Research Article

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Association between serum vitamin D level and liver MRI T2 star in patients with β-thalassemia major

β-Talasemi Major Hastalarında Karaciğer MRI T2 skoru ile Serum Vitamin D düzeyleri arasındaki İlişki

https://doi.org/10.1515/tjb-2018-0120
Received March 29, 2018; accepted November 27, 2018; previously published online June 11, 2019

Abstract

Background: Iron overloaded Beta Thalassemia major (β-TM) patients have a high risk of liver problems. In recent years studies revealed that vitamin D level is decreased in chronic liver diseases. The present study was designed to find the association between the serum vitamin D levels and the liver iron deposition in patients with β-TM.

Materials and methods: A total of 101 patients with a diagnosis of β-TM were included into this study. The patients were divided into four groups according to liver T2* MRI scores (group 1: normal, group 2: mild iron load, group 3: moderate iron load and group 4: severe iron load). Serum vitamin D was measured by chemiluminescence immunoassay method.

Results: The vitamin D level was median 14 (4–91) ng/mL. There was a positive correlation between vitamin D levels and liver T2* MRI scores (r = 0.31, p < 0.05). There is a significant difference between groups 1 and 4 for vitamin D level (p < 0.05). Vitamin D deficiency (<20 ng/mL) was observed 71% in group 1, 67% in group 2, 80% in group 3 and 100% in group 4.

Conclusions: Vitamin D monitoring and supplementation should be routine in β-TM patients to prevent both skeletal and non-skeletal complications.

Keywords: Vitamin D; Liver T2* MRI scores; Beta-thalassemia major.

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Conclusions: Vitamin D monitoring and supplementation should be routine in β-TM patients to prevent both skeletal and non-skeletal complications.

Keywords: Vitamin D; Liver T2* MRI scores; Beta-thalassemia major.

Öz


Bulgular: Ortalama T2* skoru 5.86 ± 7.68 ms idi. Vitamin D düzeyleri ortanca 14 (4–91) ng/mL olarak ölçüldü. Vitamin D düzeyleri ile karaciğer T2* MRI skoru arasında pozitif korelasyon olduğu tespit edildi (r = 0.31, p < 0.05). Grup
1 and 4 are vitamin D levels in the normal range (p<0.05). Vitamin D deficiency (<20 ng/mL) was found in group 1 (71%), group 2 (67%), group 3 (80%) and group 4 (100%).

**Sonuç:** β-TM patients showed vitamin D deficiencies in all groups. Vitamin D deficiency is common in β-TM patients. Vitamin D deficiency is common in β-TM patients.

**Anahtar Sözcükler:** Vitamin D; karaciğer MRI T2* skoru; Beta-talasemi major.

**Introduction**

Vitamin D, which has multiple functions, is a fat-soluble sterol derivative [1]. The regulation of the mineral and skeletal homeostasis is the primary and most well known physiological function of vitamin D [2]. Vitamin D deficiency alters bone development and causes diseases such as rickets, osteomalacia, and osteoporosis [3]. Recently studies have aimed to search the extraskeletal manifestations of vitamin D in human organism. In many cancer types (e.g. colon, prostate, breast) deficiency of vitamin D may be responsible for the raised prevalence. Also autoimmune diseases, cardiovascular diseases, type II diabetes may be caused deficiency of vitamin D [4, 5].

Beta thalassemia major (β-TM), in which ineffective erythropoiesis and hemolysis cause to the remarkable anemia, is a common genetic disorder [6]. Repeated blood transfusion to treat anemia lead to iron overload and deposition in vital organs particularly heart and liver. Iron overload interferes the normal physical functions of the affected organs leading to heart and liver problems, delayed physical and sexual development and finally decreased life expectancy [7]. T2-star magnetic resonance imaging (T2* MRI) is used to detect the high molecular weight iron complexes deposited in the tissues. Thus T2* MRI can quantify the amount of iron deposited, which is very helpful for early diagnosis of liver hemosiderosis before clinical signs are apparent [8].

Liver and kidney participate in the synthesis of vitamin D [9]. In recent years some studies revealed that vitamin D level is decreased in chronic liver diseases (hepatitis B virus infection, cirrhosis, etc.) [10]. Many studies emphasized the importance of low levels of vitamin D in growth retardation, heath problems, and osteoporosis seen in β-TM patients [11–14]. Vitamin D deficiency may be responsible for the liver problems in β-TM patients. Because of liver iron deposition, chronic liver disease leading to cirrhosis is seen in these patients [15]. Our study was planned to figure out the relation between vitamin D levels and the liver iron deposition in β-TM patients.

**Materials and methods**

One hundred and one (57 females and 44 males) patients with diagnosis of β-TM were included into the study. β-TM patients receive blood transfusion according to their blood counts (15–30 days intervals). T2* MRI of liver obtained within last year was used to evaluate the liver iron load (cut off points of liver T2*: normal >6.3 ms, mild: 2.8–6.3 ms, moderate: 1.4–2.7 ms, severe <1.4 ms). The patients were divided into four groups according to T2* scores (group 1: 31 patients with normal T2* score, group 2: 18 patients with mild iron load, group 3: 41 patients with moderate iron and group 4: 11 patients with severe iron load). The study protocol was approved by the institutional Ethics Committee (09.06.2016 and 11/5) and informed consent was obtained from all patients. Peripheral blood samples were taken just before receiving the transfusion. Serum vitamin D was quantified by chemiluminescence immunoassay method in Liason (Diasorin, Saluggia, Italia) instrument. Measurement of serum ferritin was carried out by chemiluminescence immunoassay method in Liason (Diasorin, Saluggia, Italia) instrument. Measurement of serum ferritin was carried out by chemiluminescence (Unicel Dxi 800 Access Analyzer, Beckman Coulter, Holliston, MA, USA). Complete blood count was performed by hematology analyzer (Coulter LH 780 Analyzer, Beckman Coulter). Liver function tests such as ALT, AST, GGT, ALP, LDH, total and direct bilirubin were measured by spectrophotometer method in AU5800 instrument (Beckman Coulter).

**Statistical analysis**

Values are showed as mean±standard deviation and median (minimum-maximum) while categorical variables are given as frequencies and percentages. The Kolmogorov-Smirnov test was used to verify the normality of the distribution of continuous variables. Since vitamin D levels were not distributed normally, Kruskal-Wallis tests was used to evaluate comparisons between the groups. Correlations were assessed with the Spearman correlation coefficient and the χ²/Fisher’s exact tests were used for categorical variables. Data were analyzed with SPSS 20 software. Statistical significance was considered as p<0.05.
Results

Demographic characteristic of patients are shown in Table 1. No patients had multivitamins or vitamin D supplementation. The mean age was 26.21 ± 7.53, with a range of 15–47 years. Hemoglobin values ranged from 6.9 to 12.4 (mg/dL) before transfusion. The T2* value was median 2 (1.12–60.35) ms. The vitamin D level was median 14 (4–91) ng/mL. There was a positive correlation between vitamin D levels and liver T2* MRI scores (r = 0.31, p < 0.05). Serum vitamin D levels were median 17 (6–91) ng/mL, median 14 (4–32) ng/mL, median 14 (4–29) ng/mL, median 10 (4–14) ng/mL for groups 1, 2, 3, and 4, respectively. There is a significant difference between groups 1 and 4 for vitamin D level (p < 0.05) (Table 2, Figure 1). Cut-off limit for vitamin D deficiency has been specified as serum levels less than 20 ng/mL and vitamin D insufficiency has been specified as serum levels between 20 and 30 ng/mL. Seventy-eight patients (77.2%) were specified as vitamin D deficient, and 20 patients (19.8%) were specified as vitamin D insufficient in the study. Three patients (2 patient in group 1 and 1 patient in group 2) had vitamin D levels within normal limits. Vitamin D deficiency (<20 ng/mL) was observed 71% in group 1, 67% in group 2, 80% in group 3 and 100% in group 4. Table 3 shows the distribution frequency of the patients according to various vitamin D cut-off values.

In the present study, serum ferritin levels were found to be markedly elevated and it was median 1939 (19–19,419) ng/mL. Total bilirubin levels were 2.53 ± 1.29 mg/dL and direct bilirubin levels were median 0.44 (0.01–1.8) mg/dL. It was observed that total bilirubin level was higher than cut-off value (<1.2 mg/dL) in 87% of patients, and direct bilirubin level was higher than cut-off value (<0.3 mg/dL) in 77% of patients. Serum ALT, AST, GGT, ALP and LDH levels were 44.69 ± 46.22 U/L, 39.37 ± 27.99 U/L, 39.08 ± 60.57 U/L, 112 ± 67 U/L and 203 ± 81 U/L, respectively. There was a moderate negative correlation between vitamin D and ferritin (r = −0.46, p < 0.05) and a weak correlation between vitamin D level.

Table 1: Clinical, hematological and biochemical characteristics of β-TM patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>26 ± 7.53</td>
<td>24 (15–47)</td>
</tr>
<tr>
<td>RBC count (10¹²/L)</td>
<td>3.42 ± 0.62</td>
<td>3 (2.5–6.9)</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>8.95 ± 1.08</td>
<td>9 (6.9–12.4)</td>
</tr>
<tr>
<td>Htc (%)</td>
<td>27.39 ± 3.36</td>
<td>27 (20.8–45.9)</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>81.37 ± 4.41</td>
<td>82 (66.7–96)</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>2902 ± 3120</td>
<td>1939 (19–19,419)</td>
</tr>
<tr>
<td>Vitamin D (ng/mL)</td>
<td>15.4 ± 10.33</td>
<td>14 (4–91)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>44.69 ± 46.22</td>
<td>31 (7–264)</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>39.37 ± 27.99</td>
<td>29 (12–199)</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>39.08 ± 60.57</td>
<td>25 (8–572)</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>112 ± 67</td>
<td>91 (50–483)</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>203 ± 81</td>
<td>188 (88–623)</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dL)</td>
<td>2.53 ± 1.29</td>
<td>2 (1–7)</td>
</tr>
<tr>
<td>Direct Bilirubin (mg/dL)</td>
<td>0.41 ± 0.51</td>
<td>0.44 (0.01–1.8)</td>
</tr>
<tr>
<td>Liver T2* (ms)</td>
<td>5.86 ± 7.68</td>
<td>2 (1.12–60.35) *</td>
</tr>
</tbody>
</table>

*p < 0.05 significant correlation between vitamin D level.

Table 2: Vitamin D levels in groups according to liver T2* MRI scores.

<table>
<thead>
<tr>
<th>Vitamin D (ng/mL)</th>
<th>Mean ± SD</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Group 1</td>
<td>19.6 ± 15.0</td>
<td>17 (6–91) *</td>
</tr>
<tr>
<td>Group 2</td>
<td>14.7 ± 8.1</td>
<td>14 (4–32)</td>
</tr>
<tr>
<td>Group 3</td>
<td>14.4 ± 6.2</td>
<td>14 (4–29)</td>
</tr>
<tr>
<td>Group 4</td>
<td>8.65 ± 3.0</td>
<td>10 (4–14) *</td>
</tr>
</tbody>
</table>

*p < 0.05 group 1 compared to group 4.

Table 3: The distribution of the patients according to liver T2* MRI scores and vitamin D cut-off levels.

<table>
<thead>
<tr>
<th>Liver T2* MRI</th>
<th>Vitamin D Levels</th>
</tr>
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<tbody>
<tr>
<td>(&lt;12)</td>
<td>(ng/mL)</td>
</tr>
<tr>
<td>(ng/mL)</td>
<td>(&lt;12)</td>
</tr>
<tr>
<td>Group 1 (n = 31)</td>
<td>9 (29%)</td>
</tr>
<tr>
<td>Group 2 (n = 18)</td>
<td>7 (39%)</td>
</tr>
<tr>
<td>Group 3 (n = 41)</td>
<td>15 (36%)</td>
</tr>
<tr>
<td>Group 4 (n = 11)</td>
<td>9 (82%)</td>
</tr>
</tbody>
</table>
negative correlation between vitamin D and ALT (r = −0.35, p < 0.05), AST (r = −0.31, p < 0.05) and GGT (r = −0.20, p < 0.05) (Table 1).

Discussion

Vitamin D insufficiency and deficiency are common in the world population. Elderly people and individuals with chronic disease are more frequently effected [16]. Recent studies revealed that the prevalence of vitamin D insufficiency and deficiency are common in patients with chronic liver disease than in healthy people up to 92% [17]. β-TM is a type of hemolytic anemia characterized by defective biosynthesis of beta globin chains [18]. Thalassemic patients need lifelong blood transfusion and this results in the deposition of iron in different organs, namely liver, heart, pancreas, lungs, and kidneys [19]. The T2* MRI technique seems to be an accurate, valid and non-invasive method for assessment of tissue iron stores. This method has radically changed strategy of the thalassemia management, especially in the tailoring of chelation regimens [20].

Low serum 25-hydroxy vitamin D levels have also been reported previously in β-thalassemic patients by many investigators [21]. Sultan et al. found a marked deficiency (72.2%) of 25-hydroxy vitamin D in β-TM [22]. This deficiency has been attributed to malabsorption of vitamin D as well as inadequate dietary intake. Many authors attributed their results to hepatic dysfunction which leads to defective hydroxylation of vitamin D and so decreased serum vitamin D level [23]. Pirinçcioğlu et al. reported that the etiology of 25-OH vitamin D deficiency might be the hepatic iron-overload rather than the dysfunction of endocrine tissues [24].

We studied the relationship between serum vitamin D levels and liver T2* MRI scores. There was no iron deposition in the liver of 30% of patients according to liver T2* MRI. But we found that 97% of patients were vitamin D deficient. Such a high ratio was a surprising finding for us. Sunlight is very important for vitamin D status of the body. The city of Antalya, in which our study was held, is located in the south of Turkey, and is sunny for approximately 9 months. Cagirci et al. in a study held in Antalya, found that vitamin D levels of healthy subjects is 24.6 ± 9.3 ng/mL. This study emphasizes that vitamin D levels of healthy people living in Antalya is more than 20 ng/mL [25]. Sunlight has ultraviolet B radiation. Interaction of ultraviolet B radiation with 7-dehydrocholesterol in the skin results in vitamin D synthesis [26]. Several factors (age, skin color) can effect vitamin D synthesis [27]. Many studies state that there is a correlation between the skin color and the vitamin D level. Dark skin color have high prevalence of vitamin D deficiency than light skin color [28]. β-TM patients suffer generalized hyperpigmentation caused by skin iron deposition and elevated bilirubin levels due to hemolysis [29–31]. In this study we did not measured the iron deposition in the skin but we found that bilirubin level was high in 87% of patients. We think that the reason for the presence of 97% of vitamin D insufficiency is skin hyperpigmentation causing insufficient 7-dehydrocholesterol synthesis. That means even β-TM patients live in sunny areas, they can not sun light for vitamin D synthesis due to their hyperpigmented skins.

In our study we found that there was a high prevalence of vitamin D deficiency in β-TM patients who had severe iron overload demonstrated by liver T2* MRI. Our results showed that a moderate correlation was present between vitamin D and liver T2* score. Also we observed that there was a negative correlation between vitamin D levels and liver function tests. So as the iron deposition in the liver increases, liver damage increases and vitamin D synthesis decreases because of defective hydroxylation in liver.

In conclusion, high prevalence of vitamin D deficiency occurs in β-TM patients that may largely contribute to their mortality and morbidity. Vitamin D insufficiency caused by skin iron deposition and hyperpigmentation progresses to vitamin D deficiency as the iron deposition in the liver increases. Vitamin D monitoring and supplement are advised for β-TM patients to prevent both skeletal and non-skeletal complications.

References