

## Cis and Trans Variants of $\alpha$ -Thalassemia Minor: “Hematological Differences and Distinction from Iron Deficiency Anemia

Bijan Keikhaei Dehdezi Fellow Ship<sup>1</sup>, Ali Khodadadi MD<sup>1</sup>, Arta Farhadi Kia MD<sup>2</sup>, Atefeh Rezaei Fellow Ship<sup>1</sup>, Saeed Bitaraf PhD<sup>1</sup>, Roya Salehi Kahyesh PhD<sup>\*1</sup>

1. Thalassemia & Hemoglobinopathy Research center, Health research institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

2. Medical Point Hospital, Izmir, Turkey

\*Corresponding author: Dr. Roya Salehi Kahyesh, Thalassemia & Hemoglobinopathy Research center, Health research institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Email: royaarta@yahoo.com.com. ORCID ID: 0000-0002-7770-6162.

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### Abstract

**Background:** Alpha thalassemia and iron deficiency anemia (IDA) are common hematological disorders characterized by microcytic red blood cells, complicating accurate diagnosis. This study investigates the genetic diversity and clinical presentation of alpha thalassemia, emphasizing the critical need for precise differential diagnosis between alpha thalassemia and IDA to ensure effective patient management and treatment.

**Materials and Methods:** In this cross-sectional study, we conducted analysis of alpha thalassemia minor and IDA, focusing on hematological indices and clinical outcomes. Patients diagnosed with either condition from 2019 to 2023 were evaluated based on key parameters, including hemoglobin (Hb), red blood cell (RBC) count, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). We also analyzed alpha thalassemia genotypes, distinguishing between cis and trans variants. This study uniquely explores hematological profiles in thalassemia minor patients.

**Results:** Patients with cis- $\alpha$ -thalassemia exhibited lower MCV ( $68.14 \pm 3.77$  fL) compared to trans- $\alpha$ -thalassemia ( $72.15 \pm 4.03$  fL;  $p = 0.143$ ). MCH was significantly lower in cis ( $20.58 \pm 1.49$  pg) than in trans ( $22.36 \pm 1.43$  pg;  $p = 0.013$ ). Mean Hb was reduced in cis ( $12.10 \pm 1.50$  g/dL) relative to trans ( $12.46 \pm 1.16$  g/dL;  $p = 0.015$ ), while RBC counts were higher in both alpha-thalassemia groups. Compared with IDA, alpha-thalassemia patients demonstrated significantly higher RBC counts ( $5.65 \pm 0.44$  vs.  $4.30 \pm 0.46 \times 10^{12}/L$ ;  $p < 0.001$ ) and Hb levels ( $12.37 \pm 1.12$  vs.  $9.60 \pm 1.12$  g/dL;  $p < 0.001$ ). Differences in MCV and MCH between thalassemia and IDA were not statistically significant.

**Conclusion:** This finding highlights significant hematological differences between alpha thalassemia variants and IDA. By elucidating the distinct features of cis and trans alpha thalassemia genotypes, this study reinforces the necessity for precise diagnosis and tailored clinical management. Further research is warranted to explore the long-term clinical implications of these genetic variants.

**Keywords:** Anemia, Differential Diagnosis, Genetic Variation, Iron Deficiency, Thalassemia Minor



## Introduction

Alpha thalassemia is a hereditary blood disorder characterized by reduced or absent production of  $\alpha$ -globin chains, essential components of hemoglobin. Affecting approximately 5% of the global population, the condition commonly presents as microcytic, hypochromic anemia. Clinical severity varies significantly, ranging from asymptomatic individuals to cases resulting in intrauterine death, depending on the number of affected  $\alpha$ -globin genes. Despite the genetic heterogeneity of  $\alpha$ -thalassemia mutations, clinical manifestations are generally categorized into four main phenotypes of increasing severity: the silent carrier state,  $\alpha$ -thalassemia trait (minor), Hemoglobin H (Hb H) disease, and Hb Bart's hydrops fetalis (1-3).

Hemoglobin consists of four  $\alpha$ -globin genes organized as linked pairs, known as the HBA1 and HBA2 genes on chromosome 16. The normal gene configuration is denoted as  $\alpha\alpha/\alpha\alpha$ . Diagnostic terminology often includes  $\alpha^+$ -thalassemia and  $\alpha^0$ -thalassemia. A single gene deletion or point mutation resulting in reduced  $\alpha$ -globin expression is classified as  $\alpha^+$ -thalassemia and may present in heterozygous or homozygous form. Diminished allelic expression may result from the deletion of a single  $\alpha$ -globin gene (deletion type) or a point mutation in one of the duplicated  $\alpha$ -globin genes (non-deletion type) (2). In contrast,  $\alpha^0$ -thalassemia refers to the absence of both  $\alpha$ -globin genes on one chromosome, typically through large deletions, rendering distinctions between deletional and non-deletional forms less relevant in these cases (4).

Alpha thalassemia minor occurs when an individual inherits two functional  $\alpha$ -globin genes and two defective ones. This can result from two main configurations: heterozygosity for an  $\alpha^0$  deletion ( $\alpha\alpha/-$ , or cis configuration) or homozygosity for an  $\alpha^+$  deletion ( $-\alpha/-\alpha$ , or trans configuration) (5). The vast majority of alpha-thalassemia mutations, about 85%, are due to seven common large deletions:  $-\alpha 3.7$ ,  $-\alpha 4.2$ ,  $--SEA$ ,  $--MED$ ,  $--FIL$ ,  $--THAI$ , and  $--20.5$ . Clinically, individuals with alpha thalassemia minor are typically asymptomatic or exhibit mild microcytic, hypochromic anemia (6).

The interaction between  $\alpha^0$ - and  $\alpha^+$ -

thalassemias can lead to a non-fatal form of the syndrome known as Hemoglobin H (Hb H) disease, which varies in severity, from mild cases to those requiring transfusions for anemia. Patients with non-deletional Hb H (genotype  $--/\alpha\alpha$ ) generally experience a more severe disease form than those with deletional Hb H (genotype  $--/-\alpha$ ). In rare cases, non-deletional Hb H may progress to Hb H hydrops fetalis. The most severe form—Hb Bart's hydrops fetalis—occurs in homozygous  $\alpha^0$ -thalassemia ( $---/---$ ), leading to complete absence of  $\alpha$ -globin chains and resulting in fetal or neonatal death (7).

Iran is located within the geographic area referred to as the “thalassemia belt,” which is characterized by a high incidence of thalassemia-related disorders. In particular, Khuzestan Province, located in the southwest and home to approximately five million people, exhibits a wide range of hemoglobinopathy genotypes due to its ethnically diverse population. An estimated two million individuals in Iran are thalassemia carriers. Prevalence rates are especially high in the northern and southern provinces, where 15–35% of the population carry  $\alpha$ -thalassemia mutations and approximately 10% carry  $\beta$ -thalassemia mutations (8).

The primary objectives of thalassemia screening programs include assessing the prevalence of genetic mutations and identifying at-risk couples, particularly in high-risk regions. Cis alpha thalassemia has been closely associated with Hemoglobin H disease, which emphasizes the importance of distinguishing between cis and trans configurations. Accurate screening plays a critical role in the prevention of severe maternal and fetal outcomes, such as Hb Bart's hydrops fetalis and other complications.

While numerous studies have described  $\alpha$ -thalassemia prevalence and general hematological patterns, limited data exist on direct comparisons between cis and trans  $\alpha$ -thalassemia minor, particularly in Middle Eastern populations where the burden is high. This study addresses that gap by evaluating hematological indices of cis and trans  $\alpha$ -thalassemia minor alongside IDA, providing genotype-specific insights relevant to screening and management.

This study aims to investigate the frequency and hematological differences between cis and trans alpha thalassemia minor in comparison with iron deficiency anemia, focusing on their impact on red blood cell indices in southwestern Iran.

## Material and Methods

### Data collecting

This was a cross-sectional study with comparative case-control elements, conducted between 2019 and 2023 at Shahid Baghaei 2 Hospital in Ahvaz, the capital of Khuzestan Province in southwest of Iran. The study population included patients aged 14 to 48 years at the time of recruitment, who had been previously diagnosed with  $\alpha$ -thalassemia minor or iron deficiency anemia (IDA). For  $\alpha$ -thalassemia cases, diagnosis was primarily established during premarital counseling or routine hematologic evaluations, while IDA cases were confirmed through laboratory testing of iron status. The primary focus was on couples identified as potential thalassemia carriers during pre-marital counseling sessions, who were subsequently referred to the hospital's genetic center for comprehensive mutation screening and genotypic classification.

### Sampling

All participants and relevant family members underwent interviews to obtain detailed medical histories, and hematological indices were recorded. Blood samples (5 mL peripheral blood in EDTA tubes) were collected and analyzed using an automated hematology analyzer (Sysmex KX-21; Sysmex Corporation, Kobe, Japan) to assess key red cell parameters. Molecular testing targeted common Mediterranean  $\alpha$ -globin gene deletions, including  $-\alpha^{3.7}$ ,  $-\alpha^{4.2}$ ,  $-\alpha^{20.5}$ , and  $-\text{MED}$ , along with point mutations, using GAP-PCR and ARMS-PCR techniques.

### Methods

A total of 272 individuals were initially diagnosed with  $\alpha$ -thalassemia minor. To ensure accurate differentiation from IDA, serum ferritin levels were assessed. Patients with ferritin levels below 30 ng/mL were excluded and referred for

treatment and re-evaluation. Additionally, 39 individuals with non-deletional  $\alpha$ -thalassemia mutations were excluded. This decision was made because non-deletional variants often display more variable clinical severity and hematological indices, making direct comparison with deletional cis and trans configurations less reliable. By focusing on deletional variants, we aimed to ensure a more homogeneous study population and clearer interpretation of hematological differences. However, this choice limits the ability to generalize findings to all forms of  $\alpha$ -thalassemia minor. This resulted in a final cohort of 233 patients with  $\alpha$ -thalassemia minor, stratified into cis and trans genotypic configurations.

For comparative purposes, a second group was selected from a pool of 323 confirmed IDA patients. A total of 125 individuals met the inclusion criteria: ferritin <10 ng/mL, age 15–50 years, hemoglobin >8 g/dL, non-pregnant status, and absence of hemoglobinopathies or other concurrent conditions. All included patients underwent evaluation of red cell indices, including hemoglobin concentration (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). These indices were compared between cis and trans  $\alpha$ -thalassemia minor groups as well as with iron deficiency anemia cases to assess distinguishing hematologic features.

### Statistical Analysis

Data analysis was performed using IBM SPSS Statistics version 26. Comparisons were made using the independent two-sample t-test, with a significance level set at  $p < 0.05$ . To assess the normality of the data distribution, the Shapiro-Wilk test was employed.

The study protocol was reviewed and approved by the Medical Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (IR.AJUMS.REC.1401.055). All participants provided written informed consent before enrollment.

## Results

Among the 272 individuals diagnosed with  $\alpha$ -thalassemia minor, 201 patients (73.9%) carried trans-type deletions ( $\alpha$ -/ $\alpha$ -), whereas 71 patients (26.1%) carried cis-type deletions ( $\alpha\alpha$ -/-). The most common genotype in the cohort was the  $-\alpha^{3.7}/-\alpha^{3.7}$  (trans) deletion, detected in 153 individuals (56.1%). Within the cis group, the  $-\text{MED}/\alpha\alpha$  genotype predominated, comprising 41 individuals (15.0%). Table I provides the detailed distribution of all genotypes and their associated hematological parameters.

Overall, 233 patients (85.7%) exhibited deletional mutations, while 39 patients (14.3%) carried non-deletional variants. Seven distinct non-deletional genotypes were identified. The most frequent of these was Hemoglobin Constant Spring (HbCS), observed in 10 individuals. Other non-deletional variants included  $-\alpha^{3.7}/\alpha\text{PolyA1}(\text{AATAAA}>\text{AATAAG})$  in 9 patients,  $-\alpha^{3.7}/\alpha^2\text{CD19}(-\text{G})$  in 5 patients, and  $-\alpha^{3.7}/\text{IVS-I-5nt}$  in 5 patients. Three individuals carried the  $\alpha\text{IVS-I}$  donor site/ $-\alpha^{3.7}$  genotype, and three rare variants were each detected in one patient:  $-\alpha^{4.2}/\alpha\text{PolyA1}(\text{AATAAA}>\text{AATAAG})$ ,  $-\alpha^{3.7}/\alpha\text{PolyA2}(\text{AATAAA}>\text{AATGAA})$ , and  $\alpha\text{CD19}(\text{G})/\alpha\text{CD142}(\text{T}>\text{C})$ . These results highlight substantial genetic heterogeneity in  $\alpha$ -thalassemia minor within this population, with deletional trans-type mutations being the most prevalent.

A comparison of hematological indices between cis and trans phenotypes demonstrated distinct trends (Table II). Mean corpuscular volume (MCV) was numerically lower in patients with the cis genotype ( $68.14 \pm 3.77$  fL) compared with those with the trans genotype ( $72.15 \pm 4.03$  fL), although this difference did not reach statistical significance ( $p = 0.143$ ). A similar pattern was observed for mean corpuscular hemoglobin (MCH), which was lower in the cis group ( $20.58 \pm 1.49$  pg) relative to the trans group ( $22.36 \pm 1.43$  pg); however, in contrast to MCV, this difference was statistically significant ( $p = 0.013$ ). Hemoglobin levels were slightly lower in the cis genotype ( $12.10 \pm 1.50$  g/dL) compared to the trans group ( $12.46 \pm 1.16$  g/dL;  $p = 0.015$ ), though the magnitude of difference was modest. The red blood cell (RBC) count showed an

opposite trend, being slightly higher in the cis group ( $5.84 \pm 0.67 \times 10^{12}/\text{L}$ ) than the trans group ( $5.59 \pm 0.65 \times 10^{12}/\text{L}$ ), consistent with expected compensatory erythrocytosis in cis deletions.

Sex-stratified analyses (Table II) showed similar patterns: males and females both demonstrated lower MCV and MCH values in the cis phenotype compared with trans, with statistical significance observed primarily in MCH for both genders. Hemoglobin differences between cis and trans groups were more pronounced in males ( $p = 0.011$ ) than in females ( $p = 0.213$ ), while RBC counts tended to be slightly higher in cis carriers across both sexes.

When  $\alpha$ -thalassemia minor patients were compared with individuals with iron deficiency anemia (IDA) (Table III), substantial hematological differences were observed. The RBC count was markedly elevated in  $\alpha$ -thalassemia minor ( $5.65 \pm 0.44 \times 10^{12}/\text{L}$ ) compared with IDA ( $4.30 \pm 0.46 \times 10^{12}/\text{L}$ ;  $p < 0.001$ ). Hemoglobin concentration was also significantly higher in  $\alpha$ -thalassemia minor ( $12.37 \pm 1.12$  g/dL) than in IDA ( $9.60 \pm 1.12$  g/dL;  $p < 0.001$ ). By contrast, MCV and MCH values were similar between groups (MCV:  $72.06 \pm 6.74$  fL vs.  $71.13 \pm 4.31$  fL,  $p = 0.124$ ; MCH:  $22.24 \pm 2.93$  pg vs.  $21.92 \pm 1.63$  pg,  $p = 0.187$ ), indicating that microcytosis and hypochromia alone cannot reliably distinguish  $\alpha$ -thalassemia minor from IDA. These findings underscore the diagnostic importance of RBC count and hemoglobin concentration in differentiating the two conditions, while emphasizing the limited specificity of MCV and MCH.

The data from Table II detail the hematological parameters across various alpha-thalassemia phenotypes, specifically comparing the trans ( $\alpha$ -/ $\alpha$ -) and cis ( $\alpha\alpha$ -/-) forms, subdivided by gender. Meanwhile, Table III provides a comparison of these indices between individuals with alpha-thalassemia minor and those with iron deficiency anemia (IDA). Mean hemoglobin was modestly higher in the trans group ( $12.46 \pm 1.16$  g/dL) than in the cis group ( $12.10 \pm 1.50$  g/dL;  $p = 0.015$ ). Although MCV was again numerically higher in the trans phenotype ( $72.15 \pm 4.03$  fL) compared to the cis phenotype ( $68.14 \pm 3.77$  fL), this difference



was not statistically significant ( $p = 0.143$ ), consistent with a similar degree of microcytosis in both forms. MCH was significantly higher in the trans phenotype ( $22.36 \pm 1.43$  pg) than in the cis phenotype ( $20.58 \pm 1.49$  pg;  $p = 0.013$ ), indicating a slightly more pronounced hypochromia in the cis group.

The RBC count in alpha-thalassemia minor is substantially higher ( $5.65 \pm 0.44 \times 10^{12}/L$ ) compared to IDA patients ( $4.30 \pm 0.46 \times 10^{12}/L$ ), with a highly significant p-value ( $< 0.001$ ). This difference reflects the typical erythrocytosis observed in thalassemia minor. Hemoglobin levels are significantly lower in IDA ( $9.60 \pm 1.12$  g/dL) versus alpha-thalassemia minor ( $12.37 \pm 1.12$  g/dL), also with  $p < 0.001$ , illustrating more profound anemia in IDA. Both conditions exhibit microcytosis; however, MCV is only slightly different ( $72.06 \pm 6.74$  fL in  $\alpha$ -thalassemia vs.  $71.13 \pm 4.31$  fL in IDA) with no statistically significant difference ( $p = 0.124$ ). Similarly, MCH values are comparable between groups ( $22.24 \pm 2.93$  pg vs.  $21.92 \pm 1.63$  pg), with no significant difference ( $p = 0.187$ ).

As depicted in Chart I, patients with  $\alpha$ -thalassemia minor exhibited significantly higher RBC counts ( $5.65 \pm 0.44 \times 10^{12}/L$ ) and hemoglobin levels ( $12.37 \pm 1.12$  g/dL) compared to those with IDA, who had mean values of  $4.30 \pm 0.46 \times 10^{12}/L$  and  $9.60 \pm 1.12$  g/dL, respectively. Although the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values were slightly lower in the thalassemia group ( $72.06 \pm 6.74$  fL and  $22.24 \pm 2.93$  pg) compared to the IDA group ( $71.13 \pm 4.31$  fL and  $21.92 \pm 1.63$  pg), these differences were not statistically significant. In contrast, the differences in RBC count and hemoglobin were highly significant ( $p < 0.001$ ).

Evaluation of effect sizes (Cohen's d) for cis and trans alpha-thalassemia and for alpha-thalassemia versus iron deficiency anemia (Table IV) showed that negative values indicate lower values in the first group (cis or trans  $\alpha$ -thal). Large effect sizes were observed for MCV and MCH in the cis vs. trans comparison, and for Hb and RBC in the  $\alpha$ -thal vs. IDA comparison. Small effect sizes were observed for Hb and RBC (cis vs. trans) and for MCV and MCH ( $\alpha$ -thal vs. IDA). Effect

sizes provide a quantitative measure of the magnitude of differences between groups. In this context, cis vs. trans comparisons showed markedly lower MCV and MCH values in the cis group, while  $\alpha$ -thalassemia vs. IDA comparisons showed substantially higher Hb and RBC values in  $\alpha$ -thalassemia minor:

-Large effect sizes:

Cis vs. Trans: MCV and MCH show considerable differences.

$\alpha$ -thal vs. IDA: Hb and RBC demonstrate significant differences.

-Small effect sizes:

Cis vs. Trans: Hb and RBC show minimal differences.

$\alpha$ -thal vs. IDA: MCV and MCH show minimal differences.

Table I: Frequency and Hematological Parameters of Alpha-Thalassemia Minor Genotypes

Genotype	N(percent)	RBC Count (10 <sup>12</sup> /L)	Hb (g/dL)	MCV (fL)	MCH (pg)
3.7 $\alpha$ /3.7 $\alpha$	153 (62.20%)	5.57 $\pm$ 0.65	12.41 $\pm$ 1.64	72.12 $\pm$ 4.15	22.36 $\pm$ 2.08
3.7 $\alpha$ /4.2 $\alpha$	18 (7.32%)	5.64 $\pm$ 0.58	12.54 $\pm$ 1.25	72.13 $\pm$ 3.19	22.24 $\pm$ 2.21
med/ $\alpha\alpha$	41 (16.67%)	5.85 $\pm$ 0.69	12.15 $\pm$ 1.51	67.98 $\pm$ 3.48	20.61 $\pm$ 1.46
(20.5 $\alpha$ )/ $\alpha\alpha$	14 (5.69%)	5.79 $\pm$ 0.63	11.87 $\pm$ 2.52	69.15 $\pm$ 4.53	20.48 $\pm$ 1.73
4.2 $\alpha$ /4.2 $\alpha$	4 (1.63%)	6.20 $\pm$ 0.63	14.02 $\pm$ 1.12	71.12 $\pm$ 3.03	22.75 $\pm$ 0.98
Unknown large deletion1 /aa	2 (0.81%)	5.81 $\pm$ 0.59	12.7 $\pm$ 0.3	66.95 $\pm$ 3.15	20.65 $\pm$ 0.49
Unknown large deletion2 /aa	1 (0.41%)	5.09	11	70.5	21.6

Table II: Characteristics of blood indices in different alpha thalassemia phenotypes

Group	Phenotype	N.	Sex (M/F)	RBC Count (10 <sup>12</sup> /L)	Hb (g/dL)	MCV (fL)	MCH (pg)	P-value
<b>Trans(<math>\alpha</math>-/<math>\alpha</math>-)</b>	$\alpha^+/\alpha^+$	175	87/88	5.59 $\pm$ 0.65	12.46 $\pm$ 1.16	72.15 $\pm$ 4.03	22.36 $\pm$ 1.43	0.015
<b>Cis(<math>\alpha\alpha</math>/--)</b>	$\alpha^+/\alpha^0$ $\alpha\alpha/\alpha^0$	57	26/31	5.84 $\pm$ 0.67	12.10 $\pm$ 1.50	68.14 $\pm$ 3.77	20.58 $\pm$ 1.49	0.143
<b>Trans(<math>\alpha</math>-/<math>\alpha</math>-)</b>	$\alpha^+/\alpha^+$	88	Female	5.22 $\pm$ 0.52	11.53 $\pm$ 1.16	71.52 $\pm$ 3.78	22.11 $\pm$ 1.49	0.013
<b>Cis(<math>\alpha\alpha</math>/--)</b>	$\alpha^+/\alpha^0$ $\alpha\alpha/\alpha^0$	31	Female	5.47 $\pm$ 0.55	11.25 $\pm$ 1.30	67.80 $\pm$ 4.53	20.48 $\pm$ 1.83	0.213
<b>Trans(<math>\alpha</math>-/<math>\alpha</math>-)</b>	$\alpha^+/\alpha^+$	87	Male	5.96 $\pm$ 0.54	13.40 $\pm$ 1.44	72.68 $\pm$ 4.21	22.60 $\pm$ 1.34	0.011
<b>Cis(<math>\alpha\alpha</math>/--)</b>	$\alpha^+/\alpha^0$ $\alpha\alpha/\alpha^0$	26	Male	6.27 $\pm$ 0.52	13.12 $\pm$ 1.02	68.56 $\pm$ 2.63	20.71 $\pm$ 0.95	0.107

Table III: Comparison of the blood indices between  $\alpha$ -Thalassemia Minor and Iron Deficiency Anemia (IDA)

Group	N	Sex (M/F)	RBC Count (10 <sup>12</sup> /L)	Hb (g/dL)	MCV (fL)	MCH (pg)
<b><math>\alpha</math>-Thalassemia Minor</b>	272	129/143	5.65 $\pm$ 0.44	12.37 $\pm$ 1.12	72.06 $\pm$ 6.74	22.24 $\pm$ 2.93
<b>IDA</b>	125	6/119	4.30 $\pm$ 0.46	9.60 $\pm$ 1.12	71.13 $\pm$ 4.31	21.92 $\pm$ 1.63
<b>P-value</b>			0.001<	.001<	0.124	0.187

Table IV: Comparison of Effect Sizes (Cohen's d) between Cis, trans, and IDA

Comparison	Hb	MCV	MCH	RBC
<b>Cis vs Trans <math>\alpha</math>-thal</b>	-0.27	-1.03	-1.22	0.38
<b><math>\alpha</math>-thal vs IDA</b>	2.47	0.16	0.14	3.00

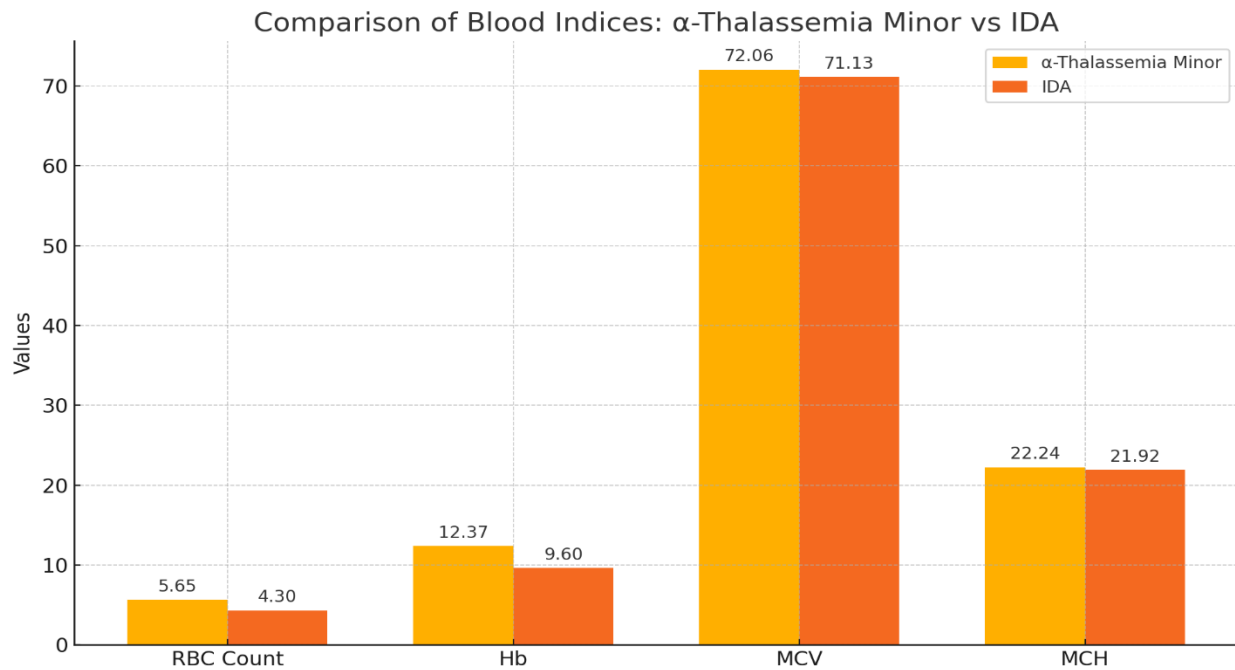


Fig 1. Comparison of blood indices between  $\alpha$ -Thalassemia Minor and Iron Deficiency Anemia (IDA)

## Discussion

Although  $\alpha$ -thalassemia and iron deficiency anemia (IDA) may present similarly on peripheral blood smears—both showing microcytosis and hypochromia—their etiologies, clinical significance, and management differ substantially. Misdiagnosis may lead to inappropriate iron therapy in thalassemia carriers, underscoring the need for accurate distinction.

In our cohort, the  $-\alpha^{3.7}/-\alpha^{3.7}$  (trans) genotype emerged as the most prevalent, consistent with Mediterranean and Middle Eastern studies (9). This observation aligns with previous reports describing the predominance of the  $-\alpha^{3.7}$  deletion in regional populations and highlights the considerable genetic diversity of  $\alpha$ -thalassemia carriers in southwestern Iran.

When comparing cis and trans variants, MCH differences were statistically significant, with the cis- $\alpha$ -thalassemia group displaying lower values. This finding underscores the influence of gene arrangement on hematological phenotype: the cis configuration ( $\alpha\alpha/-$ ) results in a more pronounced reduction in  $\alpha$ -globin chain synthesis than the trans configuration, leading to greater hypochromia. Hemoglobin levels also differed modestly, with higher mean concentrations observed in the trans group. In contrast, although mean MCV values were numerically lower in the cis group than in the trans group, this difference did not reach statistical significance in our dataset and should therefore be interpreted cautiously.

When compared with IDA,  $\alpha$ -thalassemia minor patients demonstrated significantly higher RBC counts and hemoglobin levels, reinforcing their diagnostic value in clinical differentiation. These indices are particularly useful in settings where access to molecular testing is limited, providing a cost-effective initial screening tool. However, MCV and MCH did not significantly differ between  $\alpha$ -thalassemia and IDA, confirming that these parameters alone are insufficient for accurate discrimination.

The lower MCV and MCH observed in the cis- $\alpha$ -thalassemia group highlight the distinct hematological characteristics associated with different  $\alpha$ -thalassemia genotypic configurations. In our analysis, the average MCV of  $68.14 \pm 3.77$

fL in the cis group was lower than the  $72.15 \pm 4.03$  fL observed in the trans group. Similarly, MCH was reduced in cis individuals ( $20.58 \pm 1.49$  pg) compared with the trans phenotype ( $22.36 \pm 1.43$  pg). These findings indicate a greater reduction in hemoglobin content per red blood cell among cis carriers. In cis- $\alpha$ -thalassemia, the deletion of both  $\alpha$ -globin genes on the same chromosome results in a more substantial reduction in  $\alpha$ -globin production, predisposing individuals to more marked microcytosis and hypochromia (10–12). Consequently, cis carriers may present with more pronounced hematologic abnormalities, consistent with their significantly lower MCH values.

This concept is further supported by Bender, who emphasized that the severity of  $\alpha$ -thalassemia is closely linked to  $\alpha$ -globin gene arrangement (13). Because both  $\alpha$ -thalassemia and IDA produce microcytic red blood cells, misdiagnosis is common, and accurate differentiation remains essential in clinical practice (13, 14).

Our findings confirmed that mean hemoglobin levels in both cis- and trans- $\alpha$ -thalassemia groups were higher than in IDA patients, with the trans group showing the highest concentrations. This highlights their distinct hematologic profiles. Despite the presence of microcytic anemia, individuals with  $\alpha$ -thalassemia maintain relatively preserved or moderately reduced hemoglobin levels due to compensatory erythropoiesis.

Patients with trans- $\alpha$ -thalassemia often exhibit more balanced  $\alpha/\beta$ -globin chain synthesis, resulting in milder anemia compared with cis- $\alpha$ -thalassemia. This relative balance allows more effective erythropoiesis and better compensation for reduced hemoglobin production. In contrast, IDA results from insufficient iron availability, leading to impaired hemoglobin synthesis, reduced red cell production, and markedly decreased hemoglobin levels overall (15–17).

Additionally, the underlying pathophysiologic mechanisms differ substantially. In IDA, iron deficiency leads to significantly reduced hemoglobin synthesis, decreased hematocrit, and lower MCV. Although both  $\alpha$ -thalassemia and IDA present with microcytic, hypochromic anemia, IDA stems from decreased iron availability, whereas  $\alpha$ -thalassemia arises from



defective globin chain production (17).

$\alpha$ -thalassemia minor is characterized by partial  $\alpha$ -globin chain deficiency, often accompanied by elevated RBC counts as a compensatory response. This results in higher RBC counts without the profound reductions in hemoglobin levels typically seen in IDA. Previous studies have demonstrated that  $\alpha$ -thalassemia minor patients may maintain near-normal or mildly reduced hemoglobin levels, despite microcytosis, particularly when compared to IDA patients, in whom iron depletion severely restricts hemoglobin synthesis (16–18).

Although both disorders share microcytic, hypochromic features, these arise for different reasons. In IDA, iron deficiency limits hemoglobin production, producing small, pale cells (18,19). In  $\alpha$ -thalassemia, elevated RBC counts may mask the severity of microcytosis and hypochromia, making MCV and MCH less reliable as sole diagnostic metrics.

This diagnostic overlap underscores the limitations of relying exclusively on MCV and MCH values, emphasizing the need for comprehensive evaluation—including ferritin levels, hemoglobin electrophoresis, and genetic analysis—when differentiating microcytic anemias (20).

Standard diagnostic protocols for IDA rely on serum iron, ferritin, and TIBC. However, differentiating microcytic anemias often requires extensive and sometimes costly laboratory investigations. Our findings suggest that elevated RBC counts in  $\alpha$ -thalassemia and decreased counts in IDA can serve as useful preliminary markers for differentiation. Additionally, the greater decline in hemoglobin seen in IDA aligns with earlier studies (21,22).

Mehdi et al. (2011) examined hematological parameters in patients with different types of thalassemia and IDA, reporting significant differences in Hb levels between  $\beta$ -thalassemia and IDA, including a large effect size. They also noted that MCV and MCH were significantly lower in IDA compared with thalassemia patients, mirroring patterns observed in the present study (23).

Taken together, these findings highlight that elevated RBC counts and relatively preserved hemoglobin levels are more reliable indicators for distinguishing  $\alpha$ -thalassemia minor from IDA than red cell indices alone. This distinction has important clinical implications:  $\alpha$ -thalassemia carriers require monitoring and genetic counseling, whereas IDA patients benefit from iron supplementation and dietary interventions. Accurate differentiation is therefore crucial, particularly in premarital screening and population-based prevention programs where misclassification could have significant reproductive and public health consequences.

## Conclusion

This study provides a comprehensive comparison between  $\alpha$ -thalassemia minor and iron deficiency anemia (IDA), emphasizing the critical role of accurate differential diagnosis in clinical practice. The risk of iron overload resulting from inappropriate iron supplementation in patients with  $\alpha$ -thalassemia highlights the necessity of targeted treatment strategies based on precise diagnosis.

Our findings support the value of detailed hematological assessments in distinguishing between cis- and trans- $\alpha$ -thalassemia phenotypes and other causes of microcytic anemia. Specific hematological parameters, particularly RBC count and hemoglobin concentration, can serve as useful tools in differentiating these conditions when MCV and MCH values alone may be inconclusive.

The genotypic classification of  $\alpha$ -thalassemia into cis and trans configurations is also essential for effective genetic counseling, clinical management, and a deeper understanding of the disease's molecular pathophysiology.

Given the high carrier frequency of  $\alpha$ -thalassemia in regions such as Khuzestan, the ability to distinguish between cis and trans genotypes using hematological indices is particularly valuable. These findings may inform more cost-effective population screening strategies, guiding targeted genetic counseling efforts and helping prevent the transmission of severe thalassemia syndromes.

Moreover, these insights hold important implications for public health planning, particularly in regions with high carrier prevalence, by informing screening programs and preventative strategies aimed at reducing the burden of severe thalassemia syndromes.

### Limitations

This study has certain limitations. First, most participants were recruited through premarital counseling programs, which may introduce selection bias and limit the generalizability of findings to the broader population. Second, the iron deficiency anemia (IDA) cohort exhibited a highly skewed sex distribution, with 95.2% female representation. This imbalance reflects the higher prevalence of IDA in reproductive-age women but may also act as a confounding factor in group comparisons. Future studies with more balanced recruitment strategies and sex-adjusted analyses are recommended to validate and extend these findings. Third, non-deletional  $\alpha$ -thalassemia cases were excluded to ensure greater homogeneity in genotype-phenotype comparisons. While this allowed clearer interpretation of cis versus trans deletional variants, it restricts generalizability to non-deletional forms of  $\alpha$ -thalassemia minor, which may exhibit different hematological patterns. Notable limitation of this study is the exclusion of non-deletion mutations from our analysis. This decision was based on the limited number of cases available for non-deletion mutations, which significantly reduced the statistical power of our findings. Without a sufficient sample size, it becomes challenging to draw reliable conclusions about the prevalence and impact of these mutations on hematological parameters. Consequently, future research should aim to include a larger cohort of patients with non-deletion mutations to provide a more comprehensive understanding of their effects in thalassemia.

### Availability of Data

All data generated or analyzed during this study, as well as the rights to publish, are fully

available to the journal for editorial and academic purposes.

### Ethical Considerations

This study was reviewed and approved by the Medical Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, with the approval code IR.AJUMS.REC.1401.055.

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### Authors' Contributions

BKD, RSK, and AR were the major contributors to conceptualizing and formulating the research question and designing the study.

BKD, RSK, and AR were the leaders of the research and project team.

AFK, Akh, and SB collected and analyzed the data.

R.S.K. and A.F.K. wrote the first draft of the manuscript.

BKD and RSK critically studied and appraised the first draft.

A F K and AR revised and developed the first draft based on a critical appraisal of their colleagues. All authors commented on the modified draft, and the final version of the manuscript was prepared. Finally, all authors approved the final manuscript.

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## Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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