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Original Article

Vitamin D, insulin-like growth factor-1, and stunting in children with transfusion-dependent thalassemia

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Abstract

Background Transfusion-dependent thalassemia (TDT) has a major impact on a child's growth and is associated with stunting, risk of vitamin D deficiency, and decreased insulin-like growth factor-1 (IGF-1). To date, the relationship between vitamin D levels and stunting in TDT remains unclear. Futhermore, the role of vitamin D and IGF-1 in mediating stunting in TDT patients is still unknown.
Objective To investigate the relationship between stunting and vitamin D as well as IGF-1 levels in children with TDT.
Methods This cross-sectional study involved 50 TDT children aged 5 to 18 years, included consecutively from the Pediatric Hemato-oncology Outpatient Clinic, Dr. Soetomo Hospital, Surabaya, East Java. Subjects were divided into two groups: stunted (S) and not stunted (NS). Vitamin D and IGF-1 were evaluated by antibody competitive immunoassay and sandwich-enzyme-linked immunosorbent assay (ELISA), respectively. Age, sex, and duration of repeated transfusion were analyzed as confounding factors.
Results Median IGF-1 levels were 91.43 (13.67-192.86) ng/mL and 161.53 (17.99-363.01) ng/mL in the S and NS groups, respectively (P=0.011). Mean vitamin D levels were 20 (+ 5.71) ng/mL and 20.46 (5.25) ng/mL in the S and NS groups, respectively (P=0.765). The correlation coefficient (r) of vitamin D and IGF-I levels was not significant. Multivariate analysis showed that low IGF-1 levels, male, and longer duration of repeated transfusions were associated with stunting in children with TDT.
Conclusion Low IGF-1 level is associated with stunting in children with TDT. Vitamin D is not significantly associated with either stunting or IGF-1 in children with TDT. **[Paediatr Indones. 2022;62:98-103; DOI: 10.14238/pi62.1.2022.998-103**].

Keywords: *transfusion-dependent thalassemia; stunting, IGF-1; vitamin D*

Transfusion-dependent thalassemia (TDT) has negative impacts on growth and has been associated with stunting.[1,2] Prior studies found an increased prevalence of stunting among thalassemic children, varying from 25 to 57.1%, depending on the age and sex.[3-5] Despite the efforts to increase vitamin D levels using supplementation, vitamin D deficiency remains prevalent in thalassemia patients.[3,6-9]

Insulin-like growth factor-1 (IGF-1) has a substantial role in growth regulation. Calcitriol (1,25-dihydroxy vitamin D3) increases IGF-1 receptors and, therefore, promotes the action of IGF-1. The IGF-1 stimulates 1α-hydroxylase and decreases 24-hydroxylase gene expression, causing an increase in calcitriol level. Calcitriol also increases insulin-like growth factor-binding protein-3 (IGFBP-3).[10] There are several mechanisms that may explain the occurrence of stunting in children with thalassemia, one of which is decreased IGF-1 level.[11] Low IGF-1 level has

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been associated with stunting in patients with TDT. However, the relationship between vitamin D levels and stunting in TDT remains unclear. Furthermore, the role of vitamin D and IGF-1 in mediating stunting in TDT patients is still unknown. To the best of our knowledge, no study in Indonesia has investigated the relationship between vitamin D levels and stunting in thalassemia patients.

Methods

This cross-sectional study aimed to explore the relationship between stunting in children with TDT and their vitamin D and IGF-1 levels. This study was approved by the Health Research Ethics Committee, Dr. Soetomo General Hospital, Surabaya, East Java, Indonesia, and conducted according to the Declaration of Helsinki principles. Written informed consent was obtained from subjects' parents before the study was conducted. This study was conducted between January and June 2019.

The inclusion criteria were children with TDT aged 5 to 18 years whose parents or guardians consented to participation. Exclusion criteria were patients with familial short stature, and those who received vitamin D supplementation or growth hormone therapy. Subjects were included consecutively from the Hematology Oncology Outpatient Clinic, Pediatrics Department, Dr. Soetomo Hospital. They underwent measurements of anthropometry, vitamin D, and IGF-1 levels. Subjects were divided into two groups: stunted and not stunted. Stunting was defined as height based on age and sex < 3rd percentile according to the *2000 CDC Growth Chart*.[12] Malnutrition was defined by mid upper arm circumference less than 85% from median in Frisancho mid upper arm circumference reference based on age and sex.[13]

The minimum required sample size was calculated to be 20 per group, using a formula for hypothesis test for mean difference in two populations ($n=2\sigma^2(Z_{1-\alpha}/2+Z_{1-\beta})^2/(\mu_1-\mu_2)^2$). This study used $\alpha=0.05$; 90% power; $\sigma^2=1,526.46$.[14] The projected mean IGF-1 difference from two population groups was 40 ng/mL.

Blood specimens (3 mL) were collected for vitamin D and IGF-1 examination and centrifuged. Serum was stored at -80°C prior to analysis. Vitamin D was evaluated by *ADVIA Centaur VitD assay* (antibody competitive immunoassay) and IGF-1 was evaluated by sandwich-enzyme-linked immunosorbent assay (ELISA) for human-IGF-1 serum quantitative (*ElabscienceR Human IGF-I ELISA Kit Catalog* #E-EL-H0086). Vitamin D levels were divided into three categories: deficient (<15 ng/mL), insufficient (15 to <20 ng/mL), and sufficient (20-100 ng/mL).[15]

Hemoglobin electrophoresis data and most recent laboratory findings (hemoglobin, blood urea nitrogen (BUN), serum creatinine (SC), aspartate transaminase (AST), alanine transaminase (ALT), and ferritin levels) were obtained from medical records. Glomerular filtration rate (in ml/minute/1.73 m^2) was calculated by using Schwartz formula (k x height in cms : SC in mg/dL; with k=0.55 for 1 to 13-year-old, k=0.7 for 13 to 21-year-old male, and k=0.57 for 13 to 21-years-old female). Glomerular filtration rate (GFR) was classified as normal (80-175 mL/minute/1.73 m^2), decreased (< 80 mL/mimute/1.73 m^2), and glomerular hyperfiltration (> 175 mL/minute/1.73 m^2).[16] Data were analyzed by *SPSS 16.0 for Windows* software. Subjects' characteristics were analyzed descriptively. Simple logistic regression was used to analyze the relationship between vitamin D levels and stunting, as well as IGF-1 and stunting in children with TDT. Spearman's rank correlation was used to analyze vitamin D and IGF-I levels. Multiple logistic regression (backward Wald method) was used to analyze vitamin D levels, IGF-1 levels, and other variables that could be contributing factors for stunting in children with TDT. Results with P values < 0.05 were considered to be statistically significant.

Results

Of 99 TDT patients screened during this study period, 49 children were excluded because of familial short stature. The 50 included children were divided into stunted (S) or not stunted (NS) groups (25 subjects each). The characteristics of subjects (age, sex, type of thalassemia, type of iron chelator used, current and previous hemoglobin levels, AST levels, ALT levels, glomerular filtration rate, ferritin levels, and transfusion interval) were similar in the S and NS groups (**Table 1**). Malnutrition was more prevalent in the S group compared to the NS group. The duration of

regular transfusion was longer in S group than NS group. The median height based on age and sex were 0.1 (range 0.1-1.36) percentile and 11.7 (range 3.1-55.7) percentile for S and NS groups, respectively.

Mean vitamin D level of all subjects was 20.23 (SD 5.43) ng/mL. Normal vitamin D level was found in 27 patients (54%), insufficient level in 13 patients (26%), and deficient level in 10 (20%) subjects. Mean vitamin D levels were 20.00 (SD 5.71) ng/mL and 20.46 (SD 5.25) ng/mL, in the S and NS groups, respectively. Vitamin D level was not associated with stunting in this study (OR 0.984; 95%CI 0.888 to 1.092; P=0.765).

Median IGF-1 levels were 91.43 (range 13.67-192.86) ng/mL and 161.3 (range 17.99-363.01) ng/mL in the S and NS groups, respectively. Stunting had a significant negative association with IGF-1 (OR 0.992; 95%CI 0.986 to 0.998; P=0.011).

Table 1. Subject characteristics in stunted and not stunted subjects

Characteristics	Stunted (S) (n = 25)	Not stunted (NS) (n = 25)	P value
Mean age (SD), years	12.28 (3.31)	10.98 (3.79)	0.203
Age group, n			
>5 to <12 years	10	16	0.093
12 –18 years	15	9	
Sex, n			
Male	15	16	0.771
Female	10	9	
Malnutrition, n			
Yes	21	12	0.010
No	4	13	
Type of thalassemia, n			
β major	5	6	0.809`
Severe HbE/β	18	18	
No available data	2	1	
Type of iron chelating agent, n			
Deferiprone	16	18	0.493
Deferasirox	8	6	
Deferasirox and desferrioxamine	1	0	
No iron chelating agent	0	1	
Current mean hemoglobin (SD), mg/dL	7.88 (1.16)	8.40 (0.85)	0.074
Previous mean hemoglobin (SD), mg/dL	7.88 (1.03)	8.15 (1.02)	0.347
AST level, n			
Increased	9	9	0.913
Normal	15	16	
No available data	1	0	
ALT level, n			
Increased	9	9	0.913
Normal	15	16	
No available data	1	0	
GFR, n (%)			
Glomerular hyperfiltration	20	16	0.133
Normal	4	9	
No available data	1	0	
Median ferritin (range), ng/mL	2,669 (229.58-9,756.69)	2,109 (437.85-7,001.91)	0.299
Mean duration of repeated transfusion (SD), years	8.32 (3.45)	5.49 (2.83)	0.003
Interval between transfusions, n			
< 4 weeks	18	12	0.087
> 4 weeks	7	13	
Mean vitamin D level (SD), ng/mL	20.00 (5.71)	20.46 (5.25)	0.765
Median IGF-1 level (range), ng/mL	91.43 (13.67-192.86)	161.3 (17.99-363.01)	0.011

Table 2. Multivariate analysis for variables associated with stunting in TDT children

Variables	B	Exp(B)	95%CI	P value
IGF-1 levels	-0.012	0.988	0.979 to 0.996	0.006
Sex (male)	-1.802	0.165	0.028 to 0.982	0.048
Duration of repeated transfusion	0.416	1.515	1.161-1.978	0.002

Multivariate analysis using multiple logistic regression (backward Wald method) showed that stunting in children with TDT had significant associations with low IGF-1 level, male sex, and longer duration of repeated transfusion (**Table 2**). Age, malnutrition, type of thalassemia, type of iron chelating agent, hemoglobin level, AST level, ALT level, GFR, ferritin level, and interval between transfusion were not associated by stunting in children with TDT.

Spearman's rank correlation analysis to investigate the relationship between vitamin D and IGF-1 levels in children with TDT revealed a correlation coefficient (r) of -0.473 (P=0.001). The correlation coefficient was -0.362 (P=0.045) in males (n=31 subjects) and -0.440 (P=0.059) in females (n=19 subjects). The associations between vitamin D and IGF-1 levels in males (r=-0.218; P=0.257) and females (r=-0.337; P=0.186) were no longer significant, after adjusting for age and nutritional status.

Discussion

Transfusion-dependent thalassemia has been known to have long-term complications, one of which is stunting. A cross-sectional study involving 367 children with transfusion-dependent β-thalassemia major in Pakistan showed that 65.4% of the total subjects had stunted growth (height-for-age Z-score<-2).[17] Other recent study on growth pattern in children with TDT showed similar results with 65.71% of subjects suffering from stunting.[18] The mechanism between TDT and stunting has been proposed to be due to altered IGF-1 regulation.

The IGF-1 mediates many physiological actions of growth hormone and is the major effector of bone growth. IGF-1 levels can be used as a predictor of height <-2 SD in adolescent patients with β-thalassemia major, with a cut-off point of < 38.51 ng/mL.[14] Prior study showed that IGF-1 levels were lower in β-thalassemia major patients with impaired growth compared to those with normal growth, but this finding was not statistically significant (P=0.096), possibly because of the small sample size of 19 and 14 children per group.[19] We found that lower IGF-1 levels were associated with higher incidence of stunting. Further study is required to evaluate factors which mediate the association of IGF-1 and stunting in children with TDT and the molecular mechanism of IGF-1 modulation.

Children with TDT have a specific growth pattern that is relatively normal until the age of 9-10 years, after which, growth gradually slows.[20] Retardation in height growth occurs after the age of 11 years in boys and 9 years in girls.[21] Height growth decreases with age, both in children who were initially stunted and children who were not stunted.[17] We also noted that male sex was inversely related to stunting. Boys with TDT experienced height growth retardation at an older age than girls.

The longer TDT patients receive transfusions, the more likely they are to experience growth problems. Continuous transfusions can lead to accumulation of iron in the body organs, resulting in organ malfunction.[22] Excessive iron interferes with maturation of osteoid and deposits in hydroxyapatite crystals, thus interfering with normal bone metabolism. Our results were consistent with this theory, in which stunted children had undergone longer duration of transfusions than the not stunted group.

In our study, vitamin D levels were not related to stunting in children with TDT. Vitamin D is a type 1 nutrient. If deficiency occurs, growth can still continue, but disrupted body function with specific clinical manifestations can lead to illness, disrupting growth in a secondary way[23] Another possible explanation on the lack of association between vitamin D level and stunting may have been that nearly half subjects in both S and NS groups suffered from vitamin D insufficiency and deficiency (46%).

The relationship between vitamin D and IGF-1 remains unclear. It is well established that IGF-1 induces synthesis of $1,25(OH)_2$ vitamin D in the kidney by stimulating 1α-hydroxylase and inhibits catabolism

by decreasing 24-hydroxylase gene expression.[10] There is no conclusive mechanism by which vitamin D can modify IGF-1 and IGFBP-3 concentration. Cholecalciferol treatment for vitamin D deficient children can increase circulating IGF-1. Vitamin D supplementation can also increase serum IGFBP-3. The possible mechanisms by which vitamin D can increase IGF-1 and IGFBP-3 in circulation are by inducing liver synthesis through transcription of relevant genes and/or enhancement of GH stimulation, as well as by augmenting absorption of calcium in the intestine, since calcium intake is associated with circulating IGF-1.[24] A study in Lebanon examined the relationship between IGF-1 and vitamin D in 8 to 18-year-old children and found that IGF-1 levels in boys were inversely correlated with 25(OH) vitamin D. The correlation between IGF-1 and 25(OH) vitamin D was absent in both sex groups after adjusting for the main confounding variables (age, BMI, and height).[25] An explanation may be that the relationship between IGF-1 and 25(OH) vitamin D is not independent. Similarly, we found an inverse relationship between vitamin D and IGF-I levels in children with TDT. However, after controlling for the main confounding variables (age, sex, and nutritional status), the correlation did not retain significance, again suggesting that the relationship between vitamin D and IGF-I is not independent. Another possible explanation is that increased IGF-1 level induces changes from 25(OH) vitamin D to $1,25(OH)_2$ vitamin D, thereby decreasing 25(OH) vitamin D levels. Further study needs to be conducted to explain this finding.

This study used the most recent results of liver function, renal function, and ferritin from patients' medical records, all of which were examined routinely every 3 months in our hospital. Such results might not accurately describe the conditions at the time of study onset. However, there was no significant correlation between liver function test and renal function test with stunting in TDT children in this study.

In conclusion, low IGF-1 levels are significantly associated with stunting in children with TDT. But we found no associations between vitamin D and stunting or between vitamin D and IGF-1 in children with TDT.

Conflict of interest

None declared.

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