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# Hematologic Parameters Cut-off Assessment of Adult Alpha-Thalassemia Patients in Iran

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## ABSTRACT

**Background:** Thalassemia is one of the most common blood disorders in Iran. Alpha-thalassemia is caused by the deletion of the alpha-globin gene. The frequency of deletions in the alpha-globin gene is associated with microcytosis and hypochromia, making hematological parameters valuable predictive tools in the initial identification of alpha-thalassemia patients. This study aimed to compare hematologic parameters such as Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), red blood cell (RBC) count, and hemoglobin (HGB) levels in silent and minor patients, whose genotypes were genetically characterized, with normal patients to establish cut-off points for these groups.

**Materials and Methods:** The study involved a total of 860 patients with alpha-thalassemia, including 267 cases of silent, 261 cases of minor, and 332 cases of normal alpha-thalassemia.

**Results:** Analysis of blood indices based on sex revealed that the male group had higher values than the female group. Assessment of alpha-thalassemia in minor patients showed that the Cis form (—/αα) had higher microcytosis than the Trans form (−α/−α) in this group. This difference was also observed between $\alpha^{-3.7}\alpha$/ $\alpha^{-3.7}\alpha$ and $(\alpha\alpha)^{-MED}$/αα as two different genetic forms in minor patients, with $(\alpha\alpha)^{-MED}$/αα being in the Cis form. Data indicated that the cut-off value was insignificant in silent patients compared to the normal group. However, minor patients with MCH≤23.7 and MCV≤74.9 had an AUC greater than 0.9 (p-value< 0.01), distinguishing them from the normal group.

**Conclusion:** Comparing hematological parameters in these groups illustrated that MCV and MCH are the best predictor parameters for distinguishing between groups.

**Keywords:** Alpha thalassemia; Anemia; Mean corpuscular volume (MCV); Mean corpuscular hemoglobin (MCH)

## INTRODUCTION

Thalassemia, a group of genetic disorders resulting in a variety of phenotypes, ranks among the top five most prevalent birth defects in humans due to a disorder of globin gene synthesis[1]. Alpha-thalassemia, an inherited autosomal recessive disease, is caused by a reduction or absence of alpha hemoglobin synthesis. This phenomenon is caused by an excess of the beta chain of hemoglobin[2]. The prevalence of alpha-thalassemia is high in Southeast Asia, the Mediterranean, the Basin, the Middle East, India, and Sub-Saharan Africa[3]. Iran is also in a high-incidence region of thalassemia, so around 2 million of the Iranian population are carriers of the disease. The most prevalent region of thalassemia in Iran is situated in the northern and southern provinces, where the carrier rate of alpha-thalassemia is around 35% [4].

Each person has a pair of two types of alpha hemoglobin genes (α1, α2), with each gene located

on one arm of chromosome 16[3]. The severity of the disease depends on the number of affected alpha hemoglobin genes. Based on this, the clinical manifestation of alpha-thalassemia is divided into four classes: 1) Silent carrier, identified as heterozygous α+-thalassemia (-α/αα). These patients have normal hemoglobin or mild hypochromic anemia. 2) α-thalassemia trait, characterized as heterozygous α0-thalassemia (--/αα) or homozygous α+-thalassemia (-α/-α). Patients with this condition typically experience mild anemia. 3) HbH disease with three inactive alpha-thalassemia genes in the heterozygous α+/α0-thalassemia format (--/-α). These patients present with moderate hemolytic anemia. 4) Hb Bart's, where patients lack all four normal alpha hemoglobin forms identified as homozygous α°-thalassemia (--/--), characterized by hydrops fetalis syndrome. Silent carriers and those with the α-thalassemia trait are usually asymptomatic and do not require treatment. Patients with HbH disease typically have moderate anemia, often accompanied by hepatosplenomegaly. Prenatally, Hb Bart's can lead to hydrops fetalis and is fatal if not treated with intrauterine blood transfusions[5]. The type of mutation in the alpha-globin gene is grouped into deletion (total deletion (α°) or partial deletion ($\alpha^{+}$)) or non-deletion forms. The deletion form of the alpha-globin gene is a more common mutation in alpha-thalassemia[5]. The most common forms of alpha-thalassemia partial deletion are $-\alpha^{3.7}$ and $-\alpha^{4.2}$, and for α°-thalassemia, they are ($--^{Med}$) and South East Asia deletion ($--^{SEA}$).

One of the fundamental symptoms characteristic of patients with alpha-thalassemia carriers is a reduction in blood indicators such as MCV (mean corpuscular volume) and MCH (mean corpuscular hemoglobin), with little to no significant changes in hemoglobin levels[6,7]. The precise identification of these hematological features could be extremely useful in selecting appropriate molecular tests to detect carrier mutations in patients[3]. Given this knowledge, this study focuses on assessing the relationship between blood indicators in silent and minor patients with alpha-thalassemia. The type of their mutations was identified through a molecular test and compared to normal levels to update the accurate cut-off ratio of these blood indicators.

## Materials and Methods

This study was conducted on 860 out of 2344 patients with alpha hemoglobin blood disorder who were treated at Baghaei Hospital in Khuzestan, the southwest province of Iran, between 2006 and 2020. The ethical code for this study was IR.AJUMS.REC.1401.029. Patients were selected based on the criteria that they did not have iron deficiency (>15µg/dl), chronic diseases, or other hemoglobinopathies. Out of the total patients, 425 were women, 435 were men, and children were not included in the study. The average age of the patients was 25.5±4.6, ranging from 17 to 42 years old. Molecular techniques such as Gap-PCR, strip-PCR, and sequencing were used to identify mutations in the study group. Additionally, routine hematological tests were performed to determine blood indicators such as hemoglobin concentration, MCV, MCH, HGB, and RBC. Statistical analysis of blood indicators was conducted using the STATA software with t-test and one-way ANOVA methods for patients in the minor, silent, and normal groups.

## RESULTS

### Assessment of blood indices among patient groups

Using molecular techniques, 860 patients were classified into normal (n=332), minor (n=261), and silent (n=267) patient groups, with each group further divided by sex (Table 1). The data showed that the distribution of sex within each group was not significant (p-value = 0.26), indicating an equal representation of females and males in each patient group. The average blood indices among patient groups displayed a significant difference in blood indices across all groups (p-value<0.0001).

Notably, the MCV value in each group had a higher Standard Deviation (SD) compared to other blood indices, suggesting a wider distribution range for this value. The ranges of MCH, MCV, RBC, and HGB for each patient group are outlined in Table S1. Specifically, the MCH and MCV indices ranged from 17.6-29.3 and 56.1-96, respectively, in normal individuals, while these ranges decreased in silent patients to 18.7-28.1 and 64.6-87. This decrease in

both MCH and MCV indices was more pronounced in minor patients, with MCH ranging from 15-26.6 and MCV from 70.9±11.7.

When comparing the HGB levels of the normal group versus minor and silent patients, a decrease in the HGB range was observed from 5.6-17.9 in the normal group to 9.7-16.6 and 12.9-16.4 in silent and minor patients, respectively. Additionally, RBC indices showed an increase in the RBC range from 1.7-6.6 in the normal group to 3.95-6.95 and 3.6-9.7 in silent and minor patients. The minimum and maximum ranges of blood indices by sex are provided in Table S2. The data indicated that, across all patient groups, the minimum blood indices in men were higher than in women, however, this observation was not seen in the maximum range. The MCV value in the normal group showed that women had an MCV maximum value of 96, while men had a maximum value of 88.9. This trend was also evident in RBC indices in the minor patient group, where women had higher RBC values (9.7) compared to men (7.2). Comparing blood indices between silent and minor patients revealed that all blood indices, except RBC, were significantly lower in minor patients than in silent patients (Table S3).

**Table 1:** Bassline characteristic

| | | Type = Normal N=332 | Type = Minor N=261 | Type = Silent N=267 | P |
|---|---|---|---|---|---|
| Sex code | Female | 165 (49.7%) | 138 (52.9%) | 122 (45.7%) | 0.26 |
| | Male | 167 (50.3%) | 123 (47.1%) | 145 (54.3%) | |
| HGB | | 13.37±1.72 | 12.30±1.64 | 13.34±1.467 | <0.001 |
| MCH | | 25.98±1.61 | 21.91±1.72 | 24.93±1.40 | <0.001 |
| MCV | | 79.69±4.20 | 70.90±4.82 | 77.92±3.95 | <0.001 |
| RBC | | 5.16±0.61 | 5.65±0.71 | 5.35±0.59 | <0.001 |

HGB: Hemoglobin, MCH: Mean Corpuscular Hemoglobin (pg), MCV: Mean Corpuscular Volume (fL), RBC: Red Blood Cell (10E6/µl)

**Blood indices assessment in silent alpha-thalassemia patient study group**

Statistical analysis of blood parameters between the normal group and silent patients with (-α/αα) revealed a significant difference (p-value <0.05) in blood indices in silent alpha-thalassemia patients compared to the normal group, except for HGB levels (Table 2). The MCH and MCV values were lower in the silent alpha-thalassemia group compared to the normal group, while the RBC indices in the silent alpha-thalassemia patients were significantly higher than in the normal group. Comparing blood parameters based on sex in these two groups showed that except for HGB, other blood parameters were significantly different (p-value <0.05) in normal and silent patients based on their sex (Table 3). Additionally, data indicated that the most significant blood parameter between the two sexes was MCH indices, with a p-value <0.001. Frequency analysis of silent alpha-thalassemia patients based on genotype revealed that $\alpha^{-3.7}\alpha/\alpha\alpha$ was the most frequent genotype (171 cases out of 267 cases). The second most frequent alpha-thalassemia genotype belonged to $\alpha^{CD19(-G)}$ α/αα (28 cases out of 267 cases). For this reason, the blood parameters of these two mutations were statistically analyzed in comparison to the normal group (Table S4). The data revealed that all blood parameters in $\alpha^{-3.7}\alpha/\alpha\alpha$ were statistically significant (p-value<0.05), except for HGB blood indices. The statistical analysis of blood parameters in the $\alpha^{CD19(-G)}$ α/αα patient group showed differences in all blood indices compared to the normal group. However, only the MCH parameter showed a significant difference (p-value<0.05), while other blood indices did not exhibit statistical variances compared to the normal group.

The analysis of cut-off values for MCH and MCV in silent patients indicated an AUC of ≤0.7(p-value<0.01). The MCH cut-off value of ≤25.8 provided a sensitivity of 75.28 and a specificity of 60.24. The MCV cut-off value of ≤78.6 provided a sensitivity of 56.66 and a specificity of 67.77.

**Table 2:** Statistical analysis of blood parameters between silent patients(-α/αα) and the normal group

| Blood indices | Normal | Silent (-α/αα) | Mean diff | P |
|---|---|---|---|---|
| MCH | 25.98±1.61 | 24.93±1.4 | -1.05 | <0.001 |
| HGB | 13.37±1.72 | 13.34±1.4 | -0.03 | 0.97 |
| MCV | 79.69±4.20 | 77.92±3.9 | -1.7 | <0.001 |
| RBC | 5.16±0.61 | 5.35±0.5 | 0.19 | 0.001 |

Data is presented as mean±SD. A p-value less than 0.05 is considered statistically significant

**Table 3:** Statistical analysis of blood parameters based on sex between the normal and silent alpha-thalassemia groups

| Blood indices | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| | Normal | Silent | Mean diff | p | Normal | Silent | Mean diff | P |
| MCH | 26.2±1.4 | 25±1.3 | -1.19 | <0.001 | 25.7±1.7 | 24.8±1.1 | -0.93 | <0.001 |
| HGB | 14.4±1.3 | 14.1±1.1 | -0.26 | 0.2 | 12.2±1.2 | 12.3±1.1 | 0.06 | 0.92 |
| MCV | 79.4±3.8 | 77.7 ±3.9 | -1.68 | 0.002 | 79.9±4.5 | 78.1±3.9 | -1.83 | 0.003 |
| RBC | 5.5±0.4 | 5.6±0.4 | 0.19 | 0.001 | 4.7±0.4 | 4.9±0.4 | 0.19 | 0.001 |

Data is presented as mean±SD. A p-value less than 0.05 is considered statistically significant

**Blood indices assessment in minor alpha-thalassemia patient study group**

A statistical analysis of blood parameters in minor alpha-thalassemia patients indicated that all blood parameters were significantly different (p-value<0.05) compared to the normal group (Table 4). When comparing blood parameters between Cis and Trans minor patients, it was found that MCH and MCV indices showed significant differences between the two groups, while HGB and RBC were not statistically significant (Table S5). Specifically, the MCV and MCH indices in Cis minor patients were lower than in Trans patients.

Analysis of blood parameters based on sex in minor patients revealed that all blood parameters in minor male patients were higher than in minor female patients (Table S6). Additionally, comparing blood parameters based on sex in minor patients to the normal group showed significant differences (p-value<0.05) between the two groups.

Frequent genetic mutation analysis in the minor patient group revealed that $\alpha^{-3.7}\alpha/\ \alpha^{-3.7}\alpha$ was the most common genetic mutation (145 cases out of 261 cases) among minor patients. The second most common genetic form in the minor group was $(\alpha\alpha)^{-MED}/\alpha\alpha$ (38 cases out of 261 cases). Statistical analysis of these two genetic mutations compared to the normal group showed significant differences in all blood parameters (p-value<0.05) (Table 5). Furthermore, comparing blood parameters between these two genetic mutations and the normal group revealed that $(\alpha\alpha)^{-MED}/\alpha\alpha$ had a lower blood parameter value compared to the $\alpha^{-3.7}\alpha/\ \alpha^{-3.7}\alpha$ mutation type.

The analysis of the MCV cut-off point showed an AUC of 0.92 (p-value <0.001) in minor patients. An MCV value ≤ 74.9 provided a sensitivity of 83.4 and a specificity of 92.47 in this group. The analysis of the MCH cut-off value yielded an AUC of 0.95 (p-value <0.001). An MCH cut-off value of ≤23.7 provided a sensitivity of 87.31 and a specificity of 93.98.

**Table 4:** Statistical analysis of blood parameters among minor patients (–α/–α), (—/αα), and the normal group

| Blood indices | Normal | Minor(–α/–α), (—/αα) | Mean diff | P |
|---|---|---|---|---|
| MCH | 25.98±1.61 | 21.91±1.72 | -4.07 | <0.001 |
| HGB | 13.37±1.72 | 12.30±1.64 | -1.07 | <0.001 |
| MCV | 79.69±4.20 | 70.90±4.82 | -9.67 | <0.001 |
| RBC | 5.16±0.61 | 5.65±0.71 | 0.53 | <0.001 |

Data are presented as mean±SD. A p-value below 0.05 is considered statistically significant.

**Table 5:** Statistical analysis of blood parameters among the normal group and two different genotypes ($\alpha^{-3.7}\alpha/\ \alpha^{-3.7}\alpha$ and $(\alpha\alpha)^{-MED}/\alpha\alpha$) of minor alpha-thalassemia.

| Blood indices | Normal | $\alpha^{-3.7}\alpha/\ \alpha^{-3.7}\alpha$ | Mean diff | P | $(\alpha\alpha)^{-MED}/\alpha\alpha$ | Mean diff | P |
|---|---|---|---|---|---|---|---|
| | | n=145 | | | N=38 | | |
| MCH | 25.98±1.61 | 22.3±1.4 | -3.6 | <0.001 | 20.6±1.0 | -5.3 | <0.001 |
| HGB | 13.37±1.72 | 12.4±1.6 | -0.95 | <0.001 | 12.0±1.4 | -1.27 | <0.001 |
| MCV | 79.69±4.20 | 72.1±4.1 | -7.4 | <0.001 | 67.8±3.1 | -11.8 | 0.004 |
| RBC | 5.16±0.61 | 5.5±0.6 | 0.41 | <0.001 | 5.8±0.6 | 0.64 | <0.001 |

The data is presented as mean±SD, A p-value of less than 0.05 is considered statistically significant.

## DISCUSSION

Alpha-thalassemia is the most common genetic disorder globally[8]. Various laboratory red blood cell parameters, along with genetic tests, play a promising role in diagnosing alpha-thalassemia[8,9]. Nevertheless, the first step in recognizing alpha-thalassemia is based on blood cell parameters[10]. Therefore, optimizing and updating the cut-off value of blood cell parameters could help reduce the substantial number of patients who need DNA analysis for recognition [11, 12].

In this study, the red blood cell parameters were evaluated in silent and minor group patients whose type of mutations have been identified by genetic tests compared to the normal group to discriminate the cut-off range of each blood parameter in each patient group. Data in this study showed that the mean blood indices parameter in the normal group were HGB 13.37±1.72, MCH 25.98 ±0.61, and MCV 76.69±4.2, which was lower than the mean blood indices study analysis in Amazon people in Brazil that reported the mean blood indices in the normal group were HGB 14.13±1.28, MCH 29.82±1.82, MCV 90.39±4.42[9] and the work of Velasco-Rodríguez et al. were HGB 14.69±1.32, MCH 28.32±0.91, MCV 83.97±1.31[8.] The only exception is the RBC mean, which was higher in the current study (5.16±0.6) compared to the Amazon group study (4.74±0.43)[9]. No differences were observed in RBC indices in the study of Velasco-Rodríguez et al.[8] and this work.

The mean of each blood indices parameter in this study following the study of Doosti Irani et al. work that found the mean of MCH and MCV in the normal group people in Shadegan were 24.90±2.36 and 77.40±6.03, respectively [13] and the study of Ahmad et al. that illustrated the mean of MCH 23.9±3.9 and MCV 75.1±10.0 in the normal group of the Malaysia population[14]. These differences could be due to genetic variation and environmental factors that affect the parameters of blood indices[15]. Although the distribution of the study group based on sex in Anselmo et al.'s work was 72.1% male and 27.9% female[9], this work had an equal distribution of patients based on sex, which could affect the mean result of blood parameters.

Comparing blood parameters in the normal group based on sex illustrated that HGB indices in males were 14.4±1.3, which was higher than HGB 12.2±1.2 in females, while other blood parameters did not

show significant differences in these two sex groups. This data was compatible with previous studies that found HGB is higher in the normal male group (14.6±1.1) than female (12.9±1.1). Comparing the blood indices parameters between normal and silent alpha-thalassemia in this study, it was discovered that MCV and MCH were the most significant parameters (p-value <0.001) to distinguish between the two groups. This output was compatible with the previous studies, which found that MCV and MCH are powerful parameters for discrimination [8, 11, 12, 14]. Assessment of blood indices parameters in frequent genetic mutation ($\alpha^{-3.7}\alpha/\alpha\alpha$ and $\alpha^{CD19(-G)}\alpha/\alpha\alpha$) in silent patient groups compared to the normal group discovered that $\alpha^{-3.7}\alpha/\alpha\alpha$ showed more significant differences in blood indices (p-value<0.001) except HGB compared to the normal group than $\alpha^{CD19(-G)}\alpha/\alpha\alpha$ genotype. The blood parameter assessment in minor alpha-thalassemia patients showed all blood indices reduced (p-value<0.001) compared to the normal group, especially MCV and MCH values, which represented drastic differences. This result is compatible with Velasco-Rodríguez et al. work that found MCV and MCH were the most powerful parameters to predict minor alpha-thalassemia[8]. Also, according to the result of this author, the Cis format (—/αα) of minor patients illustrated a higher reduction in MCV and MCH value than the Trans format (–α/–α)[8]. The output of Velasco-Rodríguez et al. work[8] is compatible with the current work, in which the MCV and MCH values of Cis minor patients were lower than the Trans form. Comparing blood indices based on sex in the minor patients group revealed that, like silent patients, male patients with minor alpha-thalassemia had higher blood index values than females. Analysis of blood indices in two prevalent genetic mutations in the minor group discovered that $(\alpha\alpha)^{-MED}/\alpha\alpha$ showed more significant alteration in blood parameter value compared to $\alpha^{-3.7}\alpha/\ \alpha^{-3.7}\alpha$ mutant, especially in MCV and MCH value, which could be due to the Cis form of $(\alpha\alpha)^{-MED}/\alpha\alpha$.

According to the statistical analysis of this study, MCH is the most significant indicator for screening alpha-thalassemia. This interpretation is compatible with previous work[8].

The cut-off value analysis indicated that the MCV (AUC<0638) and MCH cut-off values were not accurate enough to detect silent patients from the normal form, which was incompatible with the result of Velasco-Rodríguez et al., who illustrated that these two parameters were excellent indices to distinguish these two groups[8]. These differences could be due to the smaller sample size of Velasco-Rodríguez et al. (normal group=40 and silent group =56) compared to the current study (normal group=332 and silent group=267), which caused the wider distribution of MCV and MCH values in each group in addition to the genetic population variation between these two studies. The cut-off value analysis in minor patients demonstrated that the MCH and MCV values had AUCs of 0.95 and 0.92, respectively, which could precisely distinguish minor patients from normal patients.

## CONCLUSION

This study aimed to determine the cut-off differences in normal, silent, and minor patients. The data revealed that blood parameter values in the normal group varied by geographic region, indicating that cut-off values of blood indices should be characterized in each region of the world. Additionally, the results showed that MCV and MCH were the most significant indicators for predicting silent and minor patients, especially MCH. The data also indicated that females had lower MCH and MCV values compared to men, suggesting that the range of MCH and MCV should be analyzed based on sex.

Although slight significant differences in blood parameters were observed between αCD19(-G)α/αα and α-3.7α/αα as two distinct genetic mutations in silent patients, in minor patients, the blood indices values in the (αα)-MED/αα group changed more significantly compared to the α-3.7α/ α-3.7α group. Therefore, predicting alpha-thalassemia patients' type of genetic mutation based on blood parameters using cut-off points in each group could be highly applicable in the clinic. This approach requires a cohort study with a large group of patients to establish a more precise cut-off to accurately characterize alpha-thalassemia patients.

## CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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**Abbreviations**

MCV (Mean corpuscular Volume), MCH (Mean corpuscular hemoglobin), RBC (red blood cell), HGB (hemoglobin)

**Declarations**

**Ethics approval and consent to participate**

This study was performed following the guidelines and approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1401.029). The written form of consent was given from all participants.

**Consent for publication**

Not applicable

**Availability of data and materials**

All data of this study is available in this article.

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