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Genotype and Phenotype in Pregnant Women with Both Alpha and Beta Thalassemia Mutations Attended at Vietnam National Hospital of Obstetrics and Gynecology

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ABSTRACT

Introduction: The number of studies that explore people with both alpha and beta thalassemia mutations is still low, not only in Vietnam but around the world as well. We conducted this study to explore genotypes and phenotypes of pregnant women who have both alpha and beta thalassemia mutations in comparison to those who don't have any thalassemia mutations and those who have either alpha or beta thalassemia mutations.

Methods: This was a cross-sectional study conducted in the National Hospital of Obstetrics and Gynecology from September 2020 to July 2021 on medical records of 6929 pregnant women who attended the hospital between 2015 and 2021. Mutation analysis was performed by the α-Globin Strip Assay (Vienna Lab, Austria) and Strip Assay MED (Vienna Lab, Austria).

Results: There were 18 pregnant women with both alpha and beta thalassemia genes, in which only SEA mutation caused alpha thalassemia and nearly 70% of beta thalassemia was caused by CD26 mutation. In comparison with pregnant women who didn't have any thalassemia genes, those with both mutations had higher RBC, RDW, HbA2 and lower HGB, HCT, MCV, MCH, MCHC, and HbA1. We only see the statistically significant difference in HbA1 and HbA2 between those with both mutations and those with either alpha or beta thalassemia mutations.

Conclusion: In conclusion, our study showed that there were differences in genotype and phenotype between pregnant women with both alpha and beta thalassemia mutations and other types of thalassemia as well as those without thalassemia.

Keywords: *Alpha thalassemia, Beta thalassemia, Genotype, Phenotype, Pregnant women*

INTRODUCTION

Thalassemia is a group of autosomal recessive hematologic disorders that cause hemolytic anemia due to decreased synthesis or complete loss of the globin chain.[1] In Vietnam, thalassemia is distributed across provinces and ethnic groups throughout the country, especially ethnic minority areas in mountainous provinces.[2] Manifestations of thalassemia vary widely, from no clinical symptoms to symptoms of transfusion-dependent anemia and complications of iron infection. Currently, the main treatment for transfusion-dependent forms is still blood transfusion and iron chelation.[3]

In Vietnam, the percentage of people carrying the beta thalassemia gene in the Kinh group is 1.2%[4]. A systematic review conducted in 2020 showed that the rate of people with thalassemia in some ethnic minorities in Vietnam is 51.5%, nearly twice higher than the average rate of thalassemia in Southeast Asia.[5] Southeast Asia is also the region with the highest rate of thalassemia carriers in the world, with the highest rate of carriers at 12.8% in Malaysia.[6] Regarding the age group with thalassemia, according to a study conducted at the Vietnam National Institute of Hematology and Blood Transfusion in 2011 on 1500 patients, there were 16.6% of children under 6 years old, 27.4% of patients from 6 up to 15 years old, 27.7% of patients aged 16 to 30 years old and 28.8% of people over 30 years old.[4]

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regular blood transfusions to maintain their body's vital activities. Patients who do not receive blood transfusions can develop jaundice and iron overload in the body, and children with severe thalassemia condition often have mental and physical retardation, and delayed puberty. If left untreated, the end result is death.[7] In the world as well as in Vietnam, there has been much research on alpha and beta thalassemia. However, research on people carrying both alpha and beta thalassemia genes at the same time in Vietnam is still not enough to provide convincing evidence about these cases. Therefore, we conducted this study to investigate the genotype and phenotype of pregnant women carrying both alpha and beta thalassemia genes in northern Vietnam.

MATERIALS AND METHODS

This is a cross-sectional study conducted from September 2020 to July 2021 at the Center for Prenatal Diagnosis at the Vietnam National Hospital of Obstetrics and Gynecology. The Vietnam National Hospital of Obstetrics and Gynecology is the central hospital of northern Vietnam, where patients from various provinces with severe conditions related to pregnancy came for examination. All pregnant women were screened for thalassemia at the hospital through complete blood count test. If they have normal MCV and MCH value, no further assessments are needed. When the women had a reduced value in either indices, both them and their partner will be given a diagnosis test, usually molecular diagnosis. In the Vietnam National Hospital of Obstetrics and Gynecology, the initial cut-off value for MCV is 80 and 28 for MCH.

We performed convenient sampling method with all pregnant women who came for pregnancy examination and had their medical records kept at the Center from 2015 to 2021. We excluded those who (1) didn't have thalassemia mutation test results and those who (2) had any diseases other than thalassemia. Blood cell count test results, serum iron, serum ferritin test results, hemoglobin analysis test results were collected as phenotypes and thalassemia mutation test results were collected as genotypes. In the end, we collected information of 6929 pregnant women.

Blood cell count indices were calculated using an automated blood cell counter (Sysmex XN-330, Japan). Serum iron, serum ferritin level was determined using automated photometric assay (Roche Cobas C501, Switzerland). Hemoglobin analysis test was performed using capillary electrophoresis method (Sebia Minicap Flex Piercing, France).

We collected peripheral blood samples of pregnant women and then extracted DNA. Thalassemia mutations analysis was performed by the reverse hybridization method: α-Globin Strip Assay (Vienna Lab, Austria) and Strip Assay MED (Vienna Lab, Austria). α-Globin Strip Assay uses two sereprate test strip (A/B) per sample to analyze simultaneously 21 common mutations of alpha thalassemia (-α3.7, -α4.2, -(α)20.5, --MED, --SEA, --THAI. – FIL, α1 cd14, α1 cd59, ααα anti-3.7, α2 initiation cd, α2 cd19, α2 IVS1, α2 cd59, α2 cd125, α2 cd142 T>C, α2 cd142 T>A, α2 cd142 A>T, α2 cd142 A>C, α2 polyA-1, α2 polyA-2). Strip Assay MED can also detect 22 β thalassemia mutations including: -31 [A>G], -29 [A>G], -28 [A>G], Cap+1 [A>C], Initiation cd [ATG>AGG], cd 8/9 [+G], cd 15 [TGG>TAG], cd 17 [A>T], cd 19 [A>G] Malay, cd 26 [G>A] HbE, cd 27/28 [+C], IVS 1.1 [G>T], IVS 1.5 [G>C], cd 41/42 [-TTCT], cd 43 [G>T], cd 71/72 [+A], cd 89/90 [-GT], cd 90 [G>T], cd 95 [+A], IVS 2.1 [G>A], IVS 2.654 [C>T], and cd 121 [G>T].

This study was approved by the Institutional Review Board at the Vietnam National Hospital of Obstetrics and Gynecology.

All data were analyzed using STATA 15 software. Qualitative data are presented as numbers and percentages, and quantitative data were presented as median and inter-quantile range. Statistical tests were performed including ANOVA, Kruskal-Wallis and Man-Whiney U-test. P-value < 0.05 is considered statistically significant.

RESULTS

1. GENOTYPES OF PARTICIPANTS

Table 1: Thalassemia carriers among participants

	n	%
Non-thalassemia carriers	6359	91.77
Alpha thalassemia carriers	447	6.45
Beta thalassemia carriers	105	1.52
Both alpha and beta thalassemia genes carriers	18	0.26
Total	6929	100

Table 1 showed that among 6929 pregnant women, 91.77% were non-thalassemia carriers, 6.45% were alpha thalassemia carriers, 1.52% were beta thalassemia carriers and the lowest was pregnant women carrying both alpha and beta thalassemia genes with a rate of 0.26% (Table 1).

Table 2: Alpha and beta thalassemia mutations in those who carry both alpha and beta thalassemia genes

		Alpha thalassemia mutation^{-SEA}	
		n = 18	%
Beta thalassemia mutation	CD17	3	13.64
	CD26	11	68.18
	CD41/42	2	9.09
	CD71/72	1	4.55
	IVS 1.1	1	4.55

Table 2 showed that all 18 pregnant women carrying both alpha and beta thalassemia genes had alpha thalassemia mutation SEA associated with 5 beta thalassemia mutations, of which one with the highest proportion is CD26 mutation with 11 carriers and the lowest CD71/72 and IVS 1.1 mutation, each had 1 gene carrier.

2. PHENOTYPIC CHARACTERISTICS OF PARTICIPANTS

Table 3: Phenotypic characteristics of different mutations in those who carry both alpha and beta thalassemia genes

	CD17 (n = 3) (median and IQR)	CD26 (n = 11)(median and IQR)	CD41/42 (n = 2) (median and IQR)	CD71/72 (β0) (median and IQR)	IVS 1.1 (β0) (median and IQR)	p -value
RBC (G/l)	4.64 (4.63 – 6.59)	5.06 (4.86 – 5.25)	4.78 (4.76 – 4.8)	4.93	4.45	0.6
HGB (g/l)	108 (103 -113)	109 (96 – 113)	108.5 (108 – 109)	116	105	0.67
HCT (l/l)	0.34 (0.29 – 0.37)	0.33 (0.29 – 0.35)	0.342 (0.341 – 0.343)	0.343	0.308	0.95
MCV (fl)	65.9 (61.9 – 73.2)	65.9 (61.4 – 71.9)	71.6 (71.5 – 71.7)	69.6	69.2	0.95
MCH (pg)	22.2 (20 – 23.2)	21.8 (18.9 – 22.4)	22.75 (22.6 – 22.9)	23.5	23.6	0.08
MCHC (g/l)	317 (302 – 359)	314 (309 – 333)	317.5 (316 – 319)	338	341	0.43
RDW (%)	16.3 (14.5 – 17.7)	14.65 (13.3 – 15.8)	15 (14.8 – 15.2)	14.8	14.8	0.9
fe (μmol/l)	10.5 (5.96 – 18.2)	15.6 (12.39 – 21.63)	19.46 (15.1 – 23.82)	29.98	16.6	0.38
Ferritin (μg/l)	16.54 (7.26 – 99.24)	57.08 (31.3 – 145)	77.96 (74.19 – 81.73)	133	53.78	0.54

The phenotypic characteristics of thalassemia mutation in those who carry both alpha and beta thalassemia genes are shown in Table 3. IVS mutation had the lowest median value in RBC, HGB and HCT indices and the highest median value in MCH and MCHC indices while CD71/72 mutation had the highest me dian value in Fe and Ferritin serum indices but these two mutations had only 1 patient each. Among CD17, CD26, CD41/42 mutations, CD41/42 mutation had the highest Fe and Ferritin serum, these two indices were the lowest in CD17 mutation. All these differences were not statistically significant.

Table 4: Phenotypic characteristics in non-thalassemia carriers and those who carry both alpha and beta thalassemia genes

	Non-thalassemia carriers (n = 6,359)(median and IQR)	Both alpha and beta thalassemia genes carriers (n = 18) (median and IQR)	p-value
RBC (G/l)	4.12 (3.87 - 4.43)	4.92 (4.64 - 5.09)	< 0.001
HGB (g/l)	120 (112 – 126)	108.5 (102 – 113)	< 0.001
HCT (l/l)	0.355 (0.337 - 0.374)	0.34 (0.306 - 0.353)	< 0.01
MCV (fl)	87.9 (83.1 - 90.9)	69.2 (63.6 - 71.9)	< 0.001
MCH (pg)	29.8 (27.8 - 30.9)	22.25 (20.5 - 22.6)	< 0.001
MCHC (g/l)	337 (329 – 344)	318 (310 – 335)	< 0.001
RDW (%)	13.3 (12.7 – 14)	14.8 (13.8 - 15.8)	< 0.001
Fe (µmol/l)	16.6 (12.9 - 20.3)	16.6 (12.39 - 21.63)	0.79
Ferrtin (µg/l)	61.2 (32.6 – 109)	58.5 (31.3 - 99.24)	0.95
HbA1 (%)	97.5 (96.1 - 97.8)	84.6 (79.45 - 94.1)	< 0.001
HbA2 (%)	2.4 (2.2 – 2.8)	4.5 (3.6 – 5.4)	< 0.01
HbE (%)	24.15 (16.5 - 25.5)	15.6 (13.4 - 17.3)	0.02
HbF (%)	0.7 (0.5 - 1.3)	0.5 (0.4 – 0.6)	0.21

Table 4 compares the phenotypic characteristics of pregnant women without the thalassemia gene and those carrying both alpha and beta thalassemia genes. In pregnant women carrying the gene for both diseases, RBC, RDW, and HbA2 were higher than in non-carriers, while HGB, HCT, MCV, MCH, MCHC, HbA1, and HBE were all lower, these differences were all statistically significant with $p < 0.001$ and 0.02 (Mann-Whitney U-test).

Table 5: Phenotypic characteristics in alpha thalassemia carriers and those who carry both alpha and beta thalassemia genes

	Alpha thalassemia carriers (n = 447) (median and IQR)	Both alpha and beta thalassemia genes carriers (n = 18) (median and IQR	p-value
RBC (G/l)	4.92 (4.65 – 5.25)	4.92 (4.64 – 5.09)	0.97
HGB (g/l)	106 (100 – 112)	108.5 (102 – 113)	0.73
HCT (l/l)	0.33 (0.31 – 0.35)	0.3 (0.3 – 0.35)	0.82
MCV (fl)	67.2 (64.7 – 70.1)	69.2 (63.6 – 71.9)	0.8
MCH (pg)	21.3 (20.7 – 22.3)	22.25 (20.5 – 22.6)	0.25
MCHC (g/l)	319 (312 – 327)	318 (310 – 335)	0.73
RDW (%)	15.7 (14.8 – 16.6)	14.8 (13.8 – 15.8)	0.12
Fe (µmol/l)	16.1 (12.66 – 20)	16.6 (12.39 – 21.63)	0.96
Ferrtin (µg/l)	52.89 (28.56 – 98.62)	58.5 (31.3 – 99.24)	0.52
HbA1 (%)	97.7 (97.4 – 97.9)	84.6 (79.45 – 94.1)	< 0.001
HbA2 (%)	2.3 (2.1 – 2.4)	4.5 (3.6 – 5.4)	< 0.001
HbE (%)	15.8 (15.2 – 19.4)	15.6 (13.4 – 17.3)	0.7
HbF (%)	0.75 (0.4 – 1.4)	0.5 (0.4 – 0.6)	0.3

Meanwhile, when comparing the group of pregnant women carrying the alpha thalassemia gene with the group of interest, we found that the hemoglobin analysis results were different, the group of pregnant women carrying alpha thalassemia gene had higher HbA1 and lower HbA2. These differences were statistically significant with $p < 0.001$ (Mann-Whitney U-test) (Table 5).

Table 6: Phenotypic characteristics in beta thalassemia carriers and those who carry both alpha and beta thalassemia genes

	Beta thalassemia carriers (n = 105) (median and IQR)	Both alpha and beta thalassemia genes carriers (n = 18) (median and IQR)	p-value
RBC (G/l)	4.67 (4.42 – 5.08)	4.92 (4.64 – 5.09)	0.09
HGB (g/l)	102 (95.5 – 109.5)	108.5 (102 – 113)	0.15
HCT (l/l)	0.316 (0.29 – 0.34)	0.34 (0.306 – 0.353)	0.82
MCV (fl)	64.75 (61.8 – 73.8)	69.2 (63.6 – 71.9)	0.8
MCH (pg)	21.1 (19.9 – 23.4)	22.25 (20.5 – 22.6)	0.25
MCHC (g/l)	326.5 (317 – 336)	318 (310 – 335)	0.73
RDW (%)	15.8 (14.4 – 17.2)	14.8 (13.8 – 15.8)	0.12
Fe (µmol/l)	19.3 (15.19 – 22.2)	16.6 (12.39 – 21.63)	0.96
Ferrtin (µg/l)	93.39 (57.82 – 147.68)	58.5 (31.3 – 99.24)	0.52
HbA1 (%)	93.5 (77 – 94.6)	84.6 (79.45 – 94.1)	< 0.001
HbA2 (%)	5.1 (3.9 – 5.7)	4.5 (3.6 – 5.4)	< 0.001
HbE (%)	24.5 (23 – 26.4)	15.6 (13.4 – 17.3)	0.7
HbF (%)	1.3 (1.05 – 2.25)	0.5 (0.4 – 0.6)	0.3

Finally, when comparing the group of pregnant women carrying the beta thalassemia gene and the group of pregnant women of interest, we found that there was a difference in both HbA1 and HbA2. Which, pregnant women carrying both alpha and beta thalassemia genes have these indexes lower than the group of pregnant women carrying only beta thalassemia gene. The differences were statistically significant with $p < 0.001$ (Table 6).

DISCUSSION

Thalassemia is a major public health problem among pregnant women in Vietnam. Prenatal screening is an effective tool to prevent birth of children with this condition. However, Vietnam haven't deployed a universal screening program due to its high cost and shortage of skilled specialists. Therefore, pre-marital counseling, hospital based screening, and preventive methods during pregnancy are being implemented. Although the Ministry of Health of Vietnam has issued guidelines and many major hospitals also have their own flowcharts for the thalassemia prenatal screening process, thalassemia has still appeared with high prevalence all over in Vietnam. One of the reasons come from its variety of genotypes due to the combination of many different mutations and each genotype has its own characteristic. Having sufficient knowledge of phenotype of patients with both alpha and beta thalassemia mutation will help not only in prediction and counseling but also detect more thalassemia carriers in prenatal screening. Among 6929 pregnant women included in the study, 6.45% carried the alpha thalassemia gene, this rate is lower than previous studies because they focused on the prevalence of people carrying the alpha thalassemia gene in ethnic minority groups.[8–11] However, this rate is higher than the study conducted on 410 pregnant women who visited health facilities in Thua Thien Hue province conducted in 2011 to 2012.[12]

Regarding the percentage of beta thalassemia carriers, our study also showed similarities with previous studies of 1.2% to 1.6%. Among pregnant women carrying both alpha and beta thalassemia genes, the only mutation occurring in the HbA gene was the --SEA mutation, while the CD26 mutation occurred most frequently in the HbB gene. Genotypic differences lead to phenotypic differences. Pregnant women carrying the IVS mutation had the lowest median value in RBC, HGB and HCT indices and the highest median value in MCH and MCHC indices while CD71/72 mutation had the highest median value in Fe and Ferritin serum indices. Among CD17, CD26, CD41/42 mutations, CD41/42 mutation had the highest Fe and Ferritin serum, these two indices were the lowest in CD17 mutation. Therefore, women who carry these mutations should have routine prenatal screening for these indices to prevent the birth of children with this mutation.

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Our results showed that the group of pregnant women carrying both alpha and beta thalassemia mutations has a much higher red blood cell count than the group of pregnant women without thalassemia genes, but the quality of red blood cells decreases when the HGB, MCV, MCH, MCHC values all decrease below the normal threshold, which is consistent with the cumulative effects of both alpha and beta thalassemia mutations. Despite this, the serum iron and ferritin levels in the group of patients with both alpha and beta thalassemia mutations were unchanged, and less than in the non-carriers, however, this difference is not statistically significant. In addition, the amount of HbA in this group of people was lower than the normal group based on the HbA1 index, while the HbA2 index increased significantly, indicating the presence of beta thalassemia.

When comparing the group of pregnant women, we were interested in with two groups of women independently harboring either alpha or beta thalassemia mutations, we did not see any statistically significant difference other than HbA1 and HbA2. This indicates a similar degree of anemia between the combination of the two alpha and beta thalassemia mutants compared with carrying the alpha and beta thalassemia mutations separately. The difference in HbA1 and HbA2 values that HbA1 was higher, HbA2 was lower in the group carrying alpha thalassemia gene alone and vice versa in the group carrying only beta thalassemia gene showed that the compensatory effects of both these mutations lead to intermediate results of the two indicators above instead of a simultaneous increase. Thus, the detection of co-existing alpha and beta thalassemia mutations in a single person can help predict less severe clinical outcome.

Our study also has certain limitations. Firstly, the study was conducted on a population of pregnant women rather than an entire group of women or a combination of men and women. Secondly, this study was conducted in the North of Vietnam, so these groups of participants are not representative of the entire population. Thirdly, due to the number of people carrying both alpha and beta thalassemia genes being very low in the population, our sample may not fully reflect the characteristics of this group of people. We, therefore, recommend further studies with a larger sample size in both genders to reach the clearest conclusions.

CONCLUSION

Our study showed that there were differences in genotype and phenotype between pregnant women with both alpha and beta thalassemia mutations and other types of thalassemia as well as those without thalassemia.

Research Ethics: The study was approved by the Ethics Committee of the National Hospital of Obstetrics and Gynecology and Hanoi Medical University.

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