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Original Article

Blood Indices of the Patients with β-Thalassemia Minor Compared to the Patients with β-Thalassemia Minor-Alpha-Thalassemia

Bijan Keikhaei Dehdezi, Najmeh Nameh Goshay Fard*

Thalassemia & Hemoglobinopathy Research Center, Health research institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

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Abstract

Objective: Thalassemia, as one of the most common genetic diseases, is a group of hereditary hemoglobin disorders due to a slight disturbance in the production of alpha and beta globin chains in the structure of hemoglobin. There are still no clear criteria for differentiating thalassemia types based on hematological findings. In the current study, we aimed to evaluate the low-grade beta-thalassemia (β-thalassemia) indices in comparison with β-thalassemia minor with alpha-thalassemia (α-thalassemia).

Methods and materials: In this descriptive-analytic study, 120 patients were enrolled, including 80 patients with minor β-thalassemia and 40 patients with minor β-thalassemia with α-thalassemia. Of all patients, 5cc blood samples were taken. The red blood cell parameters including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and hemoglobin were determined. The level of MCV> 80 and A2> 3.5 β-thalassemia minor and MCV <80 and A2 <3.5 were considered as elevated thalassemia or iron deficiency anemia.

Results: The results showed that the mean of hemoglobin, HCT, MCH, MCHC, and MCV in the β-thalassemia group was significantly lower than that of the β-thalassemia with α-thalassemia group (P <0.0001). On the other hand, the level of these indices in the control group was significantly higher than in the two groups of patients (P<0.0001). The results showed that the percentage of hemoglobin A2 in the β-alpha-thalassemia group was 4.5 ± 0.91, significantly higher than the β-thalassemia group. The rate of hemoglobin and MCV was significantly lower in the β-thalassemia group compared to the silent and trait β+αthalassemia group. Also, the rate of hematocrit was significantly lower in the β-thalassemia group compared to the trait, although had no significant difference with the silence group.

Conclusion: Based on our findings, despite the difference between some hematocrit indices in the patients with β-thalassemia and β-αthalassemia, these indices cannot be used as differential indices.

Keywords: β-Thalassemia; β-α-Thalassemia; Mean Corpuscular Volume (MCV); Mean Corpuscular Hemoglobin (MCH)

***Corresponding Author:** Najmeh Nameh Goshay Fard, MD
Thalassemia & Hemoglobinopathy Research Center, Health research institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
E-mail: najmehnamehgoshayfard@gmail.com

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Introduction

Thalassemia is categorized by abnormal production or reduction in the rate of formation of normal α- or β-globin subunits of hemoglobin (Hb) A (1, 2). Mutations in genes encoding αand βglobin chains cause αand βthalassemia, respectively(3). β-thalassemia major is an inherited hemoglobinopathy that requires lifelong red blood cell (RBC) transfusions, and iron chelation therapy to prevent complications due to iron overload(4).

Currently, many mutations have been reported, including types of deletions and point mutations. Thalassemia manifests with symptoms such as anemia, enlarged spleen, and bone changes. According to the type of mutation and the severity of the involvement, the patient's clinical symptoms vary from asymptomatic to severe and fatal anemia(5).

In addition to clinical symptoms, laboratory findings are also important for the final diagnosis of thalassemia. The most important laboratory findings of screening for thalassemia diagnosis are the changes in RBC indices. The severity and clinical manifestations of the disease change depending on the type of thalassemia, blood indicators such as MCV, and MCH. The change of these indicators is especially important for the identification of carriers(6, 7). B-thalassemia major is easily diagnosed based on physical findings as well as laboratory parameters, but the differential diagnosis of β-thalassemia minor is not easy based on disease presentation or common laboratory findings alone(8). The diagnosis of thalassemia minor is usually based on the presence of microcytic hypochromic anemia according to RBC distribution width (RDW), MCV, MCH, and other parameters(9), But other microcytic anemias can present with symptoms similar to β-thalassemia minor, which is important in their differential diagnosis(10). The use of electrophoresis or chromatography methods in evaluating the amount of hemoglobin A2 (HbA2) has been suggested as a specific indicator in the diagnosis of β-thalassemia minor(11).

Considering that failure to diagnose β-thalassemia cases or misdiagnosis with α-thalassemia cases can increase the risk of birth of babies with β-thalassemia major or intermedia. The diagnosis of β-thalassemia types among thalassemias is more significant. Hence, in the current study, we aimed to evaluate the low-grade β-thalassemia indices compared with β-thalassemia minor with α-thalassemia in Shafa Hospital in Ahvaz, Iran.

Method and Materials

In this descriptive-analytical study, 120 children over 10 years of age referred to the hematology clinic of Shafa Ahvaz Hospital, whose diagnosis of microcytic hypochromic anemia was based on genetic testing, were included. Exclusion criteria were multifactor anemia such as chronic disease anemia because of hemoglobinopathies, iron deficiency anemia along with minor thalassemia, moderate, and severe anemia. Demographic and hematological data (hemoglobin, RBC count, MCV, MCH) of each patient were recorded in a checklist. The data were based on the clinical and laboratory symptoms and examination results in the patients' files. Patients were divided into two groups, including 80 patients with minor β-thalassemia, and 40 patients with minor β-thalassemia with α-thalassemia. 5 ml of blood was taken from all patients and evaluated by cellulose acetate electrophoresis with alkaline pH, and RBC indices including MCV, MCH, MCHC, and hemoglobin were determined. Serum iron binding capacity, ferritin, and serum hemoglobin A2 were checked in all patients. In order to determination of the ratio of the alpha and beta chains, werdal methods were used and its analysis was performed with HPLC. Children suspected to have iron deficiency anemia were treated with iron, and the genetic examination of alpha and beta chains was applied for all patients. Two groups were compared in terms of hematocrit parameters (hemoglobin, hematocrit, RBC, TRBC, MCV, MCH, MCHC). The cut-off index was evaluated between β-thalassemia and β-thalassemia with α-thalassemia, and the sensitivity, positive, and negative predictive value of this index were evaluated in these patients. The study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Ethics number: IR.AJUMS.REC.1397.431). Written informed consent was obtained from all patients or their parents.

Statistical analysis:

Statistical analysis was carried out by SPSS version 22. The quantitative and qualitative variables were indicated as mean±SD and number (percentage), respectively. Differences were compared by using the chi-square/Fisher's exact tests as appropriate. Also, ROC analysis was used in order to calculate sensitivity and specificity. P-value less than 0.05 was considered statistically significant.

Results

In our study, 120 patients including 80 patients with β-thalassemia minor and 40 patients with βthalassemia minor + α-thalassemia were examined. Male gender in the β-thalassemia group and β-thalassemia + α-thalassemia group were 36 (45%) and 26 (65%), respectively. There was no significant difference in the gender of the two groups (P>0.05). The mean hemoglobin, hematocrit, MCH, MCHC, and MCV in the

β-thalassemia group were significantly lower compared to the β-thalassemia + α(P<0.0001), and these indicators were significantly higher in the control group than both groups(P<0.0001). There was no significant difference in the average number of RBCs in the two studied groups (P>0.05). More details are provided in **Table 1**.

According to **Table 2**, the evaluation of hematocrit parameters based on gender showed that in both groups, the level of RBC, hematocrit, and hemoglobin in boys are significantly higher than in girls. Hematocrit, MCH, and MCV showed a significant difference in boys with β-thalassemia minor and β-thalassemia + α-thalassemia patients (P<0.05).

Table 1. Comparison of gender and hematocrit parameters in both groups

Parameter	Control group (n=40)	β-thalassemia group (n=80)	β+α-thalassemia group (n=40)
Gender: Male, n, (%)	20 (50%)	36 (45%)	26 (65%)
RBC	5.61 ± 0.11	5.90 ± 0.07	5.78 ± 0.14
Hb	14.71 ± 1.03	10.63 ± 0.09ab	11.84 ± 0.20 ab
HCT	43.19 ± 2.16	36.06 ± 0.47ab	38.75 ± 0.93ab
MCV	87.6 ± 2.34	62.80 ± 0.51ab	68.48 ± 0.87ab
MCH	22.63 ± 1.14	19.59 ± 0.17ab	20.87 ± 0.32 ab
MCHC	33.40 ± 1.7	29.09 ± 0.41a	30.73 ± 0.56

a, indicates the significant difference with the control group; b, indicates the significant difference with involved groups.

Table 2. Comparison of the rate of hematocrit parameters in studied patients based on gender

Parameter	Control		β-thalassemia		β+α-thalassemia	
	Girl	Boy	Girl	Boy	Girl	Boy
RBC	5.13 ± 0.58	5.35 ± 0.91	5.63 ± 0.52ab	6.25 ± 0.56b	5.22 ± 0.87a	6.08 ± 0.76a
HCT	42.2 ± 3.4	41.3 ± 4.6	34.77 ± 3.88ab	37.64 ± 4.07abc	34.79 ± 4.04ab	40.88 ± 5.59 abc
Hb	13.1 ± 8.6	15.2 ± 6.4	10.34 ± 0.89a	10.99 ± 0.63 ac	10.84 ± 0.96 ab	12.38 ± 1.04 abc
MCV	88.7 ± 2.5	86.7 ± 8.0	63.08 ± 5.56a	62.46 ± 3.06 ac	68.09 ± 4.23 a	68.69 ± 6.15ab
MCH	29.2 ± 0.5	29.2 ± 2.6	20.03 ± 1.15a	19.04 ± 1,27ac	21.59 ± 2.45a	20.48 ± 1.68 ac
MCHC	32.2 ± 9.2	32.3 ± 79.4	29.55 ± 3.87	28.53 ± 3.33 a	31.79 ± 3.51	30.15 ± 3.46a

a, indicates a significant difference with the control group based on gender; b, indicates the intra-group significant difference based on gender; c, indicates the significant inter-group difference based on gender.

Determination of βand α-thalassemia mutations

Based on **Table 3**, the evaluation of mutation of the alpha chain of the β+α-thalassemia group showed that 32 patients (32%) had (α α /– α) type, 6 patients (15%) had cys (α α /––), and 4 patients (10%) had trans (α –/ α–) type, also, the most common mutation of the beta chain was CD 36/37 in patients of both groups.

According to **Table 4**, the rate of hemoglobin and MCV is significantly lower in the β-thalassemia group compared to the silent and trait β+α-thalassemia group, and the rate of hematocrit was significantly lower in the β-thalassemia group compared to the trait, although had no significant difference with silence group. Moreover, the MCH of the β-thalassemia group was significantly lower than that of the silent group (P<0.05).

Table 3. Type and frequency of mutations in β+α-thalassemia group

Type of mutation	β-thalassemia group	β+α-thalassemia group		
	Beta chain	Beta chain	Mutation in the alpha chain	
CD 36/37	43	16	(α α /– α)	32
IVS II-I	13	12	cys (α α /––)	4
CD39	4	1	trans (α –/ α–)	4
IVS I-110	8	2		
CD8/9	5	-		
IVSI 16	1	-		
IVS II-745	2	-		
ATG-ACG	1	-		
CD 44	3	-		
-57 A>T	-	1		
Fr 8-9 (+G)	-	1		
28 (C-A)	-	1		
88 (C-A)	-	1		
IVSI-S	-	1		
IVSI-b (C-T)	-	1		
CDS (-CT)	-	1		
CD1S(TGG-TGA)	-	1		
IVS II 848	-	1		

Table 4. Blood signboard of studied groups based on a mutation in the alpha chain

Parameter	β-thalassemia group	β+α-thalassemia Trait	β+α-thalassemia Silent
RBC	5.9 ± 0.07	5.61 ± 0.11	5.78 ± 0.14
Hb	10.63 ± 0.09ab	12.18 ± 1.74	11.76 ± 1.11
HCT	36.06 ± 0.47a	40.88 ± 8.34	38.22 ± 5.08
MCV	62.80 ± 0.51ab	68.69 ± 9.01	68.36 ± 4.34
MCH	19.59 ± 0.17b	20.98 ± 2.46	20.84 ± 1.95
MCHC	29.09 ± 0.41b	29.75± 2.81	30.97 ± 3.67

a, indicates the significant difference between beta and trait group; b, indicates the significant difference between β-thalassemia and silent; c, indicates the significant difference between silent and trait.

Discussion

The results of our study showed that the hematocrit indices in patients with β-thalassemia and β+α-thalassemia showed that the mean hemoglobin, hematocrit, MCH, MCHC, and MCV in the β-thalassemia group were significantly lower compared to the β+α-thalassemia group. Several studies comparing blood indices in different types of beta thalassemia showed different results. In the study by Khatami et al., which was conducted with the aim of synthesizing globin chains in order to differentiate β-thalassemia carriers from α-thalassemia, the results showed that mean hemoglobin, mean hematocrit, MCV, MCH, and MCHC were significantly reduced in β-thalassemia compared to the α-thalassemia and delta-thalassemia(12). The results of this study were similar to our study. In the study of Khatami et al., it was shown that the average of the above indices was significantly higher in all types of thalassemia in males compared to females. Also in separate evaluations, blood indices were lower in minor β-thalassemia in both genders compared to the α-thalassemia, this difference can be because of differences in evaluated samples, such a way that in our study minor β-thalassemia is compared with β+α-thalassemia. The type of mutation in thalassemia has shown wide variations in the range of MCV.

Limited data are reported in the literature for the prevalence of α gene deletion in patients with microcytosis(13). In the study of Mehdi et al., 991 patients were inserted to study to evaluate the hematologic parameters to differentiate between β-thalassemia and α-thalassemia. In this study, microcytic anemia was the commonest one. Also(-α/-α) and (--/αα) mutations were the most common type of α-thalassemia. This study was not parallel with our study, the reason for this can be because of differences in geographical region and sample size(3). In the study by Joola et al., overall 35 BT mutations were found, which were the most common mutations based on prevalence rateIVSII-1(G>A) (26.1%), cd36/37(-T) (18.4%), IVSI-110(G>A) (9.9%) and IVSI-5(G>C) (6.8%), respectively (14). In the study of Saki et al. in Ahvaz, the most common mutation in the alpha chain was (α α /– α) type similar to our study, which the reason for this similarity can be related to the similar geographical distribution of both studies. On the other hand, the most common mutation in the beta chain were CD-6 HbS and IVS II-I in Saki et al. study(15, 16).

In the study by Mehdi et al., hematological parameters in patients with α-thalassemia were compared with those in patients with β-thalassemia. Individuals with the single-gene deletion had lower levels of hemoglobin, MCV, and MCH as compared to normal controls. The carriers of α-thalassemia have mild microcytic hypochromic anemia as observed by other authors(17).

However, their MCV and MCH were better than those of patients with iron deficiency anemia. MCH is a better discriminator than other red cell indices in the diagnosis of α-thalassemia(18, 19). Since there is no definitive hematological marker that can give the diagnosis of α-thalassemia, molecular analysis remains the only diagnostic approach in microcytic hypochromic anemia patients. Our findings are in accordance with previous reports, where microcytosis was explained based on the number of α-gene deletions(18, 20).

Conclusion

The results of the present study showed that there are some differences between some hematocrit indices of patients with β-thalassemia and β+α-thalassemia, but these indices are not suitable to use as a differentiation index. The comparison of these parameters based on gender showed better results. this issue, therefore, needs more research in higher sample sizes. The identification of α and β-thalassemia carrier status is important before going for expensive investigations to define the etiology of anemia, as well as to prevent unnecessarily prolonged iron supplementation. Thus, screening for thalassemia should be considered during genetic counseling of couples at high risk of thalassemia, for prenatal and premarital diagnosis. Detection of rare thalassemia gene mutations in our individuals was essential because of consanguineous marriages in Iran.

Conflict of interests

There is no conflict of interest.

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