THE PREVALENCE OF BETA-GLOBIN GENE MUTATION TYPES AND DISEASE TYPES IN BETA-THALASSEMIA PATIENTS AT KIEN GIANG GENERAL HOSPITAL

Pham Thi Ngoc Nga[1*], Le Quang Vu[2], Au Xuan Sam[1], Trinh Minh Thiet[1], Dang Quang Phu[1]

1. Can Tho University of Medicine and Pharmacy

2. Department of hematology and blood transfusion, Kien Giang general hospital

**Corresponding author: pttnga@ctump.edu.vn*

ABSTRACT

Background: *At Kien Giang general hospital, more and more Beta-thalassemia (β-Thal) patients are discovered with different Beta globin (β-globin) gene mutation types and disease types.* ***Objectives:*** *1. Identify the prevalence of β-globin gene mutation types in β-Thal patients at Kien Giang general hospital from 1/2018 to 12/2018; 2. Identify the prevalence of disease types in β-Thal patients at Kien Giang general hospital by using the β-globin sequencing technique.* ***Materials and methods****: A cross-sectional study descriptive design was applied from January 2018 to December 2018 at Kien Giang general hospital. The population was all patients were diagnosed with β-Thal in three departments. Blood samples of β-Thal patients were extracted DNA with QIAamp DNA Blood Mini Kit®. The β-globin gene mutation types were identified by a sequence of the β-globin gene at the Center for Molecular Biology, University of Medicine and Pharmacy in Ho Chi Minh City. The predominant β-globin gene mutation types were identified based on molecular genetic testing results. The prevalence of disease types was identified based on Hb electrophoresis results combined with β-globin gene mutation types.* ***Results:*** *In 187 β-Thal patients, mostly are Kinh*

(67.9%) and come from the department of Pediatrics (56.7%). 10 β-globin gene mutation types are identified with 263 alleles, 8 of them are common in Vietnam and account for 97.7%. Base on Hb electrophoresis results combined with β-globin gene mutation types, there are 6 disease types are identified with different rates, the highest is HbE heterozygous (39%) and the lowest is β-Thal homozygous (0,5%). ***Conclusions:*** *β-Thal testing for anemic patients is very necessary. Besides, screening of prenatal carriers, genetic counseling, and diagnosis is recommended to prevent β-Thal homozygous or HbE/β-Thal compound heterozygotes appear in the next generations. That contributes to improving the quality of the population in Kien Giang province.*

Keywords: *β-Thal, Kien Giang general hospital, β-globin gene mutation types, disease types.*

I. INTRODUCTION

Beta-thalassemia (β-Thal) is one of the most commonly inherited genetic disorders in the world and caused by a mutation in the Beta globin (β-globin) gene [1], [5], [11]. Vietnam is one of the countries with a high prevalence [5]. According to a report of the Association of Congenital Hemolysis of the Southwest region, in 2015, about 2,105 people were diagnosed with Thalassemia at hospitals in the Mekong Delta region. β-Thal patients will experience serious consequences on body development, life expectancy by hemolysis, and its complications. The treatment is mainly blood transfusion, iron chelation is very expensive, less effective, and only to maintain life temporarily. This is the reason why thalassemia becomes a burden on families and society [5].

In Kien Giang (a province in the Mekong Delta), the number of people infected with β-Thal is increasing, about 257,113 patients per year had a blood transfusion (data from the General planning department of Kien Giang general hospital). However, the study about the prevalence of β-globin gene mutation types and disease types of β-Thal is limited. Therefore, we perform this study with the following objectives: (1) Identify the prevalence of β-globin gene mutation types in β-Thal patients at Kien Giang general hospital from 1/2018 to 12/2018; (2) Identify the prevalence of disease types in β-Thal patients at Kien Giang general hospital by using the β-globin sequencing technique.

II. MATERIALS AND METHODS

2.1. Study design and population

A cross-sectional study descriptive design was applied. All data were collected from January 2018 to December 2018 at Kien Giang general hospital, Kien Giang province, Viet Nam.

Table 1. The β-Thal diagnostic criteria based on Hb electrophoresis results and study of Nguyen, K.H.H. [1]

Disease types	HbA	HbA_2	HbF	$HbE+HbA_2$
β-Thal homozygotes	-	≥3,5%	92-95%	-
β-Thal heterozygotes	10-30%	Normal	70-90%.	-
HbE homozygotes (HbEE)	Normal	-	-	12.6-30.3%
HbE heterozygotes (HbAE)	-	-	-	>90%.
β-Thal/HbE compound	5-60%	Normal	6-50%	25-80%

The population was all patients were diagnosed with β-Thal in three departments (the Hematology clinic, the Department of Pediatrics, and the Department of Internal Medicine). They or their guardian agreed to participate in this study, regardless of age, gender, ethnicity. The diagnostic criteria are based on MCV and/or MCH index (MCV<80fL, MCH<28pg), Hb electrophoresis results, and study of Nguyen KHH *et al* (2010) (**Table 1**).

The exclusion criteria were they or their guardian refused to participate in this study, in total, 187 patients were selected.

2.2. Data collection

The prevalence of β-globin gene mutation types in β-Thal patients

Blood samples of β-Thal patients were extracted DNA with QIAamp DNA Blood Mini Kit®. Cells were disrupted and lysed with binding buffer and proteinase K. The lysate was filtered through a filter column with a high salt concentration. The silica gel membrane in the column traps the DNA and allows other substances to pass through. Purified DNA was dissolved in a buffer of low salt concentration. The purified DNA solution was measured at 260/280nm with BioMate 3 machine and must ensure the purity of OD260/OD280 ≥ 1.7 before use in PCR. The β-globin gene mutation types were identified by a sequence of the β-globin gene at the Center for Molecular Biology, University of Medicine and Pharmacy (Ho Chi Minh City). Based on the principle of the modified Sanger method, the ddNTPs are labeled with different colored fluorescents. The automatic sequencer consists of the following main components: capillary system, laser illumination system, signal receiving, and processing system. The electrophoresis lines in the capillary will be illuminated when passed through a laser beam. The color signal recognition system will record and encode into nucleotides A, T, C, G [1]. The predominant β-globin gene mutation types were identified based on molecular genetic testing results.

The prevalence of disease types in Beta thalassemia patients

The prevalence of disease types was identified based on Hb electrophoresis results combined with β-globin gene mutation types (**Table 2**).

Table 2. Disease types of β-Thal patients base on Hb electrophoresis results combined with β-globin gene mutation types [5].

Disease types	Mutations 1	Mutations 2
β-Thal homozygous	β-globin gene (X)	β-globin gene (X)
β-Thal compound heterozygous	β-globin gene (X)	β-globin gene (Y)
β-Thal heterozygous	β-globin gene (X) or (Y)	-
β-Thal/HbE compound heterozygotes	Cd26 G>A	β-globin gene (X) or (Y)
HbE homozygotes (HbEE)	Cd26 G>A	Cd26 G>A
HbE heterozygotes (HbAE)	Cd26 G>A	-

Notes: X, Y is an example of one β-globin mutation type

2.3. Statistical analysis

All data were analyzed by using SPSS version 16.0.0. Descriptive statistics in terms of frequency, rate, percent, mean, standard deviation of quantitative value, and range were used to examine demographic characteristics, MCH index, MCV index… present the results with tables, graphs, and diagrams.

2.4. Ethical issues

This study was conducted based on the patient's voluntary participation, subjects have the right to refuse to participate in any phase of the study, they will still be received treatment as usual. In addition, patient-related information will be kept strictly confidential and only for research purposes. Nha Trang University council approved this study by 966/QĐ-ĐHNT.

III. RESULTS

In 187 β-Thal patients at Kien Giang general hospital participate in this study, mostly are Kinh (67.9%) and come from the department of Pediatrics (56.7%). 10 β-globin gene mutation types are identified with 263 alleles, 8 types of them are common in Vietnam and account for 97.7%. Base on Hb electrophoresis results combined with β-globin gene mutation types, there are 6 disease types are identified with different rates, the highest is HbE heterozygous (39%), β-Thal/HbE compound heterozygotes (31%), and the lowest is β-Thal homozygous (0,5%), specific results are as follows:

3.1. Baseline clinical characteristics of the study population

Table 3. Baseline clinical characteristics of β-Thal patients (n=187)

No	Characteristics	Mean ± SD or n (%)
1	Age ≤ 15 years, n (%)	107 (57.2%)
2	Male sex, n (%)	87 (46.5%)
3	MCV (fL ± SD)	68.5 ± 11.7
4	MCH (pg ± SD)	20.6 ± 4.7
5	Ethnic - Kinh - Hoa - Khmer	187 (100%) 127 (67.9%) 5 (2.7%) 55 (29.4%)
6	Department - Department of Pediatrics - Department of Internal Medicine - Hematology clinic	187 (100%) 106 (56.7%) 65 (34.7%) 16 (8.6%)

The age ≤ 15 years account high rate (57.2%), male sex has lower rate (46.5%) than female sex (53.5%), average MCV index (68.5%), MCH index (20.6%). About ethnic, there are 3 main groups: The highest rate is Kinh (67.9%) and the lowest is Hoa (2.7%). Most of the patients from the Department of Pediatrics (56.7%) and the Department of Internal Medicine (34.7%), the lowest in the Hematology clinic (8.6%).

3.2. The prevalence of β-globin gene mutation types in β-Thal patients

Table 4. The prevalence of β-globin gene mutation types of each ethnic

β-globin gene mutation types		Kinh	Hoa	Khmer	Total (%)
Common mutation types in Vietnam					257 (97.7%)
1	Cd26 G>A	89	3	53	145 (55.1%)
2	Cd 41/42 TTCT	29	3	9	41 (15.6%)
3	Cd 17 A>T	32	-	6	38 (14.4%)
4	IVS 2.654 C>T	11	-	-	11 (4.2%)
5	Cd 71/72 +A	6	-	-	6 (2.3%)
6	IVS 1.1 G>T	5	-	1	6 (2.3%)
7	Cd 95+A	4	-	1	5 (1.9%)
8	-28 A>G	4	-	1	5 (1.9%)
Uncommon mutation types in Vietnam					6 (2.3%)
9	Cd 15 G>A	5	-	-	5 (1.9%)
10	Cd 26 G>T	1	-	-	1 (0.4%)
Total alleles (%)		186 (70.7%)	6 (2.3%)	71 (27%)	263 (100%)

10 gene mutation types are identified in 187 patients, 8 of them are common in Vietnam (97.7%) and 2 rare types are only found in Kinh (2.3%). Cd 26 G>A has a high rate (55.1%), other way, Cd 95+A, -28 A>G, Cd 15 G>A have the same low rate (1.9%). Kinh has full 10 types with 186 alleles are approximately triple Khmer with 71 alleles, Hoa only has 2 types Cd 26 G>A and Cd 41/42 TTCT (2.3%).

3.3. The prevalence of disease types in β-Thal patients

Table 5. The prevalence of disease types was identified based on Hb electrophoresis results combined with β-globin gene mutation types

No	Disease types/Ethnic	Kinh	Hoa	Khmer	Total (%)
1	β-Thal homozygous	0	0	1	1 (0.5%)
2	β-Thal compound heterozygous	9	0	1	10 (5.4%)
3	β-Thal heterozygous	32	2	4	38 (20.3%)
4	β-Thal/HbE compound heterozygotes	47	1	10	58 (31%)
5	HbE homozygotes (HbEE)	3	0	4	7 (3.8%)
6	HbE heterozygotes (HbAE)	36	2	37	73 (39%)
Total	6 types	127 (67.9%)	5 (2.7%)	55 (29.4%)	187 (100%)

Base on Hb electrophoresis results combined with β-globin gene mutation types, 6 disease types are identified with different rates, the highest is HbE heterozygous (39%), followed by β-Thal/HbE compound heterozygotes (31%) and the lowest is β-Thal homozygous (0,5%). Especially, in β-Thal homozygous, only Khmer has 0.5%. In addition, Kinh has 5/6 types with 67.9% is double Khmer with 29.4%, Hoa only has 3 types with 2.7%.

IV. DISCUSSION

4.1. General characteristics of the study population

In 187 β-Thal patients, 57.2% belonged to the group ≤ 15 years **(Table 3)**, which is explained by the fact that severe β-Thal is often detected in the first years of life, which also leads to a high rate of patients from the Department of Pediatrics (56.7%). According to the medical literature, β-Thal is an autosomal recessive genetic disease, so both men and women are at risk for the disease, and in this study, the ratio is 0.87 male: 1 female. Most of the study population are Kinh (67.9%), the lowest is Hoa (2.7%), similar to other study participants, among 410 participants of study of Nguyen, V.H. *et al* (2013) [2], there are 86.8% Kinh ethnicity, 13.2% are from the Ta-Oi and Co-Tu ethnic groups. A study by Nguyen, H.N. (2019) conduct the same on 59 males, 45 females; 71 are Kinh, 33 ethnic minorities (12 Thai, 10 Tay, 5 other ethnic groups including Muong, San Diu, Dao, Bo Y) [4].

4.2. The prevalence of β-globin gene mutation types in β-Thal patients

10 β-globin gene mutation types are identified in 187 patients, except for Cd26 G>A causing HbE with a high percentage, the two most common mutations are Cd 41/42 TTCT and Cd 17 A>T (**Table 4**). This result is similar to Pham, T.N.N. (2017), Cd 26 G>A (33.8%), Cd 41/42 TTCT (23.3%), and Cd 17 A>T (21%) [5]. A study by Pham, T.L. (2014) also found 8 types of mutations, Cd 41/42 TTCT (30.4%) and Cd 17 A>T (21.4%) still accounted for the highest proportion [3]. In addition, the study of Nguyen, H.N also gave similar results when studied on 104 children (2019) [4]. Thus, the results of this study once again confirm that in Vietnam, the mutations found in β-Thal patients mostly fall into 8

common categories similar to many previous studies by many authors and the rate of each mutation type is different and no new mutations have been found.

Table 6. The prevalence of 8 β-globin gene mutation types popular in Viet Nam of this study when compared with other studies

β-globin gene mutations types	**The prevalence (%)**			
	Pham, T.L. (2014) [3] n = 28	*Pham, T.N.N. (2017) [5] n = 341*	*Nguyen, H.N. (2019) [4] n= 104*	*This study n=187*
Cd 26 G>A	17.8	33.8	23.5	55.1
Cd 41/42 TTCT	30.4	23.3	30.3	15.6
Cd 17 A>T	21.4	21	30	14.4
IVS 2.654 C>T	1.8	4.2	2.9	4.2
Cd 71/72 +A	8.9	4.6	4.8	2.3
IVS 1.1 G>T	8.9	3.6	1	2.3
Cd 95+A	1.8	2.1	1	1.9
-28 A>G	5.4	4.6	2.9	1.9
Others	3.6	2.8	3.6	2.3

There are 2 rare β-globin gene mutation types found and published by several domestic or foreign studies. Cd26 G>T has a very low rate (0.4%) in a female patient of Kinh ethnicity who could combine HbE/β-Thal. In 2010, when searching for mutations for 498 pregnant women and their husbands at Tu Du hospital, Nguyen, K.H.H. also found this mutation with 0.1% [1]. A study by Maria G. Doro also found of 6.3% this mutation in 22 patients with severe transfusion-dependent β-Thal [10]. Currently, according to the Ithanet database, Cd 26 G>T was first found in a family living in Northern Thailand, it also appeared in the Thai community with 0.12% [8].

There are 1.9% Cd15 G>A recorded in the study, appearing in Kinh patients, not recorded in other ethnicities. In Vietnam, not many studies have found this mutation, Pham, T.N.N. (2017) found 0.8% in one Kinh patient out of 341 patients [5]. In the world, this mutation is also recorded but with a very low rate in some countries such as Japan 0.95%, Portugal 11.79%, and Russia 6.45%, typically in the study of Reza, A. et al (2019) in Hamadan Province (Iran) found 0.35% of this mutation in only one β-Thal patient [11]. The results of this study show that Cd15 G>A detected in the Mekong Delta is a rare mutation, not a common mutation in Southeast Asia. Therefore, the use of mutation detection techniques common in Southeast Asia will miss this mutation.

4.3. The prevalence of disease types in β-Thal patients

In this study, diseases are classified based on Hb electrophoresis results and identified gene mutation types. There are 6 types of β-Thal disease, which are combined from 10 β-globin gene mutation types, including 0.5% β-Thal homozygous, 20.3% β-Thal heterozygous, 5.4% β-Thal compound heterozygous, 31% β-Thal/HbE, 3.8% HbEE, and 39% HbAE (**Table 5**). This result of study different with Pham, T.L. (2014), in 28 β-Thal patients: 19.2% HbE/β-Thal, 13.4% β-Thal compound heterozygous, 9.6% β-Thal homozygous, 31.7% β-Thal heterozygous, the remaining patients have not identified the mutation types, so the disease form is unknown [3]. Compared with other countries in the region, the HbE/β-Thal rate of the study was lower, according to Boonyawat, B., (2014) in Thai children up to 71.2% β-Thal/HbE compound heterozygotes, 18.8% β-Thal heterozygous, 3.1% β-Thal homozygous and 6.5% β-Thal compound heterozygous [6].

Another study in Malaysia (2013) by Hassan, S., on 208 β-Thal patients: 81.2% β-Thal heterozygous, 7.7% β-Thal homozygous, 5.3% β-Thal compound heterozygous, 5.7% β-Thal/HbE compound heterozygotes [9].

Almost all studies in Southeast Asia have always shown a high prevalence of HbE gene mutation types (Cd26 G>A), so in these countries, HbE/β-Thal is always the most common disease type. According to the medical literature, β-Thal is a recessive disease, but not all homozygotes will cause severe anemia in β-Thal patients. Level of anemia of a patient dependent on the nature of the mutations constituting that genotype. So finding genetic mutations, or identifying β-Thal disease types can help know the level of anemia of the patient so that doctors have appropriate and effective treatments. In prenatal diagnosis, it can help limit the birth of severe β-Thal children.

The prevalence of disease types by ethnicity is different. In Kinh ethnic group, β-Thal/HbE compound heterozygotes accounted for the most 37%, this result is different with Nguyen, V.H. (2013), in 30 Kinh thalassemia patients, there were 16.7% β-Thal heterozygous, 16.7% HbAE, and 0.03% HbEE [2]. In the Khmer ethnic group, HbAE accounted for the most with 67.3%, this result is similar to the study of Bunthupanich, R., et al (2020), performed in a total of 545 blood specimens of Laos, Khmers, Suay, or Yer ethnic group. In 145 Khmer blood specimens in Lower Northeastern Thailand, HbAE has 22.07% and HbEE has 6.21% [7]. The difference in the rate of disease types by the ethnic group has reflected the specificity of disease types of this ethnic, therefore the treatments in each ethnic group also different.

V. CONCLUSIONS

Identifying the prevalence of gene mutation types and disease types can help know the level of anemia of the patient so that doctors have appropriate and effective treatments. Therefore, the research indicated that β-Thal testing for anemic patients is very necessary. Besides, screening of prenatal carriers, genetic counseling, and diagnosis is recommended in many married couples to prevent β-Thal homozygous or HbE/β-Thal compound heterozygotes appearing in the next generations. Otherway, the prevalence of different disease types proves that β-Thal treatments are different among ethnic groups. That contributes to improving the quality of the population in Kien Giang province in particular, the whole of Vietnam in general. In addition, the data obtained from this study can contribute to the global data about β-Thal.

REFERENCES

1. Nguyen KHH, Nguyen TB, Nguyen THN, Quach THO (2010), "Research on applying molecular biology techniques to screening and prenatal diagnosis of thalassemia genetic disease", *Science and technology projects*, Ho Chi Minh city.
2. Nguyen VH, Sanchaisuriya K, Nguyen D, Phan TTH, Siridamrongvattana K, Sanchaisuriya P, Fucharoen S, Fucharoen G, Schelp FP (2013), "Thalassemia and Hemoglobinopathies in Thua Thien Hue Province, Central Vietnam", *Hemoglobin*, Vol. 37(4), pp. 333-342.
3. Pham TL (2014), *Detection of mutations in the gene causing β-thalassemia by Multiplex ARMS-PCR*, Master's thesis, Ha Noi Medical University.
4. Nguyen HN (2019), *Phenotype and genotype study in Beta-thalassemia patients*, Ph.D. thesis, Ha Noi Medical University.
5. Pham TNN (2017), *Study on the inheritance of disease-causing mutations in Beta-thalassemia patients in Mekong Delta region by molecular biology technique*, Ph.D. thesis, Can Tho University.

6. Boonyawat B, Monsereenusorn C, Traivaree C (2014), "Molecular analysis of beta-globin gene mutations among Thai beta-thalassemia children: results from a single-center study", *Appl Clin Genet*, 7, pp. 253-8.
7. Bunthupanich R, Karnpean, R, Pinyachat A, Jiambunsri N, Prakobkaew N, Pakdee N, Fucharoen S, Fucharoen S (2020), "Micromapping of Thalassemia and Hemoglobinopathies Among Laos, Khmer, Suay and Yer Ethnic Groups Residing in Lower Northeastern Thailand", *Hemoglobin*, 44(3), pp. 162-167.
8. Fucharoen S, Winichagoon P (1997), "Hemoglobinopathies in Southeast Asia: Molecular Biology and Clinical Medicine", *Hemoglobin*, 21, pp. 299-319.
9. Hassan S, Ahmad R, Zakaria Z *et al.* (2013), "Detection of beta-globin Gene Mutations Among β-thalassemia Carriers and Patients in Malaysia: Application of Multiplex Amplification Refractory Mutation System-Polymerase Chain Reaction", *Malays J Med Sci*, 20, pp. 13-20.
10. Maria GD, Giuseppina C, Laura F, Ivana P, Le PMT, Phan TTH, Nguyen HH, Monica P, Paolo M, Francesco C, Bruno M (2017), "Molecular Characterization of β-Thalassemia Mutations in Central Vietnam", *Hemoglobin*, 41(2), pp. 96-99.
11. Reza A, Keivan M, Mozaffar A, Azam A, Samane S, Mostafa B (2019), "The Spectrum of β-Thalassemia Mutations in Hamadan Province, West Iran", *Hemoglobin*, 43(1), pp.18–22.

(Received: 14/11/2019 – Accepted:6/8/2021)