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Original article

Nomogram-based prediction of growth retardation in pediatric deletional α-thalassemia: Evidence from the Mekong Delta, Vietnam

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Abstract. *Background and aim:* Growth retardation is a common complication in children with deletional α-thalassemia. Limited epidemiological data exist on growth retardation in α-thalassemia patients from low-resource settings such as Vietnam. This study investigates the prevalence and associated factors of growth retardation in pediatric patients with α-thalassemia to inform targeted interventions. *Methods:* A multicenter cross-sectional study was conducted involving children with confirmed deletional α-thalassemia from August 2022 to June 2023. Clinical, laboratory, and genetic data were analyzed to identify predictors of growth retardation. *Results:* Growth retardation affected 17.1% of the study population. Key predictors included splenomegaly ≥ grade II (OR = 12.5; 95% CI, 1.69–92.25; p = 0.013), hemoglobin levels <7 g/dL (OR = 7.67; 95% CI, 1.12–52.32; p = 0.038), and having siblings with thalassemia (OR = 13.5; 95% CI, 1.57–115.9; p = 0.018). A predictive nomogram was developed, demonstrating excellent discrimination with an area under the curve of 0.92 (95% CI, 0.82–1.0; p = 0.001) and good calibration (Hosmer–Lemeshow test, $\chi^2(df)$ = 0.139 (1); p = 0.709). *Conclusions:* This study highlights the prevalence and associated factors of growth retardation in pediatric patients with α-thalassemia in the Mekong Delta, Vietnam. By identifying key predictors and developing a practical predictive tool for early risk assessment, these findings provide a foundation for targeted interventions aimed at improving clinical outcomes, particularly in resource-limited settings. (www.actabiomedica.it)

Key words: alpha-thalassemia, child, growth disorders, risk factors, predictive model, nomograms, Vietnam, Mekong Delta, pediatrics

Introduction

Alpha-thalassemia (α-thalassemia), an autosomal recessive hereditary disorder, is caused by impaired or absent synthesis of α-globin chains in hemoglobin molecules (1). As one of the most widespread inherited disorders globally, α-thalassemia represents a significant public health concern (1). Approximately 5% of the global population carries pathogenic variants associated with thalassemia, with an estimated one million individuals directly affected by α-thalassemia syndromes (2–4). These mutations result in reduced or absent synthesis of α-globin chains. While mild forms (silent carriers and α-thalassemia trait) are often incidentally detected through microcytosis, individuals with moderate-to-severe forms exhibit a wide range of

clinical phenotypes, ranging from asymptomatic anemia to hydrops fetalis (5). Diagnostic advancements, such as multiplex gap-polymerase chain reactions (gap-PCR), have significantly improved the detection of common deletional mutations, including $-\alpha^{3.7}$, $-\alpha^{4.2}$, $-\alpha^{20.5}$, $--^{THAI}$, $--^{FIL}$, $--^{MED}$ and $--^{SEA}$ (6). Understanding genotype-phenotype correlations helps clinicians identify genetic contributions to clinical outcomes and provides essential guidance for disease management (7,8). However, despite these advances, integrating diagnostic molecular tools into routine clinical practice in Vietnam remains inadequate. Growth retardation is one of the well-known complications of α-thalassemia and is often associated with chronic anemia, oxidative stress, and endocrine dysfunction (9,10). Moreover, high rates of growth delay and failure have been reported among patients with α-thalassemia major (11). Specifically, growth retardation in pediatric deletional α-thalassemia results from interconnected hematologic and endocrine pathologies exacerbated by chronic transfusion regimens (12,13). In particular, iron overload from repeated transfusions induces pituitary dysfunction through preferential deposition in gonadotrophs and thyrotrophs (13,14), manifesting as growth hormone deficiency and delayed pubertal development (15). This endocrinopathic cascade is compounded by transfusion-induced suppression of hepcidin (14), perpetuating intestinal iron absorption despite chelation therapy (15). However, data on growth patterns in pediatric deletional α-thalassemia are limited, especially in low-resource regions like Vietnam, where mechanisms underlying growth impairment remain poorly understood (16). Most previous studies have primarily focused on clinical and laboratory characteristics of α-thalassemia, with limited attention to the relationship between associated factors and growth retardation in high-prevalence regions such as the Mekong Delta, Vietnam (17,18). This knowledge gap is particularly concerning in socioeconomically disadvantaged regions where access to advanced care and optimal management strategies is limited. Therefore, early identification of growth retardation and preventive strategies, including effective management of hypogonadism through timely and well-coordinated therapies, are essential to improving quality of life for these patients (19). This study aims to address these critical gaps by investigating the prevalence of growth retardation and its associated factors in pediatric patients with deletional α-thalassemia in the Mekong Delta, Vietnam. The development of a predictive model for growth retardation based on these factors is intended to provide pediatricians with a practical tool for use in resource-limited regions. Integrating this model into routine clinical practice could significantly improve patient outcomes and optimize resource allocation in underserved settings.

Materials and Methods

Study design and subjects

This multicenter cross-sectional study was conducted from August 2022 to June 2023 at Can Tho Children's Hospital and Can Tho Hematology and Blood Transfusion Hospital in the Mekong Delta, Vietnam. The sample size was calculated using the formula for estimating a population proportion. Based on a previous prevalence of growth retardation in children with α-thalassemia of 7.8%, reported in a multicenter study by Surapolchai et al. in Thailand (20), a significance level of 0.05, and a margin of error of 9%, the required sample size was determined to be 35. A non-probability sampling method was used, and all eligible pediatric patients meeting the inclusion criteria were enrolled during the study period. Eligible participants for this study were children with a clinical suspicion of α-thalassemia who had not undergone prior genetic testing. Inclusion criteria required both clinical and hematological findings suggestive of α-thalassemia, including microcytic and/or hypochromic anemia identified in a complete blood count (CBC) and/or the presence of target cells or inclusion bodies on a blood smear. Hematological confirmation was further supported by hemoglobin electrophoresis results consistent with α-thalassemia. To exclude iron-deficiency anemia, participants were required to have normal or elevated serum ferritin and iron levels. For cases fulfilling both clinical and hematological criteria for α-thalassemia, genetic validation was subsequently performed to confirm deletional α-thalassemia (21). Children were excluded from the study if they had

diagnostic results consistent with other hematological disorders, including iron-deficiency anemia, β-thalassemia, or anemia of chronic disease. Participants were also excluded if they had insufficient diagnostic evidence to confirm α-thalassemia, such as incomplete laboratory data or inconclusive molecular testing. Finally, any child whose parents or legal guardians did not provide consent, or those who withdrew from the study, were excluded.

Data collection

Clinical data, along with a full medical history, family history, and laboratory results, were collected. Growth retardation was defined using height-for-age z-scores (HAZ) based on the WHO Child Growth Standards, with values less than negative two standard deviations (HAZ < −2 SD) indicating stunting (22,23). Spleen size was graded according to Hackett's spleen size classification. For example, in Grade I, the spleen is palpable below the costal margin, typically during deep inspiration. In Grade II, the spleen is palpable but does not extend beyond a horizontal line halfway between the costal margin and the umbilicus, measured vertically from the left nipple. Splenomegaly was defined as Grade II or higher (24). α-Thalassemia is classified into four primary types: silent carrier, α-thalassemia trait (or minor), HbH disease, and Hb Bart's hydrops fetalis (25).

Hematological and molecular analysis

Peripheral blood samples were collected and analyzed within four hours using automated hematology analyzers (Sysmex, Japan; Pentra XLR, France) for complete blood count (CBC). Hemoglobin analysis was performed via capillary electrophoresis (MINICAP, Sebia, France). Samples were stored at 2–8°C and transported to an ISO 15189:2012-certified laboratory within 24–48 hours. Pre-existing electrophoresis data were included where available. Genomic DNA was extracted using a silica column-based kit (ABT®, Vietnam), following a lysis, ethanol precipitation, and column purification protocol. DNA concentration and purity (A260/A280 ratio: 1.7–2.2) were assessed by spectrophotometry (BioDrop Lite) (26). Gap-PCR was performed to detect four common α-thalassemia deletions (-$\alpha^{3.7}$, -$\alpha^{4.2}$, --^{SEA}, and --^{THAI}) using a 50 μL reaction mix containing Multiplex PCR Master Mix (Qiagen), primer mix, Q-Solution, RNAse-free water, and DNA template. While the HbH inclusions test was not performed, the combination of hematological parameters and molecular analysis provided sufficient diagnostic accuracy for the study (27).

The gap-PCR reaction was specifically designed to detect four prevalent α-thalassemia deletions using the primer sequences listed in Table 1 (28).

The thermocycler conditions included an initial denaturation at 95°C for 15 minutes, followed by 34 cycles of 98°C for 45 seconds, 60°C for 90 seconds,

Table 1. The sequence of primers in the Gap-PCR reaction.

Name	5' → 3' sequence	GenBank ID: nucleotides	Concentration
LIS1-F	GTCGTCACTGGCAGCGTAGATC	HSLIS10: 407→428	0.5 μM
LIS1-R	GATTCCAGGTTGTAGCGGACTG	HSLIS10: 2909→2887	0.5 μM
α2/α3.7-F	CCCCTCGCCAAGTCCACCC	HUMHBA4: 5676→5694	0.2 μM
α3.7-R	AAAGCACTCTAGGGTCCAGCG	HUMHBA4: 11514→11494	0.2 μM
α2-R	AGACCAGGAAGGGCCGGTG	HUMHBA4: 7475→7457	0.2 μM
α4.2-F	GGTTTACCCATGTGGTGCCTC	HUMHBA4: 3064→3084	0.5 μM
α4.2-R	CCCGTTGGATCTTCTCATTTCCC	HUMHBA4: 8942→8920	0.5 μM
SEA-F	CGATCTGGGCTCTGTGTTCTC	HSGG1: 26120→26140	0.2 μM
SEA-R	AGCCCACGTTGTGTTCATGGC	HSCOS12: 3817→3797	0.2 μM
THAI-F	GACCATTCCTCAGCGTGGGTG	HSGG1: 9592→9612	0.3 μM
THAI-R	CAAGTGGGCTGAGCCCTTGAG	HSCOS12: 1241→1221	0.3 μM

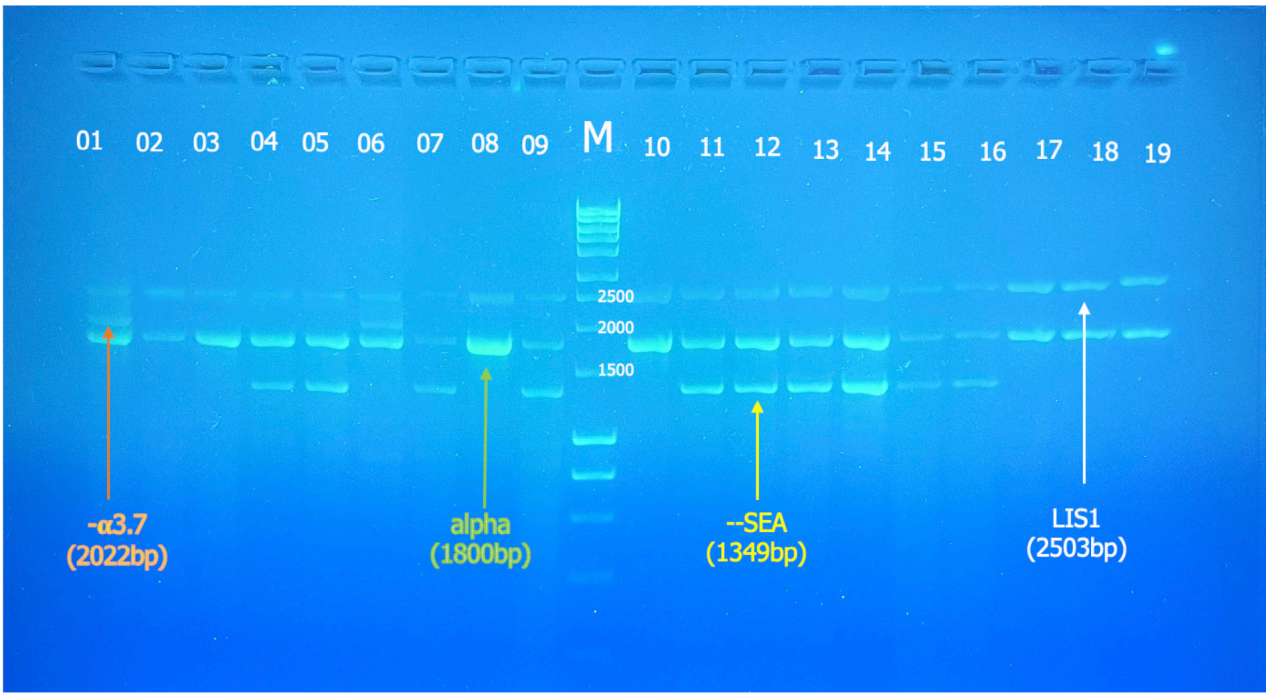

Figure 1. Representative gel image for α deletional mutations. Lane M: 1kp HyperLadder; Lane 1, 6: heterozygous $-\alpha^{3.7}$ mutation ($-\alpha^{3.7}/\alpha\alpha$); Lane 2, 3, 8, 10, 17, 18, 19: normal; Lane 4, 5, 7, 9, 11, 12, 13, 14, 15, 16: heterozygous $--^{SEA}$ mutation ($--^{SEA}/\alpha\alpha$).

and 72°C for 150 seconds. PCR products were then electrophoresed on 1.5% agarose gels in 1X TAE buffer, stained with Safe Dye, and visualized under UV light. The results were interpreted using a molecular weight ladder as a reference (29). The representative gel image for α-deletion mutations is shown in Figure 1.

Statistical analysis

All statistical analyses were conducted using R software version 4.4.2 (R Foundation for Statistical Computing, Vienna, Austria). Quantitative variables are presented as the mean (standard deviation) for normally distributed data and as the median (interquartile range) for non-normally distributed data. Categorical variables are expressed as frequencies and percentages. Fisher's exact test was used to analyze categorical data. For continuous variables, comparisons were made using Student's t-test for normally distributed data or the Wilcoxon rank-sum test for non-normally distributed data. Logistic regression analysis was performed to identify factors associated with growth retardation, with results presented as odds ratios (ORs) with their 95% confidence intervals (CIs) and corresponding *p*-values. Variables that were statistically significant in the univariable logistic regression analysis were included in the multivariable logistic regression to develop the final predictive model. To ensure the validity of our logistic regression model, multicollinearity among independent variables was assessed using the variance inflation factor (VIF) to confirm the absence of significant collinearity. The model's performance was evaluated in terms of discrimination using receiver operating characteristic (ROC) curve analysis and calibration using the Hosmer-Lemeshow test. A *p*-value of <0.05 was considered statistically significant.

Results

Of the 41 patients initially enrolled in the study, genetic testing identified α–thalassemia gene deletion mutations in 35 children. Consequently, the final study cohort included a total of 35 pediatric patients.

Table 2. General characteristics of study subjects.

Characteristics		N (%), or Mean ± SD	Median (IQR)
Age (years)		7.0 ± 4.5	6.0 (3.0–11.0)
Age at diagnosis (years)		5.2 ± 4.1	4.0 (2.0–7.0)
Female		21 (60.0)	
Weight-for-age WHO z-score		–0.33 ± 1.2	–0.2 (–0.83 to 0.01)
Height-for-age WHO z-score		–0.7 ± 1.5	–0.46 (–1.29 to 0.05)
BMI-for-age WHO z-score		–0.09 ± 1.46	–0.03 (–0.73 to 0.84)
α–thalassemia classification	Silent carrier ($-\alpha^{3.7}/\alpha\alpha$)	3 (8.6)	
	Minor ($--^{SEA}/\alpha\alpha$)	14 (40.0)	
	Non–deletional HbH ($--^{SEA}/\alpha^{T}\alpha$)	16 (45.7)	
	Deletional HbH ($--^{SEA}/-\alpha^{3.7}$)	2 (5.7)	
Hepatomegaly (liver > 1 cm below costal margin)		3 (8.6)	
Splenomegaly ≥ grade II		8 (22.9)	
Type of thalassemia	TDT	7 (20.0)	
	NTDT	28 (80.0)	

Abbreviations: TDT, transfusion-dependent thalassemia; NTDT, Non-transfusion-dependent thalassemia.

As outlined in Table 2, most participants were female (60%), with a mean age of 7.0 ± 4.5 years at the time of the study and an average age at diagnosis of 5.2 ± 4.1 years. The average z-score, including weight-for-age was –0.33± 1.2, height-for-age was –0.7± 1.5, and BMI-for-age was –0.09 ± 1.46. The majority are HbH disease cases (non–deletional: 45.7%, deletional: 5.7%), and a proportion with more severe phenotypes. A prevalence of 22.9% and 80%, respectively, of children with moderate to severe splenomegaly (≥ grade II) and non-transfusion-dependent thalassemia.

Figure 2 presents the height-for-age z-scores (HAZ) among 35 children with deletional α-thalassemia, stratified by age groups from 0 to 16 years. Most data points fall within the normal range (–2 SD to +2 SD), indicating typical growth patterns. However, as illustrated in the figure, six children (17.1%) exhibit growth retardation, defined by HAZ scores below –2 SD. These cases are distributed across various age groups, with no evident clustering. The results suggest that while the majority of children maintain growth within standard limits, a significant proportion shows notable growth impairment.

The prevalence of growth retardation was higher in transfusion-dependent individuals compared to non-transfusion-dependent individuals (42.9% vs. 10.7%).

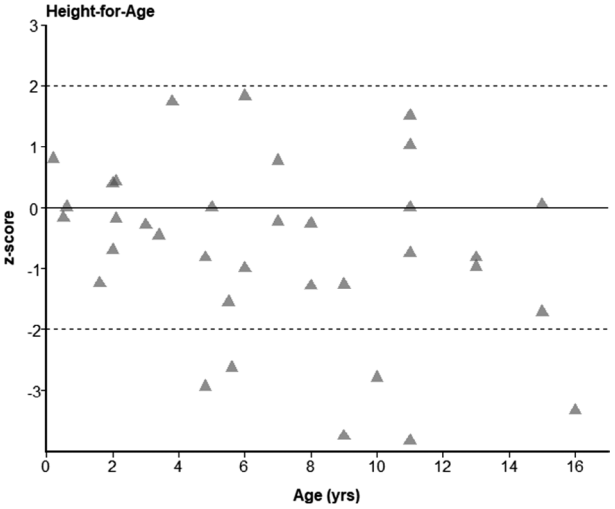

Figure 2. Growth z–score for children with deletional α–thalassemia.

Regarding gender, a higher prevalence of growth retardation (19.0%) was observed among females compared to males (14.3%), with an OR of 1.41 (95% CI: 0.22–8.99), though this result was also not statistically significant (p = 0.715) (Table 3).

The results of the univariable logistic regression analyses of factors associated with growth retardation

Table 3. Factors associated with growth retardation in children with deletional α–thalassemia.

Factors		Growth retardation		Logistic regression	
		Yes, n (%)	No, n (%)	OR (95% CI)	*p*
Age	> 10 years	2 (20.0)	8 (80.0)	1.31 (0.20–8.62)	0.777
	≤ 10 years	4 (16.0)	21 (84.0)	Ref	
Age at diagnosis	< 2 years	1 (14.3)	6 (85.7)	0.77 (0.08–7.86)	0.823
	≥ 2 years	5 (17.9)	23 (82.1)	Ref	
Gender	Female	4 (19.0)	14 (81.0)	1.41 (0.22–8.99)	0.715
	Male	2 (14.3)	12 (85.7)	Ref	
Duration of disease (years), median (IQR)		2.9 (0.9–5.3)	0.1 (0.0–2.8)	1.26 (0.9–1.74)	0.174
Siblings with thalassemia	Yes	3 (60.0)	2 (40.0)	13.5 (1.57–115.9)	**0.018**
	No	3 (10.0)	27 (90.0)	Ref	
Splenomegaly	≥ grade II	4 (50.0)	4 (50.0)	12.5 (1.69–92.25)	**0.013**
	< grade II	2 (7.4)	25 (92.6)	Ref	
Transfusion	Dependent	3 (42.9)	4 (57.1)	6.25 (0.92–42.5)	0.061
	Non dependent	3 (10.7)	25 (89.3)	Ref	
α–thalassemia classification	HbH disease	4 (23.5)	13 (76.5)	2.46 (0.39–15.63)	0.340
	Carrier and minor	2 (11.1)	16 (88.9)	Ref	
Hb at steady state	< 7 g/dL	4 (40.0)	6 (60.0)	7.67 (1.12–52.32)	**0.038**
	≥ 7 g/dL	2 (8.0)	23 (92.0)	Ref	
Serum ferritin	> 2000 ng/mL	2 (33.3)	4 (66.7)	3.13 (0.42–23.06)	0.264
	≤ 2000 ng/mL	4 (13.8)	25 (86.2)	Ref	

are presented in Table 3. It is shown that children with siblings diagnosed with thalassemia had 13.5 times higher odds of experiencing growth retardation compared to those without affected siblings (OR = 13.5; 95% CI, 1.57–115.9; p = 0.018). A hemoglobin level at steady state < 7 g/dL was significantly associated with growth retardation, with a 7.67-fold increase in odds compared to children with hemoglobin at steady state ≥ 7 g/dL (OR = 7.67; 95% CI, 1.12–52.32; p = 0.038). Children with splenomegaly graded ≥ II were found to have 12.5 times higher odds of growth retardation compared to those with splenomegaly < grade II (OR = 12.5; 95% CI, 1.69–92.25; p = 0.013. A prognostic nomogram was developed to simplify the early recognition of children at risk of progressing to growth retardation (Figure 3). This tool was established using multivariable logistic regression analysis, which identified three associated risk factors. Multicollinearity among these predictors was assessed using the variance inflation factor (VIF), with all values approximately equal to 1, indicating no significant collinearity between the included variables. These factors were incorporated into an individualized nomogram model for predicting growth retardation in children. Each risk factor was assigned a score ranging from 0 to 100. Specifically, having siblings with thalassemia was assigned 100 points, moderate to severe splenomegaly (≥ grade II) was assigned 80 points, and hemoglobin at steady state < 7 g/dL was assigned 67 points.

By aggregating these variables, a cumulative score can be calculated and subsequently represented on the nomogram to assess the likelihood of growth retardation. For example, a pediatric patient presenting with risk factors such as an older brother diagnosed with thalassemia and a hemoglobin level of 6.5 g/dL at steady state would yield a total score of 167 points, corresponding to approximately a 75% probability of developing growth retardation. This predictive modeling framework enables timely and targeted therapeutic interventions, thereby improving clinical outcomes

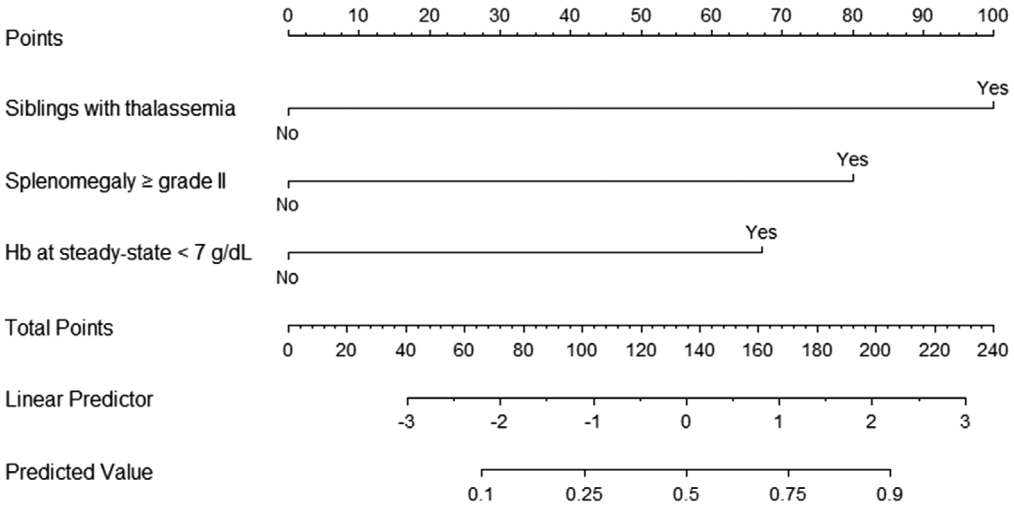

Figure 3. Nomogram–based model to predict growth retardation in children with deletional α-thalassemia.

through early recognition and management of patients with thalassemia. The nomogram model demonstrates excellent predictive capability, with an area under the curve (AUC) of 0.92 (95% CI: 0.82–1.0, p = 0.001), indicating high discrimination between children with and without growth retardation. The Hosmer–Lemeshow test results ($\chi^2(df)$ = 0.139(1), p = 0.709) confirm that the model fits well, with no significant deviation between observed and predicted probabilities.

Discussion

Our study provides a comprehensive analysis of the prevalence and factors contributing to growth retardation in children with deletional α-thalassemia in the Mekong Delta region of Vietnam.

In the current study, with a cohort of 35 children diagnosed with α-thalassemia gene deletion mutations, most participants were female (60%), with a mean age of 7.0 ± 4.5 years at the time of the study and an average age at diagnosis of 5.2 ± 4.1 years. A similar study in Thailand reported that the age at diagnosis ranged from 1 to 7 years in the deletional α-thalassemia group and from <1 to 5 years in the non-deletional α-thalassemia group (5). In our cohort, the mean height-for-age z-score was −0.7 ± 1.5 SD, and we found that 17.1% of the participants experienced growth retardation, defined as height-for-age Z-scores (HAZ) below −2 standard deviations. Previous studies in China and Thailand on pediatric patients with α-thalassemia have reported a variable prevalence of growth retardation, ranging from 7.8% to 68.5% (20,30). The pathogenesis of growth retardation in thalassemia is multifactorial, primarily driven by chronic anemia and hypoxia, chronic liver disease, zinc and folic acid deficiency, iron overload, intensive chelation therapy, emotional stress, endocrinopathies (including hypogonadism, delayed puberty, and hypothyroidism), and dysregulation of the GH–IGF-1 axis (19,31). Zinc deficiency, frequently observed in thalassemic patients due to chronic hemolysis, desferrioxamine therapy, and increased urinary losses (32), contributes to growth retardation by impairing IGF-I synthesis and disrupting bone metabolism (33). Its role in delaying linear growth is further exacerbated by concurrent undernutrition and chronic illness (34). Notably, zinc supplementation has been shown to enhance IGF-I production and improve growth outcomes in these patients (35). The association between growth retardation and disease severity is supported by evidence from related studies. For example, Moiz et al. (2018) studied 367 children with transfusion-dependent β-thalassemia major and reported a 65.4% prevalence of growth retardation, with a median HAZ of −2.69 (IQR: −3.80 to −1.46, p < 0.001) (36). Unlike our study, which focuses on children with deletional and mutational α-thalassemia, their cohort consisted of children aged 5–17 years with

severe β-thalassemia. Notably, 63% of their participants required monthly transfusions of two red blood cell units, and 50.41% had ferritin levels exceeding 5000 ng/mL (36). A significant negative correlation between HAZ and ferritin levels ($p < 0.001$) may explain their higher prevalence of growth retardation compared to our findings (36). In our cohort, body weight and height tended to decrease proportionately, resulting in a relatively stable BMI-for-age with a mean of −0.09 ± 1.46 SD, which remained largely within the normal range. This suggests that linear growth may be disproportionately affected compared to overall body mass. While the observed prevalence of growth retardation is consistent with previous studies on thalassemia-related growth issues, it is important to highlight that the majority of children in our cohort exhibited normal growth patterns. This highlights the potential benefits of early diagnosis and ongoing management in limiting the progression of severe growth impairment. Gap-PCR is a rapid and effective screening method for detecting seven common deletional mutations in α-thalassemia, including $--^{THAI}$, $--^{SEA}$, $--^{FIL}$, $--^{MED}$, $-\alpha^{20.5}$, $-\alpha^{3.7}$ and $-\alpha^{4.2}$ (37). In this study, the gap-PCR assay was used to detect four common mutations ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{SEA}$, and $--^{THAI}$). Among the 35 children in our cohort, two cases (5.7%) of deletional HbH disease ($--^{SEA}/-\alpha^{3.7}$) were identified. The $-\alpha^{3.7}$ deletion, accounting for approximately 95% of deletional α-thalassemia cases, is particularly prevalent in Thailand and Southeast Asia compared to other genotypes (38). Previous studies have highlighted the significant clinical differences between deletional and non-deletional forms of α-thalassemia. For instance, a case-control study found higher rates of hypogonadism, growth retardation, and hypoparathyroidism in patients with α-thalassemia compared to the control group. Moreover, non-deletional α-thalassemia (HbH-CS) was associated with more severe growth retardation and endocrine dysfunction compared to deletional α-thalassemia (30). The severity and clinical manifestations of thalassemia are influenced by multiple factors, including the type of thalassemia, the number of affected globin genes, and individual genetic modifiers (39). The greater severity observed in non-deletional α-thalassemia (HbH-CS) compared to deletional α-thalassemia is primarily attributed to an imbalance in globin chain synthesis. This imbalance arises from the production of unstable or dysfunctional globin proteins, rather than being solely determined by the number of deleted globin genes. In our study, several key clinical and laboratory factors were identified as significant predictors of growth retardation and were systematically incorporated into a high-accuracy predictive model. One of the most important predictors was the presence of siblings with thalassemia, which was associated with a 13.5-fold higher likelihood of experiencing growth retardation (OR = 13.5; 95% CI, 1.57–115.9; $p = 0.018$). This association may reflect shared genetic predispositions or environmental factors within families, underscoring the importance of family-centered approaches in the management of hereditary conditions like thalassemia. The role of genetic factors in familial growth impairment is further supported by findings in conditions such as ATR-X syndrome, where two affected siblings exhibited significant developmental delay, growth retardation, and hematological abnormalities due to an inherited mutation in the ATR-X gene (40). Similar genetic influences may contribute to growth disturbances in thalassemia, reinforcing the need for genetic counseling and early intervention in families with multiple affected individuals. Splenomegaly is a common complication in patients with HbH disease, particularly in non-deletional forms, and is often significantly enlarged in patients with hemoglobin Bart's hydrops fetalis (α-thalassemia major) (41). A spleen that is palpable below the left costal margin is almost always considered enlarged (42). We observed that pediatric patients with splenomegaly graded ≥ II had 12.5 times higher odds of experiencing growth retardation compared to those with milder or no splenomegaly (OR = 12.5; 95% CI, 1.69–92.25; $p = 0.013$). Our results corroborate findings from Asian cohorts, with multivariable analysis confirming that splenomegaly >3 cm was independently associated with growth failure (OR = 4.28; 95% CI, 1.19–15.39; $p = 0.026$) (43). In a related study, splenomegaly was observed in 82% of patients, with massive splenomegaly reported in 14.7% of 94 patients with thalassemia (44). Routine evaluation of splenic size and volume is crucial, as splenomegaly can predict changes in transfusion requirements (44). Additionally, splenomegaly was found to be more

common in children with -α/-α compared to normal children, with a relative risk of 1.5 (95% CI, 1.2–1.9; p = 0.004) (45). Early identification and appropriate management of splenomegaly, potentially through splenectomy or medical therapy, could help alleviate its detrimental effects on growth. In patients with non-deletional HbH disease, where splenomegaly is often associated with hypersplenism or severe anemia, splenectomy is frequently performed (46,47). The procedure has been shown to effectively increase hemoglobin levels, typically by 20–30 g/L in patients with HbH disease (9,48). Our study also revealed that hemoglobin levels at steady state < 7 g/dL were significantly associated with growth retardation, with a 7.67-fold increased risk compared to children with hemoglobin levels ≥ 7 g/dL (OR = 7.67; 95% CI, 1.12–52.32; p = 0.038). This finding aligns with previous research that identified Hb < 7 g/dL as a critical threshold for assessing disease severity and the need for transfusion in patients with non-deletional HbH disease. Through univariate and multivariate logistic regression analyses, low hemoglobin was shown to be a key indicator of disease severity and transfusion requirements, earning a score of 4 points in predictive models (49). Low hemoglobin levels contribute to reduced oxygen delivery to tissues, exacerbating metabolic stress and impairing growth. Persistent anemia can hinder skeletal and muscular development, emphasizing the importance of timely and adequate transfusion therapy. By maintaining hemoglobin levels above the critical threshold, complications related to growth retardation can be minimized, improving overall outcomes for affected children (49).

In our study, the predictive model was developed using multivariable logistic regression, and the nomogram was constructed as a practical tool in medical practice due to its ability to translate complex predictive models into an easy-to-use graphical representation. The predictive model was evaluated based on its discrimination and calibration performance. With an area under the curve (AUC) of 0.92, the model demonstrated remarkable discriminatory power in identifying children at risk for growth retardation. The model's calibration was validated using the Hosmer–Lemeshow test ($\chi^2(df)$ = 0.139 (1); p = 0.709), confirming a strong alignment between observed and predicted probabilities. This nomogram is particularly significant as it simplifies complex clinical data into an accessible, user-friendly format. Each predictor is assigned a point value reflecting its relative contribution to the risk of growth retardation. For instance, the presence of siblings with thalassemia is assigned the highest score (100 points), highlighting its strong association with the outcome. Splenomegaly (≥ grade II) and low hemoglobin at steady state contribute 80 and 67 points, respectively. This scoring system enables clinicians to rapidly assess a child's risk profile and make informed decisions about appropriate interventions. One of the model's greatest strengths is its simplicity, making it highly applicable in resource-constrained settings. In regions like the Mekong Delta, where healthcare resources are limited, this nomogram can be seamlessly integrated into routine clinical workflows without requiring advanced diagnostic technologies. By enabling the early identification of high-risk patients, clinicians can implement timely and targeted interventions, including nutritional supplementation, frequent growth monitoring, and optimized transfusion regimens. The model's flexibility also allows for validation and adaptation in other populations with high thalassemia prevalence, further extending its clinical utility.

Strength and limitations

This study employed a robust multicenter cross-sectional design to evaluate a comprehensive range of clinical, laboratory, genetic, and socio-demographic variables for identifying predictors of growth retardation. A key strength of this research is the development of a prognostic model that integrates critical predictors into an accessible and practical nomogram. The model demonstrated high predictive accuracy and substantial clinical utility, particularly in resource-limited settings like the Mekong Delta. Rigorous validation, including both calibration and discrimination analyses, further supports its reliability and applicability. However, the study's small cohort of 35 pediatric patients limits the precision and generalizability of the findings. Despite significant associations (e.g., splenomegaly ≥grade II: OR = 12.5; 95% CI: 1.69–92.25), wide confidence intervals indicate substantial uncertainty in effect sizes. The limited statistical power increases the risk of

Type II errors and potential overestimation of odds ratios. Additionally, despite the multicenter approach, the relatively small sample size constrains the generalizability of the results. The observed prevalence of growth retardation (17.1%) reflects the characteristics of the study cohort and may not be fully representative of the broader population. Furthermore, the exclusion of non-deletional HbH variants was due to resource constraints in genetic testing, which may have led to their underrepresentation in our cohort. This limitation could have influenced the accuracy of observed associations, as non-deletional mutations often present with more severe phenotypes. We acknowledge that the inability to detect these variants may have resulted in potential misclassification and suggest future studies incorporate comprehensive genetic analysis to address this gap. Although the nomogram demonstrated excellent discrimination, its broader applicability requires external validation, particularly in non-deletional HbH cohorts. External validation across diverse populations is also essential to confirm the model's robustness and ensure its clinical utility beyond the study region. While our study primarily focused on hematological and genetic factors, other contributors, such as zinc deficiency, iron overload, and endocrine dysfunction (including GH-IGF-1 axis abnormalities), may also play important roles. Future studies should investigate these factors to provide a more comprehensive understanding of growth impairment in α-thalassemia.

Clinical implications

Understanding the predictors of growth retardation in pediatric deletional α-thalassemia is crucial for improving patient outcomes. This study provides valuable insights that may help pediatricians refine their approach to surveillance and early intervention for high-risk children, particularly in resource-limited settings. Firstly, the development of a predictive nomogram with an AUC of 0.92 offers an important tool for stratifying pediatric patients based on clinical parameters such as hemoglobin levels <7 g/dL, splenomegaly ≥grade II, and having siblings with thalassemia. This model could enable early identification of children at risk for growth retardation, allowing for targeted monitoring and timely intervention to prevent irreversible growth delays. Regular growth monitoring should be prioritized for children with severe anemia to facilitate early detection of growth impairment. Secondly, the strong association between affected siblings and an increased risk of growth impairment highlights the importance of genetic counseling and sibling screening. In areas with high thalassemia prevalence, implementing family-based screening protocols could help identify asymptomatic carriers and ensure early surveillance and intervention for growth problems, ultimately improving long-term outcomes for the next generation. Additionally, the finding that hemoglobin levels <7 g/dL are strongly associated with growth retardation suggests that this threshold could serve as a practical biomarker for initiating more frequent growth assessments in non-transfusion-dependent patients. Clinicians in low-resource settings may benefit from using this threshold to guide monitoring intervals, particularly when more advanced diagnostic tools are unavailable. Optimizing transfusion regimens in at-risk children may help prevent severe growth delays, and endocrine evaluation should be considered for those with persistent growth impairment to assess the need for hormonal therapy, particularly for GH-IGF-1 axis dysfunction.

Conclusion

Our study highlights the significant prevalence of growth retardation among pediatric patients with deletional α-thalassemia in the Mekong Delta and identifies key associated factors, including splenomegaly, hemoglobin steady-state <7 g/dL, and having siblings with thalassemia. The predictive nomogram developed demonstrates high accuracy and clinical utility, providing a practical tool for early risk assessment in resource-limited settings. These findings emphasize the need for targeted interventions to improve growth outcomes and underscore the importance of integrating predictive models into routine care to optimize management. Future studies should explore growth patterns in non-deletional HbH disease, as it may exhibit different clinical trajectories. External validation in a larger cohort

could establish the nomogram as a valuable tool for clinical use, particularly in low-resource settings.

Ethics Approval: The study was approved by the Ethics Committee in Biomedical Research at Can Tho University of Medicine and Pharmacy (IRB approval No. 22.149.HV/PCT–HĐĐĐ, dated July 29, 2022).

Conflict of Interest: Each author declares that he or she has no commercial associations that might pose a conflict of interest in connection with the submitted article.

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