Alpha-thalassemia genotypes in Vietnam: a report of 12,030 pregnant women and their husbands performing prenatal screening for alpha-thalassemia

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pISSN: 0853-1773 • eISSN: 2252-8083
https://doi.org/10.13181/mji.oa.236635
Med J Indones. 2023;32:13–8

Received: December 11, 2022
Accepted: April 12, 2023
Published online: July 12, 2023

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ABSTRACT

BACKGROUND Alpha (α)-thalassemia is a global health concern, and improving screening methods is crucial for disease prevention. This study aimed to assess α-thalassemia genotypes and evaluate the effectiveness of various thresholds for mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in prenatal screening for α-thalassemia.

METHODS This cross-sectional study included pregnant women and their husbands who underwent prenatal screening for thalassemia at the National Hospital of Obstetrics and Gynecology, Vietnam from January 2012 to August 2021. Blood samples were collected and analyzed using the strip assay technique, which can detect 21 common mutations in the α-globin gene and 22 common mutations in the beta-globin gene.

RESULTS Of the 12,030 participants, 931 were identified as having α-thalassemia, with $--^{SEA}$, $-\alpha^{3.7}$, and $-\alpha^{4.2}$ being the most common mutations. When examining different thresholds of MCV and MCH, MCV <85 fL and MCH <28 pg had a lower missing rate than MCV <80 fL and MCH <27 pg, respectively. MCH <28 pg showed the highest sensitivity in screening for α-thalassemia. MCV <85 fL showed the lowest positive predictive value (PPV). The combination of MCV <80 fL and MCH <27 pg showed the lowest sensitivity in screening for α-thalassemia but the highest PPV among all thresholds.

CONCLUSIONS Optimizing the screening methods for α-thalassemia is important for preventing and managing the disease in the community. These findings have important implications for thalassemia prevention and management programs and may contribute to reducing the burden of thalassemia in the global population.

KEYWORDS alpha-thalassemia, hemoglobin, mutation, prenatal diagnosis

Thalassemia refers to a hereditary group of blood disorders or hemoglobinopathies that cause anemia and hemolysis. According to the 2013 Thalassemia International Federation (TIF) report, approximately 7% of the world's population carries the hemoglobin (Hb) disease gene, with over 200 countries and territories affected by the disease, including Vietnam, where an estimated 5–7% of the population are carriers of thalassemia gene mutations.[1]

There are two primary types of thalassemia: alpha (α)- and beta (β)-thalassemia. α-thalassemia is caused by mutations in the α-globin genes, resulting in a deficiency in the production of the α-globin chain, with a carrier frequency of approximately 20% of the population worldwide. The phenotypes of α-thalassemia vary from asymptomatic to severe, depending on the number of α-globin gene deletions/inactivations. Among these, hemoglobin H (HbH)

and hemoglobin Bart's (Hb Bart's) hydrops fetalis syndrome are two clinically significant forms. Hb Bart's, the most severe form of α-thalassemia, is caused by deletion/inactivation of all four α-globin genes (--/--), and fetuses with Hb Bart's usually die in pregnancy or shortly after birth.[2] Meanwhile, HbH is caused by the deletion/inactivation of three α-globin genes (--/-α), with patients typically producing less than 30% of the normal α-globin chains.[3] Severe HbH patients have hepatosplenomegaly, mild jaundice, and sometimes thalassemia-like bone changes and require a blood transfusion.[4] β-thalassemia is caused by mutations in the β-globin genes, resulting in a deficiency in β-globin chains. There are three phenotypes of β-thalassemia: β-thalassemia trait, β-thalassemia intermediate, and β-thalassemia major, which are distinguished by recurrent or regular blood transfusions.

Regular blood transfusion and continual iron chelation are the leading therapeutic strategies for transfusion-dependent thalassemia treatment; however, the accumulation of excess iron can adversely affect organ functions and cause morbidity and mortality. This treatment also imposes a significant economic burden, with treatment costs ranging from 629 to 2,300 USD per patient depending on age groups, and the lifetime healthcare costs reaching approximately USD 606,665.[5,6]

Prenatal screening for thalassemia based on complete blood count (CBC) and serum iron and ferritin levels is the most effective and common method to prevent severe forms of thalassemia. Screening aims to identify asymptomatic individuals whose offspring are at risk of thalassemia and provide appropriate counseling for couples to reduce the number of children born with severe thalassemia. A good understanding of genotype characteristics and indices in the prenatal diagnosis of thalassemia is essential for the precise and accurate analysis of laboratory results and for providing proper medical indications and advice to patients, thereby minimizing the birth of infants with the fatal Hb Bart's hydrops fetalis syndrome.

The mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are common thalassemia screening parameters used in Vietnam, as recommended by TIF. The Vietnamese Ministry of Health raised the MCV threshold to 85 fL in 2020 to increase the sensitivity of thalassemia screening and expand the screening population. This study aimed to evaluate the effectiveness of the new MCV threshold for thalassemia screening in Vietnam, along with the potential benefits and challenges of this policy change.

METHODS

Subjects and sampling

This descriptive cross-sectional study used convenience sampling technique in pregnant women and their husbands undergoing thalassemia screening at the National Hospital of Obstetrics and Gynecology, Vietnam, from January 2012 to August 2021. This hospital is the central hospital of northern Vietnam, where pregnant women and their husbands from various provinces with pregnancy-related conditions are examined. This study included 12,030 pregnant women and their husbands with a family history of thalassemia who underwent CBC tests. Those with normal MCV and MCH values did not require further assessment. However, if either of these indices were reduced, both the participants and their partners underwent molecular diganostic testing. High-risk participants were defined as those with at least one of the following: (i) MCV <80 fL and/or MCH <28 pg; (ii) a history of Hb Bart's hydrops fetalis syndrome; and (iii) a history of children diagnosed with thalassemia. In total, 1,344 high-risk participants underwent thalassemia mutation testing (Figure 1). The exclusion criteria were patients with other diseases affecting CBC and Hb variant analysis results, which included iron deficiency anemia, chronic kidney failure, aplastic anemia, systemic lupus erythematosus, Crohn's disease, and other chronic diseases.

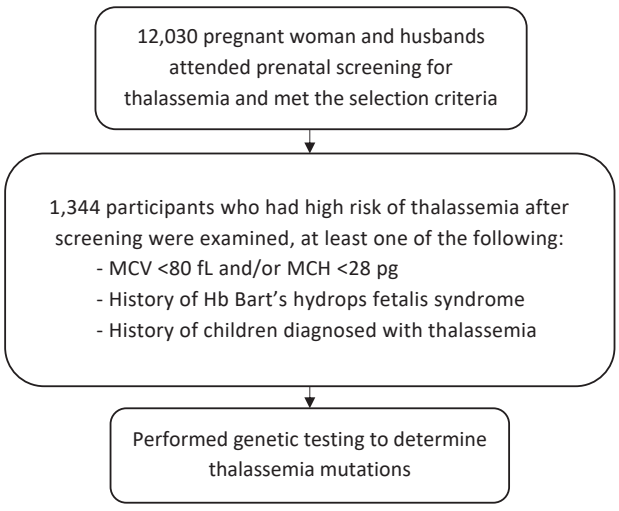

Figure 1. Participant recruitment process

Blood sample collection and analysis

To perform the CBC test, 3 ml of venous blood was collected in K2 EDTA tubes and analyzed using automated cell counters. Hb variant analysis was performed using high-performance liquid chromatography. The strip assay technique was used to detect 21 common mutations ($-\alpha^{-3.7}$, $-\alpha^{-4.2}$, $--^{MED}$, $--^{SEA}$, $--^{THAI}$, $--^{FIL}$, $-\alpha^{-20.5}$, anti $-\alpha^{-3.7}$, α^{-1} CD14 [G>A], α^{-1} CD59 [G>A], α^{-2} int CD [T>C], α^{-2} CD19 [-G], α^{-2} IVS1 [-5nt], α^{-2} CD59 [G>A], α^{-2} CD125 [CTG>CCG] Qs, α^{-2} CD142 [TAA>CAA] Cs, α^{-2} CD142 [TAA>AAA], α^{-2} CD142 [TAA>TAT], α^{-2} CD142 [TAA>TCA], α^{-2} polyA-1 [AATAAA>AATAAG], and α^{-2} polyA-2 [AATAAA>AATGAA]) in the α-globin gene and 22 common mutations ($^{-31}$ [A>G], $^{-29}$ [A>G], $^{-28}$ [A>G], Cap+1 [A>C], initiation CD [ATG>AGG], CD8/9 [+G], CD15 [TGG>TAG], CD17 [A>T], CD19 [A>G] Malay, CD26 [G>A] HbE, CD 27/28 [+C], IVS 1.1 [G>T], IVS 1.5 [G>C], CD41/42 [-TTCT], CD43 [G>T], CD71/72 [+A], CD89/90 [-GT], CD90 [G>T], CD95 [+A], IVS 2.1 [G>A], IVS 2.654 [C>T], and CD121 [G>T]) in the β-globin gene, including both heterozygous and homozygous classifications.

Ethical considerations

This study was approved by the Ethics Committee of the National Hospital of Obstetrics and Gynecology (register number 1042/CN-PSTW), and all participants provided written informed consent.

Data analysis

Data were analyzed using the SPSS software version 20 (IBM Corp., USA), and descriptive statistics were used to summarize the data.

RESULTS

Of 12,030 participants, 1,344 were at high risk of thalassemia, with 931 (7.7%) had α-thalassemia. The most frequent genotype observed was $--^{SEA}/\alpha\alpha$, accounting for 91.9% of the total patients, followed by $-\alpha^{3.7}/\alpha\alpha$ (1.7%) and $-\alpha^{4.2}/\alpha\alpha$ (1.5%). Nineteen patients had mutations in three genes, mainly $--^{SEA}$ mutation, that can cause HbH. The $--^{SEA}$ mutation was the most common mutation (91.8%), followed by $-\alpha^{3.7}$ (2.8%), $-\alpha^{4.2}$ (2.0%), and α^{CS} (1.1%) (Table 1).

Table 2 shows α-thalassemia genotypes that could be undetected if using only one of the two cut-off values of MCV: <80 fL and <85 fL. Most patients had MCV <80 fL, but some had values above, suggesting the presence of other factors affecting red blood cell size. The difference between the two MCV thresholds was clear. By using MCV <80 fL, 19 cases of α-thalassemia were missed. However, by using MCV <85 fL, only five cases were missed.

Table 1. Distribution of genotypes and mutations in α-thalassemia

	n (%)
Genotypes, n = 931	
$--^{SEA}/\alpha\alpha$	856 (91.9)
$-\alpha^{3.7}/\alpha\alpha$	16 (1.7)
$-\alpha^{4.2}/\alpha\alpha$	14 (1.5)
$--^{SEA}/-\alpha^{3.7}$	9 (1.0)
$\alpha^{CS}\alpha/\alpha\alpha$	5 (0.5)
$--^{SEA}/\alpha^{CS}\alpha$	5 (0.5)
$--^{SEA}/\alpha\alpha\alpha^{anti\text{-}3.7}$	5 (0.5)
$--^{THAI}/\alpha\alpha$	4 (0.4)
$\alpha\alpha\alpha^{anti\text{-}3.7}/\alpha\alpha$	4 (0.4)
$\alpha^{QS}\alpha/\alpha\alpha$	4 (0.4)
$--^{SEA}/-\alpha^{4.2}$	3 (0.3)
$\alpha^{init\ CD\ [T>C]}\alpha/\alpha\alpha$	2 (0.2)
$-\alpha^{3.7}/-\alpha^{4.2}$	2 (0.2)
$--^{SEA}/\alpha^{CD14\ [G>A]}\alpha$	1 (0.1)
$--^{THAI}/\alpha^{CS}\alpha$	1 (0.1)
Mutations, n = 957	
$--^{SEA}$	879 (91.8)
$-\alpha^{3.7}$	27 (2.8)
$-\alpha^{4.2}$	19 (2.0)
α^{CS}	11 (1.1)
$\alpha\alpha\alpha^{anti\text{-}3.7}$	9 (0.9)
$--^{THAI}$	5 (0.5)
α^{QS}	4 (0.4)
$\alpha^{init\ CD\ [T>C]}$	2 (0.2)
$\alpha^{CD14\ [G>A]}$	1 (0.1)

Table 3 shows the MCH values in patients with different α-thalassemia genotypes based on two different MCH thresholds (MCH <27 pg and MCH <28 pg). Most patients had MCH values <27 pg, which is consistent with the expected hypochromic phenotype in thalassemia. Nine α-thalassemia cases had an MCH ≥27 pg, and MCH ≥28 pg was seen in only three cases.

The highest number of detected α-thalassemia patients had MCH <28 pg (686/689 patients), followed by MCV <85 fL (684/689 patients). The lowest number comprised patients with a combination of MCV <80 fL and MCH <27 pg (668/689 patients). When looking at specific α-thalassemia genotypes, MCH <28 pg also detected most types of detectable mutations, and missed only three cases, including $-\alpha^{3.7}/\alpha\alpha$, $--^{SEA}/\alpha\alpha$, and $\alpha^{CS}\alpha/\alpha\alpha$. Meanwhile, the combination of MCV <80

Table 2. MCV in α-thalassemia patients

Genotypes	MCV ≥80	MCV ≥85
$-\alpha^{3.7}/\alpha\alpha$	3	1
$-\alpha^{4.2}/\alpha\alpha$	6	0
$--^{SEA}/\alpha\alpha$	5	2
$--^{THAI}/\alpha\alpha$	1	1
$\alpha\alpha\alpha^{anti\text{-}3.7}/\alpha\alpha$	2	0
$\alpha^{CS}\alpha/\alpha\alpha$	1	1
$--^{SEA}/\alpha^{CS}\alpha$	1	0
Others	0	0
Total	19	5

MCV=mean corpuscular volume

Table 3. MCH in α-thalassemia patients

Genotypes	MCH ≥28	MCH ≥27
$-\alpha^{3.7}/\alpha\alpha$	1	2
$-\alpha^{4.2}/\alpha\alpha$	0	3
$--^{SEA}/\alpha\alpha$	1	2
$--^{THAI}/\alpha\alpha$	0	0
$\alpha\alpha\alpha^{anti\text{-}3.7}/\alpha\alpha$	0	1
$\alpha^{CS}\alpha/\alpha\alpha$	1	1
Others	0	0
Total	3	9

MCH=mean corpuscular hemoglobin

fL and MCH <27 pg only detected 3/9 cases of $-\alpha^{4.2}/\alpha\alpha$ and no case of $\alpha\alpha\alpha^{anti\text{-}3.7}/\alpha\alpha$. All thresholds of MCV and/or MCH showed detection rates of α-thalassemia above 70%, of which the lowest was MCV <85 fL (71.3%), whereas the highest was with MCV <80 fL combined with MCH <27 pg (73.3%) (Table 4).

DISCUSSION

α-thalassemia is a common cause of hydrops fetalis, accounting for 60–90% of cases in Southeast Asia.[7] The geographical distribution of α-thalassemia mutations is particularly concentrated in Africa, Southeast Asia, the Mediterranean region, and Middle East, with up to 40% of carriers.[8,9] Vietnam also has a high frequency of α-thalassemia because of its geographical location. Diagnosing α-thalassemia is important in patients with unsolved hypochromic microcytic anemia. This study found a high prevalence of the α-thalassemia gene carriers (69.3%), consistent with the findings of Nguyen et al[10] in 2015. This high prevalence may be attributed to the large number of women with a history of Hb Bart's hydrops fetalis syndrome or those whose babies with Hb Bart's hydrops fetalis syndrome during their current pregnancy visited the hospital for

Table 4. Number of detected thalassemia patients by different thresholds

		MCV <85	MCH <28	MCV <85 and MCH <28	MCH <27	MCV <80	MCV <80 and MCH <27	Normal
α-thalassemia, N	$-\alpha^{3.7}/\alpha\alpha$	7	7	7	6	5	5	8
	$-\alpha^{4.2}/\alpha\alpha$	9	9	9	6	3	3	9
	$--^{SEA}/\alpha\alpha$	634	635	634	634	631	631	636
	$--^{THAI}/\alpha\alpha$	2	3	2	3	2	2	3
	$\alpha\alpha\alpha^{anti\text{-}3.7}/\alpha\alpha$	3	3	3	2	1	0	3
	$\alpha^{init\ CD\ [T>C]}\alpha/\alpha\alpha$	1	1	1	1	1	1	1
	$\alpha^{QS}\alpha/\alpha\alpha$	3	3	3	3	3	3	3
	$\alpha^{CS}\alpha/\alpha\alpha$	3	3	2	3	3	2	4
	$-\alpha^{3.7}/-\alpha^{4.2}$	1	1	1	1	1	1	1
	$--^{SEA}/-\alpha^{3.7}$	8	8	8	8	8	8	8
	$--^{SEA}/-\alpha^{4.2}$	3	3	3	3	3	3	3
	$--^{SEA}/\alpha^{CS}\alpha$	5	5	5	5	4	4	5
	$--^{THAI}/\alpha^{CS}\alpha$	1	1	1	1	1	1	1
	$--^{SEA}/\alpha\alpha\alpha^{anti\text{-}3.7}$	4	4	4	4	4	4	4
	Total	684	686	683	680	670	668	689
	Others	275	266	264	248	252	243	
Total, N		959	952	947	928	922	911	
α-thalassemia (%)		71.3	72.1	72.1	73.3	72.7	73.3	
Other types of mutations (%)		28.7	27.9	27.9	26.7	27.3	26.7	

MCH=mean corpuscular hemoglobin; MCV=mean corpuscular volume

medical checkups, thalassemia screening tests, and preventive measures.

This study identified nine different α-thalassemia mutations, creating 15 different genotypes in pregnant women and their husbands in Vietnam. Among 21 mutations screened, $--^{SEA}$ deletion was the most prevalent mutation (91.9%), as also shown in another study in Southeast Asia.[11] Those carrying the $--^{SEA}$ deletion in heterozygous form are conventionally named α-thalassemia trait and typically have mild or no obvious clinical symptoms.[12] Couples, both of whom carry this mutation, have a 25% chance of having a fetus with Hb Bart's hydrops fetalis syndrome, which accounts for 44.8% of the total causes of placental edema, based on a study in Vietnam.[13] Evaluation of the α-globin gene mutation pattern showed that the $--^{SEA}$ mutation had the highest rate (51.4%), followed by the $^{3.7}$, $^{4.2}$, and α^{CS} mutations. The $--^{SEA}$ deletion was also found to be the most common α-globin gene defect in studies conducted in other Southeast Asian countries and China.[14–19] In contrast, surveys in Malaysia showed that α-thalassemia 2 with the 3.7 kb deletion was the most common α-globin gene defect, indicating ethnic heterogeneity of the α-thalassemia gene.[20]

MCV and MCH are two parameters recommended by the TIF for prenatal diagnosis of thalassemia[21] and are commonly used in Vietnam. Microcytosis with MCV <80 fL and hypochromia with an MCH <27 pg are laboratory features of α-thalassemia 1 carriers, homozygous α-thalassemia 1 carriers, and α-thalassemia disease (HbH disease in particular). Table 2 showed that the MCV <85 fL threshold was more effective in detecting mutations than the MCV <80 fL threshold because there were fewer missed mutations (five mutations compared to 19 mutations), indicating that it could detect more α-thalassemia carriers. However, MCV <85 fL still missed five cases of α-thalassemia carriers, with three cases having large deletion mutations. Similarly, MCH <28 pg could better detect thalassemia mutation than MCH <27 pg but still missed some carriers, including the $--^{SEA}$ mutation. We found a similar trend with both the indices indicating that despite boosting their thresholds to the highest level (MCV <85 fL and MCH <28 pg), α-thalassemia cases were still missed, including α-thalassemia trait mutation in 2/4 α genes, as shown in Tables 2 and 3, which still left a potential risk of having a baby with HbH disease and fetal edema. This suggests that attempting to raise the threshold as much as possible may not be an optimal strategy for α-thalassemia screening.

MCH <28 pg showed the highest sensitivity, whereas the combination of MCV <80 fL and MCH <27 pg showed the lowest sensitivity in screening for α-thalassemia (Table 4). However, different genotypes may have different optimal detection thresholds, and further research is required to determine the most effective threshold for each genotype. Different combinations of index thresholds also represent different screening efficiencies, with the positive predictive value (PPV) varying among populations. In this study, the PPV was limited to participants attending antenatal care and prenatal screening. Despite having the lowest sensitivity, the combination of MCV <80 fL and MCH <27 pg had the highest predictability of α-thalassemia among all thresholds. Using this combination for screening could detect fewer α-thalassemia carriers but higher possibility of α-thalassemia mutation in high-risk population.

This study did not evaluate the sensitivity and specificity of the tests, as the participants had been screened for MCH and MCV indices before genetic testing. Instead, we evaluated the minimum missed rate to compare different screening thresholds. The detection rate of α-thalassemia mutation in one (silent carriers) and two genes (α-thalassemia traits) varied among the different thresholds, while that in three gene mutations was similar in all testing methods. The severity of the disease forms may cause this difference. Silent carriers of α-thalassemia can be mostly normal or mildly anemic.[22,23] α-thalassemia traits are mostly microcytic or mild hypochromic anemia, whereas HbH disease had moderate to severe hypochromic microcytic anemia.

In addition, this study showed significant differences in the current use of common screening thresholds in Vietnam, providing reliable evidence to improve screening for α-thalassemia using clinical units. Among all the indices examined, MCH <28 pg had the lowest missing rate of α-thalassemia and is recommended for α-thalassemia screening in pregnant women and their husbands.

Countries with a high prevalence of thalassemia should consider implementing prenatal screening programs using the optimal screening thresholds identified in this study. Continuous monitoring and updating screening methods are needed to ensure the best possible detection rates for thalassemia. The

results of this study may also guide future research on α-thalassemia genotypes and screening methods in other populations.

In conclusion, the prevalence of the α-thalassemia gene is high among pregnant women and their husbands in Vietnam, with the $--^{SEA}$ mutation being the most common mutation. This study evaluated the effectiveness of various thresholds for MCV and MCH in prenatal screening for α-thalassemia and found that MCV <85 fL and MCH <28 pg had a higher detection rate but still missed a small number of cases. This study highlighted the need to optimize screening methods for α-thalassemia in Vietnam.

Conflict of Interest

The authors affirm no conflict of interest in this study.

Acknowledgment

We would like to thank our colleague from the National Hospital of Obstetrics and Gynecology for supporting us during carrying out the research.

Funding Sources

This study is a part of a state-level scientific research project: "Research on building an artificial intelligence system to support prenatal screening for some common abnormalities in Vietnam." Under the program KC-4.0/19-25.

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