The Association between Serum Ferritin Levels and 25(OH)D Levels in Adult Patients with Transfusion Dependent Thalassemia

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ABSTRACT
Deficiency and insufficiency of 25(OH)D in thalassemia patients are still high even in countries with abundant sun exposure or with vitamin D supplementation. Long term routine blood transfusions in thalassemia patients cause iron overload which is characterized by increase in serum ferritin levels. Liver damage due to iron overload is thought to disrupt synthesis of 25(OH)D in the liver. This study aimed to determine the association between serum ferritin levels and 25(OH)D levels in adult patients with transfusion dependent thalassemia. This was a cross-sectional observational analytic study. The blood sample were collected from adult patients with transfusion dependent thalassemia to measure the levels of serum ferritin using the electrochemiluminescence immunoassay (ECLIA) method and 25(OH)D levels using the chemiluminescence microparticle immunoassay (CMIA) method. There were 45 subjects, 24 males and 21 females with median age of 23 years; duration of transfusions was 16.3 ± 6.2 years. 23 subjects used deferasirox and 22 subjects used deferiprone iron chelator. The median of serum ferritin levels was 4,467 ng/mL and the mean of 25(OH)D levels was 12.69 ± 4.84 ng/mL. There was significantly negative association between serum ferritin levels and 25(OH)D levels (r = -0.41, p = 0.003). In this study, significantly negative association was found between serum ferritin levels and 25(OH)D levels. High serum ferritin levels may decrease 25(OH)D levels in adult patients with transfusion dependent thalassemia.

KEYWORDS
Ferritin
25(OH)D
Transfusion dependent thalassemia

GRAPHICAL ABSTRACT
Introduction
Thalassemia is a group of congenital anemia that occurs due to impaired synthesis of one or more globin subunits from normal human hemoglobin [1]. Transfusion dependent thalassemia group requires routine blood transfusions throughout life to maintain optimal hemoglobin levels [2]. Long term routine blood transfusions cause iron overload which is characterized by increase in serum ferritin levels [3]. One of the complications of iron overload in thalassemia is impaired liver, an organ that plays an essential role in synthesis of 25(OH)D [4,5].

Studies in USA and India show that 25(OH)D deficiency in thalassemia patients are 32.8% and 80.6%, respectively [6,7]. 25(OH)D deficiency and insufficiency in thalassemia patients are found to be high even in countries with abundant sun exposure or with vitamin D supplementation [6,8]. 25(OH)D deficiency in adult patients with thalassemia is thought to decrease in bone mineral density reported by 60.5% [9]. Therefore, detecting 25(OH)D deficiency in adult patients with thalassemia is very important.

Iron overload in thalassemia causes excessive labile iron in hepatocytes which triggers the formation of reactive oxygen species (ROS) [5]. Superoxide causing the iron-sulfur [2Fe-2S] of ferredoxin becomes unstable and damaged which causes enzymatic activity of 25-hydroxylase to be disrupted [10,11]. Increased ROS also causes necrosis or apoptosis of hepatocyte so that the quantity of 25-hydroxylase in hepatocyte decreases. The reduction of 25-hydroxylase enzyme in hepatocyte causes the disruption of vitamin D3 hydroxylation to 25(OH)D [4,5].

There are still limited studies about 25(OH)D levels in adult patients with thalassemia. Studies of the association between serum ferritin levels and 25(OH)D levels in patients with transfusion dependent thalassemia are also obtained with inconsistent results. Accordingly, this study aimed to determine the association between serum ferritin levels and 25(OH)D levels in adult patients with transfusion dependent thalassemia.

Material and methods
This study was a cross-sectional observational analytic attempt conducted at Dr. Soetomo Hospital, Surabaya, Indonesia from June to July 2019. This study was approved by the ethics committee of Dr. Soetomo Hospital with ethical clearance number 1185/KEPK/V/2019. The sample was collected by consecutive sampling technique. Informed consent was obtained from all subjects. This study involved 45 subjects with inclusion criteria of age ≥ 18 years old, male or female and diagnosed with thalassemia who had received routine blood transfusions at Dr. Soetomo Hospital. The exclusion criteria in this study were history of infection in 3 weeks before, malignancy, hepatitis B, hepatitis C, alcoholism, smoking, body mass index (BMI) ≥ 25 kg/m2, malabsorption, chronic kidney disease, pregnancy or breastfeeding and history of consuming vitamin D, vitamin A, vitamin C, vitamin E, N-acetylcysteine, phenytoin, phenobarbital, carbamazepine, isoniazid, rifampicin, pyrazinamide or corticosteroid drugs in 4 weeks before.

Basic data including gender, age, duration of transfusions, iron chelator, sun exposure score and BMI were collected. Sun exposure score was calculated using weekly sun exposure score questionnaire. There were three choices for the amount of time spent outdoors each day (0 = < 5 minutes, 1 = 5-30 minutes, 2 = > 30 minutes) and four choices for clothing or skin exposure while outdoors (1 = face and hands only, 2 = face, hands and arms, 3 = face, hands and legs, 4 = “bathing suit”). The multiplication product of the amount of time spent outdoors and the amount of skin exposed was calculated for each day (minimal = 0, maximal = 8), then all seven days scored were summed (minimal = 0, maximal = 56) [12]. Pre-transfusion blood samples were collected to measure the serum ferritin and 25(OH)D levels. Serum collected using serum separator tubes. Samples containing precipitates were centrifuged before performing the assay. Serum ferritin level was measured using electrochemiluminescence immunoassay (ECLIA) with Ferritin kit from Roche Diagnostics. Serum 25(OH)D was measured using chemiluminescence microparticle immunoassay (CMIA) with ARCHITECT 25-OH vitamin D Kit from Abbott Laboratories. The resulting chemiluminescent
reaction was measured as relative light units detected by the ARCHITECT iSystem optics. Serum ferritin and 25(OH)D levels were quantitatively measured in ng/mL.

Characteristic data were presented descriptively in the form of frequencies and percentages for categorical data, medians and ranges or mean and standard deviations for numerical data. Normality data was analyzed by Shapiro Wilk. The association between independent variables and dependent variables was analyzed using Pearson correlation test for normally distributed data and Spearman correlation test for abnormally distributed data. p value < 0.05 was considered significant (95% confidence interval).

All statistical analysis was undertaken using SPSS 23.0 (IBM, Chicago, USA).

**Result and Discussion**

The data of 45 subjects are presented in Table 1. 24 subjects (53.3%) were male and 21 (46.7%) were female. The subjects had a median age of 23 years. The mean duration of transfusion was 16.3 years. All subjects were on iron chelation therapy, 23 subjects (51.1%) used deferasirox and 22 subjects (48.9%) used deferiprone. None of them used deferoxamine or iron chelator combination. The median sun exposure score of subjects was 14. The mean BMI of subjects was 18.7 kg/m² with the lowest BMI by 14.4 kg/m² and the highest BMI by 24.1 kg/m².

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n = 45</th>
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<tbody>
<tr>
<td>Gender, n (%)</td>
<td></td>
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<tr>
<td>Male</td>
<td>24 (53.3)</td>
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<tr>
<td>Female</td>
<td>21 (46.7)</td>
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<tr>
<td>Age, median (years)</td>
<td>23</td>
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<tr>
<td>Duration of transfusions, mean (SD) (years)</td>
<td>16.3 (6.2)</td>
</tr>
<tr>
<td>Iron chelator, n (%)</td>
<td></td>
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<tr>
<td>Deferiprone</td>
<td>22 (48.9)</td>
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<tr>
<td>Deferasirox</td>
<td>23 (51.1)</td>
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<tr>
<td>Sun exposure score, median</td>
<td>14</td>
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<tr>
<td>BMI, mean (SD) (kg/m²)</td>
<td>18.7 (1.9)</td>
</tr>
</tbody>
</table>

The median of serum ferritin levels was 4,467 ng/mL, the lowest level was 1,360 ng/mL and the highest level was 11,646 ng/mL. The mean of 25(OH)D levels was 12.69 ± 4.84 ng/mL, the lowest level was 3.5 ng/mL and the highest level was 26.6 ng/mL. The results of serum ferritin and 25(OH)D levels are presented in Table 2. In this study, most of the subjects (93.3%) were 25(OH)D deficiency (≤ 20 ng/mL) and 6.7% subjects were 25(OH)D insufficiency (21-29 ng/mL).

<table>
<thead>
<tr>
<th>Table 2: Serum ferritin and 25(OH)D levels of subjects</th>
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<tbody>
<tr>
<td>Serum ferritin levels</td>
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<tr>
<td>Median (ng/mL)</td>
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<tr>
<td>Range (min – max) (ng/mL)</td>
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<td>25(OH)D levels</td>
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<tr>
<td>Mean ± SD (ng/mL)</td>
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<td>Range (min – max) (ng/mL)</td>
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There was a significant negative association between serum ferritin levels and 25(OH)D levels (r = - 0.41, p = 0.003) [Figure 1], meaning the higher serum ferritin levels, the lower 25(OH)D levels. Some factors that influence 25(OH)D levels were age, BMI and sun exposure score. In this study, there was no significant association between age (r = - 0.101, p = 0.511), BMI (r = 0.253, p = 0.093) or sun exposure score (r = 0.256, p = 0.09) with 25(OH)D levels.
The majority of subjects (53.3%) in this study were male. This result was similar to that of the study in Indonesia where the majority of subjects (59.3%) were male [13]. However, another study on 90 subjects showed more female subjects (58.9%) than male subjects [14]. Thalassemia is a genetic disorder characterized by decreased or absent synthesis of normal globin chains encoded by genes in autosomal chromosome so it can affect all gender [5]. The subjects in this study had a median age of 23 years and mean duration of transfusion of 16.3 years. A study in Italy on 111 subjects showed that the age of thalassemia was older with mean of 32.6 ± 6 years and duration of transfusion of 17.4 years [15]. Another study showed that life expectancy of thalassemia patients was significantly increased during the last decade due to adequate regular blood transfusion and iron chelation [16]. All the subjects received iron chelation therapy where the majority of subjects used deferasirox. This result was similar to other studies where the majority of subjects also used deferasirox [15,17]. This is most likely due to better chelating drug compliance for deferasirox than deferiprone [18].

The serum ferritin levels were found high in this study with median of 4,467 ng/mL. The majority of subjects (93%) had serum ferritin levels ≥ 2,500 ng/mL, indicating severe iron overload. Routine blood transfusions are the major cause of iron overload in transfusion dependent thalassemia [5,19]. High serum ferritin levels in this study was most likely due to long duration of transfusions and poor chelating drug compliance. In this study, there was positive association between duration of transfusions and serum ferritin levels (r = 0.364, p = 0.014) and majority of subjects (68.9%) had poor chelating drug compliance. Other studies by Baldini et al., and Ezzat et al., found lower serum ferritin levels by 1,359 ± 1,040 and 1,182 ± 702, respectively [15,17]. This might be due to differences of iron chelating drugs used and better compliance in both of the last studies. The 25(OH)D levels were found low in this study with mean of 12.69 ± 4.84 ng/mL. This result was lower than that of other studies in Italy and Canada with mean of 18.72 ± 11.72 and 27.04 ± 11.44, respectively [15,17]. This was probably due to vitamin D supplementation and lower serum ferritin levels found in both of the last studies.

In this study, there was a negative association between serum ferritin levels and 25(OH)D levels (r = - 0.41, p = 0.003). This result was supported by Fadilah et al., (r = - 0.368, p < 0.01) and Napoli et al., (r = - 0.31, p < 0.05) [13,14].
The negative association between serum ferritin levels and 25(OH)D levels indicates the role of iron overload in 25(OH)D deficiency. Iron overload is toxic to many organs including heart, liver and several endocrine organs [5,20,21]. Liver is the main organ where iron is stored and is the main target organ for complications of iron overload in thalassemia [5,22]. In condition of iron overload, the labile iron in cells will be excessive [23]. High labile iron levels in hepatocyte have a tendency to produce ROS that can damage hepatocytes or impair its function [5,24-26].

Theoretically, liver plays an important role in production of 25(OH)D by hydroxylation of vitamin D3/D2. ROS causes the iron-sulfur [2Fe-2S] of ferredoxin becomes unstable and damaged, causing enzymatic activity of 25-hydroxylase to be disrupted [4,10,11,27]. Increased ROS also causes activation of caspase, lipid peroxidation, DNA and protein structure damage, and necrosis or apoptosis of hepatocyte so that the quantity of 25-hydroxylase enzyme in hepatocyte decreases. The reduction of 25-hydroxylase enzyme in hepatocyte causes the disruption of vitamin D3/D2 hydroxylation to 25(OH)D [4,5,28-32].

However, other investigations have found that serum ferritin levels were not associated with 25(OH)D levels in thalassemia patient [15,17]. This was probably due to vitamin D supplementation that could influence 25(OH)D levels not excluded in those studies. Serum ferritin levels in this study were moderately associated with 25(OH)D levels. It may be explained by the fact that serum ferritin is an indirect measure of iron overload, serum ferritin levels may not correlate closely with liver iron concentration. Serum ferritin is also an acute phase reactant and can rise with inflammation [19].

Several factors that can influence 25(OH)D levels were sun exposure, age, BMI and dietary intake. Sun exposure that contain ultraviolet B is influenced by duration, area, intensity of exposure and skin pigmentation. The intensity of sun exposure is influenced by time of exposure, latitude, season and climate [33]. All subjects in this study were examined during dry season and domiciled in the East Java region, which is geographically within 7o12 to 8o48 south latitudes. The duration and area of exposure were calculated based on the sun exposure score. In this study, sun exposure scores were not associated with 25(OH)D levels. While, a study showed significant association between sun exposure scores and 25(OH)D levels in summer (r = 0.59, p = 0.003), this study was conducted in adult normal population [12]. Thalassemia patients also tend to have a darker skin, bronze skin due to deposition of bilirubin and iron [34]. This is possible to disrupt the process of photosynthesis of vitamin D3 in the skin.

Age influences the level of 7-dehydrocholesterol in the skin, and will decrease in elderly. BMI also influences levels of 25(OH)D where vitamin D can be absorbed and sequestered in body fat [33,35]. In this study, there was no significant association between age with 25(OH)D levels (r = -0.101, p = 0.511) or BMI with 25(OH)D levels (r = 0.253, p = 0.093). This was probably due to the fact that the serum ferritin levels in this study were high, also due to other factors including no subjects with elderly and obesity was also excluded in this study. Further studies with examination of dosimeter and nutrition analysis are needed.

**Conclusion**

High serum ferritin levels with low 25(OH)D levels can be found in adult patients with transfusion dependent thalassemia. High serum ferritin levels may decrease 25(OH)D levels in adult patients with transfusion dependent thalassemia. Therefore, serum ferritin levels should be optimized to prevent further decrease of 25(OH)D levels.

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**Authors’ contributions**

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.
Conflict of Interest
Authors declare that they have no conflict of interest.

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