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Original Article

A Comparison between Serum 25-Hydroxyvitamin D3 Levels and Serum Ferritin in Children and Adolescents with Iron Deficiency Anemia, Thalassemia Minor, Thalassemia Major and Healthy People

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Abstract

Background: We aimed to determine the level of serum vitamin D and serum ferritin in four groups' children and adolescents.

Methods: This descriptive/analytical study was conducted in 2019 on children and adolescents with thalassemia aged 7-18 years in Ahvaz, Iran. Overall, 160 patients with the target inclusion and exclusion criteria were assigned into four groups (n=40) of people with thalassemia minor, thalassemia major, iron deficiency anemia and healthy individuals. The level of ferritin and vitamin D was assessed. Mann-Whitney test, Wilcoxon test and Spearman's correlation test were used as well as SPSS Statistics V21.

Results: The mean age of the participants was 13.07 ± 3.82 yr and 100 people (62.5%) were male. In terms of vitamin D deficiency, 16 people (40%) had severe deficiency in iron deficiency group. The highest level of ferritin was found in thalassemia major group and the lowest in iron deficiency group. Regarding the level of vitamin D and ferritin in the healthy group, there was a statistically significant difference between mean vitamin D and serum ferritin levels (P=0.027). In iron deficiency group, this level of ferritin was observed with mild deficiency (P=0.017). In thalassemia major group, the increase in ferritin was associated with severe vitamin D deficiency (P=0.05.

Conclusion: Severe vitamin D is seen in thalassemia major due to the increase in ferritin, but moderate deficiency of this vitamin in healthy people and mild deficiency in iron deficiency anemia group was observed considering the normal levels of ferritin.

Keywords: Vitamin D; Ferritin; Thalassemia minor; Thalassemia major; Iron deficiency

Introduction

Iron deficiency is the most common cause of anemia (1). According to the WHO, iron deficiency anemia accounts for 50% of anemias, which is around 30% in Iran (2). Patients with IDA may cause major consequences such as premature birth, low birth weight and developmental disorders in children (3-5). The risk factors of this anemia include the use of non-steroidal anti-inflammatory drugs, frequent blood donation, vegetarian diet, vitamin deficiency and thalassemia (6).

Thalassemia is one of the most common genetic disorders in humans, includes two types of minor and major (7). Thalassemia syndromes caused by an imbalance in the synthesis of one or more globin chains in hemoglobin (7, 8). The total annual incidence of symptomatic individuals is 1 in 100,000 in the world. Beta thalassemia is common in Mediterranean countries, and the prevalence of thalassemia major in Iran is reported to be 4%-6% (9). More than two million patients with thalassemia minor live in Iran, the highest frequency is 10% in the coast of Caspian Sea and Persian Gulf, and its frequency is 8%-10% in the south of Iran and in Fars Province (10).

Iron deficiency anemia (IDA) and thalassemia minor are both common in Iran, and the diagnosis and differentiation of the two is very important (7). Osteopenia and osteoporosis are among the common side effects that can be seen parallel to the progress of treatment methods and life expectancy in patients with thalassemia, and in various studies, bone density disorder has been confirmed in adults and children with thalassemia major (9). Overall, 77% of the Iranian population suffers from vitamin D deficiency, and this percentage is affected by occupation, nutrition, gender, age, and underlying diseases (11). Vitamin D levels in Asian children are less than optimal, but few studies have been done in Asia, especially in children, about the level of vitamin D and the factors affecting it (12). Vitamin D deficiency has many complications, including dizziness, headache, digestive problems, weakness, muscle pain, and frequent infections, and one of the most important complications is iron deficiency anemia, associated with osteoporosis (13, 14). In iron deficiency anemia, the body's iron reserves are depleted and the amount of serum iron decreases, then the total iron binding capacity of transferrin increases and serum ferritin, the size of red blood cells, and the amount of hemoglobin in each red blood cell decrease (15). The accumulation of iron in thalassemia patients with impaired intestinal absorption as well as hepatic 25-hydroxylation makes the person susceptible to vitamin D deficiency (16).

Different and sometimes conflicting results have been reported in several studies on vitamin D levels in thalassemia major patients (9). There is an inverse relationship between ferritin and vitamin D levels in children with thalassemia major (9). Moreover, most patients with thalassemia major have low serum D levels (16). Vitamin D levels were lower in patients with iron deficiency anemia compared to patients with thalassemia minor and healthy people (7). The number of studies conducted on children and adolescents, especially in the field of thalassemia minor, is limited. According to the carried-out studies, this study aimed to determine serum vitamin D level and serum ferritin in four groups of healthy people, thalassemia major and minor and iron deficiency anemia in children and adolescents with thalassemia in Khuzestan Province in order to investigate the relationship between vitamin D deficiency and iron level, and ferritin among these patients.

Materials and Methods

This is a descriptive/analytical study conducted in 2019 on children and adolescents with thalassemia aged 7-18 yr. The research population of this study includes all the children and teenagers who are suffering from thalassemia major and have a medical record in Khatam Al-Anbiya Hospital in Shoushtar and Shafa hospital in Ah-

vaz, southern Iran. Moreover, people referring to the children's clinic in Shoushtar whose thalassemia minor and iron deficiency anemia were examined and diagnosed, were selected as part of the research population.

This project has been approved with the ethics code IR.AJUMS.REC.1397.517 in the Research Council of Jundishapur University of Medical Sciences, Ahvaz. After obtaining the necessary written permissions, the researcher referred to the research site and started the research by making arrangements with the officials of the relevant unit. After introducing himself/herself and obtaining the consent of all the parents of children and teenagers participating in this project, the importance of the issue and how study would be conducted were explained to them.

According to the sample size formula (7), 160 patients were selected based on inclusion and exclusion criteria. The first group consisted of 40 people with thalassemia minor without iron deficiency, the second group included 40 people with iron deficiency anemia without thalassemia, the third group included 40 people with thalassemia major, and the fourth group included 40 healthy people without iron deficiency and thalassemia minor.

Inclusion criteria

1. Selection of patients was based on iron deficiency cases according to MCV values less than 80 femtoliters, serum iron less than 45 micrograms/dl and ferritin less than 12 µg/l and TIBC more than 400 µg/dl in accordance with red blood cell indices including MCH, MCV and RDW.
2. Group of thalassemia minor patients (Hb A2 higher than 3.5%, MCV less than 80, normal serum iron and ferritin)
3. Group of thalassemia major patients (normal or increased serum iron and ferritin levels)
4. Normal people (with normal hemoglobin, MCV, serum iron and ferritin levels).

Exclusion criteria

Taking vitamin D supplements (oral or injectable), history of taking anticonvulsants, salicylates, non-steroidal anti-inflammatory drugs, taking iron and folic acid supplements in the last few days, or any disease interfering with vitamin D absorption or metabolism. A history of chronic liver disease, kidney disease and digestive malabsorption, digestive tract ulcers or intestinal, colorectal problems, and infection with parasitic infections such as schistosomes and hookworms.

The groups were matched in terms of age and sex, all people participated in the study with the knowledge of the goals of the project following the announcement of the voluntary call. In patients treated with calcium supplements or multivitamins, these products were discontinued for one week. Patients with iron deficiency anemia, thalassemia minor and thalassemia major were selected according to the inclusion criteria. Then, sampling was done after making arrangements with the laboratory of Shafa hospital and the laboratory of Khatam Al-Anbiya hospital in Shoushtar. All patients referred to the laboratories on a specific day, and 5 cc blood was collected, 2 cc of used for counting tests. Cells and hemoglobin were poured into closed tube containing ethylenediaminetetraacetic acid and the rest was poured into the acid-washed and coded tube to separate the serum and carry out other tests.

Hematology parameters were evaluated in blood samples containing EDTA anticoagulant using Sysmex KX21 device.

Iron and TIBC tests were performed using BT3000 biochemistry autoanalyzer. Ferritin and vitamin D were measured by ELISA method using a kit (IDS Co.) and ELISA Reader (BioTek). Electrophoresis was done by microcapillary method using Sebia capillary electrophoresis.

Values higher than 30 ng/dl are considered normal, between 30 and 20 ng/dl are insufficient, and less than 20 means vitamin D deficiency (17). Ferritin was measured based on the patients' serum samples within 24 h after collection. Using this kit, ferritin less than 10 g/Lµ is considered as low and 10-120 is considered normal (9). 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 normal range were considered healthy people.

No fees were charged to the patient during these tests. To check the normality of the data, Kolmogorov–Smirnov test was used, and the data did not have a normal distribution. Mann-Whitney tests were used to compare the groups and compare the mean levels of the variables. The Wilcoxon test and Spearman's correlation coefficient were used to compare the variables. Data analysis was done using SPSS ver.21 (IBM Corp., Armonk, NY, USA). A level above 0.05 was considered as a significant level.

Results

The mean age of the patients was 13.07±3.82, and 100 patients (62.5%) were male. In terms of vitamin D deficiency, 16 people (40%) had severe deficiency in iron deficiency group, 26 people (65%) in thalassemia major group had moderate deficiency, 26 people (65%) in thalassemia minor group had mild deficiency, and 22 people (55%) in the healthy group were normal. The highest mean level of vitamin D was seen in healthy group and the lowest in thalassemia major group. According to the Kruskal-Wallis test, there was a statistically significant difference between the groups in terms of mean vitamin D levels (P<0.001) (Table 1).

Table 1: Frequency and mean levels of vitamin D in the studied groups

Variable		***Group***				***Significance level***
		Frequency (%)				
Vitamin D Ng/dl		Healthy n=40	Iron deficiency n=40	Minor n=40	Major n=40	
	Severe deficiency <10	-	16 (40%)	4 (10%)	6 (15%)	
	Moderate deficiency 10-20	4 (10%)	8 (20%)	2 (5%)	26 (65%)	P < 0.001
	Mild deficiency 20-30	14 (35%)	8 (20%)	26 (65%)	4 (10%)	
	Normal >30	22 (55%)	8 (20%)	8 (20%)	4 (10%)	
		Mean ± SD				
		36.93±14.72	18.99±13.65	31.46±17.24	18.70±13.89	P < 0.001

Moreover, according to the Mann-Whitney test, there was a difference between vitamin D levels in healthy people and people with iron deficiency and thalassemia major (P<0.001). This difference was also seen between thalassemia minor versus major (P<0.001), and iron deficiency versus thalassemia minor (P=0.031). However, there was no difference between healthy individuals versus thalassemia minor (P=0.138), and iron deficiency versus thalassemia major (P=0.998)(Table 1).

Regarding ferritin, 30 people (41%) from the healthy group, 24 people (32%) from the iron deficiency anemia group, and 20 people (27%) from the thalassemia minor group are in the normal range. The highest amount of ferritin was seen in the thalassemia major group and the lowest in the iron deficiency group. According to the

Kruskal-Wallis test, there was a statistically significant difference between the mean of the groups in terms of Ferritin (P<0.001) (Table 2).

Table 2: Frequency and mean level of ferritin in the studied groups

Variable		***Group***				***Significance level***
		Frequency (%)				
Ferritin g/Lμ		Healthy n=40	Iron deficiency n=40	Minor n=40	Major n=40	
	deficiency <10	2 (7%)	16 (53%)	12 (40%)	0 (0%)	P < 0.001
	Normal 10-120	30 (41%)	24 (32%)	20 (27%)	0 (0%)	
	high 120<	8 (14%)	0 (0%)	8 (14%)	40 (72%)	
		Mean ± SD				
		74.47±14.72	10.45±2.01	98.75±134.28	19.1929±0.5591	P < 0.001

Furthermore, according to the Mann-Whitney test, there was a difference in the ferritin level between healthy people and those with iron deficiency and thalassemia major (P<0.001). This difference was also seen between thalassemia minor versus major and both groups versus iron deficiency group (P<0.001), but there was no difference between the amount of ferritin in healthy people versus thalassemia minor (P=0.802). (Table 2)

Regarding the level of vitamin D and ferritin, based on the Kruskal-Wallis test, there was a statistically significant difference between the mean vitamin D deficiency and the level of serum ferritin in the healthy group (P=0.027). In the iron deficiency group, this amount of ferritin was seen with a slight deficiency (P=0.017). In the thalassemia major group, increased ferritin was associated with severe vitamin D deficiency (P=0.05), but this significant difference between vitamin D and ferritin levels was not seen in thalassemia minor group (P=0.970) (Table 3). The data in Table 3 also suggest that there was no significant difference between vitamin D and ferritin levels in thalassemia minor (P=0.970).

Table 3: Ferritin index according to different levels of vitamin D in the study groups

Variable	***The level of Ferritin g/Lμ***			
Vitamin D levels (ng/dl)	Healthy	Iron deficiency	Thalassemia minor	Thalassemia major
		Mean ± SD		
severe deficiency (<10)	-	10.25±3.13	80.05±85.73	6400.0±1109.08
moderate deficiency (10-20)	108.95±77.88	10.75±0.88	58.10±0.03	5835.38±2125.44
Mild deficiency (20-30)	97.01±69.38	11.00±0.02	110.47±155.13	4960.00±138.56
normal (>30)	53.86±63.87	10.00±0.08	80.15±98.90	3420.0±161.65
Significance level with Kruskal-Wallis test	* P=0.027	* P=0.017	P=0.970	P=0.05

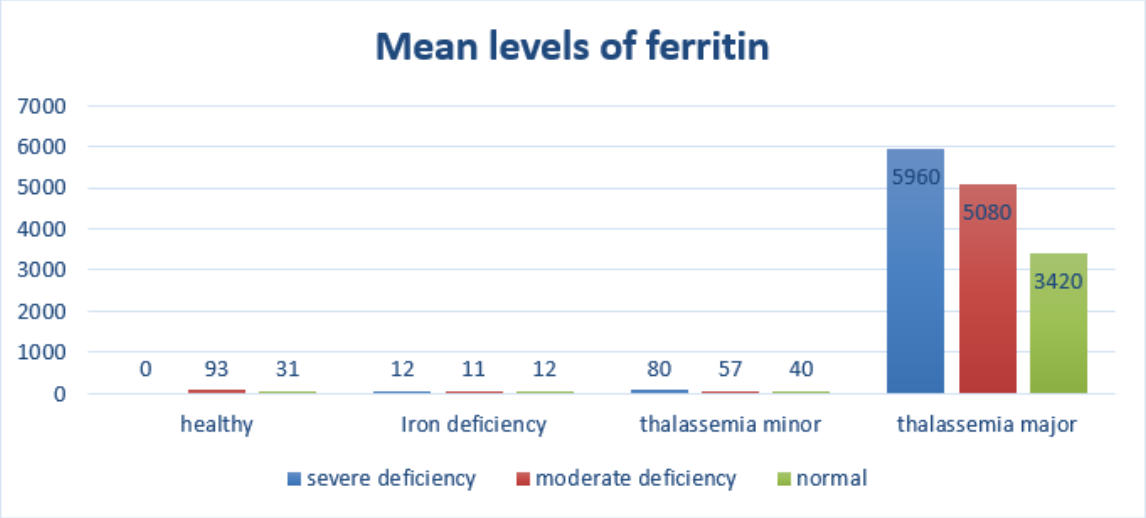

Fig. 1: Comparison of ferritin levels between different groups according to vitamin D levels

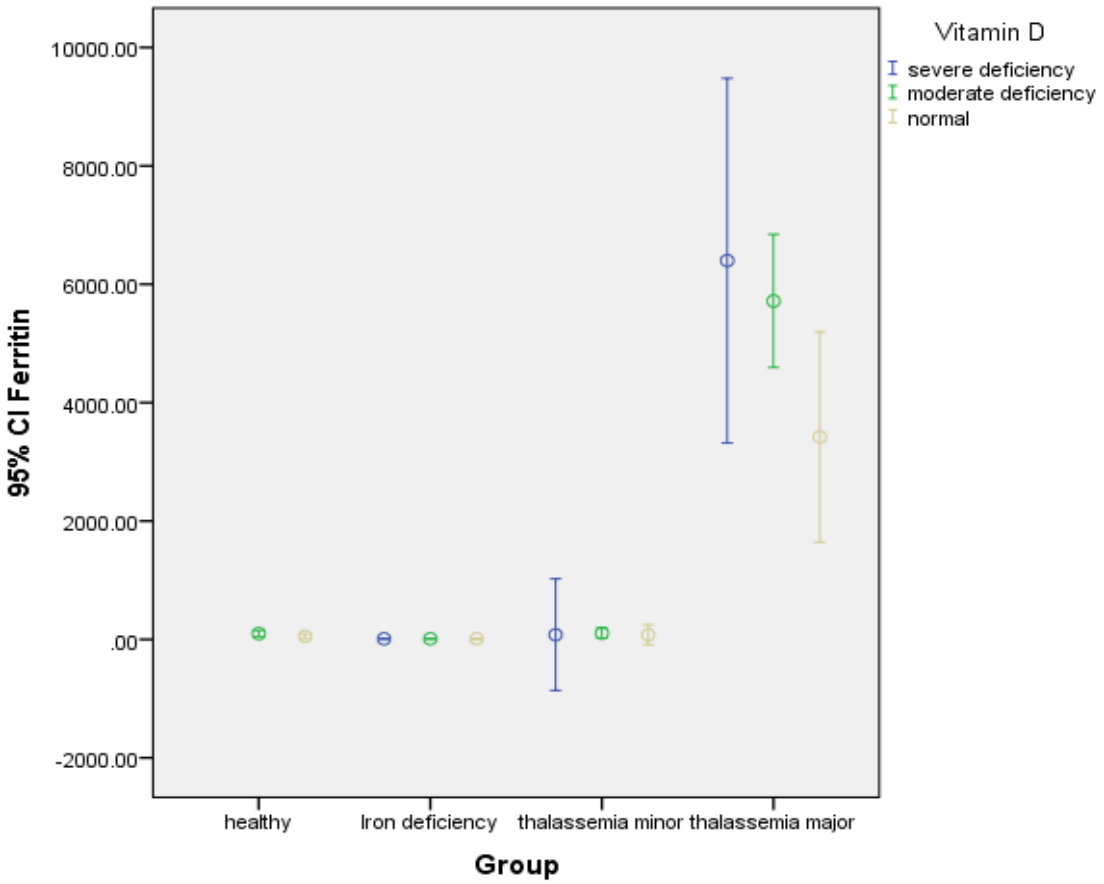

Fig. 2: Error diagram Ferritin levels between different groups according to vitamin D levels

According to Spearman's correlation coefficient test, there was a significant inverse relationship between serum vitamin D level and serum ferritin level in healthy people (R=-0.344, *P*=0.030), thalassemia major (R=-0.516, *P*=0.001) and iron deficiency (R=-0.320, *P*=0.044). However, no significant relationship was found in people with thalassemia minor (R=-0.177, *P*=0.273).

In terms of examining demographic variables using the Mann-Whitney test, the vitamin D level of men in the iron deficiency group was higher than that of women (*P*<0.001).

Table 4: Vitamin D and ferritin indices according to gender in the study groups

Group	***Variable (Vitamin D)***		***Significance level using Mann-Whitney test***
	Man	Woman	
	Mean ± SD		
Healthy	40.80±21.21	36.25±13.59	*P*=0.726
Iron deficiency	27.11±12.39	12.34±10.90	**P*=0.000
Thalassemia minor	36.05±19.47	29.50±16.18	*P*=0.738
Thalassemia major	17.80±14.91	20.06±12.54	*P*=0.07
	Variable (ferritin)		
Healthy	129.30±85.24	64.80±62.79	**P*=0.0010
Iron deficiency	10.77±0.80	10.18±2.61	*P*=0.563
Thalassemia minor	158.65±174.40	73.07±106.66	**P*=0.045
Thalassemia major	5798.33±1885.31	5280.00±2013.60	*P*=0.157

Moreover, the level of ferritin in the group of healthy people (*P*=0.010) and the group of thalassemia minor was significantly higher in men (*P*=0.045) (Table 4).

Discussion

The results of the present study showed that the highest mean level of vitamin D was seen in the healthy group and the lowest in the thalassemia major group, which is similar to other results (18). The level of vitamin D in patients with thalassemia major was low, and similar to the present study, most deficiency was reported at an average level (19). Meanwhile, the mean level of vitamin D in patients with thalassemia major indicates an insufficient level or a mild deficiency of this vitamin, and the reason for the difference between these results and the present study can be due to the lifestyle and nutrition of the patients in the mentioned study (20).

In this study, the level of vitamin D in healthy people was significantly higher than that of people with iron deficiency, which is similar to other results (21). There was a significant relationship between vitamin D levels and anemia in 17-year-old children (22). Iron deficiency anemia significantly impairs the intestinal absorption of fat-soluble vitamins, such as vitamin D (21). These findings have been confirmed using correlation analysis. Vitamin D deficiency leads to increased hepcidin synthesis (directly or indirectly by increasing pro-inflammatory cytokines). This hormone is synthesized in the liver and controls the metabolism of iron, so the absorption of iron from the intestinal epithelium is reduced and segregation of macrophages is increased. Nearly one-third of the body's iron is bound to FER. Therefore, changes in body iron reserves are reflected by its concentration in serum (21).

The results of the present study showed that the highest level of ferritin was seen in the thalassemia major group and the lowest in the iron deficiency group. In this regard, the ferritin level of thalassemia major patients was higher than 1000, which is similar to the present study (19). In the current study, the level of ferritin was significantly higher in healthy people than in people with iron deficiency, which is in line with another results (21). Furthermore, the amount of ferritin in

the thalassemia major group was higher than that of healthy subjects, which is similar to another study (18).

Regarding the level of serum vitamin D and ferritin in this study, according to the normality of the serum ferritin level, in the healthy group, there was a significant moderate deficiency of vitamin D and in the group of iron deficiency, a mild deficiency of this vitamin was seen. However, in the thalassemia major group, the increase in ferritin was significantly associated with severe vitamin D deficiency. In this regard, a significant relationship was seen between the severe decrease in vitamin D and the increase in ferritin plasma level in thalassemia major patients compared to the healthy group, which is similar to the present study (23). Meanwhile, the increase in parathyroid hormone activity along with the increase in ferritin in thalassemia major patients was associated with an increase in vitamin D levels (24). The level of vitamin D in patients with thalassemia major was increased. High serum levels of vitamin D in patients with thalassemia despite primary hyperparathyroidism or extrarenal regulation may occur due to the activity of alpha-1-hydroxylase, an enzyme that turns 25D into 1.25D. This enzyme is present in kidneys and non-specific defense cells such as macrophages (25). There was no significant correlation between vitamin D and ferritin levels in thalassemia major patients (26, 27).

In terms of demographic variables, the amount of vitamin D in the iron deficiency group and ferritin in the thalassemia minor group was higher in men than in women. In this regard, the level of vitamin D was lower in females (28). Several factors affect the amount of ferritin and vitamin D (exposure to sunlight, nutrition, and the use of supplements affect the amount of vitamin D).

Among the limitations of this study, we can mention the difference in the age of the studied samples, and lack of examination of other risk factors.

Conclusion

The lowest level of vitamin D and the highest level of ferritin were seen in the thalassemia major group, which indicates an inverse relationship between these two variables. However, in the group of healthy people, a moderate deficiency of vitamin D and in the group of iron deficiency, a mild deficiency of this vitamin was seen considering the normal level of ferritin, and in the group of thalassemia minor, there was no correlation between the level of vitamin D and ferritin.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interests.

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