei B. Prevalence and Genetic Analysis of alpha- and beta-Thalassemia and Sickle Cell Anemia in Southwest Iran. J Epidemiol Glob Health. 2018;8(3-4):189-95.
4. Laghmich A, Alaoui Ismaili FZ, Barakat A, Ghailani Nourouti N, Khattab M, Bennani Mechita M. Alpha-Thalassemia in North Morocco: Prevalence and Molecular Spectrum. Biomed Res Int. 2019;2019:2080352.
5. Al-Allawi NA, Jalal SD, Ahmed NH, Faraj AH, Shalli A, Hamamy H. The first five years of a preventive programme for haemoglobinopathies in Northeastern Iraq. J Med Screen. 2013;20(4):171-6.
6. Shamoon RP, Al-Allawi NA, Cappellini MD, Di Pierro E, Brancaleoni V, Granata F. Molecular Basis of beta-Thalassemia Intermedia in Erbil Province of Iraqi Kurdistan. Hemoglobin. 2015;39(3):178-83.
7. Weatherall DJ, Williams TN, Allen SJ, O'Donnell A. The population genetics and dynamics of the thalassemias. Hematol Oncol Clin North Am. 2010;24(6):1021-31.
8. Yang Z, Zhou W, Cui Q, Qiu L, Han B. Gene spectrum analysis of thalassemia for people residing in northern China. BMC Med Genet. 2019;20(1):86.
9. Vichinsky EP. Changing patterns of thalassemia worldwide. Ann N Y Acad Sci. 2005;1054:18-24.
10. Aziz A, Ahmed W, Sarward G. Molecular analysis of Hb-E and Beta-thalassemia major patients among Bangladeshi population. Austin J Biotechnol Bioeng. 2017;4(4):1085.
11. Al-Allawi NA, Jalal SD, Nerwey FF, Al-Sayan GO, Al-Zebari SS, Alshingaly AA, et al. Sickle cell disease in the Kurdish population of northern Iraq. Hemoglobin. 2012;36(4):333-42.
12. Farashi S, Harteveld CL. Molecular basis of alpha-thalassemia. Blood Cells Mol Dis. 2018;70:43-53.
13. Farashi S, Vakili S, Garous NF, Ashki M, Forouzesh Pour F, Zeinali F, et al. Identification of Mutations Causing Aberrant Termination and Deficient Splice Donor Site on the HBA1 Gene. Hemoglobin. 2016;40(1):38-43.
14. Liebhaber SA, Kan YW. Differentiation of the mRNA transcripts originating from the alpha 1- and alpha 2-globin loci in normals and alpha-thalassemics. J Clin Invest. 1981;68(2):439-46.