

Vitamin D and Parathyroid Hormone Levels and Their Relation to Serum Ferritin Levels in Children with Thalassemia Major: One-Center Study in Western Indonesia

ABSTRACT

Background. Children with thalassemia major need regular blood transfusions to survive. These multiple transfusions put them at risk for iron overload, which result in organ damage such as parathyroid gland. The aim of this study is to determine levels of calcium, phosphorus, vitamin D and parathyroid hormone in children with thalassemia major and compare them with controls, and analyze their correlation with serum ferritin level.

Methodology. This was a cross-sectional study in 37 patients with thalassemia major in Department of Pediatrics, Dr. M. Jamil Hospital Padang and 37 age- and sex-matched healthy controls. Calcium ion, inorganic phosphorus, vitamin D (25-hydroxycholecalciferol), parathyroid hormone (PTH) and serum ferritin level were measured.

Results: There were 18 boys and 19 girls in each group. Their age ranged between 2 and 16 years, with 20 (54%) children aged more than 10 years. Median of ferritin level in patients and controls were 2981 (IQR 3662) ng/mL and 36.6 (IQR 27.5) ng/mL respectively. Mean level of calcium ion and phosphorus levels in patients were 1.17 (SD 0.07) mmol/L and 5.42 (SD 0.76) mg/dL respectively. Median values of vitamin D and PTH in patients were 54.3 (IQR 21.1) ng/mL and 127.5 (IQR 183.1) pg/mL respectively. Compared to healthy controls, calcium ion level in patients was significantly lower ($P = .001$). On the contrary, phosphorus, vitamin D and PTH levels were not significantly different. Vitamin D and PTH levels had no significant correlation with serum ferritin levels ($P = 0.147$ and $P = 0.2$ respectively).

Conclusion. Vitamin D and PTH levels have no significant correlation with serum ferritin level. The calcium ion level has a moderate negative correlation with serum ferritin level.

Keyword: Parathyroid hormone, vitamin D, ferritin, thalassemia, children

BACKGROUND

Thalassemia is an autosomal recessive inherited blood disorder caused by defect of globin chain synthesis. Thalassemia major is a severe form of disease that requires lifelong blood transfusion, resulting in excess iron level in body. Excess iron can cause interference function of various organs, including parathyroid gland. ¹Parathyroid hormone (PTH) is a hormone secreted by the parathyroid gland that plays a role in calcium hemostasis, together with vitamin D and calcitonin. Parathyroid hormone keep blood calcium levels within normal range by calcium reabsorption and

35 phosphate excretion in the kidney, increasing calcium absorption from bone, and also plays a role in
36 the formation of active vitamin D 1,25-dihydroxyvitamin D in the kidney.²

37 Some studies have reported that some of thalassemia patients had significantly low levels of
38 PTH, vitamin D and calcium, and high level of phosphorous compared with healthy controls.³⁻⁵ Hagag
39 AA et al in their study in Egypt reported that there were significant negative correlation between
40 PTH level with serum ferritin level.⁵ In contrast to this, some have reported that there were no clear
41 relationship between PTH and ferritin serum level.⁶⁻⁸ In the pediatric ward of Dr. M. Jamil hospital
42 Padang, there are 37 children with thalassemia major who get routine transfusion every month.
43 These children are at risk of high iron load because of not optimal iron chelation treatment,
44 whether due to lack of drug availability or bad compliance to medication. With this background we
45 want to determine the levels of calcium, phosphorus, vitamin D and parathyroid hormone in children
46 with thalassemia major in our center, compare them with controls, and analyze their correlation
47 with serum ferritin level.

48 **METHODS**

49 This study was a correlational study with a cross-sectional design, taken place in Dr. M. Jamil hospital
50 Padang from July to October 2016. The samples included 37 children with thalassemia major and
51 equal amount of age- and sex-matched healthy children as controls. Present study had been
52 approved by the research ethics committee of Faculty of Medicine, Andalas University Padang. All
53 subjects were fasted for 8 hours before blood collection. Blood was taken aseptically through
54 peripheral veins by trained personnel. A total of ± 6 ml of blood was taken and the serum was
55 collected for laboratory investigation of calcium ion, phosphorus inorganic, ferritin, vitamin D (25-
56 hydroxyvitamin D), and intact parathyroid hormone (PTH). Calcium ion investigation performed by
57 ion selective electrode (ISE) method by using kit from SnapPak Roche. Phosphorus inorganic
58 investigation was performed by Phosphomolybdate Colorimetric method by using kit from Siemens
59 healthcare diagnostic. Investigation of ferritin (using Diagnostic Biochem Canada), vitamin D (using
60 Diagnostic Biochem Canada), and PTH (using Bioassay Technology Laboratory) were performed by
61 Enzyme-linked immunosorbent assay (ELISA) method.

62 All parametric results were expressed as mean $\pm$ SD for each group when data normally distributed
63 and as median $\pm$ IQR when data not normally distributed. Statistical analyses were performed using
64 the student t test to compare concentration among the patients and controls when data were
65 normally distributed, and Mann-Whitney test were used when data were not normally distributed.
66 For correlation analysis, Pearson coefficient of correlation was used if data were normally

distributed, and Spearman's correlation test if not normally distributed. A probability value of less than 0.05 was assumed as significant.

RESULT

Of 37 children with thalassemia in this study, the number of boys is almost the same as girls and children aged 10-16 years are the most age group (54%). Nutritional status of children with thalassemia major by body weight per body height (BW/HW) is particularly good nutrition (81%), but much more appear as short stature (56.8%). (Table 1)

Table 1. Characteristic of Subject Study

Parameters	Patients (%) n = 37	Controls (%) n = 37
Sex		
- Boy	18 (48,6)	18 (48,6)
- Girl	19 (51,4)	19 (51,4)
Age		
- 2- 4 years old	4 (11)	4 (11)
- 5 – 9 years old	13 (35)	13 (35)
- 10-16 years old	20 (54)	20 (54)
Nutritional Status (BW/HW)		
- Undernourished	7 (19)	9 (24,3)
- Well-nourished	30 (81)	22 (59,5)
- Overweight	0	6 (16,2)
Stature		
- Short	21 (56,8)	7 (19)
- Normal	16 (43,2)	30 (81)

The mean of calcium in thalassemic patients is lower than that of healthy controls, and these results differ significantly ($P = 0.001$). Similarly, the median serum ferritin level of children with thalassemia major was significantly higher than that of healthy controls ($P = 0.000$). In contrast, levels of phosphorus, vitamin D and PTH of children with thalassemia major did not differ significantly with healthy controls (table 2).

Table 2. Comparison of calcium ion, phosphorus inorganic, vitamin D, PTH and serum ferritin between patients and controls group

Parameter	Patients N = 37	Controls N = 37	P
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Calcium ion (mmol/L)			
Mean (SD)	1.17 (0.07)	1,21 (0.04)	0.001*
Phosphor (mg/dL)			
Mean (SD)	5.42 (0.76)	5,3 (0.63)	0.417*
Vitamin D (ng/mL)			
Median (IQ)	54.3 (21.1)	53,3 (47)	0.563 [#]
PTH (pg/mL)			
Median (IQ)	127.5 (183.1)	155,8(69.1)	0.165 [#]
Ferritin (ng/mL)			
Median (IQ)	2981 (3662)	36,6 (27.5)	0.000 [#]

*Student t – test[#]Mann-Whitney test

By using Spearman's correlation test, there was negatively significant correlation between calcium with serum ferritin levels ($r_s = -0.44$, $P = 0.007$). There were no significant correlation between inorganic phosphorous, vitamin D, PTH level with serum ferritin(table 3).

Table 3. Correlation between serum ferritin levels with calcium, phosphorus, vitamin D and PTH levels in children with thalassemia major

		Calcium ion	Inorganic Phosphorous	Vitamin D	PTH
Ferritin	r_s	-0,44	0,31	-0,24	-0,16
	P	0.007	0.06	0.147	0.2

DISCUSSION

This cross sectional study included 37 children with thalassemia major at M. Djamil hospital Padang and equal number of healthy children as a control group. The number of female and male patients is almost the same. This is in agreement with Hagag et al who found that of 60 children with thalassemia major in their study, there were 32 (53.5%) females and 28 (46.7%) males. This revealed that thalassemia is inherited in an autosomal recessive fashion, not through sex chromosome.⁵

The levels of PTH in children with thalassemia in our study is not different significantly to healthy controls. This result is in accordance with a study in Egypt that reported the level of PTH in both patients and controls groups were not significantly different with a higher level in patients group.⁹ This study is also in agreement with Zoga J¹⁰, but is not in agreement with some studies that found PTH levels of children with thalassemia major were significantly lower than healthy controls.^{3,5,8}

Vitamin D is a prohormone that plays an important role in calcium hemostasis. Liver and kidneys play an important role in vitamin D metabolism. Liver iron overload may interrupt its metabolism. Vitamin D level in this study do not differ significantly with healthy controls. This result disagrees with some studies.^{5,10,11}

107 Calcium is a mineral that has an important role in many physiological processes of the body,
108 including the contraction of striated muscles, heart and smooth muscle; blood clots and nerve
109 impulse transmission. In present study calcium levels of children with thalassemia major was
110 significantly lower than that of healthy controls. This result is consistent with Hagag AA et al. Similar
111 results were also obtained by Soboor M, et al in Pakistan, Modi AS, et al and Goyal M in India.^{3-5,12}

112 Phosphorus, along with calcium, is a major structural component of bone in the form of a
113 calcium phosphate salt called hydroxyapatite. In addition, phosphorus is an important component of
114 cell membranes, energy production, phosphorylation reactions, and acid-base hemostasis.
115 Phosphorus levels in the body, similar to calcium are regulated by vitamin D and PTH.² In present
116 study, the average phosphorus levels in children with thalassemia major were higher than healthy
117 controls, but not statistically significant. This result was similar to Mahachoklertwattana P¹³ and
118 Fahim FM¹¹ who reported that phosphorous level of thalassemic patients were nearly the same with
119 controls. This result is in contrast to Modi AS in India, Soboor M in Pakistan and Hagag AA in Egypt,
120 who found that phosphorus levels in children with thalassemia major were significantly higher than
121 those in healthy controls.^{4,5,12}

122 In this study, PTH level has no correlation with ferritin serum level. This result is in
123 agreement with Sleem et al⁶ and Basha N KP⁸ et al, while not in agreement with Hagag
124 AA.⁵ Furthermore, in this study there is no significant correlation between vitamin D level and ferritin
125 serum. This result agree with those study by Zoga J¹⁰ and Fahim FM¹¹, but disagree with Fadilah TF¹⁴.
126 Calcium is the only parameter that has significant correlation with ferritin serum.

127 There is interesting result in our study that found only calcium is lower compared to control,
128 while PTH and vitamin D that control hemostasis of calcium are almost similar to control. How this
129 happen is not clearly understand whether this lower level of calcium only because of citrate toxicity
130 due to repeated blood transfusion or might be calcitonin has important role in this condition.
131 Further study should carry out to answer this question.

132 Based on this study, the administration of calcium supplementation in children with
133 thalassemia major in Dr. M. Jamil Padang hospital should be recommended, while vitamin D
134 supplementation might not be given. Further study should be performed to evaluate whether calcium
135 supplementation alone can increase calcium levels of children with major thalassemia.

136 We conclude that calcium levels of children with thalassemia major significantly lower than
137 healthy controls, while phosphorus, vitamin D and PTH level are not differ significantly. There is

138 moderatenegative correlation between ferritin serum and calcium, while there are not significant
139 correlation between ferritin serum and phosphorus, vitamin D and PTH level.

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