In His Name

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Clinical and genetic characteristics of hemoglobin H disease in Iran

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1	Pediatric Hematology and Oncology ISSN/ISBN: 0888-0018, 1521-0669	Pediatrics Neonatology +2 more	Taylor & Francis	1.200	Q3	2.60	Q2	48	ISI, Scopus, PubMed, Embase	

Introduction

- Hb H disease is a type of alpha thalassemia with moderate severity caused by an imbalance in alpha-globin chains. This condition arises from deletions or mutations in the alpha-globin genes and is classified into two main types.
- Deletion Type: It is the most common type and is caused by the deletion of three alpha-globin genes on the chromosomes. Its symptoms are relatively milder.
- Non-deletion type: Resulting from the combination of the deletion of two alpha globin genes and a point mutation. This type has more severe symptoms such as severe anemia, enlargement of the liver and spleen, and a greater need for blood transfusions.
- This disorder is primarily characterized by microcytic hypochromic hemolytic anemia, mild jaundice, splenomegaly, leg ulcers, gallstones, and occasionally the appearance of thalassemia.
- In Iran, especially in Khuzestan Province, there is a high diversity of this disease in genotype and phenotype.

Materials and methods

- Patient selection
- Between January 2016 to February 2020, medical records of 202 cases of Hb H disease from various geographic regions of Iran who were referred to the thalassemia centers, with the age range of 4–70 years (55.4% female and 44.6% male) were reviewed
- Based on diagnostic criteria that include genetic and diagnostic tests, only individuals who are definitively considered ill are included in this study, which comprises 101 people.
- The age, sex, age at diagnosis, spleen size, history of splenectomy and blood transfusion, hematologic, electrophoretic, and genetic data were extracted for each patient
- Patients were divided into two main groups [transfusion dependent (TD) or non-transfusion dependent (NTD)]

DNA extraction

- Genomic DNA was extracted from peripheral blood samples using DNA isolation kit (QIAamp DNA Blood Mini Kit, Qiagen)
- Features and advantages of the QIAamp DNA Blood Mini Kit
- High quality and purity: The DNA obtained from this kit has high purity and is very suitable for use in sensitive applications such as PCR, enzymatic reactions, and sequencing.
- Compatible with various samples: In addition to whole blood, this kit is capable of extracting DNA from plasma, serum, saliva, cerebrospinal fluid, and other bodily fluids.
- Convenience and speed: The steps to work with this kit are simple and quick, and it does not require complex techniques or special equipment. Typically, DNA can be extracted in less than an hour.
- No use of phenol-chloroform: The method of working with this kit does not require hazardous chemical solvents like phenol and chloroform, which ensures user safety and DNA stability.

Steps to work with the kit

- Cell lysis: At this stage, the sample cells (such as white blood cells in the blood) are lysed with the help of specific kit buffers, and their DNA is released.
- Binding to the spin column: In the presence of specific buffers and suitable salt conditions, DNA binds to the silica matrix present in the spin column.
- Washing: After the DNA has bound, contaminants, proteins, and other impurities are removed from the column using several wash buffers.
- DNA elution: Finally, the pure DNA is separated from the column using an elution buffer and is ready for use.

Main applications

- PCR and Real-Time PCR: The DNA extracted with this kit is highly suitable for PCR techniques.
- Sequencing: The high purity of DNA allows for its use in genome sequencing or specific fragments.
- Genetic and forensic investigations: Due to the high quality and purity of DNA, it is also widely used in forensic and research tests.

Multiplex Gap-PCR

- Main applications of Multiplex Gap-PCR
- Detection of deletions and insertions in DNA sequences: This method is particularly useful in detecting large gene deletions, which occur in some genetic diseases. For example, in diseases such as Duchenne muscular dystrophy and beta thalassemia, deletions occur in specific genes that can be detected by Gap-PCR.
- Rapid mutation identification: This method can be used to identify mutations in various samples and can be utilized for the initial screening of patients.
- Application in gene therapy and genetic research: For studying the effects of gene deletions and insertions in gene therapy, as well as research focused on specific genes, the use of Gap-PCR is appropriate.

How Multiplex Gap-PCR Works

- In Multiplex Gap-PCR, multiple pairs of primers are used simultaneously in a single PCR reaction. Each of these pairs identifies and replicates a specific region of DNA. If a deletion has occurred in a region, that region will not replicate, while regions without deletion replicate normally.

Key steps in Multiplex Gap-PCR

- Primer design: Accurate primer design is crucial for identifying deletion and insertion regions. Primers should be designed in such a way that they only amplify the target regions.

- PCR reaction: After adding the primers, the amplification process begins. During this process, the non-deleted regions are amplified and observed in agarose gel or other identification systems.

- Analysis of results: After the completion of the PCR reaction, the results are examined using gel electrophoresis. The resulting bands indicate the presence or absence of target areas.

Advantages and disadvantages of Multiplex Gap-PCR

- Advantages:
- High speed: The process of detecting deletions is carried out more quickly.
- Low cost: Compared to other methods like sequencing, it is less expensive.
- Ability to detect large mutations: Gap-PCR can identify larger mutations such as deletions of several thousand base pairs.
- Disadvantages:
- Need for precise primer design: Designing suitable primers for any type of deletion or insertion requires expertise.
- Low sensitivity for small mutations: Not suitable for detecting small or single-nucleotide mutations.
- Multiplex Gap-PCR is widely used in human and animal genetics laboratories for screening large mutations and genetic analyses, and it is one of the efficient tools for clinical diagnostics.

Reverse hybridization strips

- The Reverse Hybridization Test Strips method, is one of the common techniques in genetic diagnosis and DNA sequence analysis, with extensive applications in medicine, genetics, and virology. This method is used to identify and distinguish between different DNA sequences and is particularly effective for identifying various viruses, bacteria, and genetic mutations

Steps and functioning of Reverse Hybridization Test Strips

- Steps and functioning of Reverse Hybridization Test Strips
- First، Extraction and amplification of DNA
- Reverse hybridization: The desired sequences are separately fixed at specific locations on the hybridization strip (Test Strip). After DNA replication, the sample is placed on the gel. If the DNA sequences of the sample match the sequences on the strip, they bind to them and hybridization occurs.

- Washing and detection: After the hybridization is complete, the strip is washed to remove sequences that have weakly or non-specifically bound. Then, using detection materials such as labeled enzymes or dyes, the hybridization sites are identified, and the final result becomes visible.

Advantages of the Reverse Hybridization method

- High speed: Compared to traditional bacterial or viral culture methods, this method is faster.
- High sensitivity and accuracy: It can detect even a small number of specific sequences.
- Application in multiplex detection: simultaneously capable of identifying multiple sequences or multiple types of pathogens in a single sample.

Disadvantages and limitations

- Need for special equipment: Requires devices such as PCR and detection equipment.
- Probability of non-specific hybridization error: If the hybridization conditions are not properly set, incorrect results will be obtained.

Main applications

- Identification of viral and bacterial infections: such as hepatitis, HIV, and human papillomavirus (HPV).
- Diagnosis of genetic mutations: in the diagnosis of genetic diseases such as cystic fibrosis, thalassemia, and some cancers.
- HLA typing: In organ transplantation to determine tissue compatibility and prevent rejection.
- In general, the Reverse Hybridization Test Strips method is an effective tool in genetic diagnostics and analyses, and due to its high speed and accuracy, it has found extensive application in the fields of medicine and disease diagnosis.

Statistical analysis

Table 1. Frequency of mutated a-globin alleles in 101 Iranian patients with Hb H disease.

Mutation	Type of mutation	Number	*N* (%)
--^{MED}	Deletion	57	32.5
-$\alpha^{3.7}$	Deletion	41	23.4
-$\alpha^{20.5}$	Deletion	20	11.4
-$\alpha^{4.2}$	Deletion	1	0.6
-α^{5NT}	Point mutation	12	6.8
$\alpha^{poly\text{-}A1}$ α	Point mutation	12	6.8
α^{CS} α	Point mutation	10	5.7
$\alpha^{poly\text{-}A6}$ α	Point mutation	6	3.4
α^{polyA4} α	Point mutation	3	1.7
α^{cd59} α	Point mutation	2	1.1
α^{cd19} α	Point mutation	1	0.6
$\alpha^{IVSII+4}$ α	Point mutation	1	0.6
α^{cd99} α	Point mutation	1	0.6
α^{cd142} α	Point mutation	1	0.6
α^{21nt}	Point mutation	1	0.6
α^{cd108} α	Point mutation	1	0.6
α^{cd90} α	Point mutation	1	0.6
α^{CD130} α	Point mutation	1	0.6
$\alpha^{IVSII\text{-}I}$ α	Point mutation	1	0.6
$\alpha^{cd36/37}$ α	Point mutation	1	0.6
$\alpha^{Hb\ Setif}$ α	Point mutation	1	0.6

Results

- In this study, demographic data, hematologic parameters, clinical characteristics and genetic data of 101 cases who were diagnosed with Hb H disease with the age range of 4–70 years were documented.
- Molecular findings including 21 allelic mutations and 30 genotypes are presented
- Based on the results of the mutation analysis, –MED double gene deletion was the most common mutation with a frequency of 32.5%, followed by -α3.7(23.4%), -α20.5 (11.4%), -α5NT (6.8%), αpoly-A1α (6.8%), αCSα (5.7%), αpoly-A6α (3.4%) among all reported α-globin mutant alleles in this study
- The –MED/-a3.7 (29.7%) was found as the most frequently encountered deletional genotype, followed by -α20.5/-α3.7 (5.9%), while -α20.5/- α5NT and αpoly-A6α/αpoly-A6α were the most common non-deletional genotypes

Table 2. Alpha globin genotypes in 101 Iranian patients with Hb H disease.

Genotype	Type of mutation	Frequency	*n* (%)
--^{MED}/-α$^{3.7}$	Deletional	30	29.7
-α$^{20.5}$/-α$^{3.7}$	Deletional	6	5.9
--^{MED}/- α $^{4.2}$	Deletional	1	1
– α$^{3.7}$ /– α$^{3.7}$	Deletional	1	1
- - /- α$^{3.7}$	Deletional	1	1
-α$^{20.5}$/-α^{SNT}	Non- Deletional	7	6.9
α$^{poly\text{-}A6}$α/α$^{poly\text{-}A6}$α	Non- Deletional	6	5.9
--^{MED}/α^{CS}α	Non- Deletional	5	4.9
α$^{poly\text{-}A1}$α/α$^{poly\text{-}A1}$α	Non- Deletional	5	4.9
--^{MED} /α^{polyA2}α	Non- Deletional	5	4.9
--^{MED} / α^{cd19} α	Non- Deletional	4	3.9
--^{MED}/α^{-SNT}	Non- Deletional	4	3.9
-α$^{20.5}$/ α^{CS}α	Non- Deletional	3	2.9
--^{MED} / α^{polyA4}α	Non- Deletional	3	2.9
-α$^{20.5}$/ α$^{poly\text{-}A1}$α	Non- Deletional	3	2.9
α^{CS}α / α^{CS}α	Non- Deletional	2	1.9
α$^{poly\text{-}A1}$α / α^{cd59} α	Non- Deletional	2	1.9
--^{MED}/ α$^{IVSII+4}$ α	Non- Deletional	1	1
-α$^{20.5}$/ α^{cd99} α	Non- Deletional	1	1
--^{MED} / α^{cd142} α	Non- Deletional	1	1
--^{MED} /α^{21nt}	Non- Deletional	1	1
--^{MED} / α^{cd108} α	Non- Deletional	1	1
--^{MED} / α^{cd90} α	Non- Deletional	1	1
-α$^{3.7}$/ α$^{poly\text{-}A1}$α	Non- Deletional	1	1
-α$^{3.7}$/ α^{-SNT}	Non- Deletional	1	1
α^{CD130} α /α^{CD130} α	Non- Deletional	1	1
α$^{IVSII\text{-}I}$ α / α$^{IVSII\text{-}I}$ α	Non- Deletional	1	1
α^{cd19} α / α^{cd19} α	Non- Deletional	1	1
α$^{cd36/37}$ α / α$^{cd36/37}$ α	Non- Deletional	1	1
α$^{Hb\ Setif}$ α / α$^{Hb\ Setif}$ α	Non- Deletional	1	1

Table 3. Laboratory and hematological parameters in 101 Hb H disease patients.

Parameter	Deletional Hb H (*n* = 39)	Non-deletional Hb H (*n* = 62)	*p* value
RBC	5.06 ± 0.67	4.53 ± 0.89	0.03
Hb (g/dl)	9.04 ± 1.89	8.99 ± 1.15	0.89
MCV (27)	60.37 ± 5.75	66.12 ± 8.16	0.00
MCH (pg)	18.03 ± 2.47	19.30 ± 3.31	0.03
Ferritin	347.02 ± 373.94	603.40 ± 715.52	0.02
Bilirubin (mg/dL)	2.44 ± 2.01	2.54 ± 1.61	0.86
HbA (%)	91.34 ± 7.85	87.32 ± 8.19	0.03
HbA2 (%)	1.62 ± 0.57	1.40 ± 0.68	0.13
HbF (%)	0.80 ± 0.58	0.98 ± 0.76	0.33
Hb H (%)	7.17 ± 6.96	11.16 ± 7.02	0.03

Note. Data are shown as mean ± SD.

Clinical presentations	Deletional Hb H ($n=39$)	H ($n=62$)	p-value
Sex			
Male	14 (36%)	28 (45%)	
Female	25 (64%)	34 (55%)	0.35
Age at diagnosis	14.85 ± 12.62	12.84 ± 13.77	0.46
Spleen size			
Not palpable	17(43.5%)	24(38.7%)	
Just palpable	4 (10%)	2 (3%)	0.11
2 cm below LCM	9 (23%)	9 (14.5%)	
≥3 cm below LCM	9 (23%)	27 (43.5%)	
Splenectomy	2 (5.1%)	11 (17.7%)	0.06
Age of splenectomy	37 ± 15.55	22.27 ± 11.25	0.13
Age of first blood transfusion	21.22 ± 15.34	16.27 ± 18.37	0.46
Transfusion			
Non-transfused	24 (61.5%)	25 (39.6%)	
Regular transfusion	2 (5.1%)	10 (16.1%)	0.07
Occasional transfusion	13 (33.3)	27 (43.5%)	
Jaundice	8 (20%)	11(18%)	0.72
Growth failure	0	2 (3.2%)	0.25
Cardiovascular disease	0	4 (6.4%)	0.10
Facial changes	2 (5.1%)	8 (12.9%)	0.20
Thrombotic event	0	2 (3.2%)	0.24
Diabetes Mellitus	2 (5.4%)	1)1.6%)	0.55
Leg ulcer	0	0	–

Note. The data are presented as mean ± SD or number (percent).

- All TD patients except one, had ferritin concentrations of more than 500 ng/ml.
- The –MED/-α3.7, αpoly-A1α/αpoly-A1α, αpoly-A6α/αpoly-A6α, –MED/αCSα, α CD130 α/α CD130 α, -α3.7/- α–5NTand -α20.5/αCSα genotypes were reported in patients with dysmorphic facial features.
- Two patients with –MED/α–5NT and -α20.5/αCSα genotypes experienced thrombotic events.
- Two siblings who were TD with -α20.5/-α5NT genotype developed renal failure for which no conclusion could be drawn

Conclusion

- Although Hb H is a growing disease in the world, it has not yet been fully understood. the exact prevalence and correlation between genotype-phenotype, as well as the natural history of this disease is not quite clear

THE END

THANK YOU FOR YOUR ATTENTION