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Comparing Hematological Indices of People with Iron Deficiency Anemia, Thalassemia Minor and Major, and Healthy People

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Abstract

Background: This study aimed to evaluate three hematological indices in children and adolescents with iron deficiency anemia, thalassemia minor and major and healthy people.

Materials and Methods: In this descriptive cross-sectional study, 164 patients were selected based on the inclusion and exclusion criteria, and divided into four groups (n=41): iron deficiency anemia, thalassemia minor, thalassemia major, and healthy people. The results obtained from the tests were recorded in the patients' checklist. Mann-Whitney test, Wilcoxon test, ROC curve and area under the curve were adopted for data analysis using SPSS Statistics V21.0.

Results: The mean age of patients was 13.07±3.82 years, of which 100 (62.5%) were men. There is a significant difference due to the hemoglobin concentration (Hb) index in two-by-two comparison of the groups (P<0.001), but this difference was not seen between iron deficiency and thalassemia minor groups (P=0.159). This difference was substantial (P<0.001) in terms of mean corpuscular volume (MCV) index, although it was only seen between the iron deficiency and thalassemia major groups (P=0.225). Finally, this mean difference was reported in Serum ferritin index (P<0.001), but no difference was observed between healthy group and thalassemia minor group (P=0.802).

Conclusion: The results showed that there is a difference in meanHb, MCV, and Ferritin levels between the studied groups, and the Hb and Ferritin indices are favorable for iron deficiency anemia, and the Hb and MCV indices are favorable to distinguish the thalassemia minor and major from healthy people.

Keywords: Ferritin, Thalassemia Minor, Beta-Thalassemia, Iron Deficiency, Hemoglobin Concentration, Mean Corpuscular Volume

Introduction

The most common hypochromic microcytic anemias are iron deficiency anemia and thalassemia. Anemia is a condition in which the number of RBCs or hemoglobin is low, resulting in reduced oxygen-carrying capacity [1]. Iron deficiency is a primary cause of anemia, which is responsible for nearly half of the global anemia population. Nevertheless, anemia can also be the result of other deficiencies, including riboflavin, vitamins A and B12, and folate [2, 3]. A number of chronic

diseases, such as tuberculosis, cancer, acquired immunodeficiency syndrome, and other inherited or acquired disorders, such as thalassemia may also lead to anemia [4-6].

Based on World Health Organization (WHO), children are the most vulnerable group for anemia, and the global prevalence of anemia among children was estimated at 42.6% in 2011, and Southeast Asia was the second group. Based on recent studies conducted in Iran, the estimated prevalence of iron deficiency anemia among children and adolescents varies between 13.9% and 31.0% [7]. Low socio-economic status, maternal anemia, and poor hygiene are the main factors in the high prevalence of childhood anemia [8].

Thalassemia is a group of disorders in which the α/β-Globin mRNA ratio changes, and these changes lead to the destruction of RBCs in the bone marrow and bloodstream. This hemoglobinopathy is prevalent throughout Africa, South Asia, and the Mediterranean area, making it the most often seen form. Genetic mutations in β-globin genes may result in decreased or absent expression of this gene, and the severity of the illness or anemia is linked to the presence of normal amounts of β-globin. Beta thalassemia is divided into two categories: transfusion-dependent thalassemia (beta thalassemia major) and non–transfusion-dependent thalassemia (beta thalassemia minor and intermedia) [9].

The frequency of beta thalassemia significantly varies in different regions in Iran. The highest frequency (10.0%) was reported in areas near Caspian Sea, the Persian Gulf and southern Iran. In other regions, such as Fars province, its prevalence was 4-8% [10].

Several factors were suggested as simple diagnostic tools to distinguish iron deficiency anemia from thalassemia. However, some of the best distinguishing factors use parameters that can only be measured in modern countries which are not always available in small laboratories. Using a factor with suitable diagnostic accuracy based only on parameters obtained from blood cell count using simple counters would be useful in clinical evaluation.

Hematological indices were assessed in various studies to distinguish thalassemia from iron deficiency anemia [11-13]. Therefore, no study examined Hematological indices between different groups and healthy people. Therefore, the present study was conducted to compare and evaluate three Hematological indices to differentiate children and adolescents with iron deficiency anemia and thalassemia minor and major from healthy people.

Materials and Methods

This descriptive cross-sectional study was conducted in 2022 on children, and adolescents with thalassemia aged 7-18 years in Shoushtar, Iran. The research population of this study includes all children and adolescents with medical records in the thalassemia clinic of Khatam al-Anbiya Hospital in Shoushtar. Furthermore, those who were sent to the Children's Clinic in Shoushtar for examination and diagnosis of iron deficiency anemia and thalassemia minor were included in the study population. After obtaining necessary written permission from Research Vice-Chancellor of University and making arrangements with Shoushtar Faculty of Medical Sciences, the researcher went to the research location (Thalassemia Clinic of Khatam Al-anbia Hospital in Shoushtar), and conducted the research within three months with the necessary arrangements with the officials of relevant unit. After introducing himself/herself and getting the consent of all the parents of children and teenagers participating in this project, the significance of the subject, as well as the research methodology were explained.

164 patients were selected based on determined inclusion and exclusion criteria according to the sample size formula [14]. The initial group consisted of 41 individuals diagnosed with thalassemia minor but without iron deficiency. The second group comprised 41 individuals diagnosed with iron deficiency anemia but without thalassemia. The third group consisted of 41 individuals diagnosed with thalassemia major. Lastly, the fourth group comprised 41 healthy individuals without iron deficiency or thalassemia minor.

Inclusion criteria: 1. In the patients with iron deficiency (MCV: for boys less than 80.8 femtoliters, for girls less than 1.82 femto liters. Hb: for boys less than 13 g/dL, for girls less than 12 g/dL. Ferritin: less than 15 micrograms/liter). 2. In the patients with thalassemia minor (HbA2 higher than 3.5%, MCV less than 80, normal serum iron and ferritin levels). 3. In the patients with thalassemia major (normal or increased serum iron and ferritin levels) cut-off point HbA2 >3/5%. 4. In normal people (normal hemoglobin, MCV, serum iron and ferritin levels) [1, 9, 11].

Exclusion criteria: History of taking anticonvulsants, salicylates, NSAIDs, and iron and folic acid supplements within the last few days, history of chronic liver disease, kidney disease and gastrointestinal malabsorption, gastrointestinal ulcers or intestinal and colorectal problems as well as parasitic infections such as Schistosoma and hookworms.

The groups were matched due to age and gender. All subjects participated in the study with the knowledge of the goals of the project following the announcement of voluntary call. Patients with iron deficiency anemia, thalassemia minor, and thalassemia major were selected according to the inclusion criteria and sampling was then performed. After making arrangements with the laboratory of Khatam al-Anbiya hospital in Shoushtar, all patients referred to the laboratory on a specific day while being NPO for 10 hours. Five mil of blood were collected, two ccs of which were poured into a tube with a lid containing EDTA for cell count and hemoglobin

tests. The rest was poured into an acid-washed and coded tube to separate the serum and perform other tests.

CBC sample was evaluated regarding cell and hemoglobin count using a Sysmax Kx21 Hematology Analyzer, and HbA2 test was performed by column chromatography with a biosystem kit.

Ferritin was measured in patients' serum samples within 24 hours after collection. Using this kit, ferritin less than 10 g/Lm was considered as low, and 10-120 was considered normal [15]. Finally, people who had no history of special diseases and were apparently in perfect health, and their electrophoresis hemoglobin, ferritin, and iron CBC were within the normal range were considered as healthy people.

No fees were charged to the patient during these tests. The patients' checklist included a record of the test findings. The data did not exhibit a normal distribution when the Kolmogorov-Smirnov test was applied to assess the data's normality. With the Mann-Whitney test, the groups were contrasted. Wilcoxon test was used to compare the mean levels of variables while the ROC curve, and its area under the curve were used to assess the efficiency of Ferritin, Hb, and MCV indices in differentiating the patients from healthy people. Data analysis was done using SPSS Statistics V21.0. P-value greater than 0.05 was considered as significant level.

Results

160 patients were examined, and one person from each group was excluded based on exclusion criteria. The results showed that the mean age of the patients (13.07±3.82) in a range of 3 to 19 years. One hundred people (62.5%) were men.

Based on Kruskal-Wallis test, there was a significant difference between the mean Hb, MCV, and Ferritin levels among the study groups (P<0.001) (Table 1).

Table 1. Mean and standard deviation of Ferritin, Hb, and MCV indices in the study groups

Group	Healthy people n=40	Iron deficiency anemia n=40	Thalassemia minor n=40	Thalassemia major n=40	P.value with Kruskal-Wallis test
Hb	13.01±1.26	10.02±1/13	10.40±1.20	7.62±0.94	*P<0.001
MCV	84.71±2.97	79.00±4.44	63.93±6.59	77.60±4.35	*P<0.001
Ferritin	74.47±14.72	10.45±2.01	98.75±134.28	5591.0±1929.19	*P<0.001

As can be seen, the data in Table 1 show that in two-by-two comparison of groups using Mann-Whitney test, there is a significant difference in terms of Hb index (P<0.001), but this difference was not seen between iron deficiency group, and thalassemia minor group (P=0.159).

Regarding MCV index, a statistically significant difference was observed among the groups (P<0.001), yet this difference was absent only between iron deficiency and thalassemia major groups (P=0.225). Finally, this mean difference regarding serum ferritin levels showed significant results between groups (P<0.001), but no difference was observed between the healthy group versus thalassemia minor group (P=0.802).

The ability of the ferritin, Hb, and MCV indices to distinguish patients from healthy individuals was assessed using the Receiver Operating Characteristic (ROC) curve and its area under the curve. The area under the curve to distinguish iron deficiency patients from healthy individuals was 0.997 (P<0.001) for Hb index, 0.868 (P<0.001) for MCV index and 0.995 (P<0.001) for serum Ferritin levels index (P<0.001) (Table 2, Fig.1).

Table 2. Contingency table of Ferritin, Hb, and MCV indices in iron deficiency patients and healthy people

Variable		Result		Total	P.value
		Healthy people	Iron deficiency		
Index Hb	**>11.75**	18(90.0)	2(10.0)	20(100.0)	**p<0.001**
	<11/75	0(0.0)	20(100.0)	20(100.0)	
Total		18(45.0)	22(55.0)	40(100%)	
Index MCV	**>82.30**	15(75.0)	5(25.0)	20(100.0)	**p<0.001**
	<82.30	2(10.0)	18(90.0)	20(100.0)	
Total		17(57.5)	23(42.5)	40(100%)	
Ferritin index	**>12/75**	19(95.0)	1(5.0)	20(100.0)	**p<0.001**
	<12/75	0(0.0)	20(100.0)	20(100.0)	
Total		19(47.5)	21(52.5)	40(100%)	

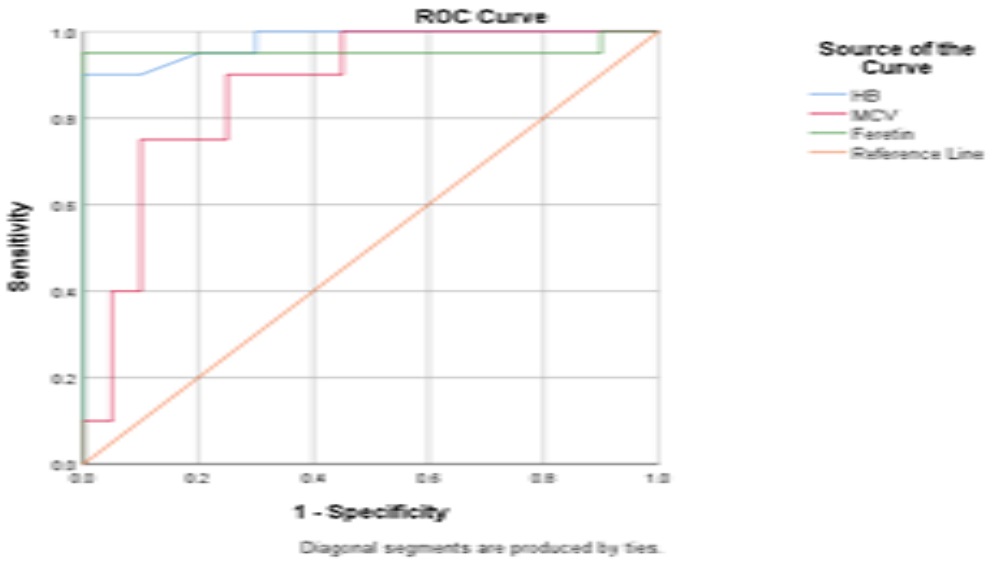

Fig.1. ROC curve of the efficiency of Ferritin, Hb, and MCV indices to distinguish iron deficiency patients from healthy people

The findings of Table 2 and Fig.1 show that in terms of sensitivity and specificity, Ferritin (0.95, 0.100) and Hb(0.90, 0.100) indices with cut-off points 12.75 and 11.75 are desirable for diagnosing iron deficiency disease.

The area under the curve to distinguish thalassemia minor patients from healthy people was 0.944 for Hb index (P<0.001), 1.000 for MCV index (P<0.001) and 0.516 for serum Ferritin levels index (p=0.860) (Table 3, Fig.2).

Table 3. Contingency table of Ferritin, Hb, and MCV indices in thalassemia minor patients and healthy people

Variable		Result		Total	P.value
		Healthy people	Thalassemia minor		
Hb index	>11.75	18(90.0)	2(10.0)	20(100.0)	**p<0.001**
	<11.75	2(10.0)	18(90.0)	20(100.0)	
Total		20(50.0)	20(50.0)	40(100%)	
MCV index	>77.80	20(10.0)	0(0.0)	20(100.0)	**p<0.001**
	<77.80	0(0.0)	20(100.0)	20(100.0)	
Total		20(50.0)	20(50.0)	40(100%)	
Ferritin index	>11.45	19(95.0)	1(5.0)	20(100.0)	**p<0.001**
	<11.45	14(70.0)	6(30.0)	20(100.0)	
Total		33(82.5)	7(17.5)	40(100%)	

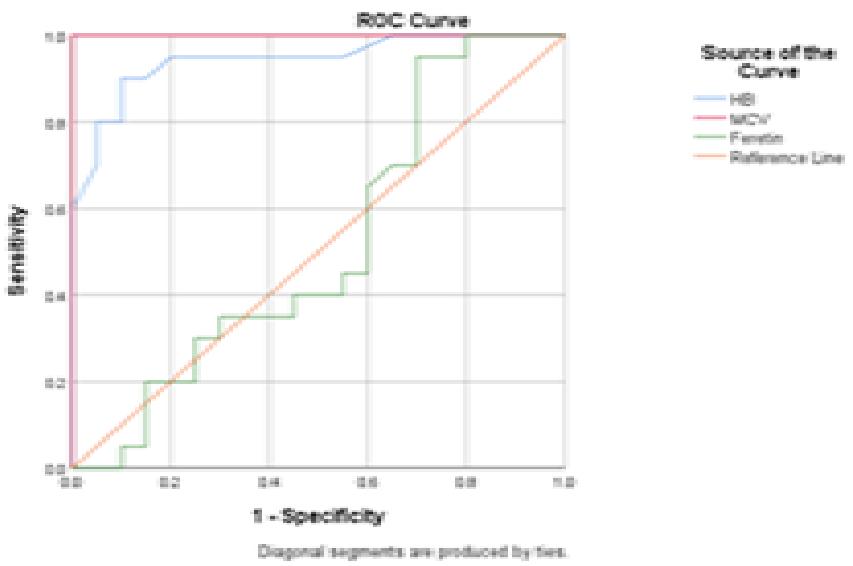

Fig. 2. ROC curve of the efficiency of Ferritin, Hb, and MCV indices for distinguishing thalassemia minor patients from healthy people

Table 4. Contingency table of Ferritin, Hb, and MCV indices in thalassemia major patients and healthy people

Variable		Result		Total	P.value
		Healthy people	Thalassemia major		
index Hb	>9.80	20(100.0)	0(0.0)	20(100.0)	**p<0.001**
	<9.80	0(0.0)	20(100.0)	20(100.0)	
Total		20(50.0)	20(50.0)	40(100%)	
MCV index	>80.80	20(100.0)	0(0.0)	20(100.0)	**p<0.001**
	<80.80	5(25.0)	15(75.0)	20(100.0)	
Total		25(62.5)	15(37.5)	40(100%)	
Index ferritin	>6.80	20(95.0)	20(5.0)	40(100.0)	**p<0.001**
	<6.80	0(0.0)	0(0.0)	0(0.0)	
Total		20(100.0)	20(100.0)	40(100%)	

The data in Table 3 show that the sensitivity, and specificity of MCV (0.100, 0.100) and Hb (0.90, 0.90) indices with cut-off points 77.80 and 11.75 are desirable for the diagnosis of thalassemia minor.

The area under the curve to distinguish thalassemia major patients from healthy people was 1.00 for Hb index (P<0.001), 0.890 for MCV index (P<0.001) and 0.000 for serum Ferritin levels index (P<0.001) (Table 4, Fig.3).

As can be seen, the data in Table 4 and Fig. 3 show that the sensitivity, and specificity of MCV (0.100, 0.80) and Hb (0.100, 0.100) indices with cut-off points 80.80 and 9.80 are desirable for the diagnosis of thalassemia major.

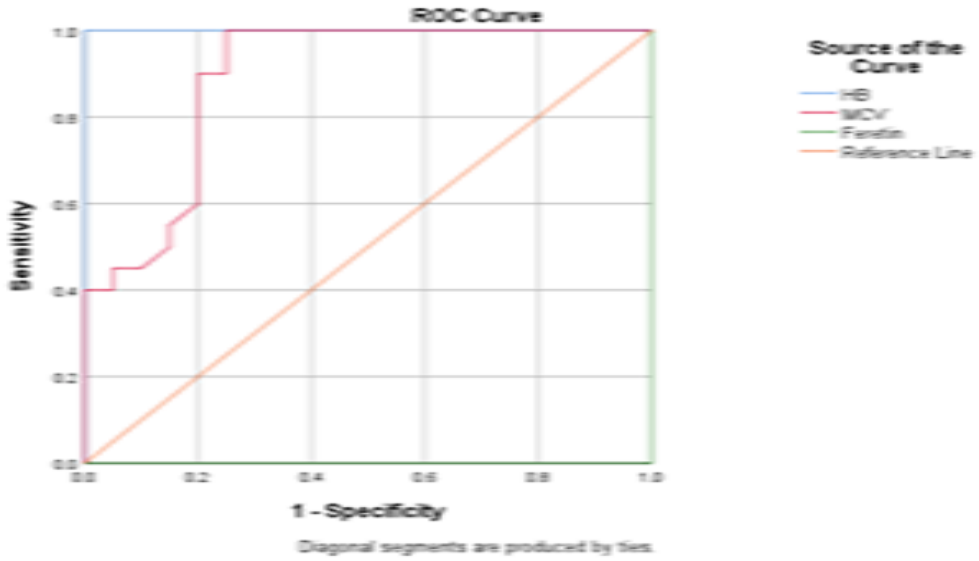

Fig.3. ROC curve of the efficiency of Ferritin, Hb, and MCV indices for distinguishing thalassemia major patients from healthy people

Discussion

Based on our results, based on two-by-two mean analysis of the groups, it was found that Hb concentration and serum ferritin level are favorable to determine iron deficiency anemia while Hb and MCV indices are favorable for distinguishing thalassemia minor and major from healthy people.

Blood indices analysis revealed that the Hb concentrations in the thalassemia minor and iron deficiency groups were substantially greater than those in the thalassemia major group. In this regard, in a study by Jameel et al. conducted on 21-36-year-old samples, the results showed that Hb concentration in iron deficiency group were higher than thalassemia major patients [12], which is consistent with the results of present study. In other studies [11, 16-18], the mean Hb concentration in thalassemia major group were reported to be significantly higher than that of iron deficiency anemia, which is not consistent with the results of the present study. The authors did not discuss why their data conflicted with other studies, but it might have been due to the severity of anemia. These data support the hypothesis that iron deficiency itself may increase Hb values regardless of anemia. According to the findings of an English research, iron deficiency anemia may be caused by underlying conditions like as diabetes, microcephaly, and a lack of other vitamins, and it may be mistaken for increased Hb concentration [19]. Differentiating between IDA and β-TT using CBC factors is a significant, accurate, quick, cheap, and easy way to identify children at risk of suffering from anemias and avoid unnecessary research costs [19].

MCV concentration in thalassemia minor was significantly lower than that of thalassemia major and iron deficiency. Similar to the present study, a study by Matos showed that this index was significantly higher in iron deficiency anemia compared to thalassemia minor [20]. However, in a study by Kramati, no significant difference was found between thalassemia minor and iron deficiency anemia. Furthermore, no significant difference was observed between iron deficiency and thalassemia major in terms of MCV index in their study [21]. The results of a study by Jameel et al. are similar to the present study [12]. Nevertheless, Rashwan's investigation revealed a notable disparity in the average MCV concentration between the thalassemia major group and the iron deficiency anemia group, with the former exhibiting a much lower concentration [11]. In the studies of Beyan [17] and Yousafzai et al. [22], results similar to the study of Rashwan have been obtained. It is noteworthy that in the study of Matos, the MCV concentration in iron deficiency anemia was significantly higher than thalassemia major [20]. The reason for the difference in the results of mentioned studies compared to the present study can be attributed to the variety of genetic mutations. Besides, the sample size, the mean age of study groups and treatment with iron supplements can influence the results [23].

The results of present study showed that serum Ferritin levels in thalassemia major group were significantly higher than that of thalassemia minor group, but there was no difference in serum Ferritin levels between healthy group and thalassemia minor group. However, in a study by Dehghani et al., Ferritin levels in healthy people were higher than that of thalassemia minor [24]. The findings of a research by Jameel et al. demonstrate, among other comparable findings, that ferritin levels in thalassemia major were substantially greater than those in iron deficiency [12]. In this regard, in a study by Tharwat, Ferritin levels in thalassemia major patients were higher than 1000 [25]. Serum Ferritin levels were significantly higher in healthy people compared to people with iron deficiency, which is in line with the results of a study by Gul [26] Moreover, Ferritin levels in thalassemia major group were higher than that of healthy subjects, which is in line with a study by Abdolmotaleb [27].

Using the area under the curve (AUC), it was determined that serum Ferritin levels and Hb are desirable indices for differentiating iron deficiency patients from healthy people, which is similar to the study of Zuther in terms of serum Ferritin levels (0.67 sensitivity and 0.75 specificity) [28]. In the study of Kablan, Hb index with sensitivity, and specificity of 21.43 and 0.100 and a cut-off point of 2.2 was recommended for diagnosing iron deficiency anemia [29], which is similar to the present study. Meanwhile, the evidence from the studies of Salvi et al. and Jimmy Perez et al. shows that Hb has a poor performance (sensitivity less than 0.61, specificity less than 0.53) in diagnosing iron deficiency among pregnant women [30, 31]. Unlike Ferritin, Hb serves as an indicator of stored iron levels. This may account for its limited effectiveness in identifying iron insufficiency, particularly during the first phases. Hb serves as a valuable marker for identifying iron insufficiency [32]. Besides, MCV index is a parameter that can be used to diagnose iron deficiency anemia. This has been confirmed by World Health Organization (WHO), which states that MCV and MCH are the most sensitive indices of iron deficiency in the CBC panel [32].

Using the area under the curve, it was determined that MCV and Hb indices are desirable for distinguishing thalassemia major patients from healthy people. Hence, these two indices with sensitivity and specificity of 0.93 and 0.100 in the study of Ullah and Hb index with sensitivity and specificity of 0.53 and 0.54 in the study of Sirdah are suitable for diagnosing thalassemia major [13, 33]. MCV index in the study of Sari with sensitivity and specificity of 87% and 87% and a cut-off point less than 20.5 pg has priority in the diagnosis of thalassemia major [34]. In the study of Kablan, Hb index with sensitivity and specificity of 0.100 and 84.6 and a cut-off point of 3.6 was suggested for the diagnosis of thalassemia major [29].

Using the area under the curve, it was determined that MCV and Hb indices are suitable for distinguishing thalassemia minor patients from healthy people.

According to a research conducted by Tripathi, the Hb index had the best sensitivity (91.89%) and the lowest specificity (22.32%) for diagnosing thalassemia minor. On the other hand, the MCV index exhibited the highest specificity (88.69%) for the same diagnosis.

Conclusion

The results showed that there is a difference in mean Hb, MCV, and serum Ferritin levels among the studied groups, and Hb and Ferritin indices are favorable for iron deficiency anemia, and Hb and MCV indices are favorable to distinguish thalassemia minor and major patients from healthy people. It is recommended that further research be done with a bigger sample size in order to precisely ascertain the sensitivity and various formulae in separating individuals with thalassemia and iron deficiency anemia from those in good health.

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Conflict of interest

None declared.

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Ethical Considerations

In this study, the data of the Hematological Indices in Children and Adolescents with Iron Deficiency Anemia, Thalassemia Minor and Major and Healthy People was used, we did not have access to the names and characteristics of the people, also for this design, the code of ethics was obtained from the Ahvaz Jundishapur University of Medical Sciences.

Code of Ethics

This study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences with code IR.AJUMS.REC.1397.517

Authors' Contributions

Akram Hemmatipour: Writing and preparing the initial draft. Razieh Jalakani: Writing and preparing the initial draft. Azam Jahangirimehr: Statistical analysis, project administration and revision. Bijan Kikhaei: Conceptualization,
Dariush Rokh Afrooz: project administration and revision, Seyedeh Moloud Rasouli Ghahfarokhi: Conceptualization.

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