Correlation of Cardiac and Liver Iron Level with T2*MRI and Vitamin D3 Serum Level in Patients with Thalassemia Major

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Introduction: Thalassemia is a microcytic hypochromic anemia characterized by congenital abnormalities in hemoglobin production. It is estimated that 3% of the world population carry one of the characteristics of beta-thalassemia traits. It is the most common genetic disease in the world.1,2 In the next 20 years, it is expected that around 900,000 thalassemic people will be born in the world, 95% of them in Asia, especially in India and the Middle East.3,4 Thalassemia is predominantly found in underdeveloped countries, and the prevalence of this disease is lower in Europe and North America.5,6 According to the Thalassemia Association in Iran, the number of patients with thalassemia in 2005 was 18,616, this number is more than 20,000 in 2016, and most of these patients are in the Mazandaran and Fars provinces.7,8 The disease is autosomal recessive and is inherited from the parents. A beta thalassemic baby is seen in about 300 cases every born in Iran. This is three times more than the highest hereditary diseases incidence, such as Down syndrome (it’s about 1 in 1000).9 Patients with beta-thalassemia major need to have a lifelong blood transfusion, since they suffer from severe anemia (hemoglobin 6 g/dL).10 Each blood unit contains 200–250 mg iron.9,11-13 Repeated blood transfusions cause iron deposition in vital organs like heart, liver and endocrine glands.14 Iron...
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in cases of increased iron load caused by thalassemia
worsens bone disease in these patients. Vitamin
D deficiency also is associated with a decrease in cardiac
function, muscle weakness and glucose intolerance. In
addition to vitamin D deficiency, PTH increases the
expression of L-type or LVGCC-induced calcium
channels, that these channels, in addition to calcium,
absorb iron. This absorption caused iron deposition
in muscles. Therefore, there was a relative correlation
between low levels of vitamin D, increased iron deposition
in liver and ventricular disorders in thalassemia. In this study, we aimed to investigate the association
between vitamin D deficiency and increased iron uptake
by liver and heart which measurable by T2 * MRI.

Materials and Methods
In this cross-sectional study, thalassemia major patients
who were older than 5 years of age were investigated
in Arak Amir Kabir Hospital, Iran. We used the
Cochran formula for sample size by the effect of the
thalassemia patients in Arak Amir Kabir Hospital, Iran.
Thalassemia diagnosis was done by hemoglobin electo-
phoresis, target cells in the blood smear, and pre-

cence of isolated microcytic anemia. Ethics Committee
approved the study (IR.ARAKMU.REC.1394.271) and
written informed consent was taken from patients or
parent(s) or by the legal representative prior to trial
participation with the Declaration of Helsinki. Forty

overload may initiate structural changes in the myocardium
which can eventually lead to heart failure which is considered
as the major cause of death in thalassemic patients who
depend on blood transfusion.1

The liver is the first organ to deposited iron and stores
about 70% of visceral iron. Therefore, liver iron reflects
the whole body of iron in the body. The heart is another
important organ that iron deposited in. So iron deposition
in this organ causes impairment of performance and is
also the most important cause of death in thalassemic
patients, measuring the amount of iron deposited is very
important. In thalassemia patients, bone diseases are one of
the causes of mortality, including osteoporosis, bone softness,
changes in the shape of the spine and other large bones,
and thinning the bone cartilage. Meanwhile, vitamin D is
considered as one of the factors involved in the absorption
of minerals which made bone matrix. Calcium and phos-
phorus are minerals that their absorption need for
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with diagnosed thalassemia major by the molecular
methods were included in study. MRI was performed
using Tesla magneto symphony Graniand 32.5/1
(Siemens Healthineers Germany).

The serum ferritin level was evaluated by ELISA
(Abcam, Cambridge, England). The method of measuring
serum vitamin D3 level was luminescence electroence-
phalography which uses polyclonal antibodies and speci-

cally antibodies against 25OH vitamin D3. The unit of
vitamin D is ng/dl and the range of measurements by the
method is from 1/1 ng/dl to 1500 ng/dl. Participants were
excluded if they had liver disorders, hepatitis B, C, HIV,
changing chelation regimen within the last 6 months, and
renal failure. Patients were discontinued from the study
for these reasons: safety, lost to follow-up and voluntary
discontinuation.

Statistical Analysis
Data were analyzed using SPSS 16 software (Chicago,
USA). Normal distribution of data was checked by
Kolmogorov–Smirnov. The results of quantitative and quali-
tative data were tested by ANOVA, T-test and linear regres-
sion. The P value <0.05 in was considered statistically
significant. The intraclass correlation (ICC) was computed
in order to determine the reproducibility of each method. For
each method, the ICC amount was close to one, and the
appropriate statistical analysis used in study.

Results
Of the 40 thalassemia major patients were transfusion
dependent (Td), 27 (67.5%) and 13 (32.5%) patients
were female and males respectively, the mean age of
the patients was 23.8 ± 10.7 years, change between 7
and 55 years old. Three patients (7.5%) had been
splenectomy.

The mean of T2 MRI values in all patients was 23.8 ±
7.7, the maximum and minimum were 38 and 7.5, respec-
tively. The serum vitamin D3 levels in 33 patients (82.5%
of cases) were less than 20 ng/dl, 2 (5%) patients were in
the range of 20–30 ng/dl, and the other five patients had
more than 30 ng/dl. The vitamin D level compared to
reference range and categorized in deficient, insufficient
and adequate categories. The mean serum vitamin D3
level in all patients was 16.1 ± 8.1 ng/dl. Ferritin and
Parathyroid hormone measurements are also carried out
and summarized in Table 1.
Relationship Between Vitamin D3 Serum Levels and Age

As shown in Table 2, we found a significant correlation between vitamin D3 serum level and age in our studied subjects. The correlation suggests a reciprocal relationship between age and vitamin D3.

The T-tests, showed no difference in Vitamin D3 serum levels in men and women (Table 3).

Correlation Between Heart T2*MRI and Ferritin, Vitamin D3, PTH

Correlation coefficient between Heart T2*MRI and ferritin levels was 0.437 and was significant. However, as presented in Table 4, there was no correlation between Heart T2*MRI, serum vitamin D3 and PTH levels.

Correlation Between Liver T2*MRI and Ferritin, Vitamin D3, PTH

A significant association between liver T2*MRI and ferritin levels was seen. However, as shown in Table 5, there was no correlation between liver T2*MRI and serum vitamin D3 and PTH levels.

Correlation Between Ferritin and, Vitamin D3, PTH

As shown in Table 6, there was a positive correlation between the two variables. In addition, there was no correlation between serum ferritin and Vitamin D3.

Discussion

We found that there was a correlation between serum ferritin and PTH levels. This can be indicative of the fact that PTH, in contrast to Vitamin D3, is a better indicator of endocrine and osteoporosis in these patients. In this descriptive analytical study, 40 blood transfusion-dependent thalassemia patients were examined, the mean Vitamin D3 serum levels were 16.1 ± 8.1 in all patients, which showed an insufficient level.

In a study in North America, the relationship between thalassemia syndrome and vitamin D deficiency was found in 361 patients, which 12.8% of subjects had less than 27 nmol/l

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### Table 1 Summarized Measured Criteria in the Patients

| Measured Index | Standard Deviation | Mean | Total |
|----------------|--------------------|------|-------|
| Heart T2*MRI   | 7.709              | 23.8 | 40    |
| Liver T2*MRI   | 7.222              | 5.5  | 40    |
| Ferritin       | 2110.800           | 1681.75 | 40 |
| D3             | 8.121              | 16.14| 40    |
| PTH            | 7.300              | 43.95| 40    |

### Table 2 Pearson Correlation Coefficient Between Age and Vitamin D3

| Age | Pearson Correlation Coefficient | Number |
|-----|----------------------------------|--------|
|     | -0.611*                          | 40     |

| Serum Vitamin D3 level | Pearson Correlation Coefficient | Number |
|-----------------------|---------------------------------|--------|
|                       | -0.611*                          | 40     |

Note: *Correlation coefficients significant under 0.05.

### Table 3 t-Test Vitamin D3 Serum Levels in Men and Women

| Gender         | Number | Mean | Standard Deviation | Minimum | Maximum | Significant Level in t-Test |
|----------------|--------|------|--------------------|---------|---------|---------------------------|
| Serum Vitamin D3 level | Women | 27   | 17.45              | 9.054   | 35      | 0.180*                    |
|                  | Men    | 13   | 13.79              | 5.673   | 29      |                           |
|                  | Total  | 40   | 16.14              | 8.121   | 35      |                           |

Note: *Correlation Coefficients significant under 0.05.

### Table 4 Pearson Correlation Coefficient Between Heart T2*MRI and Vitamin D3, Ferritin, PTH

| PTH     | Vitamin D3 | Ferritin | Heart T2*MRI |
|---------|------------|----------|--------------|
| -0.162  | 0.018      | -0.437   |              |
| 0.318   | 0.916      | 0.005*   |              |

Note: *Correlation Coefficients significant under 0.05.

### Table 5 Pearson Correlation Coefficient Between Liver T2*MRI and Vitamin D3, Ferritin, PTH

| PTH     | Vitamin D3 | Ferritin | Liver T2*MRI |
|---------|------------|----------|--------------|
| -0.107  | -0.022     | -0.335   |              |
| 0.509   | 0.896      | 0.035*   |              |

Note: *Correlation Coefficients significant under 0.05.

### Table 6 Pearson Correlation Coefficient Between Ferritin and, Vitamin D3, PTH

| PTH     | Vitamin D3 | Ferritin | Heart T2*MRI |
|---------|------------|----------|--------------|
| -0.162  | 0.018      | -0.437   |              |
| 0.318   | 0.916      | 0.005*   |              |

Note: *Correlation Coefficients significant under 0.05.
and 82% had less than 75 nmol/l vitamin D. Another study on 96 thalassemic patients showed that 43% of patients had a deficiency of vitamin D, and 30% of them had insufficient level of vitamin D. In addition, a study on 220 thalassemia patients in showed that 37.2% of patients have serum vitamin D levels, less than 23 nmol/L. The percentage of patients with vitamin D deficiency in these studies varies with the present study, and it may be partially explained by underlying illness of patients, the difference in average age and the type of climate where they live. In this study, there was a correlation between heart and liver T2*MRI and serum ferritin levels. This finding correlates with the results of the study by Taghizadeh Sarvestani et al, which showed a negative correlation between serum ferritin levels and liver iron levels, measured by T2*MRI. Of note, serum ferritin levels and iron heart level there had no correlation.

In our study, there was no correlation between ferritin and vitamin D3 in serum. This finding is consistent with the findings of nakavachara et al, in which 109 children between 5.9 to 14.1-year-old with HbE/B thalassemia were investigated. In our study, age increase caused a drop in the serum level of Vitamin D3. It suggests that old patients may require more Vitamin D3 supplementation.

Conclusion
Vitamin D measurement in thalassemic patients indicate a deficiency of this vitamin, though they get enough sunlight, this may suggest to administer vitamin D3. Also, deficiency of this vitamin is associated with an imbalance in the absorption of essential minerals for bone matrix and on the other hand, disruption of the endocrine in the thalassemic patients due to iron overload. The measurement of PTH level can be used to assess the degree of endocrine disruption in such patients. Therefore, periodic monitoring of other hormones is recommended. Accordingly, it can be concluded that a periodic and regular assessment of PTH levels is necessary in such patients.

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Disclosure
The authors report no conflicts of interest.

Table 6 Pearson Correlation Coefficient Between Ferritin, Vitamin D3, PTH

| Vitamin D3 | PTH   | Pearson Correlation Coefficient | p-value | Number |
|-----------|-------|---------------------------------|---------|--------|
| 0.535     | 0.006 | 0.430                           |         |        |
| 0.099     |       |                                  |         |        |

Note: *Correlation Coefficients significant under 0/05.

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