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Nutritional intake, sun exposure and vitamin D level in childrens with thalassemia major

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ABSTRACT

Children with thalassemia major generally experience growth retardation. One of the growth parameters is serum vitamin D levels. The main source of vitamin D comes from endogenous synthesis with sun exposure and dietary sources. This cross sectional study involved 84 children with thalassemia major aged 4-14 years old taken through concecutive sampling. Nutritional intake was obtained through a semi quantitative food frequency questionnaire, while the duration of sun exposure was calculated at peak UVB intensities at 11.00-15.00. The enzyme-linked immunosorbent assay (ELISA) method was used to examine vitamin D levels. The data was analized by Spearman Rank corelation dan multiple linier regresion. The results were 4.8% of subjects had vitamin D insufficiency and 95.2% had deficiency. Daily average intake of nutrients compared to their requirements were energy intake 70.5% (1538.7 Kcals), protein 55,4 % (45.5 grams), fat 79,2% (57.7 grams), carbohydrates 68,6% (204.9 grams) and vitamin D 15,8% (2.4 μg). The mean (SD) of sun exposure was 158.3 (91.2) minutes. Energy, protein, and fat intake were significantly associated with vitamin D levels ($r = 0.27$; 0.31; 0.38 and $p < 0.025$). Sun exposure was strongly associated with vitamin D levels ($r = 0.80$; $p < 0.025$), while vitamin D intake was associated with vitamin D levels ($p > 0.025$). Fat intake and sun exposure have a strong effect on vitamin D levels, with a regression equation of vitamin D levels = 5.62 + 0.22 * fat intake + 0.12 * sun exposure.

***Keywords*:** nutritional intake, sun exposure, vitamin D, thalassemia major

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1. INTRODUCTION

Thalassemia is the most common genetic disorder in Indonesia, with 3-10% carriers of thalassemia-β [1]. WHO (World Health Organization) states that 7% of the world population is a carrier of thalassemia genes and an estimated 300-400 thousand babies are born with thalassemia every year[2]. The Health Ministry of Republic Indonesia[1] records that 2,500 babies born each year with thalassemia in Indonesia. Indonesian Thalassemia Foundation reports [1] that 9,028 people with thalassemia in Indonesia in 2018 and 3,636 people (40.3%) were in West Java. If there is no prevention, the amount of people with thalassemia will reach 25 thousands in Indonesia at 2020.

Children with thalassemia major generally experience growth retardation and metabolic abnormalities [3][4]. Several studies before reported that growth disturbance was about 20% to 60%, depend on the severity of disease. This condition was caused by many factors, including hypoxia due to chronic anemia, chelation poisoning, low serum zinc levels, excess liver iron, lack of nutritional support and growth hormones [3][5].

A lot of country in Asia report case of vitamin D deficiency (VDD) in thalassemia children. A study in India on 2017[6] stated that 41% had vitamin D deficiency, 46% had insufficiency and only 13% had normal vitamin D levels, whereas in Thailand [7] reporterd VDD reached 90% .VDD in thalassemia was related to inadequate of nutritional intake and vitamin D hydroxylation disorders in the liver due to hemochromatosis that caused high serum ferritin levels [7].

Inadequate of vitamin D intake is the most common problem found. Nutrients needed for the synthesis of vitamin D are not only derived from dietary sources of vitamin D, but are also other nutrients such as protein, fat, and carbohydrates. Cohort studies in the United States had [8] reported that children with thalassemia major with well nourished, 97% consume inadequate vitamin D, and 39% consume energy <80% of Estimated Energy Requirements (EER).

Protein is required in the metabolism of vitamin D, because protein in form of vitamin D binding protein (DBP) is responsible for carrying 95-99% of the total 25(OH)D3, the other small portion is carried by albumin and lipoprotein through weak nonspecific bond [9-11]. Protein is also required to produce enzymes and vitamin D receptors (VDR) in metabolism of vitamin D. A lot of research on thalassemia children found that intake of energy and protein were at lower level than recomended.

Vitamin D is a fat soluble vitamin derived from cholesterol and stored in body fat. Cholecalciferol, which is produced in the skin or from diet will be sequetrated in body fat[12]. In condition of inadequate fat intake, vitamin D synthesis get slower so that serum vitamin D levels are low. This study intend to know the correlation between nutritional intake including energy, protein, fat, carbohydrate, vitamin D and sunlight exposure with vitamin D levels in children with thalssemia major.

2. MATERIALS AND METHODS

2. 1. Design

The design of this study was cross-sectional, carried out in October to November 2019, with consecutive sampling.

2. 2. Participans

Subjects were pediatric thalassemia major outpatients at Polyclinic of Thalassemia Hematology-Oncology Division, Department of Pediatrics, Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin Hospital. Subject was approved by their parents or guardians after getting explanation and signed an informed consent. Based on sampling formula, 84 subjects was obtained. Exclusion criteria were children with thalassemia major who had undergone splenectomy, kidney failure, and malnutrition (Body Mass Index for age <-3 SD).

2. 3. Informed Consent and ethical clearance

Ethical clearance was obtained from ethics commitee of Health Research Dr. Hasan Sadikin Hospital Bandung, Indonesia number LB.02.01/X.6.5/312/2019. Written informed consent was obtained from all subjects or parents prior to study.

2. 4. Nutritional intake

Data of nutritional intake was obtained through interviews using semi quantitative - food frequency questionnaires focused to get estimation quantity of dietary intake by calculating mean of total energy carbohydrate, protein, fat, Fe and vitamin D intake. The results were analyzed with the nutrisurvey software 2017, then compared to individual requirements.

2. 5. Body Mass Index (BMI)

Height was measured barefoot using *Microtoise Stature Meter 2M.* Weight was measured using electrodigital weight scale. Then, we calculated the average of these two measurements. The formula of BMI is body mass in kilograms divided by the square of the body height in metre, and then calculated Z score of BMI for age base on World Health Organization (WHO) growth references.

2. 6. Mid Upper Arm Circumference (MUAC)

MUAC measurements was carried out on the left arm using the MUAC measuring instrument (tape), then calculated persentage of gold standard.

2. 7. Sun exposure

Duration of exposure to sunlight obtained through interviews about outdoor activities at peak time UVB intensities from 11:00 to 15:00 (minutes/day). Then, calculated amount in one week.

2. 8. Vitamin D level

Assesment of vitamin D levels using the enzyme-linked immunosorbent assay (ELISA) method conducted in the clinical laboratory of Hasan Sadikin Hospital Bandung. Vitamin D suficiency (normal) was expressed when the level of serum vitamin D >12-150 ng/mL, insufficiency when the level of serum vitamin D 12-20 ng/mL, and deficiency when the level of serum vitamin D < 12 ng/mL.

2. 9. Data Analysis

Association between nutrient intake and sun exposure was analyzed by the Rank-Spearman correlation test. Association was significant if $p < 0.025$. Variables that affect vitamin D levels were obtained from multiple linear regression tests with $p < 0.05$.

3. RESULTS

Based on the results of the test of serum vitamin D levels, 4 subjects (4.8%) had insufficiency and 80 subjects (95.2%) had deficiency.

3. 1. Subject Characteristics

The subjects of this study consisted of 49 boys (58%) and 35 girls (42%). Most subjects were school children with ages 7-14 years (73.8%), while the onset of transfusion age were the most <12 months and 97% subjects have got blood transfusion > 6 months. Most subjects had ferritin levels 1000 mg / mL, while subjects who had Hb level of pre-transfusion ≥7 g / dL much more than <7 g / dL, and 83.3% of subjects well exposed to sunlight (Table 1).

Table 1. General Characteristics of subjects (n = 84).

Variables	Numbers (%)
Gender	
Boy	49 (58.0)
Girl	35 (42.0)
Age (years) (mean ± SD)	9,4 ± 2.9
4 – 6	22 (26.2)
7 – 14	62 (73.8)
Age of onset transfussion (months) (mean ± SD)	15.0 ± 19.2
< 12	58 (69.0)
≥ 12	26 (31.0)
Length of transfussion (months) (mean ± SD)	96.5 ± 39.7
< 6	2 (2.4)
≥ 6	82 (97.6)
Level of ferritin (mg/mL) (mean ± SD)	3413,79 ± 177,12
< 1000	5 (5.9)

≥ 1000	79 (94.1)
Level of Hb pre-transfusion (g/dL) (mean ± SD)	7.22 ± 1.05
< 7	29 (34.5)
≥ 7	55 (65.5)
Exposure to sunlight (menutes/week) (mean ± SD)	158.3 ± 91.2
Less exposed (<120)	14 (16.7)
Well exposed (≥ 120)	70 (83.3)

The nutritional status of subjects with indicators height for age Z-score, percent of Mid upper arm circumference (MUAC) to standards, and BMI for age Z-score can be seen in Table 2. Based on height by age data obtained that most of the subjects had stunted and very short stature (severely stunted) with a percentage of 76.2%. Based on the upper arm circumference, 51.2% of subjects experienced acute malnutrition.

Different results were shown in the nutritional status using BMI for age, most subjects were in well nourished (89.3%) and only 10.7% were malnutrition. From the three indicators of nutritional status, it can be seen that height and MUAC more sensitive in filtering the incidence of malnutrition than BMI.

Table 2. Characteristics of subjects based on nutritional status (n = 84).

Indicators of nutritional status	**Number(%)**
Height for age (Z-score) (mean ± SD)	-2.81(1.26)
Normal (-2 SD sampai 2 SD)	20 (23.8)
Stunted/severely stunted (<-2 SD)	64 (76.2)
MUAC for age (% standard) (mean ± SD)	85.86(11.68)
Well nourished (> 85%)	41 (48.8)
Acute malnutrition (≤85%)	43 (51.2)
BMI for age (Z-score) (mean ± SD)	-0.4 (1.35)
Well nourished (-2 to 2 SD)	75 (89.3)
Malnutrition (<-2 to -3 SD)	9 (10.7)

Nutritional intake was obtained through a semi quantitative food frequency questionnare. Total energy requirement are calculated based on Estimated Energy Requirements (EER) with physical activity factors 1.26 (active) with a composition protein : fat : carbohydrate were 15% : 30% : 55% from EER. The requirement Fe and vitamin D using Recommanded Dietary Allowance (RDA) for Indonesia [13] in 2019.

Table 3. Intake and nutrient requirements of the subjects (n = 84).

Nutritional intake	Intake		Requirement	
	Mean (SD)	**Range**	**Mean (SD)**	**Range**
Energy (Kcals)	1538.7(379.9)	451-2728.9	2212(228.3)	1823-2733
Protein (g)	4.,5 (12,9)	17-74	82.5(8.5)	68-102
Protein (% E)	11.5(2.4)	5-17		
Fat (g)	69.4(25.2)	18-147	73.3(7.6)	60-91
Fat (% E)	33.6(6.0)	23-61		
Carbohydrate	204.8(64.6)	60-383	303.7(31.4)	250-375
Carbohydrate (% E)	52.5(8.1)	21-66		
Fe (mg)	7.1(3.3)	0.5-16.5	9.7(1.8)	8-15
Vitamin D (μg)	2.4(2.9)	0-15.6	15(0)	15

%E = percentage specific nutrient intake from total energy intake

The mean energy intake of the subject is 1538.7 Kcals from the average requirement of 2212 Kcals. The proportion of fat looks good with an average of 33.6% of total energy, whereas protein and carbohydrates are lower than required (tabel 4). Nutritional intake compared requirement is categorized as inadequate (<80%) and adequate (≥80%), except Fe intake is said to be good if <80% of requirement because it must be limited.

Figure 1 shows that the majority of subjects experienced inadequate intake of all nutrients, 73.8% of subjects lacked energy intake, 96.4% lacked protein intake, 53.6% lacked fat intake, and 70.2% lacked carbohydrate intake. Iron intake still exceeds RDA by 19.1%, and almost all subjects (98.8%) had inadequate vitamin D intake.

Energy, protein, fat and carbohydrate intake compared to individual requirements. Fe and vitamin D intake compared with RDA for Indonesia in 2019

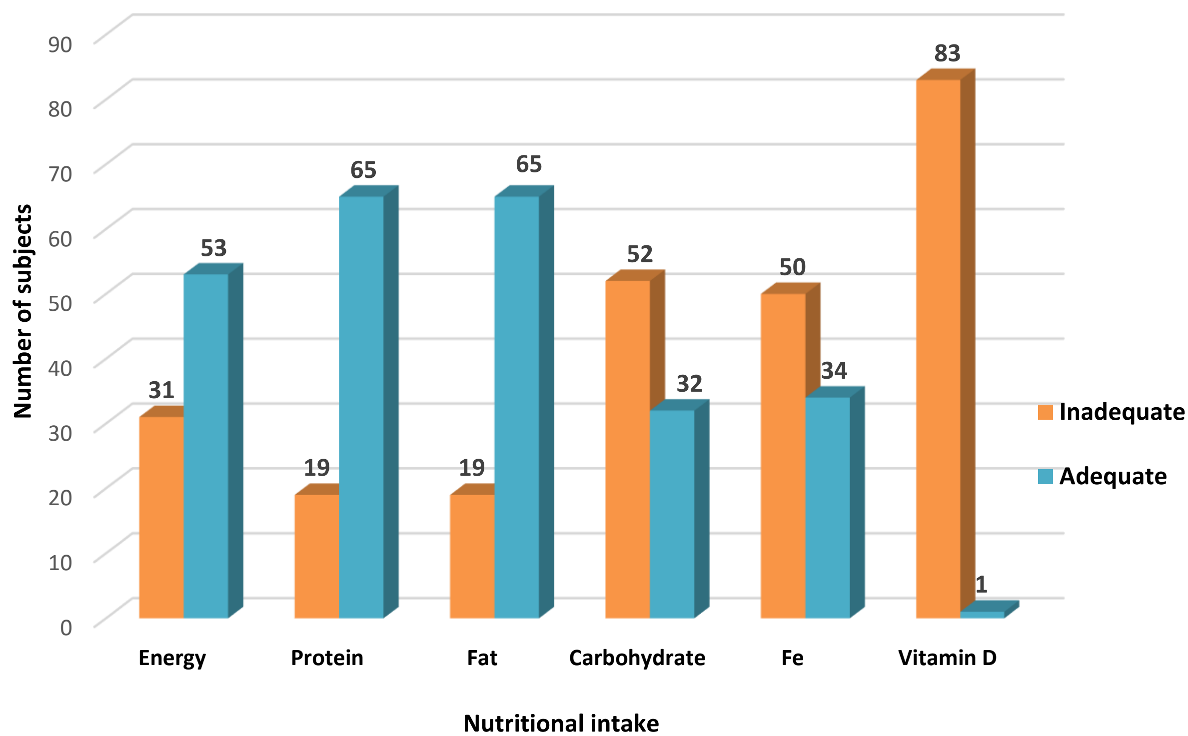


Figure 1. Graph of subject characteristics based on nutritional intake (n = 84)

3. 2. Association of nutritional intake with vitamin D levels

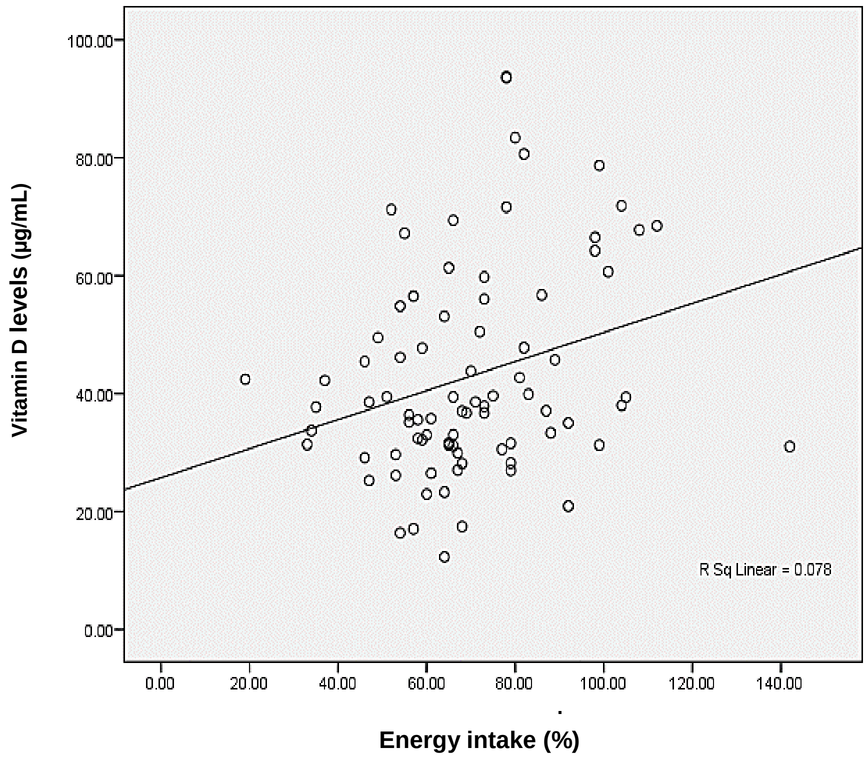
Energy, protein, and fat intake were significantly associated with vitamin D levels (Spearman r = 0.27, 0.31, and 0.38 ; p <0.025), whereas carbohydrate, Fe and vitamin D intakes were no associated with serum vitamin D levels (p> 0.025).

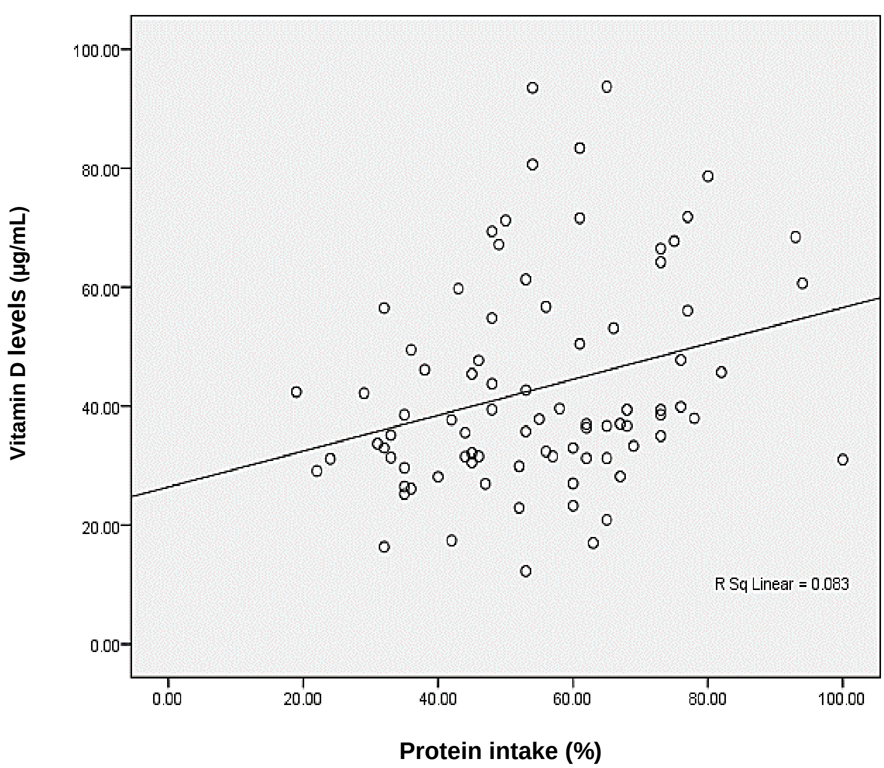
Table 4. Association between nutritional intake and vitamin D levels.

Percentage of nutritional intake compared to requirements	r_s value	p value*
Energy (%)	0,27	0,013
Protein (%)	0,31	0,004
Fat (%)	0,38	0,000
Carbohydrate (%)	0.16	0.148
Fe (%)	0.04	0.754
Vitamin D (%)	-0.01	0.977

Note: * Spearman correlation test, significant if the p value <0.025

Association between energy intake, protein, and fat was positive, meaning that the greater intake of the three nutrients would be greater the vitamin D levels obtained. The graph of the association of energy, protein and fat intake with vitamin D levels can be seen as follows:





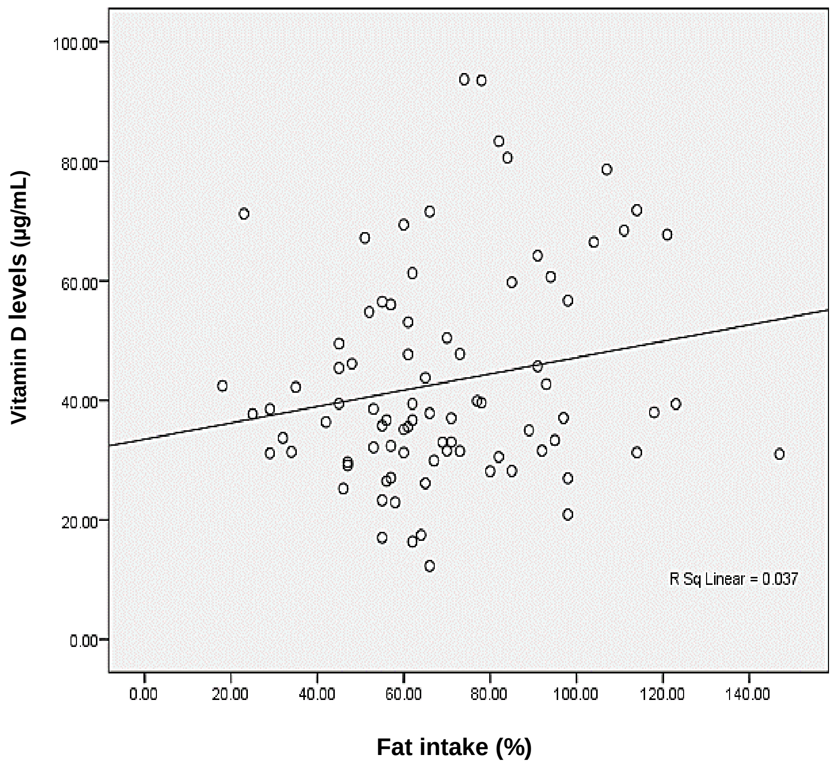


Figure 2. The graph of association between nutritional intake with vitamin D levels

3. 3 Association between sun exposure and vitamin D levels

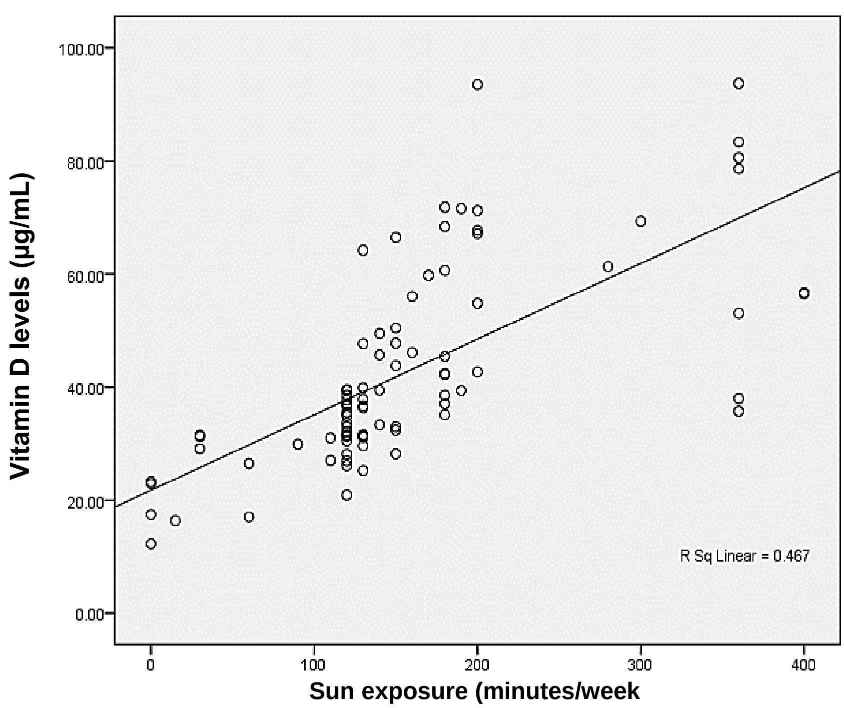


Figure 3. The graph of association between sun exposure and vitamin D levels

Based on the Spearman Rank correlation test, the results show that sun exposure associated very strongly with vitamin D levels ($r = 0.80$, $p < 0.025$). This association was positive, meaning that longer time exposed to sunlight would be higher of vitamin D levels. The association graph of sun exposure with vitamin D levels can be seen as follows:

3. 4 Association of all variables with vitamin D levels

Table 5 illustrates asociation of all variables with vitamin D levels. Based on the previous correlation test, there are five variables related to serum vitamin D levels. In addition, multiple linear regression tested with the backward method. There were only two variables that can predict serum vitamin D levels. These variables were fat intake and sun exposure which can be made the following regression equation:

Vitamin D levels = 5,62 + 0,22*Fat intake (%) + 0,12*sun exposure (minute/week)

Table 5. Association of all variables with vitamin D levels.

Variabel	ß	SE (B)	p value*
1. Model 1			
Constant	-1.77	9.86	0.858
Total energy intake	-0.23	0.14	0.104
Protein intake	0.01	0.01	0.955
Fat intake	0.34	0.14	0.003
Level of Hb pre-transfusion	1.70	0.11	0.204
Sun exposure	0.12	0.02	0.000
2. Model 2			
Constant	5.62	5.11	0.274
Fat intake	0.22	0.06	0.000
Sun exposure	0.12	0.02	0.000

Note: Multiple linier regression test, significant if p value <0,05

5. DISCUSSION

Repeated and routine blood transfusions in children with thalassemia major cause iron overload, if deposited in the liver will disrupt vitamin D hidroxylation in the liver, thereby reducing the amount of vitamin D circulating in the body[14]. In this study only 4.8% subjects experienced vitamin D insufficiency, different with a study in Egypt (2013) [3] stated that 37% of thalassemia major children had vitamin D deficiency and 54% had vitamin D. insufficiency. Some studies in India [15] [7] also showed the majority of vitamin D deficiency in children with thalassemia major. There may be other factors causes vitamin D synthesis to go well. Energy, protein, and fat intake, and sun exposure were associated with vitamin D levels in this study.

Children with thalassemia major have a risk lack of nutrient intake. Low Hb levels cause children to get tired easily, causing decreased appetite and inadequate food intake. In addition, eating habits of children who like to consume low-calorie foods, food intolerance (such as lactose), avoiding certain foods, especially foods high in iron, and side effects of chelation drugs (nausea, cramps, and loss of zinc) can reduce dietry intake of children with thalassemia [16].

Ineffective erythropoiesis and rapid red cell turnover due to the short life span of red blood cells in thalassemia causes an increase in energy expenditure and other nutrients to maintain normal erythropoiesis. It has been reported that thalassemia children have increased energy expenditure and protein turnover [17]. Vitamin D is an important component in controlling Respiratory Quotient (RQ) through glucose utilization and is therefore highly positively related to basal and bone metabolism [18].

In this study most of the subjects experienced a lack of energy intake. The average of total energy intake is 70.5% of EER, under individual requirement. If there is a lack of energy for a long time, it can cause malnutrition which can interfere with the child's growth. Children with thalasemia who underwent blood transfusions since infancy have confirmed stunted growth, and can be proven in this study most stunted.

Protein intake related to serum vitamin D levels was demonstrated in this study. Protein is an important nutrient in cells and is the main source of nitrogen for the body. Protein will be hydrolyzed into enzymes, hormones, hemoglobin and as a source of energy. Vitamin D is often referred to as hormones and in the process of metabolism requires a lot of protein in the form of enzymes or vitamin D receptors (VDR). Protein is also needed in the form of vitamin D binding protein (VDP) to carry 95-99% of the total 25 (OH) D3, the other small portion in the form of albumin and lipoprotein through weak non-specific bonds. Vitamin D is a group of fat-soluble secosteroids, and dietary sources of protein are the same with fat sources. The richest sources of protein and fat come from animals. In this study is found an association between protein intake and vitamin D levels. These results are also supported by the research of Nicolas et al. [19], stating that protein-rich foods such as fish, fortified milk and meat are the main sources of natural cholecalciferol (vitamin D3) can suppress *DHCR7* activity, a gene making enzyme that converts 7-dehydrocholesterol into cholesterol in human skin cells thereby affecting vitamin D levels.

Fat is a nutrient that has various functions, including as a constituent of cell membranes, as a reserve of energy, solvents of fat soluble vitamins (vitamins A, D, E, K), and as a hormone to regulate communication between cells, and functions as vitamin D. World Health Organization (WHO) recommends consuming fat as much as 15-30% of total calories. This amount meets the needs of essential fatty acids and helps the absorption of fat-soluble vitamins, one of which is vitamin D.

The fat intake of the subjects in this study ranged from 23.1% -44.1% of the total calories consumed, with an average of 34.1 %. Most subjects consume fat according to WHO recommendations. This study also proves the association between fat intake and vitamin D levels. These results are consistent with the research of Jungert et al. [20] in 2013 which states that fat intake is positively correlated with vitamin D intake and has an effect on vitamin D levels.

Vitamin D absorption is best with a low to moderate amount of fat, compared to very low or high fat amount. Specifically, researchers have shown that 11 grams of fat leads to higher absorption than 35 grams or 0 grams [21]. The cause of large amounts of fat can inhibit the absorption of vitamin D can not be explained. Possibility that vitamin D becomes almost too soluble and remains in fat clots, making some fat clots in the intestine too large to efficiently pass through the intestinal lining . In this study, almost all of subjects had low vitamin D intake so that vitamin D will also be absorbed a little. We believe that the fat consumed is converted to cholesterol as sources for endogenous synthesis of vitamin D.

In this study, almost all subjects experienced vitamin D intake with a mean of 2.4 μg per day, still far below the RDA recommendations. Previous studies on Indonesian children aged 2 - 12 years, found that the average intake of vitamin D was 3.7 μg per day, under the recommendation of the Institute of Medicine (IOM) [9] [22][10] of 15 μg per day. The lack of vitamin d intake in this subject likely due to the low intake of vitamin D sources, vitamin D fortified foods, limited availability of foods containing vitamin D and the lack of public purchasing power for vitamin D sources because the price is relatively expensive. The main sources of vitamin D from food are salmon, mackerel, tuna, mushrooms, egg yolks and orange juice. Vitamin D can also be obtained from foods fortified with vitamin D, including cereal products, bread products, milk, butter, cheese, margarine. Most subjects in this study rarely consume these foods, only chicken eggs and cereal are often consumed.

No significant association between vitamin D intake with vitamin D level was found in this study. Likewise, cohort studies in Germany [20] which stated that low vitamin D intake did not affect levels vitamin D. It can be concluded that the synthesis of vitamin D does not rely on dietary sources of vitamin D, but uses an endogenous source with sun exposure.

Carbohydrates were not associated to vitamin D levels because food sources of vitamin D are abundant in animals, while carbohydrate sources derived from plants. Most subjects experienced a lack of macro nutrient intake, so carbohydrates prioritized for energy sources.

Most of the subjects Fe intake was low, according to dietary recommendations for thalassemia. Low Fe intake can prevent iron buildup in the liver, so that vitamin D synthesis is not interrupted. Iron requirements for healthy children range from 8-15 mg / day [13]. The average iron intake of subjects is 73% of the needs of healthy children. Thalassemia children should be educated about foods that should be avoided with high iron content such as liver, red meat or other products. Iron from animal sources is more easily absorbed than other sources such as cereals and bread. Fish is a source of protein with low iron content. Parents are also given education not to use iron cookware, because the iron from the cookware can move to food. Drinks with a high vitamin C content such as orange juice can increase iron absorption, while tea and coffee can inhibit iron absorption when consumed at meals or 1 hour after eating [23].

This iron plays an important role in the enzymatic system including those needed for the activation of Vitamin D. CYP27B1 requires the compounds ferredoxin reductase and ferredoxsin. Both of these enzymes contain heme, so vitamin D metabolism depends on iron. If it less, can interfere with the activation of vitamin D [24].

Sun exposure is the best source of vitamin D, because 80% of vitamin D synthesis comes from endogenous. Individuals living near the equator exposed to sunlight without the use of sunblock and sunscreen-type protectors have levels of 25 (OH) D above 30 ng / mL. There was a significant association between sun exposure and serum vitamin D levels in this study. The subjects of this study were children with thalassemia major children with an average age of 9 years and most of the men with a lot of activities were done outdoors. In addition to going to school on foot, he also spent a lot of time outdoors playing. Most subjects play bicycle, soccer and others from 1:00 to 3:00 p.m. Based on Hollick's research [25], sun exposure for 5-15 minutes at 10:00 - 15:00 during spring, summer, and fall can produce vitamin D 1000 IU 32 and UVB exposure for 2 hours / week and prevent vitamin D deficiency. Average subjects in this study were exposed to UVB 135 minutes (> 2 hours) a week. The amount of UVB exposure has exceeded the recommended 15 minutes / day or 2 hours / week to meet the needs of vitamin D. Therefore vitamin D deficiency can be prevented even though vitamin D intake is low.

Several studies have shown that an increase in ferritin levels is followed by a decrease in vitamin D levels, in this study vitamin D levels were mostly normal. This study prove that vitamin D synthesis is not interrupted or only slightly disturbed by high ferritin levels if prolonged sun exposure at peak UVB intensities ≥ 2 hours / week. Several limitations were found in this study, namely the use of iron chelation drug is not seen so that the compliance of its use cannot be known. This might affect vitamin D levels.

4. CONCLUSIONS

Total energy intake, protein, fat and sun exposure are factors related to vitamin D levels, whereas Fe and vitamin D intake are not related to vitamin D. Fat intake and sun exposure can predict serum vitamin D levels, so that children with thalassemia major are advised to meet nutrients intake, especially fat and sufficient of sun exposure at the peak of high-intensity UVB. In further studies, it is best to examine bone density and calcium levels to determine the risk of osteoporosis in thalassemia children and monitoring the use of iron chelation and gradation of splenic enlargement associated with vitamin D levels.

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