Molecular characterization of alpha globin and beta globin genes in patients with hemoglobinopathies in Central Vietnam

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Abstract

Background: Hemoglobinopathy is the most common monogenic disease worldwide. The aims of the current study were: (1) to investigate some hematological characteristics of patients with hemoglobinopathies; and (2) to detect the mutation of α-globin and β-globin genes, as well as the association between genotype and degree of anemia. **Materials and method:** 251 patients with hemoglobinopathies were examined for the α-globin or β-globin gene mutations. **Results:** 51% were the carriers, and 49% were thalassemia intermedia or thalassemia major. Hematological characteristics were suitable for α-thalassemia or β-thalassemia. Eleven β-globin gene mutations were observed. The β^0/β^A, β^E/β^A, β^E/β^E, β^E/β^+, β^+/β^+ genotypes were only found in β-thalassemia intermedia individuals; the β^0/β^0 genotype was limited to β-thalassemia major patients; the β^+/β^0 and β^E/β^0 genotypes were seen in both types. Four α-globin gene mutations were observed. All α-thalassemia patients were intermedia, the most common genotype was $--^{SEA}/-\alpha^{3.7}$. **Conclusion:** There were differences in anemia degree between β-globin genotypes.

Key words: *hemoglobinopathies, α-globin, β-globin.*

1. INTRODUCTION

Hemoglobinopathies are among the most common monogenic diseases, with approximately 7% of the worldwide population being carriers and one of the major health problems. Hemoglobinopathies include two main groups as thalassemia and structural hemoglobin variants, both are caused by mutations and/or deletions in the α- or β-globin genes. Thalassemia is characterized by decreased or absent synthesis of normal globin subunits, and reduced or absent synthesis of α-globin or β-globin chains lead to α-thalassemia or β-thalassemia, respectively. Whereas mutations changing the molecular structure of hemoglobin cause abnormal hemoglobin or structural hemoglobin variants [1].

The combination of thalassemia and structural variants of hemoglobin results in different abnormal genotypes, affecting the clinical manifestations of the disease [2]. According to the number of mutated α-globin genes, α-thalassemia is divided into four types, including silent carrier state (single α-globin gene deletion) with no anemia and normal red blood cell indices; α-thalassemia trait (two α-globin genes deletion or single non-deletional mutation) with mild hypochromic and microcytosis; HbH disease (deletions or abnormalities of three α-globin genes) with moderate hemolytic anemia, splenomegaly; and HbBart's hydrops foetalis (absent of all four α-globin genes) with severe foetal anemia and death in utero. β-thalassemia includes β-thalassemia minor, β-thalassemia intermedia and β-thalassemia major [3]. Accurate prediction of a mild phenotype may avoid unnecessary transfusions and their complications, while early diagnosis of a severe type will allow for early transfusion, therefore preventing hypersplenism and red cell antigen sensitization [4]. Also, studying the genetics of hemoglobin disorder lays the groundwork for understanding the clinical and hematologic characteristics and for treatment, prevention, prenatal diagnosis, and genetic counseling.

Hemoglobinopathies are Southeast Asia's most common genetic disorders with a high prevalence of α-thalassemia, β-thalassemia, HbE, and HbCS. The gene frequencies of α-thalassemia reach 30-40% in North Thailand and Laos, 4.5% in Malaysia, and 5% in Philippines, whereas β-thalassemia varies from 1 - 9%. HbE is the hallmark in Southeast Asia, accounting for 50-60%, particularly in the border regions of Thailand, Laos, and Cambodia. HbCS varies between 1 and 8% [2].

In Vietnam, hemoglobinopathies were described

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as being distributed all over the country. However, in the North, South, and Central of Vietnam, there are differences in the frequency and molecular characteristics of the α-globin and β-globin genes mutations [5 - 9].

Hence, the aims of the current study were:

(1) To investigate some hematological characteristics of patients with hemoglobinopathies.

(2) To detect the mutation of α-globin and β-globin genes, as well as the association between genotype and degree of anemia.

2. SUBJECT AND METHODS

2.1. Subject

A total of two hundreds and fifty one patients with hemoglobinopathies from January, 2020 to June, 2022 were enrolled in this study. The patients were determined as thalassemia carriers based on MCV < 80 fL and/or MCH < 28 pg; or had been diagnosed with thalassemia according to the diagnostic criteria of the Ministry of Health, including chronic anemia syndrome, chronic hemolytic syndrome, microcytic hypochromic anemia, and the presence of abnormal hemoglobin or change in hemoglobin composition [10]. The severity of α-thalassemia, β-thalassemia was classified according to the standards of the Ministry of Health and the Thalassemia International Federation [10, 11].

2.2. Methods

Step 1: Collection of samples

The samples were collected at the Department of Hematology, Hue University of Medicine and Pharmacy Hospital; Department of Hematology Laboratory and Department of Clinical Hematology, Hematology - Blood Transfusion Center, Hue Central Hospital. 2 ml whole blood sample was collected using EDTA as an anticoagulant.

Step 2: Hemoglobin analysis

Hemoglobin components were detected by electrophoresis.

Step 3: DNA extraction

DNA was extracted from whole blood samples by Wizard Genomic DNA Purification (Promega) kit. The concentration and quality of extracted DNA was measured by NanoDrop 2000. Then, the DNA was stored at -20°C for further analysis.

Step 4: Detection α-globin and β-globin genes mutations

* *Detection β-globin genes mutations*

- Four PCR reactions were performed that amplified the complete β-globin gene, encompassed the proximal promoter region to the IVS I (-293 → +237); exon 1, IVS I, exon 2 and part of IVS II (+34 → +628); part of IVSII (+622 → +1155); the final part of IVS II and the exon 3 (+1099 → +1674). List of primers:

+ Reaction 1: B1-F: CTTACCAAGCTGTGATTCCA
B1-R: GTCAGTGCCTATCAGAAACC
+ Reaction 2: B2-F: AACCTCAAACAGACACCATG
B2-R: ACTTCCACACTGATGCAATC
+ Reaction 3: B3-F: TGGAAGTCTCAGGATCGTTT
B3-R: GCTATTGCCTTAACCCAGAA
+ Reaction 4: B4-F: GCCTCTTTGCACCATTCTAA
B4-R: TTTAAATGCACTGACCTCCC

- PCR components included 12.5 µl GoTaq Green MasterMix (Promega), 1 µl each primer (10 pmol/µl), 1 µl DNA (100 ng/µl), 9.5 µl deionized H2O up to 25 µl final reaction volume. The amplification conditions were as followed: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C or 58°C for 30 seconds, extension at 72°C for 1 minute; and a final extension cycle at 72°C for 10 minutes.

- PCR products were loaded on a 1% agarose gel stained with SafeView. Electrophoresis was performed under 80V in 1 hour, with a ladder 100bp. The bands were observed under the ultraviolet light.

- PCR products were sent for sequencing at 1st BASE company located in Malaysia.

- Sequencing results were exported as .ab1 file and analyzed by BioEdit software. Then, using NCBI BLAST to compare the samples' sequence to the reference sequences on Genbank to identify mutations.

* *Detection α-globin genes mutations*

- Gap-PCR was performed to detect common α-thalassemia deletions in Vietnam, such as $-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{SEA}$ mutations.

Table 1. Primer sequences for detecting common α-thalassemia deletions

Primer	Sequence (5' → 3')	Amplicon size
LIS1-F	GTCGTCACTGGCAGCGTAGATC	2503 bp
LIS1-R	GATTCCAGGTTGTAGACGGACTG	
α2/3.7-F	CCCCTCGCCAAGTCCACCC	2022/2029 bp
3.7-R	AAAGCACTCTAGGGTCCAGCG	

α2/3.7-F	CCCCTCGCCAAGTCCACCC	1800 bp
α2-R	AGACCAGGAAGGGCCGGTG	
4.2-F	GGTTTACCCATGTGGTGCCTC	1628 bp
4.2-R	CCCGTTGGATCTTCTCATTTCCC	
SEA-F	CGATCTGGGCTCTGTGTTCTC	1349 bp
SEA-R	AGCCCACGTTGTGTTCATGGC	

PCR products were loaded on a 1% agarose gel stained with SafeView. Electrophoresis was performed under 80V for 2 hours, with a ladder 1kb. The bands were observed under the ultraviolet light.

- Applied allele-specific PCR was applied to identify HbCS mutation. Two PCR reactions were performed to determine the normal and mutant alleles for HbCS, respectively. The forward primer CS-F: 5'– CCT GGG CCG CAC TGA CCC TAT T – 3' was used for both reaction, the reverse primer CS-N: 5' – AGG AGG AAC GGC TAC CGA GGC TCC AGA TTA – 3' or CS-M: 5' – AGG AGG AAC GGC TAC CGA GGC TCC AGA TTG – 3' was used for reaction of the normal allele (N) or mutant allele (M), respectively. The PCR products were loaded on a 1% agarose gel stained with SafeView. Electrophoresis was performed under 80V in 1 hour 30 minutes, with a ladder 100 bp. The bands were observed under the ultraviolet light.

2.3. Research ethics

Patients in the trial were informed and consented; patient data and study findings were kept confidential. The outcomes are intended solely for medical and scientific study and treatment.

3. RESULTS

3.1. Hematological characteristics of patients with hemoglobinopathies

Table 2. Red blood cell indices in patient groups

RBC indices	Total n = 251	α-thalassemia carrier (1) n = 25	β-thalassemia carrier (2) n = 103	HbH disease (3) n = 22	β-thalassemia intermedia (4) n = 76	β-thalassemia major (5) n = 25	p
Hb (g/dL)	9.4 ± 2.2	11.7 ± 1.5	10.8 ± 1.7	8.7 ± 1.6	7.8 ± 1.4	7.5 ± 1.3	p(1)(2)=0.015 p(3)(4)=0.009 p(4)(5)=0.475
MCV (fL)	68.4 ± 8.8	66.9 ± 5.9	66.2 ± 8.4	73.4 ± 7.9	68.5 ± 8.6	74.3 ± 9.9	p(1)(2)=0.647 p(3)(4)=0.020 p(4)(5)=0.007
MCH (pg)	21.2 ± 3.1	22.4 ±2.2	21.0 ± 3.1	19.3 ± 1.9	20.6 ± 2.6	23.7 ± 4.3	p(1)(2)=0.012 p(3)(4)=0.045 p(4)(5)=0.001

There were differences in Hb and MCH index between α-thalassemia and β-thalassemia carrier groups. Patients with HbH (α-thalassemia intermedia) had higher Hb, MCV, and lower MCH than β-thalassemia intermedia; the difference was statistically significant. There was no difference in Hb index between β-thalassemia intermedia and β-thalassemia major.

Table 3. Hemoglobin components in patient groups

Hb (%)	α-thalassemia carrier (1) n = 25	β -thalassemia carrier (2) n = 103	HbH disease (3) n = 22	β –thalassemia intermedia (4) n = 76	β -thalassemia major (5) n = 25	p
HbA	97.8 ± 0.8	86.1 ± 10.7	89.9 ± 4.9	28.4 ± 32.2	54.7 ± 33.9	p(1)(2)<0.0001 p(4)(5)=0.001
HbA_2	2.2 ± 0.7	5.6 ± 1.2	1.0 ± 1.6	6.1 ± 2.6	4.6 ± 2.5	p(1)(2)<0.0001 p(4)(5)=0.013

HbF	0.02 ± 0.12	0.9 ± 2.2	-	26.9 ± 18.2	22.9 ± 23.3	p(1)(2)=0.014 p(4)(5)=0.167
HbE	-	24.5 ± 3.9	-	42.1 ± 18.1	31.5 ± 13.1	p(4)(5)=0.04
HbH	-	-	9.3 ± 3.9	-	-	
Hb Bart's	-	-	1.1 ± 0.6	-	-	
HbCS	-	-	2.3 ± 0.7	-	-	

**Among HbH patients, there were 19 patients with HbH, 9 patients with HbBart's, 6 patients with HbCS.*

Among β-thalassemia patients, there were 32 β-thalassemia carriers, 69 β-thalassemia intermedia and 14 severe β-thalassemia patients had HbE.

The hemoglobin components were typical for α-thalassemia and β-thalassemia. α-thalassemia carriers had a normal hemoglobin component. HbH disease had reduced HbA, normal HbA2 and presence of HbH, HbBart's and HbCS. β-thalassemia group was characterized by decreased HbA, increased HbA2 and HbF, and the presence of HbE in patients with HbE/β-thalassemia.

3.2. Characterisation of α-globin, β-globin gene mutations and association to the degree of anemia

3.2.1. Characterisation of α-globin, β-globin gene mutations

Table 4. The distribution of α-globin, β-globin gene mutations

Type allele	Mutation	Number of alleles	(%)
α- thalassemia		**69**	**100**
α^0	$--^{SEA}$	45	65.2
α^+	$-\alpha^{3.7}$	16	23.2
	$\alpha^{cs}\alpha$	6	8.7
	$-\alpha^{4.2}$	2	2.9
β-*thalassemia*		**297**	**100**
β^E	cd 26 (G>A) (HbE)	119	40.07
β^0	cd 17 (A>T)	87	29.29
	cds 41/42 (-TTCT)	37	12.46
	IVS-I-1 (G>T)	20	6.73
	cds 71/72 (+A)	11	3.70
	cd 95 (+A)	6	2.02
	cd 26 (G>T)	5	1.68
β^+	-28 (A>G)	9	3.03
	-50 (G>A)	1	0.34
	-72 (T>A)	1	0.34
	IVS-II-654 (C>T)	1	0.34

4 α-globin mutations were detected, in which the $--^{SEA}$ and $-\alpha^{3.7}$ mutations were the most common, accounting for up to 88.4%. 11 mutations in the β-globin gene were identified, in which the highest proportion was cd 26 (G>A) (HbE), cd 17 (A>T), and cds 41/42 (-TTCT), respectively, accounting for 81.82%.

3.2.2. The association between genotype and disease severity and anemia level

All patients with HbH disease were intermediate, the most common genotype was $--^{SEA}/-\alpha^{3.7}$ accounting for 63.6% (14/22), then $--^{SEA}/\alpha^{CS}\alpha$ and $--^{SEA}/-\alpha^{4.2}$ genotypes were observed as 27.3% (6/22) and 9.1% (2/22), respectively.

Table 5. β-globin genotype và β-thalassemia

β-genotype	Thalassemia intermedia		Thalassemia major	
	n	%	n	%
β^0/β^A	5	100	-	-
β^E/β^A	3	100	-	-
β^E/β^E	4	100	-	-
β^E/β^+	3	100	-	-
β^+/β^+	2	100	-	-
β^+/β^0	1	50	1	50
β^E/β^0	58	79.5	15	20.5
β^0/β^0	-	-	9	100
Total	**76**		**25**	

The genotype with the highest proportion was β^E/β^0 with 72.3% (73/101). β^0/β^A, β^E/β^A, β^E/β^E, β^E/β^+, β^+/β^+ genotypes only found in patients with thalassemia intermedia, β^0/β^0 only found in patients with thalassemia major, while β^+/β^0 and β^E/β^0 were found in both thalassemia intermediate and major patients.

Table 6. The association between genotype and anemia level

	Genotype	Non- anemia	Mild anemia	Moderate anemia	Severe anemia	p
α- thalassemia						
α-thalassemia carrier	-α/αα	-	2 (100%)	-	-	0.310
	--/αα	7 (30.45%)	7 (30.45%)	9 (39.1%)	-	
HbH disease	--/-α	-	3 (18.8%)	10 (62.4%)	3 (18.8%)	0.116
	--/α^Tα	-	-	2 (33.3%)	4 (66.7%)	
β-thalassemia						
β-thalassemia carrier	β^E/β^A	11 (34.4%)	10 (31.2%)	11 (34.4%)	-	0.044
	β^+/β^A	2 (66.7%)	-	1 (33.3%)	-	
	β^0/β^A	10 (14.7%)	17 (25%)	40 (58.8%)	1 (1.5%)	
β-thalassemia intermedia	β^E/β^A	-	-	2 (66.7%)	1 (33.3%)	0.0004
	β^0/β^A	-	-	4 (80%)	1 (20%)	
	β^E/β^E	-	1 (25%)	3 (75%)	-	
	β^E/β^+	-	-	3 (100%)	-	
	β^+/β^+	-	-	2 (100%)	-	
	β^+/β^0	-	-	-	1 (100%)	
	β^E/β^0	-	-	20 (34.5%)	38 (65.5%)	

β-thalassemia major	β^+/β^0	-	-	-	1 (100%)	
	β^E/β^0	-	-	4 (26.7%)	11 (73.3%)	0.284
	β^0/β^0	-	-	9 (36%)	16 (64%)	

There was no difference between the α-globin genotype and the degree of anemia. There was a significant difference between the β-globin genotype and the degree of anemia in patients with β-thalassemia carrier and β-thalassemia intermedia.

4. DISCUSSION

4.1. Hematological characteristics of patients with hemoglobinopathies

The hematological indices in the study were specific to thalassemia patients, presenting hypochromic microcytic anemia including hemoglobin 9.4 ± 2.2 g/dL, MCV and MCH were 68.4 ± 8.8 fL and 21.2 ± 3.1 pg, respectively (Table 2). Our results were similar to Nguyen Khac Han Hoan (2013) studied on the HbH group, Vo The Hieu et al. (2013) performed on thalassemia intermedia and thalassemia major patients [12, 13].

Hemoglobin components were varied depending on the type of disease. For β-thalassemia, β^0 refers to the complete absence of β-globin chain, β^+ refers to reduced production of β-globin chain (around 10%), while β^E is specific for HbE mutation. Meanwhile, the α-globin chain is still producing normally, stimulating the production of δ-globin and γ-globin chains. The δ-globin and γ-globin chains will combine with α-globin, thereby increasing the amount of HbA_2 ($\alpha_2\delta_2$) and HbF ($\alpha_2\gamma_2$). HbA_2 index > 3.5% is a specific marker for β-thalassemia, while HbH (β_4), HbBart's (γ_4), HbCS are typical for α-thalassemia.

Our study's hemoglobin composition was consistent with the disease forms (Table 3). However, the thalassemia major patients had higher HbA levels than intermediate patients (p = 0.001). This could be explained by the fact that the thalassemia major patients required frequent blood transfusions so that this result could include both the patient's hemoglobin component and the previously transfused blood.

Nguyen Khac Han Hoan (2013) reported in HbH patients, the HbA, HbA_2 and HbH were 93.0 ± 7.7%, 2.1 ± 1.5%, and 8.6 ± 76%, respectively, similar to our study, however, HbBart's was lower, only 0.6 ± 0.3% [12]. Doro et al. (2017) in their research on transfusion dependent β-thalassemia patients showed decreased HbA, average of 68.4 ± 28.8%, increased HbF and HbE with 17 ± 21.3% và 22.9 ± 16.7%, respectively [5]whereas limited data were available concerning the central area of the country. In this study, we describe the molecular characterization and frequency of β-globin gene mutations in the Thua Thien Hue Province of Central Vietnam as the result of a first survey conducted in 22 transfusion-dependent patients, and four unrelated heterozygotes. Nine different known mutations were identified (seven of the β0 and two of the β+ type. This result was similar to our study's indexes on patients with β-thalassemia major (p > 0.05).

4.2. Characterisation of α-globin, β-globin gene mutations and association to the degree of anemia

4.2.1. Characterisation of α-globin, β-globin gene mutations

The most frequent mutations observed in α-globin gene are deletions, while in β-globin gene, the single nucleotide substitution, small insertion, or deletions are predominant. The spreading of mutations is heterogeneous, geographically and ethnically specific.

We recorded 4 α-globin gene mutations, in which $--^{SEA}$ was the most common at 65.2%, the $-\alpha^{3.7}$, HbCS and $-\alpha^{4.2}$ mutations occupied 23.2%, 8.7%, and 2.9%, respectively (Table 4). Ngo Diem Ngoc (2018) in the North also noted that $--^{SEA}$ was the most common mutation, accounting for 50%. However, HbCS mutations are more common than $-\alpha^{3.7}$, accounting for 27.3% and 10.8%, respectively. Bach Quoc Khanh et al. (2021) studied 16 ethnic groups in the South Central Coast and Central Highlands reported that $-\alpha^{3.7}$ and HbCS had high rates, 66.8%, and 31.3%, respectively, while $--^{SEA}$ accounted for only 0.9% [14].

Eleven β-globin gene mutations were identified, in which the three most common mutations accounted for 81.82%, including cd 26 (G>A) HbE, cd 17 (A>T), and cds 41/42 (-TTCT) with 40.07%, 29.29%, and 12.46%, respectively (Table 4). The remaining eight mutations account for a low rate, of which cd 26 (G>T), -50 (G>A), -72 (T>A) were

rare, only recently recorded in Central Vietnam [15]. Nguyen Hoang Nam (2019) in the North and Nguyen Khac Han Hoan (2013) in the South also observed cd 26 (G>A) HbE, cd 17 (A>T), and cds 41/42 (-TTCT) were the most common. The remaining mutations had different rates between regions, as IVS-I-1 (G>T) in the Central area had a higher prevalence than in the South and North, while IVS-I-5 (G>C) had only been observed in the North [16].

4.2.2. The association between genotype and disease severity and anemia level

The patients with HbH disease all belong to the thalassemia intermediate; the most common gene type is $--^{SEA}/-\alpha^{3.7}$ accounting for 63.6%, $--^{SEA}/\alpha^{cs}\alpha$ and $--^{SEA}/-\alpha^{4.2}$ genotypes accounting for 27.3% and 9.1%, respectively. Our study did not record any difference in the degree of anemia between the α-globin genotypes (Table 6). However, some studies have shown that patients with deletional type (--/-α) have milder symptoms than patients with non-deletional type (--/$\alpha^{T}\alpha$ or --/$\alpha\alpha^{T}$). Ngo Diem Ngoc (2018) research on 97 HbH patients reported 31.9% as deletional type and 68.1% as non-deletional type. Non-deletional HbH disease had more severe clinical manifestations than deletional type, and the clinical expression level of genotypes decreases with $--^{SEA}/\alpha^{cs}\alpha$ > $--^{SEA}/-\alpha^{4.2}$ > $--^{SEA}/-\alpha^{3.7}$ ($p<0.05$) [17]. Fucharoen's (2009) study on 355 HbH patients in Thailand also showed that the non-deletional type had more anemia and splenomegaly than the deletional type [18]. This difference may be due to the small sample size of our study.

The degree of globin chain imbalance is the main determinant of the severity of thalassemia syndromes. Thus, the combination of α-thalassemia mutations can alter the phenotype of β-thalassemia disease.

Table 5 presents the β-globin genotype of the β-thalassemia intermediate and major groups. The results showed that there were eight genotypes including β^0/β^A, β^E/β^A, β^E/β^E, β^E/β^+, β^+/β^+, β^+/β^0, β^E/β^0, and β^0/β^0.

Patients with β^0/β^A or β^E/β^A genotypes were all intermediate. These β-thalassemia heterozygous cases may be associated with mutations that result in excess of the α-globin gene (an excess of two α-globin genes such as ααα/ααα or αααα/αα or an excess of one α-globin gene such as ααα/αα). A combination of mutations like the above will increase the degree of imbalance of α-globin and β-globin chains, thereby increasing the severity of the disease [19]. In addition, the combination of HbH disease and β^E/β^A genotype can reduce the severity of α-thalassemia, resulting in patients with an intermediate phenotype. A Prayalaw's study (2015) in Thailand on 312 non-transfusion-dependent thalassemia patients showed that 20.2% of patients had heterozygous β^E/β^A genotype associated with HbH [20].

In our study, the patients with β^E/β^E, β^E/β^+, and β^+/β^+ genotypes were all intermediate, and none of them were major. Meanwhile, patients with β^+/β^0, β^E/β^0 genotypes appeared in both groups. Although these genotypes have both mutated alleles, but while the β^0 allele completely fails to produce the β-globin chain, the β^+ allele still produces 10% of the normal β-globin chain and β^E with the phenotype of β^+ [21]. Therefore, patients with β^E/β^E, β^E/β^+ and β^+/β^+ genotypes have a milder clinical presentation than β^+/β^0 and β^E/β^0. Traivaree et al. (2018) researched β-thalassemia patients in Thailand and also showed that patients carrying β^E/β^+ genotype were thalassemia intermediate [22].

β^+/β^0, β^E/β^0 genotypes were observed in both thalassemia intermediate and major patients. The degree of imbalance between the two globin chains is a significant determinant of the severity of thalassemia. Some studies have shown that the loss of two α-globin genes is associated with a milder clinical phenotype in most β-thalassemia patients; while the loss of an α-globin gene in patients with β^0/β^0 genotype has less clinical mitigating effect than the remaining genotypes. Notably, for patients with the HbE/β-thalassemia genotype, the α-thalassemia combination significantly reduced clinical severity even with the loss of only one α-globin gene [23]. Moreover, increased production of HbF may also reduce the severity of β-thalassemia by reducing α-like and non α-like globin chain imbalances. Many studies have shown that the *XmnI* polymorphism in the $^{G}\gamma$-globin gene and the *BCL11A* gene polymorphism are associated with increased HbF synthesis and reduced severity in patients with HbE/β-thalassemia [19].

In our study, 100% of patients with β^0/β^0 genotype belong to the group of β-thalassemia major. This result is similar to Shoujaa's (2019) study, which showed that patients with β^0/β^0 genotype were blood transfusion dependent [24].

There was a difference between the β-globin genotype and the level of anemia in patients with β-thalassemia carrier and β-thalassemia intermedia. The degree of anemia was consistent with the respective genotypes.

5. CONCLUSION

Through the study of 251 patients with hemoglobinopathies, we draw some conclusions as

follows:

- The red blood cell index decreased: Hb 9.4 ± 2.2 g/dL, MCV 68.4 ± 8.8 fL, MCH 21.2 ± 3.1 pg. The hemoglobin composition was consistent with α-thalassemia or β-thalassemia.

- Four α-globin gene mutations were identified. Patients with HbH disease are all intermediate, the most common genotype was $--^{SEA}/-\alpha^{3.7}$, accounting for 63.6%. Eleven β-globin gene mutations were detected. β-thalassemia patients included 75.2% thalassemia intermediate, and 24.8% thalassemia major. The most common β-genotype was β^E/β^0, which occupied 72.3%. β^0/β^A, β^E/β^A, β^E/β^E, β^E/β^+, β^+/β^+ genotypes only found in patients with thalassemia intermedia; β^0/β^0 was only seen in thalassemia major; β^E/β^0 and β^+/β^0 were found in both groups. There was a difference in the degree of anemia among the β-globin genotypes.

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