Research article

Molecular characterization of HBB gene mutations in beta-thalassemia patients of Southern Iraq

Ali Habeeb Odah Al-Musawi[1], Hussein Mohammed Aziz[2], Safa Khudair[3], Tahreer Hadi Saleh[4]

[1]Deparatement of Biology, College of Education for Women, University of Thi-Qar, Thi-Qar, Iraq
[2]Department of Anesthesia Techniques, College of Medical Science Technology, The University of Masherq, Baghdad, Iraq
[3]Department of Dental Prosthetic Technology, Medical Technical College, Al-Farahidi University, Al-Jadiriyah Bridge, Baghdad, Iraq
[4]Biology Department, College of science, Mustansiriyah University. Baghdad, Iraq

(Received: August 2022 Revised: September 2022 Accepted: October 2022)

Corresponding author: **Ali Habeeb Odah Al-Musawi.** Email: ali.habeeb2020@utq.edu.iq, alim.bio93@gmail.com

ABSTRACT

Introduction and Aim: Beta-thalassemia is a serious inherited genetic disorder and an increasing health burden globally. Beta -thalassemia is caused by genetic globin abnormalities within the hemoglobin beta (HBB) gene. This study aimed to characterize the HBB gene mutations in beta -thalassemia among southern Iraqi patients.

Materials and Methods: The study included 30 beta -thalassemia patients referred to the Thi-Qar Center for Genetic Diseases, Iraq and 15 control samples from a random group of apparently healthy individuals. Genomic DNA was isolated from blood sample collected from each individual. The DNA was amplified for specific regions of the HBB gene and the amplified products sequenced. The sequences generated were analysed for mutations using sequence analysis tools.

Results: Molecular analysis revealed several mutations in the HBB gene including translocation, deletion and substitution mutations in the population tested positive for the beta -thalassemia trait.

Conclusion: Thalassemia major is a serious concern in southern Iraq and therefore this study emphasizes a need for complete mutation profiling of the beta -globin gene as a strategy for screening of carriers within the population. Such examinations could be useful in pre-marital genetic counseling and for undertaking prevention and treatment measures.

Keywords: beta -thalassemia; mutation; PCR.

INTRODUCTION

In the Middle East, thalassemia is the most common hereditary disorder and a major health concern. Thalassemia results from abnormalities in the globin beta gene, which is inherited. It is one of the primary contributors of chronic hemolytic anemia which worsens over the first year of life, a hallmark of thalassemia, particularly the beta major form (1). There are over two hundred known distinct mutations within this gene, the majority of which are point mutations that influence the way the genes are expressed (2, 3). These mutations are found in the regulatory elements during the processes of linking exons, which results in abnormal splicing of the mRNA and the encoded regions of the HBB gene. Due to this, a screening approach of the entire gene is necessary to detect β-thalassemia (4,5). HBB gene is located on the short arm of chromosome 11, consisting of three exons encoding for 146 different amino acids (6,7). HBB gene mutations in addition to decreased production of hemoglobin leading to chronic anemia can also be potentially fatal (8,9). Worldwide, changing patterns of thalassemia has been recorded, with the highest rate of sickle cell disease prevalence reported for extreme south and far north regions (10,11). Thalassemia major is a condition that is regarded to be a significant issue in many parts of Iraq, particularly in the country's north and southern regions (12). In this study, we undertook a random screening of individuals from the southern Iraqi population for the prevalence of β-thalassemia trait (BTT) and analyse the HBB gene of each BTT positive individual for the type of mutational pattern and their distribution in the population.

MATERIALS AND METHODS

Subjects

This analysis included 35 thalassemia patients from the Genetic Diseases Center in the province of Thi-Qar, southern Iraq and 15 healthy individuals as control samples from the same region. All participants were aged between 2 to 28 years. The study conducted was from January 2022 to May 2022. Baseline data of each individual was collected by using a formal questionnaire. Venous blood (5 ml) collected from

each individual was transferred to tubes containing EDTA was further used for genomic DNA extraction.

Genomic DNA isolation

Genomic DNA was extracted from 200 µl EDTA blood sample using The AccuPower® gsync DNA kit (Bioneer, South Korea) following the manufacturer's instructions. The DNA extracted was checked for its concentration and purity by measuring the optical density at 260 nm using ND 2000 spectrophotometer (Thermo Scientific Inc. USA). DNA extracted was stored at -20 ºC until further use.

Polymerase Chain Reaction (PCR) amplification

The DNA extracted was subjected to PCR amplification of Exon1 and Exon3 and a part of start of Gene (intron number 1) and intron number 2 regions of the HBB gene. The PCR reactions were carried out using the AccuPower® PCR pre-Mix (Bioneer, South Korea). Primers used were as follows: Exon 1: Fwd: 5'-GCCTAGTACATTACTATTTGG-3' and Rev: 5'-GCACACAGACCAGCACGTTG-3' ; Exon3: Fwd: 5'-GCCGTTACTGCCCTGGGGC-3' and Rev:5'-CCCTGTTACTTATCCCCTTCC-3'; and Medal of the Gene (Exon number 3) Fwd:5'-GCCTAGTACATTACTATT TGG-3' and Rev: 5'-GCA CACAGACCAGCACGTTG-3'.

PCR was performed in a programmable thermocycler (Thermo Fisher, USA) using the following program: an initial delay at 95°C for 5 minutes followed by 30 cycles of denaturation of 95°C for 30 seconds, annealing at 56°C for30 seconds, extension at 72°C for 30 seconds, and a final extension of 5 minutes at 72 °C. The PCR products were electrophoresed on 1.5% agarose gel. The PCR products were excised, purified and outsourced (Macrogen, South Korea) for sequencing. The HBB sequences obtained were subjected to sequence analysis to find mutations.

RESULTS

The Primers used in amplifying regions of the β-globin gene, amplified 500b of the Exon 1, and 557bp of the Exon3 and intron 2 regions of the HBB gene (Fig. 1 and Fig. 2).

Sequence analysis of the amplified fragments identified several SNPs (single nucleotide polymorphisms) within the gene sequence. Our analysis showed a wide spectrum of known and novel mutations within the Iraqi population. In total 14 mutations were identified, the results of which are presented in Table 1. Three of these mutations occurred in the Exon region and 11 were seen to be in the intron2 region. Mutations occurring within the exon regions were of the transversion type (G>C or G>T) (Table1).

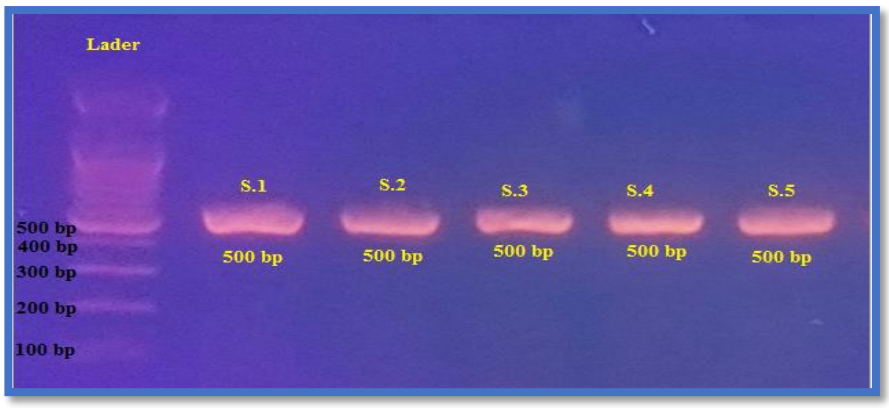

Fig.1: PCR for Exon 1 region of the HBB gene. Lane 1:100bp DNA ladder; Lanes2-5: positive for Exon1 (500 bp).

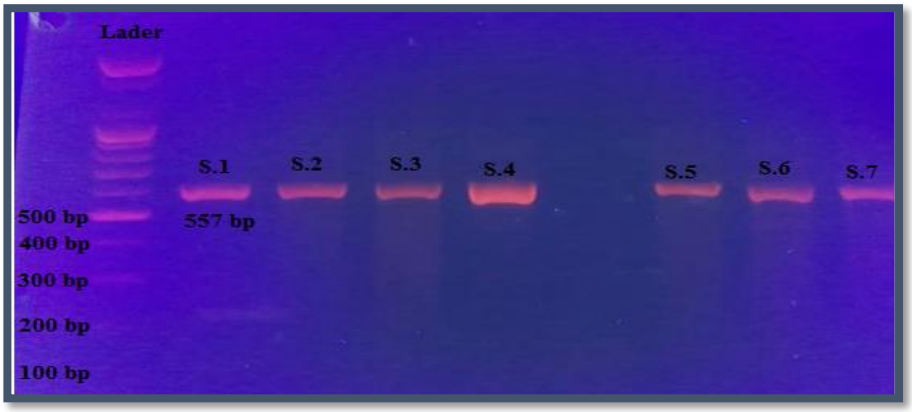

Fig.2: PCR for Exon 3 and Intron 2 region of the HBB gene. Lane 1: 100bp DNA ladder; Lanes 2-5: positive for Exon3 (557 bp); Lanes 7-9: positive for intron2 (557 bp).

Table 1: HBB mutations observed in β-thalassemia population in this study

S.no	Mutations	Location	Type	Effect	Originality
S1	g.401 G > A	Intronic	Transversion	-	Novel
S2	g.401 G > A	Intronic	Transversion	-	Novel
S3	g.938 *del* T	Intronic	deletion	Frame shift	Novel
S3	g.1448 *del* C	Glutamine > Arginine	Transversion	Point. M	Novel
S4	g.947*del* A	Intronic	deletion	Frame shift	Novel
S4	g.952 *del* A	Intronic	deletion	Frame shift	Novel
S5	g.934 G > C	Intronic	transition	-	Novel
S5	g. 938 *del* A	Intronic	deletion	Frame shift	Novel
S6	g.947 *del* A	Intronic	deletion	Frame shift	Novel
S7	g.1450 G > T	Glutamic acid > Glutamic acid	Transversion	Silent. M	Novel
S8	g.1458 G > T	Glutamic acid > Lysine	Transversion	Point . M	Novel
S9	g.1458 G > T	Glutamic acid > Lysine	Transversion	Point . M	Rs 600612.1
S10	g.1233 G > C	Intronic	transition	-	Novel
S11	g.1236 G > A	Intronic	transition	-	Novel
S12	g.1262 A > T	Intronic	Transversion	-	Rs 600616.1
S13	g.952 *del* A	Intronic	deletion	Frame shift	Novel

DISCUSSION

Consanguineous marriage is more common in the East, which contributes to a higher concentration of gene variants that are potentially harmful since related individuals are more likely to marry within their own family. Over the course of the past several years, this has directly contributed to a sizeable increase in the number of persons who are affected with the condition. β- thalassemia is an inherited disorder that, in some people, might result in death. This condition affects a disproportionately large number of people all over the world. Hence in this study, we investigated the most significant genetic mutations, particularly the mutations that occurred more than once in the samples that were analyzed, as well as the factors that led to their occurrence. According to the findings, there were many mutations that were repeated in the samples that were investigated. These mutations included replacement, deletion, and displacement mutations. This suggests that there are fragile areas in the gene that are prone to mutations, which in turn affects the nature of the product protein.

Among the novel variants identified majority were within intronic2 region and this assumes significance as mutations in introns are known to have a significant effect on the gene expression of the β-globin gene, RNA splicing and mRNA stability (13,14). Similarly, several novel mutations identified in this study were located in the non-coding regions of the HBB gene. Further investigation on the mutations occurring in the exon regions of the HBB gene is required for further understanding of the functioning of the β-globin protein. There are a variety of medical applications for the PCR technique, such as the diagnosis of CML (15), Adenocarcinoma (16), *Brucella melitensis* (17), the detection of MRSA (18), the identification of methicillin-resistant *Staphylococcus aureus* (18), the genotyping and evaluation of some parameters in toxoplasmosis (19), and the genotyping of *Clostridium perfringens* toxins (19). In addition, the PCR technique can be used for the (20).

CONCLUSION

Results in this study emphasize the need for complete mutation profiling of the β-globin gene as a strategy for screening of β-thalassemia carriers within the population. Such screening could be useful in pre-marital genetic counseling and for undertaking prevention and treatment measures in southern Iraq where Thalassemia major is a serious concern.

ACKNOWLEDGEMENT

The authors extend their gratitude to the laboratory technicians at Salahaldeen Hospital, Salahaldeen province, Iraq for their assistance in collecting samples and data for this study. The authors also express their appreciation to the faculty and staff of Biology Department, College of Science, Tikrit University for their advice and support throughout the research process.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

REFERENCES

1. Saleh, K.K.,Kakey, E.S. Some molecular characterization of beta-thalassemia major in Koya City. In International Conference on Pure and Applied Sciences.2018; 4(1):64-68.
2. Galanello, R. Origa, R. Beta Thalassemia. Orphanet journal of rare diseases, 2010; 5(1):1-15.
3. Chaudhary, S., Dhawan, D., Sojitra, N., Chauhan, P., Chandratre, K., Chaudhary, P.S., 2017. Whole gene sequencing based screening approach to detect beta-thalassemia mutations. Biol Med (Aligarh), 2017, 9(383):2.
4. Mandal, P.K., Maji, S.K. and Dolai, T.K. Present scenario of hemoglobinopathies in West Bengal, India: An analysis of a large population. International Journal of Medicine and Public Health,2014; 4(4):496.
5. Higgs, D.R., Engel, J.D., Stamatoyannopoulos, G. Thalassaemia. The lancet. 2012;379 (9813): 373-383.
6. Hamed, E.A., ElMelegy, N.T. Renal functions in pediatric patients with beta-thalassemia major: relation to chelation therapy: original prospective study. Italian journal of pediatrics,2010; 36(1):1-10.
7. Mashi, A., Khogeer, H., Abalkhail, H., Khalil, S. Molecular patterns of beta-thalassemia mutations of Saudi patients referred to King Faisal Specialist Hospital and Research Center. Journal of Applied Hematology, 2017;8(3):99-104.
8. Cao, A. Galanello, R. Beta-thalassemia. Genet Med, 2010; 12(2):61-76.
9. Aziz, A., Ahmed, W., Sarwardi, G., Ara Naznin, R., Rehena, J., Ferdoushi, A. Molecular Analysis of Hb-E and Beta-Thalassemia Major Patients among Bangladeshi Population. Austin J Biotechnol Bioeng, 2017; 4(4):1085.
10. Vichinsky, E.P. Changing patterns of thalassemia worldwide. Annals of the New York Academy of Sciences, 2005;1054(1):18-24.
11. Al-Allawi, N.A., Jalal, S.D., Ahmed, N.H., Faraj, A.H., Shalli, A.,Hamamy, H. The first five years of a preventive programme for haemoglobinopathies in Northeastern Iraq. Journal of Medical Screening, 2013; 20(4):171-176.
12. Al-Allawi, N.A., Jalal, S.D., Nerwey, F.F., Al-Sayan, G.O., Al-Zebari, S.S., Alshingaly, A.A., *et al.* Sickle cell disease in the Kurdish population of northern Iraq. Hemoglobin, 2012; 36(4):333-342.
13. Kazazian HH, Antonarakis SE. The varieties of mutation Prog Med Genet. 1988; 7:43- 67.
14. Warsy AS, ElHazmi MA, Al Momin AK, *et al.* Extensive polymorphisms in Saudi beta thalassaemia patients. Biosci Biotechnol Res Asia. 2013;10: 127-132.
15. Ahmed, A.A., Khaleel, K.J., Fadhel, A.A., Al-Rubaii, B.A.L. Chronic Myeloid Leukemia: A retrospective study of clinical and pathological features. Bionatura, 2022, 7(3), 41. DOI. 10.21931/RB/2022.07.03.41.
16. Ali, S.M., Lafta, B.A., Al-Shammary, A.M., Salih, H.S. In vivo oncolytic activity of non-virulent newcastle disease virus Iraqi strain against mouse mammary adenocarcinoma. AIP Conference Proceedings, 2021, 2372, 030010.
17. Awadh, H.A., Hammed, Z.N., Hamzah, S.S., Saleh, T.H., Al-Rubaii, B.A.L. Molecular identification of intracellular survival related *Brucella melitensis* virulence factors. Biomedicine (India), 2022, 42(4):761–765.
18. Fakhry, S.S., Hammed, Z.N., Bakir, W.A.-E., ALRubaii, B.A.L. Identification of methicillin-resistant strains of *Staphylococcus aureus* isolated from humans and food sources by use mecA 1 and mecA 2 genes in Pulsed-field gel electrophoresis technique, 2022, 7(2):44. DOI. 10.21931/RB/2022.07.02.44.
19. Jiad, A.L., Ismael, M.K., Salih, T.A., Malik, S.N., Al-Rubaii, B.A.L. Genotyping and evaluation of interleukin-10 and soluble HLA-G in abortion due to toxoplasmosis and HSV-2 infections. Annals of parasitology. 2022, 68(2):385–390.
20. Hashim, S.T., Fakhry, S.S., Rasoul, L.M., Saleh, T.H., Alrubaii, B.A.L. Genotyping toxins of *Clostridium perfringens* strains of rabbit and other animal origins. Tropical Journal of Natural Product Researchthis link is disabled, 2021, 5(4): 613–616.