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Original Article

Relationship Between the Plasma Levels of Vitamin D and Magnesium and Cardiac Involvement and Iron Overload in Thalassemia Major Patients Referred to Ganjavian Hospital of Dezful in 2018

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ABSTRACT

Background: Thalassemia is a hematologic and genetic disorder prevalent both in Iran and the world. As these patients receive frequent transfusions, they are exposed to iron overload in different tissues such as the liver and the heart. Vitamin D and magnesium deficiencies have been known as important risk factors for cardiovascular diseases. We studied the association between the plasma levels of vitamin D and magnesium and different levels of heart involvement in thalassemia major patients.

Methods: Sampling was performed by the convenience method based on the inclusion/exclusion criteria of the study. Patients' data, including age, sex, vitamin D plasma levels, magnesium plasma levels, cardiac iron overload based on MRI T2*, echocardiographic information, and electrocardiographic (ECG) information, were collected.

Results: In this study, 43 patients, composed of 20 men (46.5%) and 23 women (53.5%) at a mean age of 31.35±7.03 years were examined. In total, 39.5% and 27/9% of them had abnormal echocardiography and ECG. Maximum cardiac abnormalities observed in echocardiography were related to left ventricular diastolic dysfunction (23.3%), right ventricular enlargement (9%), reduced left ventricular ejection fractions (9%), and more-than-mild tricuspid valve regurgitation (9%). Moreover, a reduction in the magnesium plasma level was associated with an increase in the number of cardiac abnormalities in echocardiography significantly (P<0.05). There was a significant relationship between echocardiography and ECG results.

Conclusions: Considering the inverse relationship between the plasma levels of vitamin D and magnesium and cardiac iron overload, we recommend that the plasma level of vitamin D and magnesium be checked periodically in these patients. ***(Iranian Heart Journal 2021; 22(4): 15-24)***

KEYWORDS: Iron, Thalassemia, Vitamin D, Magnesium, Heart disease

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Thalassemia encompasses a spectrum of hereditary anemia induced by a reduction or a lack of 1 or more globin chain syntheses.[1] According to data from the Thalassemia Association, there are 18 616 thalassemia patients in Iran, most of whom reside in the provinces of Mazandaran and Fars.[1] Based on a synthesis-disrupted chain, thalassemia is divided into different types, namely α, β, γ, δ, δβ, and εγδβ.[2] Most patients with hemizygote β-thalassemia suffer from severe anemia, they are transfusion-dependent, and only a few of them do not need a blood transfusion.[3] The decisive treatment of thalassemia major is bone marrow transplantation, but it is associated with high mortality rates.[4] The Other treatment option is frequent blood transfusions, leading to blood transfusion dependence.[5]

Iron overload, especially in the liver, endocrine organs, and the heart, is a serious complication in frequent blood transfusions.[6] Iron overload in the heart causes systolic and diastolic dysfunction. The main cause of death in many patients is congestive heart failure; nonetheless, in some patients, arrhythmias cause sudden death.[7] Although to a greater extent myocardial complications of iron deposition can be reduced by iron chelation therapy, cardiac siderosis remains high, and it is considered the most important cause of death in patients with major thalassemia.[8] The most straightforward way to estimate iron overload is a myocardial biopsy. Nevertheless, the main disadvantage of this method is its invasiveness.[9] In recent years, magnetic resonance imaging (MRI) T2* has been drawn upon as a simple noninvasive method to show iron concentrations in the organs.[10] According to parametric studies, MRI T2* is associated with cardiac biopsy results, and it is a reliable guide for noninvasive assessments of iron storage in the heart.[11]

A review of available studies indicates that the progressive accumulation of iron in these patients, accompanied by intestinal malabsorption and hepatic hydroxylation, renders these patients susceptible to vitamin D deficiency. In various studies on the plasma level of vitamin D in patients with thalassemia major, different and often contradictory results have been reported.[12] Further, the relationship between a reduction in the vitamin D level and the incidence of many cardiovascular diseases has been reported.[13] vitamin D deficiency can lead to ventricular hypertrophy, atherosclerosis, heart failure, and rhythm disorders.[14] In thalassemia major patients, usually the symptoms of vitamin D deficiency are confused with the symptoms of anemia, which includes joint/low back pain, myasthenia, and osteoporosis. It is worthy of note that joint/low back pain relief has been reported after treatment with vitamin D.[15]

The second intracellular cation in terms of frequency is magnesium, which plays a significant role in cell activities.[16] Studies have shown that lower serum levels of magnesium are related to the incidence of inflammation,[17] complicating vascular tone control and endothelial function. It appears that these mechanisms contribute to atherosclerosis advancement and potentially cause coronary artery disease.[18,19] Severe magnesium malnutrition can lead to various pathological complications in animal models. These complications, including cardiac muscle necrosis, arrhythmias, hemorrhagic dermatitis, increased oxidative stress, increased heart sensitivity to ischemia/perfusion,[20] and cardiac systolic dysfunction, have been reported previously.[21] Although iron deposition in the heart is the main cause of cardiac dysfunction in patients with thalassemia under frequent transfusions, it seems that some other causes such as vitamin D and magnesium deficiencies are also involved in cardiac

complications in these patients, especially those who do not receive transfusions. Considering the high incidence of thalassemia in Iran and because the bulk of mortality and morbidity in these patients is induced by cardiac causes, it seems necessary to study cardiac involvement and its causes with a view to correcting these elements. Thus, the present study investigated the plasma levels of vitamin D and magnesium and their relationships with cardiac involvement in thalassemia major patients.

METHODS

In this cross-sectional study, 43 thalassemia major patients were screened in Ganjavian Hospital in Dezful, Iran, in 2018. These patients had medical files in the thalassemia center of the hospital, and they came to the hospital frequently to undergo examinations or to receive a transfusion. Patients who fulfilled the entry criteria were chosen by the convenience method. None of the patients enrolled in the current investigation had a history of smoking, alcohol consumption, supplementary vitamin D and magnesium consumption, and other medications except for iron chelators. According to the trials and self-reports, patients with congenital heart diseases, hepatic or renal failure, thyroid disorders, or any other chronic diseases were excluded from the study. This study had no additional costs for the patients, and no interventions were conducted outside the routine therapeutic/diagnostic procedures. Written informed consent was provided by the patients, and their information was secured by our research group. The study protocol was approved by the Ethics Committee of Dezful University of Medical Sciences (IR.DUMS.REC.1397.009).

The patients' demographic data, including age and sex, were obtained from the thalassemia center of the hospital. Tests to determine the hematologic levels of vitamin D and magnesium were done on blood samples prepared for other routine experiments, and no additional blood samples were taken from the patients. A vitamin D plasma level <30 ng/mL and a magnesium plasma level <1.8 mg/dL were considered deficient. Cardiac iron overload was assessed by the MRI T2* technique, whereby the amount of heart iron storage is assessed based on relaxation time in milliseconds (ms). A lower secondary relaxation time, T_2*, shows a higher iron storage level in the heart. In this study, T_2* was valued as the index of iron overload using the CMRtools software package, and T_2* of the heart was categorized as normal (>20 ms), low (14–20 ms), moderate (10–14 ms), and severe (<10 ms).[10] For the evaluation of cardiovascular involvement in the patients, echocardiography was performed by an echocardiography fellow with a Vivid 3 device (USA). In echocardiography, the cavities were measured in the M mode. An ejection fraction <50% was considered reduced left ventricular contraction. A tricuspid valve gradient >30 mm Hg in echocardiography, equal to a systolic pulmonary artery pressure >35 mm Hg, was considered abnormal. Electrocardiography (ECG) was performed for all the patients, and the results were analyzed by a cardiologist, who reported abnormal results.

The data were analyzed with the SPSS software, version 22. Qualitative variables were reported as frequencies and percentages and quantitative variables as the mean ± the standard deviation (SD). For data analysis, the Spearman correlation, Mann–Whitney *U*, and Fisher exact statistical tests were employed. The significance level in all the tests was considered to be 0.05.

RESULTS

The present study assessed 43 patients, consisting of 20 men (46/5%) and 23 women

(53.5%) at a mean age of 31.35±7.03 years (min 12 and max 44). By ECG, 27.9% of the patients had abnormal results in the form of repolarization abnormalities (a negative T wave). Based on MRI T2*, 62.8% of the patients were normal in terms of iron deposition in the heart tissue (Table 1).

The frequency distribution of the observed abnormalities in echocardiography was as follows: left ventricular diastolic dysfunction in 23.3% of the study population, a reduced ejection fraction in 9.3%, right ventricular dilatation in 9.3%, and more-than-mild tricuspid regurgitation in 9.3% (Table 2).

The plasma levels of vitamin D (P<0.05) and magnesium (P<0.01) in patients with normal echocardiography were significantly higher than those in patients with abnormal echocardiography. The T2* time was significantly higher in the normal echocardiography group than in the abnormal echocardiography group (higher iron deposition in the heart of the abnormal echocardiography group) (P<0.01) (Table 3). The relationship between the plasma levels of vitamin D and magnesium and the number of abnormalities in echocardiography was evaluated, and a significant inverse relationship was observed between the number of echocardiographic abnormalities and the plasma level of magnesium (P<0.05), whereas the number of echocardiographic abnormalities was not related to the plasma level of vitamin D (P>0.05).

Table 1. Frequency distribution for the abnormal cases of echocardiography, electrocardiography, plasma magnesium, plasma vitamin D, and iron deposition in the heart based on magnetic resonance imaging T2*

Variables		Frequency	Percentage
Echocardiography	Normal	26	65.5
	Abnormal	17	39.5
Electrocardiogram	Normal	31	72.1
	T inverse	12	27.9
Iron deposition in the heart	Normal	27	62.8
	Low	8	18.6
	Moderate	6	14.0
	Severe	2	4.7
Plasma magnesium	Normal	33	76.7
	Deficient	10	23.2
Plasma vitamin D	Normal	18	43.8
	Deficient	25	58.1

Table 2. Frequency distribution of echocardiographic abnormalities

Echo Abnormalities	Frequency	Percentage
Left ventricular diastolic dysfunction	23.3	10
Reduced ejection fraction	9.3	4
Right ventricular dilatation	9.3	4
More-than-mild tricuspid regurgitation	9.3	4
Right ventricular dysfunction	6.9	3
More-than-mild mitral regurgitation	4.7	2
Systolic pulmonary hypertension in the absence of pulmonary disease	4.7	2

Table 3. Comparison of the averages of plasma vitamin D, plasma magnesium, and iron deposition in the heart based on magnetic resonance imaging T2* in the patients with cardiac involvement based on echocardiography results

Variables	Echocardiography mean±SD		Mann–Whitney *U*
	Normal	Abnormal	
Plasma level of vitamin D	38.85±14.31	30.06±14.31	*P* =0.012
Plasma level of magnesium	2.12±0.23	0.92±0.14	*P* =0.003
T2* time (Iron deposition in the heart)	31.03±8.36	19.13±9.98	*P* =0.001

Table 4. Correlation between the levels of plasma vitamin D and magnesium and the rate of iron deposition in the heart based on magnetic resonance imaging T2* results

Variables	T2* Time (Iron deposition in the heart)
Plasma level of vitamin D	The Spearman correlation coefficient =0.334 *P* =0.033
Plasma level of magnesium	The Spearman correlation coefficient =0.461 *P*=0.002

Table 5. Relationship between echocardiography and electrocardiography results

Electrocardiography Results	Echocardiography Results Frequency (%)		The Fisher exact Test
	Normal	Abnormal	
Normal	26 (100.0)	5 (29.4)	*P*=0.000
Abnormal	0 (0.0)	12 (70.6)	

Table 6. Relationship between electrocardiography findings and the mean of iron deposition in the heart based on magnetic resonance imaging T2* and the plasma levels of vitamin D and magnesium

Variables	Electrocardiography Findings		Mann–Whitney *U*
	Normal	Abnormal	
T2* time (Iron deposition in the heart)	29.43±12.01	18.32±9.40	*P*=0.001
Plasma level of vitamin D	36.39±14/80	32.77±21.94	*P* =0.124
Plasma level of magnesium	2.07±0.24	1.96±0.13	*P*=0.208

Table 4 shows that there was a positive significant correlation between the plasma levels of vitamin D and magnesium and the T2* time (ie, a significant negative correlation with the iron deposition level in the heart) ($P<0.05$). Hence, an increase in the plasma levels of vitamin D and magnesium led to a drop in the level of iron deposition.

A significant relationship was observed between echocardiography and ECG results ($P<0.001$) such that all patients with abnormal echocardiography had abnormal ECG and most patients with normal echocardiography had normal ECG (Table 5).

According to Table 6, the mean T2* time was significantly higher in the patients with normal ECG than in those with abnormal ECG (ie, higher iron deposition in the heart of the patients with abnormal ECG than in the normal group) ($P<0.01$). According to this table, no significant differences were observed between the plasma levels of vitamin D and magnesium in patients with normal and abnormal ECGs ($P<0.05$).

The mean left ventricular ejection fraction showed a significant difference between the male and female subjects ($P<0.05$) in that the value was higher in women. However,

the averages of other parameters were not significantly different between men and women (P>0.05).

DISCUSSION

Thalassemia is the most common recessive monogenic disorder worldwide.[22] Iran is located on the thalassemia belt, and it contains about 25 000 thalassemia major patients.[23] Thanks to recent medical advancements, the survival rate in these patients has increased. These are multiple transfusion-dependent patients,[24] and each blood unit contains approximately 250–300 mg iron, which is equal to 2 years of iron uptake from the circulatory system. Consequently, multiple blood transfusions in thalassemia patients over time lead to the accumulation of iron in different organs of the body like the heart.[25,26] Thus, organic involvement in hemochromatosis patients is an important problem, which needs further studies. MRI T2* is a simple noninvasive method to determine iron concentrations in organs.[10] A relationship between reduced plasma levels of vitamin D and magnesium and manifestations of many cardiovascular diseases has been previously reported.[13] Accordingly, in the present study, we investigated the association between the hematological levels of vitamin D and magnesium and different cardiac involvements using echocardiography, MRI T2*, and ECG in patients with thalassemia major.

In this study, ECG in patients with thalassemia major was normal in 72.1% of the cases. This figure was reported in studies by Chehkandi et al[27] and Malekan et al[28] as 70.6% and 82.0%, respectively, which are similar to the values reported in the present paper. In this study, the most prevalent abnormality observed in ECG was a negative T wave. Moreover, we observed a significant relationship between abnormal ECG and heart involvement based on echocardiography or MRI T2*. It seems that repolarization abnormalities are predictors of cardiac iron deposition, which is consistent with the findings of other studies.[29,30]

Myocardial siderosis is a slowly progressive phenomenon, in which patients show symptomatic cardiac dysfunction over time, and accurate ECG analysis shows patients susceptible to arrhythmias and sudden death.[29] Moreover, in centers where MRI or echocardiography is not available, ECG can help show cardiac involvement in thalassemia patients. Still, ECG at the early stages of cardiac siderosis lacks the same screening value as echocardiography, and it is usually useful in advanced age.[31-33] The absence of a relationship between the plasma deficiency of vitamin D and magnesium and ECG results is probably due to multifactoriality and nonspecific ST-T changes in ECG.

We found that echocardiography was abnormal in 39.9% of our patients with thalassemia. Oztarhan et al[34] studied 100 patients with thalassemia major and compared them with 93 healthy individuals matched in terms of sex and age. They reported that 41.2% of their patients had abnormal echocardiography, which is similar to our study. Our findings showed that the vitamin D level was significantly higher in patients with normal echocardiography than in patients with abnormal echocardiography.

Malek et al[12] stated that vitamin D deficiency was prevalent in children with thalassemia major. Wang et al[35] showed that vitamin D3 deficiency was associated with an increased risk of cardiovascular diseases and that the consumption of vitamin D3 supplements, along with calcium, could reduce the risk factors for cardiovascular diseases such as hypertension, metabolic syndromes, and diabetes. Considering the inverse significant relationship between the

vitamin D level and cardiac iron overload based on MRI T2* in our study and similar ones, perhaps a probable reason for this is secondary vitamin D dermal synthesis impairment and skin darkening due to iron deposition. Additionally, iron deposition in the epithelium of the intestines and the liver, respectively, by disrupting the vitamin D circulatory uptake and hepatic hydroxylation of this vitamin can lead to vitamin deficiency. In addition, malnutrition and anorexia in these patients and feeding constraints such as the limited usage of chicken's yolk and liver, which are rich in vitamin D, are also influential in the deficiency of this vitamin.[21] In the current study, chiming in with a study by Wood et al[36] on thalassemia major patients, the deficiency of vitamin D was related to cardiac iron deposition based on echocardiography and MRI T2*. In a comprehensive study in the United States on 4962 persons, a 39.9% rate of vitamin D deficiency was found in individuals ≥20 years old.[37] In another study on thalassemia major patients in the Iranian city of Kerman, 86% of the study population had vitamin D deficiency, which was inversely related to the serum ferritin level of the patients.[38] In this study, 58.1% of the patients had plasma vitamin D deficiency, which was related to the cardiac iron storage according to MRI T2*. The reason for this different plasma vitamin D deficiency can be geographical differences, nutrition, or genetics.

In the present study, we showed that the mean serum level of magnesium in patients with normal echocardiography was significantly higher than that in patients with abnormal echocardiography. Additionally, a higher number of abnormal findings in echocardiography and higher cardiac iron deposition were significantly correlated with reduced plasma magnesium levels. Previous studies have shown that hypomagnesemia increases the risk of ventricular tachyarrhythmias.[39,40]

Fal Solaiman et al[41] reported that 55% of their patients with congestive heart failure suffered from hypomagnesemia, which caused the incidence of atrial fibrillation and ventricular premature beats. In some studies, the plasma level of magnesium in thalassemia patients is lower than that in the control group.[42,43] In our study, 23% of the patients had reduced plasma magnesium levels (12.5%–20%) considering blood magnesium deficiency in our country. However, as the magnesium ion is intracellular, checking the plasma level of magnesium in our study and other investigations cannot show overall magnesium deficiency in the body, which especially occurs in chronic patients. This requires more exact methods of measuring intracellular magnesium. Moreover, it has been noted that magnesium deficiency impairs vitamin D and calcium metabolism, and it is necessary to remedy the deficiency of these 2 ions in the body.[44]

CONCLUSIONS

In this study, the plasma levels of vitamin D and magnesium in patients with abnormal echocardiography and ECG were significantly lower than those in patients with normal echocardiography and ECG. Moreover, the level of cardiac iron overload was higher in patients with abnormal echocardiography and ECG than in the normal group. On the other hand, there was an inverse significant relationship between the plasma levels of magnesium and vitamin D and the rate of iron overload in cardiac tissue. Considering the inverse association between the plasma levels of vitamin D and magnesium and iron overload, we recommend that vitamin D and magnesium supplements, along with iron chelators if necessary, be prescribed periodically for thalassemia major patients to reduce iron

overload complications as much as possible. We also suggest that similar studies on a wider level with larger patient populations be conducted in the future. Our results failed to specify whether the deficiency of vitamin D and magnesium is related to cardiac iron overload casually. Therefore, more studies are needed in this field to determine the accurate mechanisms of the association between vitamin D and magnesium and iron storage in patients with thalassemia.

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