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Prevalence of Ten Common Iranian β-thalassemia Mutations Among β-thalassemia Patients in Kashan Using ARMS-PCR

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ABSTRACT

Thalassemia is a prevalent inherited hematological disorder that affects erythrocyte production. This chronic condition is caused by mutations in the globin gene, resulting in either a reduction or complete absence of one of the two globin chains- alpha or beta. The β-thalassemia is an inherited hematological disorder with an autosomal recessive pattern. In Iran, this disease can be caused by at least 47 different mutations in the β-globin gene, with approximately 10 common mutations accounting for over 80% of β-thalassemia cases. This study aimed to identify the spectrum of common β-thalassemia mutations among β-thalassemia patients in Kashan, located in central Iran. In present study 40 β-thalassemia patients (major and intermedia) were analyzed. DNA was extracted from whole blood samples utilizing the salting-out method. Screening for causal mutations was conducted using the amplification refractory mutation system PCR (ARMS-PCR) technique. Among the 10 common mutations, IVS II-I was the predominant mutation (30%), followed by Fr 36-37 (20%), Fr 8-9 (15%), IVS I-110 (12.5%), codon 8 (10%), IVS I-6 (7.5%), and IVS I-5 (5%). The mutations IVS I 3´end (-25 del), codon 44, and codon 39 were not detected in this study. The findings indicate that the Kashan population exhibits a diverse range of thalassemia allelic distributions. IVS II-I and Fr 36-37 were identified as the most prevalent mutations. These results are consistent with findings from other regions in central Iran and can serve as a foundation for β-thalassemia screening and prenatal diagnosis programs.

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Introduction

Thalassemia encompasses a group of inherited hematological disorders characterized by the aberrant production of hemoglobin, resulting in chronic anemia. This condition arises from mutations in the globin genes, leading to diminished or absent synthesis of either the alpha or beta globin chains (Bajwa and Basit, 2023). Approximately 3% of the global population is carriers of β-thalassemia. The disorder is inherited in an autosomal recessive pattern and affects millions globally (Rao *et al*., 2024). Clinical manifestations of β-thalassemia major typically emerge within the first two years of life, presenting as severe anemia, jaundice, skeletal deformities (notably in the facial and cranial regions), hepatosplenomegaly, and growth retardation. Without regular blood transfusions and iron chelation therapy, affected individuals may face life-threatening complications. The disease was first clinically characterized by Thomas B. Cooley in 1925, who identified it as erythroblastic anemia (Cooley and Lee, 1925). In 1932, Whipple and Bradford introduced the term thalassemia to more accurately reflect its prevalence among Mediterranean populations, thereby supplanting the previously used, less specific terminology

(Whipple and Bradford, 1932). Over 350 mutations have been identified as causative of β-thalassemia. The condition is highly prevalent in the Mediterranean region (including Italy, Greece, and Cyprus), the Middle East (including Iran, Iraq, and Saudi Arabia), and parts of Asia (including India, Pakistan, and Bangladesh) (De Sanctis *et al*., 2017) Prenatal diagnosis (PND) programs in Iran have detected more than 52 thalassemic mutations with varying ethnic heterogeneity (Alizadeh *et al*., 2014; Strauss and Bernard, 2009). Africa, the Mediterranean basin, the Middle East, the Indian subcontinent, Southeast Asia, Melanesia, and the Pacific islands are among the most affected areas. These regions have a carrier frequency of between 1% and 20% for β-thalassemia. The prevalence can range between 10% and 20% in sub-Saharan Africa, up to 40% in certain Middle Eastern and Indian populations, and as high as 80% in northern Papua New Guinea and isolated groups of North-East India (Kiani *et al*., 2024). It is the most prevalent single-gene disorder in Iran, with over 2 million carriers and 25,000 patients identified in the country (Miri *et al*., 2013).

The symptoms of this disease include severe anemia, hepatosplenomegaly, and deformation of facial and cranial bones. Patients with this condition require frequent blood transfusions throughout their lives (Khanahmad *et al*., 2007). Given the high prevalence of this disease worldwide, the most effective prevention strategy is identifying carriers and performing prenatal diagnosis. Implementing mandatory genetic counseling and premarital screening represents a critical step in this regard. However, determining the distribution pattern of alleles provides valuable assistance in rapid diagnosis and identifying the type of mutation in the fetus. Thus, considering the ethnic and racial diversity in Iran, this aspect is of particular importance (Miri *et al*., 2013; Saleh-Gohari and Bazrafshani, 2010). Mutations are being investigated across various provinces and counties, and this study focuses on analyzing the prevalent mutations in the Kashan population.

Materials and methods

Study population and setting

This study was conducted on patients with β-thalassemia major in Kashan, Iran. All individuals diagnosed with β-thalassemia major and registered at the Kashan Thalassemia Center were invited to participate. The center was selected because it serves as the sole registry for all patients in the region, allowing comprehensive coverage of the entire affected population. Before enrollment, the study objectives and procedures were clearly explained to participants, and written informed consent was obtained from each patient or their legal guardian. Kashan was specifically chosen as the study site not only for the centralized registry but also because of the limited molecular data available on the mutation spectrum in this region, highlighting the importance of a localized investigation.

Data and samples collection

Demographic and clinical information, including age, sex, clinical manifestations, and family pedigrees, were carefully recorded. The study population consisted of 40 patients (21 males and 19 females) with an age range of 6–22 years. Given the ethnic diversity of Kashan, it was hypothesized that the mutation pattern might differ from that reported in other parts of Iran. Ten common β-globin mutations were investigated using the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) technique, which is a targeted and cost-effective molecular method. Since the total number of registered patients was limited to 40, a census sampling approach was applied to include all eligible individuals in the analysis.

A total of 40 patients were identified at this center, and 10 mL of blood was collected from each individual in sterile, screw-capped Falcon tubes containing EDTA-Na_2. The samples were stored at -20°C in a freezer pending further analysis.

Ethical approval

All procedures in this study complied with the ethical standards of the University of Kashan's Human Ethics Committee (Off line code: 1386-03-22-Meeting44), under the Iran Ministry of Health and Medical Education.

DNA extraction

Genomic DNA was extracted from peripheral blood samples using a modified salting-out protocol (Miller *et al.*, 1988). Briefly, 2 mL of whole blood was mixed with 4 mL of buffer A (10 mM Tris-HCl, 11% sucrose, 5 mM $MgCl_2$, and 1% Triton X-100) to lyse red blood cells. The mixture was incubated on ice for 5 minutes and subsequently centrifuged at 4°C for 10 minutes at 3,000 rpm (approximately 805 ×g with a rotor radius of 8 cm). The supernatant was discarded, and the white cell pellet was retained.

To lyse the white cells and extract DNA, 600 µL of Buffer P (10 mM Tris-HCl, 150 mM NaCl, 10 mM EDTA) was added to the pellet, followed by the addition of 30 µL of 10% SDS and 25 µL of proteinase K (20 mg/mL). The tubes were gently mixed and incubated at 56°C for 2 hours to ensure complete digestion of proteins. After incubation, 250 µL of saturated NaCl was added to precipitate proteins, and the samples were placed on ice for 5 minutes before being centrifuged at 13,000 rpm (approximately 15,000 ×g with a rotor radius of 8 cm) for 10 minutes at 4°C.

The resulting supernatant containing DNA was transferred to a clean microcentrifuge tube, and DNA was precipitated by adding 550 µL of cold isopropanol. After gentle inversion, DNA strands were visualized as a white precipitate and washed once with 70% ethanol. The DNA pellet was air-dried and resuspended in 50 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Extracted DNA was stored at -20°C for further analysis.

ARMS-PCR

Allele-specific amplification was performed using the ARMS-PCR to detect specific point mutations associated with β-thalassemia. Based on previous studies, twenty mutations have been reported as the most common in Iran. Among these, ten of the most prevalent mutations were selected for this study (Table 1), and the corresponding primers were procured from Zist Pajoohan Pishro Co. (Iran). Primer sequences were designed based on known β-thalassemia mutations and validated using Primer-BLAST to ensure specificity.

For ARMS-PCR, the reaction was carried out in a final volume of 25 µL, containing approximately 100 ng of genomic DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.5 µM of each primer (mutation-specific and control primers), and 1 U of Taq DNA polymerase (Thermo Fisher Scientific, USA). The PCR amplification was conducted under the following conditions: initial denaturation at 94°C for 5 minutes; followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 67°C for 1 minute, and extension at 72°C for 1 minute; with a final extension at 72°C for 10 minutes.

PCR products were separated by electrophoresis on a 2% agarose gel containing ethidium bromide (0.5 µg/mL) and visualized under transilluminator. The expected product size was compared to a 100 bp DNA ladder (Fermentas, USA). Positive and negative controls were included in each run to ensure the specificity and validity of the reaction.

Table 1. The list of primers for 10 common mutations in patients with beta-thalassemia.

Mutation	Oligomer (5'→3')
IVS II-I	M: AAGAAAACATCAAGGGTCCCATAGACTGAT
	N: AAGAAAACATCAAGGGTCCCATAGACTGAC
IVS I-5	M: CTCCTTAAACCTGTCTTGTAACCTTGTTAG
	N: CTCCTTAAACCTGTCTTGTAACCTTGTTAG
IVS I-110	M: ACCAGCAGCCTAAGGGTGGGAAAATACACT
	N: ACCAGCAGCCTAAGGGTGGGAAAATACACC
C 39	M: CAGATCCCCAAAGGACTCAAAGAACCTGTA
	N: CAGATCCCCAAAGGACTCAAAGAACCTGTG
Fr 8-9	M: CCTTGCCCCACAGGGCAGTAACGGCACACC
	N: CCTTGCCCCACAGGGCAGTAACGGCACACT
IVS 3 end (-25 del)	M: CTCTGGGTCCAAGGGTAGACCACCAGCATA
	N: GCAGCTAAGGGTGGGAAAATAGACCCAATA
Fr36-37	M: GGTAAGGACTCAAAGAACCTATGGGTCCAG
	N: GGTAAGGACTCAAAGAACCTATGGGTCCAA
C 8	M: ACACCATGGTGCACCTGACTCCTGAGCACG
	N: ACACCATGGTGCACCTGACTCCTGAGCACA
IVS I-6	M: TCTCCTTAAACCTGTCTTGTAACCTTCATG
	N: TCTCCTTAAACCTGTCTTGTAACCTTCATA
C 44	M: CAGCATCAGGAGTGGACAGATCCCCAATGA
	N: CAGCATCAGGAGTGGACAGATCCCCAATGG
Common C	ACCTCACCCTGTGGAGCCAC
Common D	GAGTCAAGGTCGAGAGATGCAAGGA

M= Mutant allele; N= Neutral (Wild type) allele

Results

In this study, 40 patients (21 males and 19 females) referred to the thalassemia center in Kashan were selected, and their mutations were analyzed utilizing the ARMS-PCR method and specific primers. Among these patients, the parents of 15 individuals were consanguineous, while the parents of 25 patients were unrelated.

Ten common mutations in Iran were examined, with IVS II-I being the most prevalent mutation

(30%). This mutation results in the substitution of G to A at the first nucleotide of intron I in the β-globin gene, leading to the inactivation of the donor splice site, improper mRNA processing, and defective splicing.

The second most common mutation was Frameshift 36-37, with a frequency of 20%. Frameshift 8-9, accounting for 15% of cases, ranked third. This mutation involves the insertion of a guanine nucleotide between codons 8 and 9 of the β-globin gene, shifting the reading frame and resulting in premature translation termination. The IVS I-110 mutation, which involves a G to A substitution at position 110 of intron I in the β-globin gene, had a frequency of 12.5%. Codon 8, with a frequency of 10%, was the fifth most prevalent mutation. The IVS I-6 mutation, which leads to the substitution of T to C, had a frequency of 7.5%. Finally, IVS I-5, causing a G to C substitution, was the least common mutation, with a frequency of approximately 5%.

Discussion

This study aimed to investigate the prevalent mutations of the beta-thalassemia gene in Kashan, located in Isfahan Province, and to compare the findings with data from other regions of Iran and the world. The prevalence of beta-thalassemia is reported to be 8% in Isfahan (Hashemizadeh and Noori, 2013) and 3.22% in Kashan (Afzali *et al*., 2000). Among the identified mutations, IVS II-1, which interferes with mRNA splicing and leads to the formation of a β^0 allele, was found to be the most frequent mutation in Kashan, with a prevalence rate of 30%. Previous research indicates that this mutation is also highly prevalent in several other provinces across Iran, including Tehran, Mazandaran, Kurdistan, Khuzestan, and the southern cities, as well as in Fars, Hormozgan, Kermanshah, Lorestan, Hamedan, and Kerman (Mousavi S. S. *et al*., 2024; Nezhad *et al*., 2018; Pooladi *et al*., 2022; Rahimi, 2013).

Supporting these findings, studies by Cürük in Azerbaijan and Raghad in Iraq have also identified IVS II-1 as a common variant (Abbas *et al*., 2024; Cürük *et al*., 1992). The widespread occurrence of this mutation in these regions may be attributed to shared genetic backgrounds and historical patterns of migration. A frameshift mutation in codons 36-37, resulting in a β^0 allele, has been identified as the second most prevalent mutation in Kashan, with a frequency of 20%. This mutation is predominantly observed in the populations of Khuzestan, Lorestan, and Kohgiluyeh and Boyer-Ahmad, likely due to genetic homogeneity and a high incidence of consanguineous marriages in these regions. It is also frequently reported in Kermanshah, Hormozgan, and southwestern Iran (Khan *et al*., 2021; Nasiri *et al*., 2020; Nezhad *et al*., 2018; Pouranfard et al., 2020; Rahimi, 2013). Another frameshift mutation, located in codons 8-9 (Fr 8-9), also leading to a β^0 allele, was found in 15% of cases in Kashan. This mutation has been reported as the second most prevalent in Kurdistan, with a frequency of 14.8%, and has been detected in Khuzestan, Kermanshah, Tehran, Sistan and Baluchestan, and Kerman (Khan *et al*., 2021; Nasiri *et al*., 2020; Rahimi, 2013).

Studies from Iraq have identified this mutation as part of the heterogeneous mutation spectrum in that country (Abbas R. A. *et al*., 2024). The IVS I-110 mutation, which results in a β^0 allele, is prevalent among Mediterranean populations. In the Middle East, this mutation has been reported with a frequency of 5.8% in Palestine, where it is the second most frequent mutation (Hammoud *et al*., 2020; Nezhad *et al*., 2018), and it has also been documented in Iraq. Its frequency in other countries includes 25.4% in Algeria, 39.53% in the former Yugoslavia, 41% in Egypt, and 62% in Lebanon. In Iran, the frequency has been reported as 16.8% in Khuzestan and 7.4% in the southwestern provinces (Khan *et al*., 2021; Nezhad *et al*., 2018). A mutation in *codon 8* resulting in the deletion of one amino acid (AA-) has been reported as the most common mutation in Kohgiluyeh and Boyer-Ahmad (19.8%) (Pouranfard *et al*., 2020) around 2% in the population of Mashhad (Khanahmad *et al*., 2007) 6.5% in Khuzestan (Hamzehloei and Mohajer, 2012) 4.93% in Mazandaran, and 2.05% in Golestan Province (Mousavi S. S. *et al*., 2024).

The IVS I-6 mutation, which results in a β^+ allele, was identified in 7.5% of cases in Kashan. This mutation has also been recognized as one of the most prevalent mutations in Fars Province

(Rahimi, 2013). Furthermore, in the Palestinian population, it has been reported as the most prevalent mutation, with a frequency of 74.5% (Nezhad *et al*., 2018). Its prevalence has also been documented as 29.5% in the former Yugoslavia (Mohammadi *et al*., 2007) and 13% in both Egypt and Iraq (Abbas *et al*., 2024). The *IVS I-5* mutation, also associated with a β^+ allele, was identified in this study as having the lowest frequency among the Kashan population. It has been documented as one of the most prevalent mutations in the provinces of Hormozgan (69%), Sistan and Baluchestan (87.2%), Kerman (66%), and Khorasan (over 50%). Additionally, its presence has been identified in Tehran and Golestan (Mousavi *et al*., 2024; Rahimi, 2013). In Arab countries, the mutation's frequency is reported to be approximately 47%, rendering it one of the most common variants (Hamamy and Al-Allawi, 2013; Khan *et al*., 2021). To provide a comprehensive comparison of the prevalent mutations across different regions of Iran, a comparative table 2 has been included. This table summarizes the frequency of the most common mutations in the beta-thalassemia gene, as observed in various Iranian populations. The data presented offer a regional perspective, facilitating a clearer understanding of the distribution patterns of these mutations within the country.

Table 2. Comparison of the frequency of common mutations of the β-thalassemia gene in different regions of Iran.

Mutations	Cities/Regions in Iran	Frequency in Kashan (%)
IVS II-1	Kashan, Tehran, Mazandaran, Kurdistan, Khuzestan, Fars, Hormozgan, Kermanshah, Lorestan, Hamedan, Kerman	30%
Frameshift codons 36-37	Kashan, Khuzestan, Lorestan, Kohgiluyeh and Boyer-Ahmad, Kermanshah, Hormozgan	20%
Frameshift codons 8-9	Kashan, Kurdistan, Khuzestan, Kermanshah, Tehran, Sistan and Baluchestan, Kerman	15%
IVS I-110	Khuzestan, southwestern Iran	Not specified
Codon 8 (AA-)	Kohgiluyeh and Boyer-Ahmad, Mashhad, Khuzestan, Mazandaran, Golestan	Fifth most frequent (~less than 15%)
IVS I-6	Kashan, Fars	7.50%
IVS I-5	Hormozgan, Sistan and Baluchestan, Kerman, Khorasan, Tehran, Golestan	Lowest frequency (unspecified)

Table 3 and the accompanying bar chart provide a clear depiction of the frequency of each mutation. The data indicate that IVS II-I is the most prevalent mutation, followed by Frameshift 36-37 and Frameshift 8-9. The reported mutation frequencies largely align with findings from other parts of Iran (Rezaee *et al*., 2012), with the exception of the codon 8 mutation, which is not widely recognized as a common mutation in other populations. Nonetheless, in the present study, it was identified as the fifth most prevalent mutation in the Kashan population.

Conclusion

Given Iran's geographical location within the thalassemia belt, the implementation of a comprehensive and long-term strategy for controlling this disease is of national importance. The high prevalence and diversity of β-globin gene mutations observed in different regions of Iran, including Kashan, underscores the need for region-specific screening programs and genetic counseling services**.** This study identified ten types of β-globin gene mutations among 40 thalassemia patients residing in Kashan County. The Fr 8-9 and IVS II-1 mutations are classified as Asian-Indian mutations, which is consistent with the findings of a 2008 study by Dr. Derakhshandeh *et al*., wherein these mutations were also reported among the most prevalent mutations in Isfahan Province.

Table 3. Frequency distribution of mutations found in patients with β-thalassemia in Kashan.

Mutations	Frequency (%)	Count (N)
IVS II-1	30%	12
Frameshift 36-37	20%	8
Frameshift 8-9	15%	6
IVS I-110	12.50%	5
Codon 8	10%	4
IVS I-6	7.50%	3
IVS I-5	5%	2
Total	100%	40

This alignment not only highlights the persistence of certain mutation patterns over time but also emphasizes the importance of continuous genetic surveillance to monitor emerging trends in mutation frequency.

Moreover, understanding the mutation spectrum in local populations such as Kashan contributes to more effective premarital screening, carrier detection, and prenatal diagnostic strategies, ultimately helping to reduce the burden of thalassemia major births. This correlation between the present study and previous regional data indicates that these mutations are significant factors in screening and prevention strategies. Integrating molecular diagnostic tools with public health initiatives is crucial for managing disease burden and enhancing patient outcomes. These findings underscore the importance of incorporating molecular diagnostics into routine screening programs, particularly in regions with high mutation diversity, such as Kashan. Establishing region-specific mutation panels can significantly improve the efficiency of carrier detection and prenatal diagnosis. Ultimately, such targeted approaches are essential for reducing the incidence of β-thalassemia major and improving public health outcomes in thalassemia-endemic areas.

Conflict of Interest

The authors declare no conflict of interest.

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References

Abbas R. A., Hassan, R. H., Taghlubee, I. M., Mohammed, S. I., Mohammed, H. H., Hasan, H. H., Judi, A. T., Ali, L. S., Mohammed, W. J., & Shihab, H. M. (2024). Prevalence and molecular characterization of β-thalassemia in Kirkuk province of Northern Iraq. *Hemoglobin*, 48(5), 308- 313. https://doi.org/10.1080/03630269.2024.2418507

Afzali, H., Moosavi, G. A., & Ekinchi, H. (2000). Prevalence of Iron deficiency anemia and minor beta-thalassemia and comparison of their red cells indices in volunteers for marriage referred to Golabchi outpatient clinic in Kashan in 1376- 77. *Feyz Medical Sciences Journal*, 3 (4), 78- 83. http://feyz.kaums.ac.ir/article-1-484-en.html

Alizadeh, S., Bavarsad, M. S., Dorgalaleh, A., Khatib, Z. K., Dargahi, H., Nassiri, N., ... & Saki, N. (2014). Frequency of beta-thalassemia or beta-hemoglobinopathy carriers simultaneously affected with alpha-thalassemia in Iran. *Clinical Laboratory*, 60(6), 941-9. https://doi.org/10.7754/clin.lab.2013.130306

Bajwa, H., & Basit, H. (2023). Thalassemia. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Available from: https://www.ncbi.nlm.nih.gov/books/NBK545151/

Cooley, T. B., Witwer, E. R., & Lee, P. (1927). Anemia in children: with splenomegaly and peculiar changes in the bones report of cases. *American Journal of Diseases of Children*, 34(3), 347-363. https://doi.org/10.1001/archpedi.1925.04120210054006

Cürük, M. A., Yüregir, G. T., Asadov, C. D., Dadasova, T., Gu, L.-H. Baysal, E., Gu, Y.-C. R., S., M. L., & Huisman, T. H. (1992). Molecular characterization of β-thalassemia in Azerbaijan. *Human Genetics*, 90, 417-419. https://doi.org/10.1007/BF00220470

De Sanctis, V., Kattamis, C., Canatan, D., Soliman, A. T., Elsedfy, H., Karimi, M., ... & Angastiniotis, M. (2017). β-thalassemia distribution in the old world: an ancient disease seen from a historical standpoint. *Mediterranean Journal of Hematology and Infectious Diseases*, 9(1), e2017018. https://doi.org/10.4084/MJHID.2017.018

Hamamy, H. A., & Al-Allawi, N. A. (2013). Epidemiological profile of common haemoglobinopathies in Arab countries. *Journal of Community Genetics*, 4, 147-167. https://doi.org/10.1007/s12687-012-0127-8

Hammoud, H., Ghanem, R., Abdalla, R., Semaan, P., Azzi, J., Prada, E. P., & Hassan, K. H. (2020). Genetic mutations of beta thalassemia in middle east countries. *World Journal of Pharmaceutical Sciences*, 9, 134-150. https://doi.org/10.20959/wjpps20202-15449

Hamzehloei, T., & Mohajer, T. F. (2012). The spectrum of mutations in 100 thalassemic carriers referred to Ghaem Hospital of Mashhad. *Iranian Journal of Pediatric Hematology and Oncology*, 2(2), 49-53. https://ijpho.ssu.ac.ir

Hashemizadeh, H., & Noori, R. (2013). Premarital screening of beta thalassemia minor in north-east of Iran. *Iranian Journal*

of Pediatric Hematology and Oncology, 3(1), 210-215. https://ijpho.ssu.ac.ir/

Khan, A. M., Al-Sulaiti, A. M., Younes, S., Yassin, M., & Zayed, H. (2021). The spectrum of beta-thalassemia mutations in the 22 Arab countries: a systematic review. *Expert Review of Hematology*, 14(1), 109-122. https://doi.org/10.1080/17474086.2021.1860003

Khanahmad, H., Azadmanesh, K., Shokrgozar, M., Niavarani, A., Karimi, M., Rabbani, B., Khalili, M., … & Zeinali, S. (2007). Design & constructing pBGGT plasmid: a carrier plasmid for betathalassaemia gene targeting. *Innovative Journal of Pediatrics*, 16 (1), 63-70. https://brieflands.com/journals/ij-pediatrics

Kiani, A., Ghorbani Aliabadi, E., Kermani, B., Rezabeigi Davarani, F., Rahmanian, V., & Daneshi, S. (2024). Assessing the success and effectiveness of the major β-thalassemia screening program in Southeast Iran: A case study of Jiroft district. *Journal of Health Sciences and Surveillance System*, 12(3), 319-326. https://doi.org/10.30476/jhsss.2023.97455.1707

Miller, S. A., Dykes, D. D., & Polesky, H. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, 16(3), 1215. https://doi.org/10.1093/nar/16.3.1215

Miri, M., Tabrizi Namini, M., Hadipour Dehshal, M., Sadeghian Varnosfaderani, F., Ahmadvand, A., Yousefi Darestani, S., & Manshadi, M. (2013). Thalassemia in Iran in last twenty years: the carrier rates and the births trend. *Iranian Journal of Blood and Cancer*, 6 (1), 11-17. http://ijbc.ir/

Mohammadi Asl, J., Samarafzadeh, A., Makvandi, M., Zandian, K., & Pedram, M. (2007). Investigation of common beta-globin gene mutations in patients with thalassemia major in Khuzestan Province using the RDB method. *Journal of Epidemiology and Global Health*, 8(3), 189-195. https://doi.org/10.5336/medsci.2008-8156

Mousavi S. S., H. Karam I, A. Tamadoni, H. Mahmoudi, R. Shekarriz, & R. Siami, e. a. (2024). Spectrum of Beta-thalassemia mutations in potential carriers with microcytic hypochromic anemia from Mazandaran and Golestan, Northern Provinces of Iran. *BioMed Research International*, 2013, 8664803. https://doi.org/10.1155/2024/8664803

Nasiri, A., Rahimi, Z., & Vaisi-Raygani, A. (2020). Hemoglobinopathies in Iran: an updated review. *International Journal of Hematology-Oncology and Stem Cell Research*, 14(2), 140- 150. https://doi.org/10.1155/2013/803487

Nezhad, F. H., Nezhad, K. H., Choghakabodi, P. M., & Keikhaei, B. (2018). Prevalence and genetic analysis of α- and β-thalassemia and sickle cell anemia in Southwest Iran. *Journal of Epidemiology and Global Health*, 8(3), 189-195. https://doi.org/10.2991/j.jegh.2018.04.103

Pooladi, A., Gheshlagh, R. G., Kahrizi, N., Roshani, D., Jalali, C., & Moradveisi, B. (2022). The spectrum of alpha and beta thalassemia mutations: a 10-year population-based study of the premarital health screening program in West of Iran. *Iranian Journal of Pediatric Hematology and Oncology*, 12 (3), 190-198. http://ijpho.ssu.ac.ir

Pourarfard, J., Vafaei, F., Afrouz, S., & Rezaeian, M. (2020). Thalassemia gene mutations in Kohgiluyeh and Boyer-Ahmad province. *Iranian Journal of Blood and Cancer*, 12(1), 18-23. http://ijbc.ir

Rahimi, Z. (2013). Genetic epidemiology, hematological and clinical features of hemoglobinopathies in Iran. *BioMed Research International*, 2013, 803487. https://doi.org/10.1155/2013/803487

Rao, E., Chandraker, S. K., Singh, M. M., & Kumar, R. (2024). Global distribution of β-thalassemia mutations: an update. *Gene*, 896, 148022. https://doi.org/10.1016/j.gene.2023.148022

Rezaee, A. R., Banoei, M. M., Khalili, E., & Houshmand, M. (2012). Beta-thalassemia in Iran: new insight into the role of genetic admixture and migration. *The Scientific World Journal*, 2012(1), 635183. https://doi.org/10.1100/2012/635183

Saleh-Gohari, N., & Bazrafshani, M. (2010). Distribution of β-globin gene mutations in thalassemia minor population of Kerman Province, Iran. *Iranian Journal of Public Health*, 39(2), 69-76. https://ijph.tums.ac.ir/index.php/ijph

Strauss, B. S. (2009). Genetic counseling for thalassemia in the Islamic Republic of Iran. *Perspectives in Biology and Medicine*, 52(3), 364-376. https://doi.org/10.1353/pbm.0.0093

Whipple, G. H., & Bradford, W. (1932). Racial or familial anemia of children: associated with fundamental disturbances of bone & pigment metabolism (Cooley-Von Jaksch). *American Journal of Diseases of Children*, 44(2), 336-365. https://doi.org/10.1001/archpedi.1932.01950090074009