Association between vitamin D levels and left ventricular function and NT-proBNP levels among thalassemia major children with iron overload

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ABSTRACT

Background : **Heart disease is the major cause of death in thalassemia patients. Repeated blood transfusions and hemolysis cause iron overload and also disrupts the hydroxylation and synthesis of vitamin D, causing vitamin D deficiency. Vitamin D deficiency is associated with cardiac dysfunction.**

Objective **The purpose of this study was to determine the association between vitamin D levels and left ventricular function and N-terminal pro-brain natriuretic peptide (NT-proBNP) levels in thalassemia major children with iron overload.**

Patients and Methods : **A cross-sectional study was conducted in March-April 2015 in the thalassemia clinic, Department of Child Health, Dr. Hasan Sadikin General Hospital, Bandung, Indonesia. Thirty-four children with thalassemia were enrolled consecutively. Serum vitamin D and NT-proBNP levels were measured with electrochemiluminescence (ECLIA) method and echocardiography was performed to assess ventricular function.**

Results : **Significant correlations were found between vitamin D levels and left ventricular ejection fraction (LVEF) (r = 0.399, P = 0.019) and fractional shortening (FS) (r = 0.394, P = 0.021). There was also significant correlation between vitamin D and NT-proBNP levels (r = -0.444, P = 0.008). Chi-square analysis also showed a relationship between vitamin D and NT-proBNP (P = 0.019) levels. There was a difference in NT-proBNP levels among thalassemia major children with iron overload (P = 0.020). Post hoc analysis showed that there was a significant difference in NT-proBNP levels between those with vitamin D deficiency and those with normal vitamin D levels (P = 0.012).**

Conclusion : **There is an association between vitamin D and left ventricular function and NT-proBNP levels in children with thalassemia major and iron overload. Vitamin D can be considered in patients with thalassemia having vitamin D deficiency.**

Keywords : Iron overload, left ventricular function, N-terminal pro-brain natriuretic peptide (NT-proBNP), thalassemia, vitamin D

INTRODUCTION

The number of patients with thalassemia major is increasing every year. The World Health Organization (WHO) reported in 2014 that 250 million people worldwide (4.5%) carry the thalassemia genes resulting

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in 300,000-400,000 new cases each year. In Indonesia 2,500 babies with thalassemia are born every year, and over 6,000 people are registered as transfusion dependent thalassemics, requiring specialist care to overcome the diseases and its complications. Heart disease due to iron overload is the major cause of death in thalassemia patients (70%).[1-3]

Hemolytic anemia coupled with ineffective erythropoiesis characterizes thalassemia major necessitating repeated blood transfusions and leading to iron overload, affecting various organs such as liver and skin, disrupting the hydroxylation and synthesis of vitamin D. This results in vitamin D deficiency in thalassemia major patients with iron overload.[4-6]

Deficiency of vitamin D reduces contractility of the heart muscle and increases production of parathyroid hormone which in turn increases heart rate and cardiac hypertrophy. In thalassemia major patients, deficiency of vitamin D also increases cardiac iron uptake, leading to iron induced cardiomyopathy.[4,7]

N-terminal pro-brain natriuretic peptide (NT-proBNP) is the amino terminal fragment of hormone proBNP, that is, mainly released in response to cardiac wall stress. NT-proBNP can be used to diagnose preclinical cardiac dysfunction.[8,9]

Currently there are many thalassemia patients who do not receive vitamin D supplementation. The purpose of this study was to determine the association between vitamin D level and left ventricular function and NT-proBNP level in children with thalassemia major and iron overload.

PATIENTS AND METHODS

A cross-sectional analytical study was conducted during March–April 2015 at the thalassemia clinic, Department of Child Health, Dr. Hasan Sadikin General Hospital, Bandung, Indonesia. Thirty-four children were enrolled consecutively, with the following inclusion criteria:

a. Thalassemia major children aged <18 years,
b. Children who received iron chelation therapy for more than 1 year,
c. Children who had in the last 3 months serum ferritin levels at ≥2,500 ng/mL, and
d. Children who had hemoglobin (Hb) level before transfusion at >8 g/dL. Exclusion criteria were the presence of congenital heart disease, diagnosis of malignancy, and pericardial effusion.

The level of vitamin D and NT-proBNP was measured with electrochemiluminescence (ECLIA) method, whereas left ventricular function was measured by echocardiography performed by pediatric cardiologist. Statistical analysis of normally distributed data was done using Pearson's correlation test, while Spearman's correlation test was used for abnormally distributed data. Statistical significance was present if $P < 0.05$. The relationship between vitamin D and NT-proBNP was analyzed with chi-square test and one way ANOVA. We normalized distribution of NT-proBNP before analyzing the data using one way ANOVA.

An explanation about the purpose of the study was given to the parents of the children who met the inclusion criteria and their consent was obtained. The levels of Hb before transfusion, serum ferritin, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), urea, and creatinine were measured. Echocardiography was performed 24 hours posttransfusion. Following the transfusion, Hb, vitamin D levels, and NT-proBNP were measured. In inclusion criteria we set Hb level before transfusion at >8 g/dL to minimalize effect of severe anemia (according to WHO criteria for severe anemia in children aged 5–14 years).[10]

Data recorded include name, gender, address, medical record number, age, serum ferritin level, Hb after transfusion, SGOT, SGPT, urea, creatinine, types of chelating agent and compliance with therapy, vitamin D, and NT-proBNP levels and left ventricular function. This study was approved by the Health Research Ethics Committee of the Faculty of Medicine, University of Padjadjaran/Hospital Dr. Hasan Sadikin and did not require any external funding.

RESULTS

The study included 34 children; mean age 10.9 years (range 7-14). Baseline urea, creatinine, alanine transaminase (ALT), and aspartate transaminase (AST) levels of the subjects in this study were as within normal limits. Characteristic of the patients and laboratory findings are shown in Table 1.

Deferiprone was the most common chelating agent used in 27 out of 34 children. All parents stated that their children were compliant with the chelation therapy.

The echocardiography results shows that mean EF 64.53% (53%-75%) and FS 34.74% (28%-43%) were lower than the normal mean values but still within normal limits [Table 2].

Statistical analysis using Pearson's correlation test showed a significant positive correlation between level of

Table 1: Patient characteristics

Characteristic	*n*	Mean (SD)	Median	Range
Sex				
Male	14			
Female	20			
Age (year)		10.9 (2.16)	10.00	7-14
Hemoglobin after transfusion		11,725 (0,86)	11.22	10-12.3
Ferritin serum (mg/dl)		4,213.88 (1,747.86)	3218	2,500-8,668
AST (U/L)		48.87 (24.48)	41	19-149
ALT (U/L)		41.94 (23.67)	37.5	9-106
Urea (mg/d)		23.37 (5.63)	22.50	12-43
Creatinine (mg/d)		0.34 (0.07)	0.34	0.23-0.55

AST: Aspartate transaminase, ALT: Alanine transaminase, SD: standard deviation

vitamin D and left ventricular function, ejection fraction (EF) (r = 0.399, P = 0.019) and fractional shortening (FS) (r = 0.394, P = 0.021) and Spearman's correlation test showed vitamin D levels had a significant negative correlation with NT-pro BNP levels (r = -0.444, P = 0.008).

There was no significant correlation between age, ferritin serum level, and Hb after transfusion, with EF and FS [Table 3] and also there was no significant correlation between age, ferritin serum level, and Hb level after transfusion with NT-proBNP level [Table 4].

Gustafsson conducted a study which showed that NT-proBNP values >125 pg/mL may indicate cardiac dysfunction and are associated with an increased risk of cardiac complications.[11] With these criteria chi-square analysis showed there is an association between vitamin D levels with levels of NT-proBNP (P = 0.019) [Table 5].

There was a difference between NT-proBNP levels among the three vitamin D levels (deficiency, insufficiency, and normal) in the iron overloaded thalassemia major children. *Post hoc* analysis showed a significant difference between NT-proBNP levels in children with vitamin D deficiency and those with normal vitamin D levels [Table 6].

DISCUSSION

In this study, mean ferritin level was over 4,000 mg/L which is associated with an increased risk of cardiac dysfunction. It has been previously reported by Anderson *et al*. that ferritin level >2,500 mg/L increased the risk of cardiac dysfunction.[12] After the introduction of chelating agent therapy, the occurrence of heart failure shifted to second decade. The mean age of our subjects is 10.9 years, therefore, left ventricular ejection fraction (LVEF) and FS is still within normal limits, but begin to decreese than mean normal values.[13]

Results of this study showed a significant positive correlation between levels of vitamin D and EF (r = 0.399, P = 0.019) and FS (r = 0.394, P = 0.021) as shown in Figures 1 and 2. There was no significant correlation between age, ferritin serum level, and Hb after transfusion, with EF and FS. This result was consistent with a previous study conducted by Wood *et al*.[4] that states that there is a correlation between vitamin D levels and LVEF.[4] Low levels of vitamin D increases the production of parathyroid hormone that in turn increases heart rate. It also disturbs contraction of cardiomyocytes and increases natriuretic peptide secretion that may lead to cardiac hypertrophy.[14,15] Therapy with an active vitamin D analog reduces left atrial hypertrophy and attenuates the rise of BNP.[16]

Vitamin D suppresses the release of tumor necrosis factor α (TNF-α) and enhances interleukin-10 (IL-10) synthesis. Some studies have shown that increasing inflammatory cytokines like TNF-α and IL-10 deficiency leads to severe atherosclerosis.[7,17,18] Schleithoff *et al*.[7] found that parathyroid hormone level was significantly lower in congestive heart failure (CHF) patients with vitamin D supplementation compared to placebo. Anti-inflammatory cytokine IL-10 was higher after supplementation, and proinflammatory cytokine TNF-α remained constant in supplementation group, while it was increased in placebo group.[7]

Table 2: LV function on echocardiography

Echocardiography results	Mean	SD	Range
EF%	64.53	4.74	53-75
FS%	34.74	3.52	28-43

EF: Ejection fraction, FS: Fraction of shortening, SD: Standard deviation

Table 3: Correlation between vitamin D level, age, ferritin serum level, and Hb after transfusion with left ventricle function

	EF		FS	
	r	*P*	*r*	*P*
Vitamin D level*	0,399	0,019	0,394	0,021
Age*	0,063	0,722	0,094	0,595
Ferritin serum level**	−0,089	0,616	−0,116	0,514
Hemoglobin after transfusion**	0,108	0,543	0,122	0,492

EF: Ejection fraction, FS: Fraction of shortening, *Pearson correlation, **Spearman correlation

Table 4: Correlation between vitamin D level, age, ferritin serum level, and hemoglobin after transfusion with NT-proBNP level

	NT-proBNP Level	
	*r***	*P*
Vitamin D level	−0,444	0,008
Age	−0,094	0,598
Ferritin serum level	0,128	0,470
Hemoglobin after transfusion	0,172	0,332

**Spearman correlation

Table 5: Association between vitamin D level and NT-proBNP

Vitamin D levels	NT-ProBNP levels		*P**
	Cardiac dysfunction# >125 pg/mL	No cardiac dysfunction# <125 pg/mL	
	n	*n*	
Deficiency	17	2	0,019
Insufficiency	7	4	
Normal	1	3	
Total	25	9	

*Chi square analysis, #Study by Gustafson 2003[10]

Table 6: Association between vitamin D level and NT-proBNP

Parameter	Vitamin D levels			*P**
	Deficiency (*n* = 19)	Insufficiency (*n* = 11)	Normal (*n* = 4)	
NT-ProBNP levels	2.4(0.29)	2.1(0.47)	1.9(0.18)	0.020

*One way Anova, *Post hoc* analysis deficiency versus insufficiency P = 0.55, Deficiency versus normal P = 0.012, Insufficiency versus normal P = 0.233

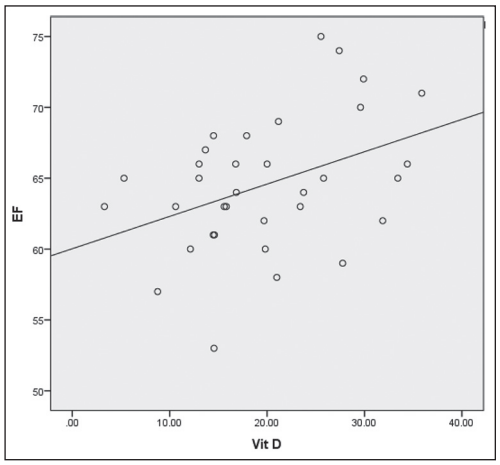

Figure 1: Correlation between vitamin D level and ejection fraction (EF)

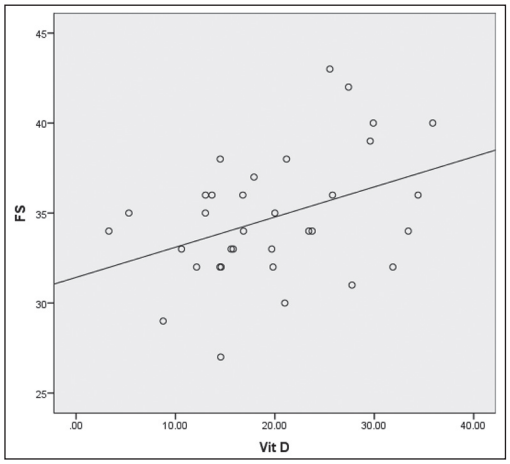

Figure 2: Correlation between vitamin D level and fraction of shortening (FS)

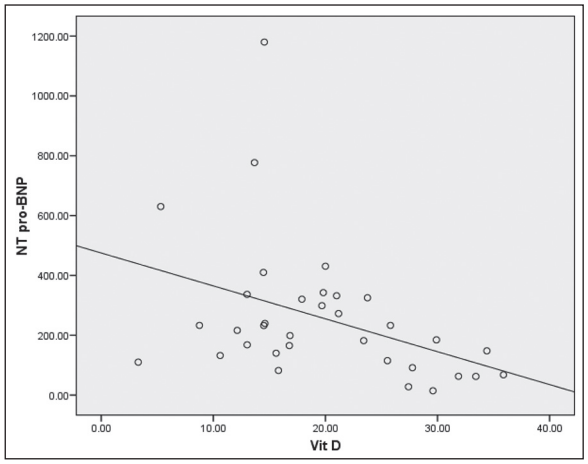

Figure 3: Correlation between vitamin D and NT-proBNP

Deficiency of vitamin D in patients with thalassemia stimulates trans-membrane calcium movement through left ventricle dependent calcium channel (LVDCC). Research conducted by Oudit *et al*.[19] stated that LVDCC plays a role in transporting nontransferring binding iron (NTBI) into the myocardium, thereby increasing cardiac iron uptake.[19] Increased cardiac iron uptake will lead to cardiomyopathy.[20] Wood *et al*.[4] have reported that D25-OH levels was positively correlated with LVEF and inversely related with Cardiac R2*. Cardiac R2* correlated directly with cardiac iron concentration and was found to predict cardiac dysfunction.[2,4,21-24]

NT-proBNP levels in this study had a significant negative correlation with the levels of vitamin D (r = –0.444, P = 0.008) as shown in Figure 3. There was no correlation between age, ferritin serum level, and Hb level after transfusion with NT-proBNP level.

Using cut-offs of >125 pg/mL based on Gustafson,[11] statistical analysis showed association between vitamin D levels with levels of NT-proBNP. The NT-proBNP levels increase before Doppler echocardiographic indexes become apparently abnormal. NT-proBNP has high sensitivity, and can potentially detect cardiac dysfunction in asymptomatic patients.[25]

Vitamin D levels were divided into three groups: >30 ng/mL (>75 nmol/L) was classified as normal, 20-30 ng/mL (50-75 nmol/L) was classified as vitamin D insufficiency, and <20 ng/mL (<50 nmol/L) was classified as vitamin D deficiency. In this study, 19 children having thalassemia major (55.8%) had vitamin D deficiency with vitamin D levels <20 ng/mL. These results were consistent with a study conducted by Fadhillah *et al*.[26] that stated that there was vitamin D deficiency in 85.5% of thalassemia children in Hasan Sadikin Hospital. Wood in 2008 also found vitamin D deficiency occurred in 55.4% of patients with thalassemia. Iron deposition in the liver and skin of patients with thalassemia major disrupts hydroxylation and synthesis of vitamin D, so most of the patients with thalassemia major have vitamin D deficiency. According to the study conducted by Fadhillah *et al*.[26] specifically in patients with thalassemia major, vitamin D deficiency is not influenced by food intake and sun exposure, but more because of interference, hydroxylation, and synthesis of vitamin D.[26]

In our study, we found a difference between NT-proBNP levels among iron overload thalassemia major children with vitamin D deficiency and those with normal vitamin D levels. Therefore, vitamin D supplementation should be considered in thalassemia major children, many of whom are vitamin D deficient to reduce the risk of heart failure. Fung *et al*.[27] reported that 43% of patients with thalassemia in USA have vitamin D deficiency, which persisted despite daily low dose supplementation of 400-1,000 IU vitamin D. Supervised high dose of vitamin D supplementation improved vitamin D status. Solinman *et al*.[5] recommended that thalassemic patients with vitamin D level <20 ng/mL should be repleted. Vitamin D status should be assessed regularly in all patients with thalassemia. Monitoring of

patients on high dose supplementation is essential to ensure adequacy of therapy and to monitor toxicity.[5,27-29]

Our study presents some limitations such as the small number of subjects and absence of data on general prevalence of vitamin D deficiency in normal population. Compliance to chelation therapy was self-reported and, therefore, prone to error. However, during every monthly visit, the importance of compliance to the chelating agent was emphasized. Further, research with more subjects is needed to evaluate cardiac function in children with thalassemia major who suffer from vitamin D deficiency, and it is necessary to follow-up the effect of supplementation on various indices of ventricular function including NT-proBNP levels.

CONCLUSIONS

There is an association between vitamin D levels and left ventricular function and NT-proBNP levels among thalassemia major children with iron overload. There is a difference between NT-proBNP levels among iron overload thalassemia major children with vitamin D deficiency and those with normal vitamin D level. Supplementation of vitamin D can be considered for thalassemia patients, particularly for those with vitamin D deficiency.

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Conflicts of interest

There are no conflicts of interest.

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