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ASSOCIATION BETWEEN *25-HYDROXYVITAMIN D* LEVELS AND GROWTH PARAMETERS IN THALASSEMIA WITH REPEATED BLOOD TRANSFUSION

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ABSTRACT

Background. Blood transfusion is a therapeutic choice for thalassemia with anemia. Hemosiderosis is the side effects of blood transfusions which cause impaired liver function, leads to low vitamin D levels in plasma. The low level of vitamin D cause delay in physical growth. The aim is to determine the relation between *25-hydroxyvitamin D* levels and growth parameters in thalassemia with repeated blood transfusion history. **Method.** In this analytical cross-sectional study, thalassemia patients with repeated blood transfusion and ages 0-18 years old were recruited. This research was held in Semarang, Purwodadi, and Rembang from May to September 2019. Thalassemia patients who met the criteria were measured the *25-hydroxyvitamin D* level, physical activity and the growth parameters (weight, height, and body mass index). **Results.** Forty thalassemia patients aged 6-18 years old were recruited in this study. The percentage of boys are 47.5% and girls are 52.5%. Most of subject had high ferritin serum (87.5%) and low vitamin D level (87.5%). Growth parameters from the patients were 52.5% weight-for-age <5 percentile, 45% height-for-age <5 percentile and 25% BMI-for-age underweight. There isn't significant result in *25-hydroxyvitamin D* with growth parameters, and a significant result between physical activity and height-for-age (*p=0.027*), but not with weight-for-age and BMI-for-age. **Conclusion.** The *25-hydroxyvitamin D* do not associate with growth parameters significantly in thalassemia with repeated blood transfusion but physical activity has significant association with height-for-age.

Keywords: thalassemia, *25-hydroxyvitamin D*, growth parameters, physical activity

INTRODUCTION

Thalassemia is a global health problem with high prevalence and mortality. Thalassemia effect men and women equally and often occur in persons of South Europe-Mediterranean, Middle East, Africa, South Asia, East Asia, Asia and Southeast Asia.[1] Indonesia is one of the countries in Southeast Asia with thalassemia carrier rates are around 3-8% or 23 newborns every 1.000 birth have thalassemia.[2] Riset Kesehatan Dasar (RISKESDAS) 2007 reported 8 provinces in Indonesia with the highest thalassemia cases, Aceh Darussalam (13.4%), Jakarta (12.3%), South Sumatera (5,4%), Gorontalo (3.1%) and Riau (3.0%).[3] Thalassemia is a group of inherited hematologic disorders caused by mutation in genes encode globin results in synthesis failure of the hemoglobin chains and manifested as microcytic anemia.[4,5] According to the type of globin chains, thalassemia is defined into two main group that is alfa thalassemia and beta thalassemia.[1,4]

Thalassemia with Hb <6-7 g/dl require regular transfusions to prevent the complications due to the anemia.[1,6] Blood transfusions decreases the rate of mortality in thalassemia but also cause iron overload which leads to hemosiderosis or accumulations of iron in body cells and tissues.[6] Hemosiderosis can occur in any organs which results in organ malfunction.[7]

If hemosiderosis occurs in the liver, it will impact the liver functions. One of the liver functions is vitamin D metabolism. Liver malfunctions will results in the low vitamin D level in plasma.[8,9] Several research reported both low and normal levels of vitamin D in thalassemia.[10,11] Vitamin D is a hormone, has a role in bone growth. Physical activity is another factor affecting physical growth especially bone and muscle growth.[12] Many research have reported the incidence of delayed growth in thalassemia patients.[10,13,14] A recent study also reported delayed growth in thalassemia were accompanied by low 25-hydroxyvitamin D plasma level. The cause of delayed growth in thalassemia stills needs further research. Relations between vitamin D and growth parameters in thalassemia has not been done in Central Java. The study aim is to determine the association between 25-hydroxyvitamin D and physical activity with growth parameters in thalassemia with repeated blood transfusion.

METHODS

This analytic cross-sectional study was carried out in Semarang, Rembang, and Purwodadi from May until September 2019. In this study, thalassemia patients both male and female age 0-18 years old with repeated blood transfusion history were recruited. We defined as repeated blood transfusion if thalassemia patient received blood transfusion more than 10 times. Patients with fever, leukocytosis, vitamin K and vitamin D supplementation were excluded. This research used consecutive sampling method and the minimum subjects was 36 children. Growth parameters, 25-hydroxyvitamin D (25(OH)D) levels, and physical activity were assessed in this study.

All patients who met the criteria were interviewed to collect the demographic data, blood transfusion history and iron chelation therapy. The physical activity in this study was assessed using Physical Activity Questionnaire for Children (PAQ-C) and Physical Activity Questionnaire for Adolescence (PAQ-A). The PAQ can be used to differentiate the physical activity levels using the questionnaire score. This study used 2.75 as cut-off point and defined into low and high levels of physical activity. Then, to measure the physical growth, we performed anthropometric measurement (weight and height), body mass index was calculated and plotted using the CDC 2000 Standard Growth Charts. We used the lowest line (5^{th} percentile) as cut-off point to identify poor growth. Blood sample was taken to assess leukocytosis through complete blood count. Patients who were not leukocytosis, were measured the level of 25(OH)D with ELISA test in GAKY FK UNDIP Laboratory. The 25(OH)D level was defined into two categories, low (≤30ng/ml) and normal (>30ng/mL).

Data were analyzed by using statistical program to determine the associations between the variables. Statistical analysis in this study was done using Chi-Square analysis with Fisher Exact as an alternative test. The p-value less than 0.05 is considered significant. This study was approved by Ethical Committee Medical Faculty of Diponegoro University, Semarang, Indonesia. (No.176/EC/KEPK/FK-UNDIP/V/2019.)

RESULTS

There were 47 thalassemia patients during the study period. Seven patients were excluded because of leukocytosis. Thus, the data were collected from 40 subjects with age ranges 6-18 years old. Blood laboratory test results that most of our patient had low levels of vitamin D (87.5%) and high ferritin serum level (87.5%). The characteristics of subjects are shown in Table I.

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Table I. Characteristic of the study population

Variable	n (%)	Mean±SD (Min-Max)
Age	-	11.03±3.34 (6-18)
Gender	-	-
Male	19(47.5)	
Female	21(52.5)	
Weight-for-age (kg)	-	28.12±9.14 (15-48.3)
< 5th percentile	21(52.5)	
≥ 5th percentile	19(47.5)	
Height-for-age (cm)	-	131.12±15.93
< 5th percentile	18(45)	(104-169.5)
≥ 5th percentile	22(55)	
BMI-for-age (kg/m^2)	-	15.89±1.99
< 5th percentile	10(25)	(12.82-20.91)
5 – 85th percentile	30(75)	
25-hydroxyvitamin D	-	18.71±7.96
Low (≤ 30 ng/mL)	35(87.5)	(5.37-38.94)
Normal (> 30 ng/mL)	5(12.5)	
Ferritin Serum	-	3,065.47±2,292.01
< 1000 ng/mL	5(12.5)	(504-10,126.00)
≥ 1000 ng/mL	35(87.5)	
Physical Activity	-	2.66±-0.71
Low	23(57.5)	(1.00-4.40)
High	17(42.5)	

Table II. Association of 25-hydroxyvitamin D and physical activity with weight-for-age

		Weight-for-age		Total	P
		< 5th percentile	≥ 5th percentile		
25(OH)D levels	Low	19	16	35	0.654
	Normal	2	3	5	
Physical Activity	Low	13	10	23	0.750
	High	8	9	17	

+ Chi-square test; ¥ Fisher-exact test

Table III. Association of 25-hydroxyvitamin D and physical activity with height-for-age

		Height-for-age		Total	P
		< 5th percentile	≥ 5th percentile		
25(OH)D levels	Low	17	18	35	0.355
	Normal	1	4	5	
Physical Activity	Low	14	9	23	**0.027***
	High	4	13	17	

+ Chi-square test; ¥ Fisher-exact test

Table IV. Association of 25-hydroxyvitamin D and physical activity with BMI-for-age

		BMI-for-age		Total	P
		< 5th percentile	≥ 5th percentile		
25(OH)D levels	Low	9	26	35	1.000
	Normal	1	4	5	
Physical Activity	Low	6	17	23	1.000
	High	4	13	17	

+ Chi-square test; ¥ Fisher-exact test

Statistical analysis showed that neither 25(OH)D levels nor physical activity had no significant association with weight-for-age (p=0.355, p=0.654), and BMI-for-age (p=1.000, p=1.000). the 25(OH)D levels had no significant association with height-for-age (p=0.355), but physical activity had (p=0.027).

DISCUSSIONS

In this study, 87.5% of our patients have low 25(OH)D level or <30ng/ml. Previous studies reported both normal and low vitamin D level in thalassemia.[10,11,15] However, other research also reported the low vitamin D level in healthy children in Indonesia.[16] The low level of vitamin D is caused by hemosiderosis. Hepatic hemosiderosis or deposition of hemosiderin in liver tissue or cell cause liver malfunctions which results as low vitamin D levels in plasma.[8] Thalassemia patients who received blood transfusion as therapy, have risk for experiencing hemosiderosis due to the iron overload.[17] Thalassemia patient usually receives blood transfusion around 500 ml packed red cell once a month and around 200-400 mg of iron accumulates each blood transfusion.[7] it leads to high ferritin serum level. To prevent hemosiderosis, thalassemia patient must receive the iron chelation therapy to maintain ferritin serum levels. Iron chelators is binding the iron in the serum, which is then excreted from the body.[7] From our study, we found that 87.5% patients had ferritin serum level above 1000 ng/ml even though all patients receive the iron chelation therapy. Many factors determine the success of therapy. One of it is patient adherence. Adherence to chelation therapy is generally poor due to the adverse effect of the long-term therapy.

Children with thalassemia with growth retardation were accompanied by low 25-hydroxyvitamin D level.[18] Vitamin D regulates and maintains the homeostasis of the bone growth. Growth failure is one of the clinical manifestation due to the low level of vitamin D. Previous research reports the high prevalence of growth retardations in thalassemia.[19] Ferritin serum levels related with the height-for-age in thalassemia.[13] This study obtained that 25(OH)D was not associate with the growth parameters in thalassemia patient with repeated blood transfusion. In others research conducted in India stated that vitamin D and calcium supplementation did not affect the physical growth although an improvement was found in bone mineral content (BMC) dan bone mineral density (BMD).[20] This insignificant results likely caused by many factors influence physical growth, such as genetic, hormonal, nutritional intake, and chronic disease.[21,22] Genetic and heredity play a role in genes expression which is controlling the hormonal secretion, intracellular signalling pathways, and paracrine signalling.[23] The adequate nutritional intake also determine the growth progression.[22] Chronic disease affects growth hormone resistance and the low level of IGF-1 and IGF-binding protein

3 which determine body growth. Thalassemia patient has anemia, iron deficiency anemia and IGF-I secretion defective which cause growth problems.[15]

Other factor affecting growth is physical activity. In this study, we found that physical activity has a significant association with height-for-age in thalassemia patients but not with other growth parameters. The data which were collected from PAQ, showed that our patients did physical activity 3 to 5 times a week, like running, walking/jogging, biking, soccer and jump rope. This is in line with previous research which state that the physical activity affects body weights and heights.[24] Physical activity effect the linear bone growth and bone mass. Physical activity also improves the skeletal muscle mass and breakdown the adipose tissue for energy productions.[25] Furthermore, physical activity results in mechanical stimulation of the bone which affect bone chemical-signalling, such as Frizzled-LRP5/6-mediated activation of Wnt that induces osteoblast expression, and RUNX2. Its increase mesenchymal stem cells which results in proliferation and differentiation of pre-osteoblasts and stimulates bone mineralization. Physical activity also has an positive effect on blood flow and bone tissues perfusion which support bone growth.[12]

The limitation of this study is the assessment tools of physical activity. Subjective data from the questionnaire has low sensitivity and specificity which is increasing the risk of bias. Objective assessment tools may provide more accurate data of patient's physical activity. The other limitation was and the vitamin D categorical which cannot differentiate the insufficiency or deficiency of vitamin D levels.

CONCLUSIONS

Low vitamin D levels and high ferritin serum is prevalent in thalassemia patients. Growth status of thalassemia patient are varying in this study. The 25-hydroxyvitamin D levels do not associate with growth parameters in thalassemia with repeated blood transfusion. Physical activity has significant association with height-for-age in thalassemia patients.

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