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***Original Research Article***

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


## ABSTRACT

**Background**. Children with thalassemia major are at risk for deficiency of vitamin D and parathyroid hormone. It is caused by the excess iron level due to repeated blood transfusion.

**Objective**. To know the levels of calcium, phosphorus, vitamin D and parathyroid hormone of children with major thalassemia and compare it with controls, and analyze its association with serum ferritin levels.

**Methodology**. A cross-sectional study of 37 children with major thalassemia hospitalized in the Pediatric Department of Dr. M. Jamil Padang and 37 healthy children according to age and sex as control. Six milliliters of blood sample was taken for laboratory investigation of calcium ion, phosphorus, vitamin D, parathyroid hormone (PTH) and serum ferritin.

**Results**. The number of boys is almost the same as girls and children over 10 years old are the most age group (54%). The mean rate of calcium ion and phosphorus of children with thalassemia major was 1.17 ± 0.07 mmol/L and 5.42 ± 0.76 mg/dL, with median values of vitamin D and PTH respectively, 54.3 ± 21.1 and 127.5 ± 183.1. Compared to healthy controls, the children's calcium ion level with thalassemia is significantly lower. In contrast, phosphorus, vitamin D and PTH levels were not significantly different. The levels of vitamin D and PTH have a weak correlation with serum ferritin levels with rs values respectively -0.24 and -0.16.

**Conclusion**. Vitamin D and PTH levels of children with thalassemia major has no significant difference compared to healthy controls. Serum ferritin level have a weak negative correlation with vitamin D and PTH. The calcium ion level has a moderate negative correlation with serum ferritin level.


**Keyword**: Thalassemia, Ferritin, children, vitamin D, calcium

## BACKGROUND

Thalassemia is an autosomal recessive inherited blood disorder caused by globin chain synthesis disorders from hemoglobin. Thalassemia major is the heaviest form of a disease that requires lifelong blood transfusion and resulting in excess iron level in body. Excess iron can cause interference function of various organs, such as liver and endocrine disorders (such as parathyroid gland, thyroid, etc.). (Porter J, 2014; Vichinsky E, 2012)

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Research conducted by ... found that in patients with thalassemia major, there are ..% with hipoparatiroid. In contrast, from research conducted by found that ...% patients have hyperparathyroid. As for calcium levels, from research conducted by ... it was found that…

In the pediatric ward of RSUP Dr. M. Jamil Padang there are 37 children with thalassemia major who get routine treatment every month. These children are at risk of high iron level due to the not optimal iron chelation treatment, lack of drug availability or not compliance to medication.

**METHOD**

This study was a correlational study with a cross-sectional design on thalassemia major children who treated routinely in the ward of RSUP Dr. M. Jamil Padang in July - October 2016. The samples in this study were 37 children with thalassemia major and healthy children according to age and sex in the same amount as control.

All subjects are fasted for 8 hours before blood collection. Blood was taken aseptically through peripheral veins by trained personnel. A total of ± 6 ml of blood was taken and the serum was collected for laboratory investigation of calcium ion, phosphorus, ferritin, vitamin D (25-hydroxyvitamin D), and intact parathyroid hormone (PTH). Investigation of ferritin (Diagnostic Biochem Canada), vitamin D (Diagnostic Biochem Canada), and PTH (Bioassay Technology Laboratory) were performed by Enzyme-linked immunosorbent assay (ELISA) method. Calcium ion investigation (Ca ion) performed by ion selective electrode (ISE) method by using kit from SnapPak Roche. Phosphorus (PO4) investigation was performed by Phosphomolybdate Calorimetric method by using kit from Siemens healthcare diagnostic.

This study had been approved by the research ethics committee of Faculty of Medicine Andalas University.

Student t-test is used to see the difference between calcium, phosphorus, ferritin, vitamin D and parathyroid hormone levels between thalassemia patients with controls. To see the relationship between vitamin D levels and parathyroid hormone levels with serum ferritin levels, Spearman correlation test was performed with relationships were significant when $p < 0.05$.

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## RESULT

Thirty-seven 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 in accordance with the of thalassemia major children much more appear as short stature (56.8%). (Table 1)

Table 1. Characteristic of Subject Study

| Characteristic | Patient (%)<br>n = 37 | Control (%)<br>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) |
| - Overwieight | 0 | 6 (16,2) |
| Stature | | |
| - Short | 21 (56,8) | 7 (19) |
| - Normal | 16 (43,2) | 30 (81) |

According to statistical tests, calcium ion and phosphorus levels of children with thalassemia major and healthy control are normally distributed, otherwise ferritin, vitamin D, and PTH levels are not normally distributed.

Table 2 shows that the mean calcium ion child content with thalassemia major is lower than that of healthy controls, and these results differ significantly. Similarly, the median serum ferritin level of children with thalassemia major was significantly higher than that of healthy controls. In contrast, levels of phosphorus, vitamin D and PTH of children with thalassemia major did not differ significantly with healthy controls.

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Table 2. Mean levels of calcium, phosphorus, vitamin D, PTH and ferritin study subjects

| Parameter | Patient<br>Mean (SD) atau Median (IQ) | Controll<br>Mean (SD) atau Median (IQ) | p |
|---|---|---|---|
| Calcium ion (mmol/L) | 1,17 (SD 0,07) | 1,21 (SD 0,04) | 0,001* |
| Phosphor (mg/dL) | 5,42 (SD 0,76) | 5,3 (SD 0,63) | 0,417* |
| Vitamin D (ng/mL) | 54,3 (IQ 21,1) | 53,3 (IQ 47) | 0,563# |
| PTH (pg/mL) | 127,5 (IQ 183,1) | 155,8(IQ 69,1) | 0,165# |
| Ferritin (ng/mL) | 2981 (IQ 3662) | 36,6 (IQ 27,5) | 0,000# |

**Student t – test* #*Mann-Whitney test*

By using Spearman's correlation test, calcium ion level was most negatively correlated with serum ferritin levels (rs = -0.44). The phosphorus was correlated weak positive (rs = 0.31), vitamin D was correlated weak negative (rs = -0.24), and PTH was correlation very weak negative (rs = -0.16).

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

| | Calcium | Phosphor | Vitamin D | PTH |
|---|---|---|---|---|
| Ferritin | -0,44# | 0,31# | -0,24# | -0,16# |

## DISCUSSION

This cross sectional study included 37 children with thalassemia major at M Djamil hospital Padang, this study also included 37 healthy children as a control group. The number of patients female equals with male. In agreement with Hagag et al who has studied 60 children with thalassemia major in Egypt with age range of 6-10 years and healthy children as control with an age range of 5-10 years. Children with thalassemia major including 32 (53.5%) females and 28 (46.7%) males which has shown that thalassemia inherited in an autosomal recessive fashion, not through sex chromosome. (Hagag AA et al., 2015; Permono B 2005).

Calcium is a mineral that has an important role in many physiological processes of the body, including the contraction of striated muscles, heart and smooth muscle; blood clots and nerve impulse transmission. In this study it was found that the calcium levels of children with thalassemia major was significantly lower than that of healthy controls. This is in line with Hagag AA et al in Egypt which found that the child's calcium level with thalassemia major was significantly lower than that of healthy controls. Similar results were also obtained by Soboor M, et al in Pakistan, Modi AS, et al and Goyal M in India. (Guyton AC, 2006; Hagag AA dkk, 2015; Soboor M dkk, 2014; Modi AS, 2012; Goyal M, 2010).

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Phosphorus, along with calcium, is a major structural component of bone in the form of a calcium phosphate salt called hydroxyapatite. In addition phosphorus is an important component of cell membranes, energy production, phosphorylation reactions, and acid-base hemostasis. Phosphorus levels in the body, similar to calcium are regulated by vitamin D and PTH. In this study the average phosphorus levels in children with thalassemia major higher than with healthy controls. However, this is not statistically significant. This result is in contrast to US Modi research in India, Soboor M in Pakistan and Hagag AA in Egypt, which found that phosphorus levels in children with thalassemia major were significantly higher than those in healthy controls(Knochel JP, 2006; Hagag AA dkk, 2015; Soboor M dkk, 2014; Modi AS, 2012)

In this study the levels of vitamin D and PTH children with thalassemia are almost equal to healthy controls. This result is different from the research done by Hagag AA which found that vitamin D and PTH levels of children with thalassemia major were lower than healthy controls. This difference may be due to the smaller number of samples in this study compared with Hagag AA and also because of the large variety of vitamin D and PTH levels obtained so that it is not normally distributed.

Based on this study, ferritin only has a weak negative correlation with vitamin D levels and is very weak with PTH. But instead have a moderate negative correlation with calcium ion levels.

Based on the results of this study, the administration of calcium supplementation in children with thalassemia major in Dr. M. Jamil Padang hospital can be recommended. In contrast to the provision of vitamin D supplementation should not be given unless it has been proven by examination of vitamin D levels have been deficient. Based on the results of the study it is necessary to do further research to see whether calcium supplementation alone can increase calcium levels of children with major thalassemia or needed a vitamin supplementation.

Vitamin D deficiency that occurs due to liver or hypoparathyroid disorders in thalassemia patients has been reported by some researchers. Research conducted by Ezzat HM et al., of 30 thalassemia major patients found 19 people (63.3%) experienced vitamin D insufficiency and deficiency. From the research found a significant association between liver iron levels> 5 mg (LIC = liver iron concentration ) with vitamin D levels <60 nmol / L and there was a significant reverse correlation between LIC and vitamin D (r = -0.33). Research conducted by Fadilah TF et al. Hasan Sadikin in Bandung in 64 thalassemia major children, obtained 55 (86%) children have vitamin D levels <50 nmol / L. From these studies there is a negative correlation between the levels of ferritin with vitamin D levels (r = -0.368; p <0.01).(Ezzat HM dkk, 2015; Fadillah TF dkk, 2012)

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Vitamin D is a prohormone that plays an important role in calcium hemostasis. The liver and kidneys play an important role in vitamin D metabolism. Vitamin D derived from the skin and food is hydroxylated in the liver to 25-hydroxyvitamin D, which then under the influence of parathyroid hormone is metabolized to the active form of 1,25-dihydroxyvitamin D in the kidneys. This active vitamin D then increases the absorption of calcium and phosphate in the intestine, as well as stimulates osteoclast proliferation and calcium resorption of bone. (Soliman A, 2013; Holick MF, 2007)

Parathyroid hormone is a hormone secreted by the parathyroid gland that plays a role in calcium hemostasis. Parathyroid hormone in principle serves to maintain blood calcium levels within normal limits by increasing calcium reabsorption and phosphate excretion in the kidney, increasing calcium absorption from bone, and also plays a role in the formation of active vitamin D 1,25-dihydroxyvitamin D in the kidney. (Guyton AC, 2006)

Thalassemia patients are at risk of impaired parathyroid hormone function due to iron deposition in the parathyroid gland. This results in a hypoparathyroid state that results in hypocalcemia that may cause neuromuscular disorders and bone mineralization. Low calcium levels with high levels of phosphorus and low parathyroid hormone levels are diagnostic for hypoparathyroid. (Hagag AA, 2015; Angelopoulos NG et al., 2006; Hamidieh AA et al., 2009)

The prevalence of hypoparathyroid in patients with thalassemia varies considerably. Chern JP gained 10.7% prevalence, while Angelopoulos et al got 13.5% of cases. A multicentre study conducted by Shamshirsaz AA in Tehran Iran found a prevalence of 7.6% of cases (Chern JP, 2002; Angelopoulos et al., 2006; Shamshirsaz AA et al., 2003).

Examination of parathyroid hormone levels is recommended to be examined annually in patients with thalassemia major, starting from 5 years of age, after 3 years of transfusion or if there is clinical indication. Hipoparatiroid in thalassemia patients generally occurs in the second decade of life. However, from the research conducted by Habeb AM et al in 81 people with thalassemia major, found 9 (11.1%) sufferers experienced hipoparatiroid and 3 of them aged between 2-9 years. In the study the mean ferritin level of the patient was 3697,7□536,9 ng / ml. Another study conducted by Hagag AA in 60 children with thalassemia major age 6-10 years, obtained a higher prevalence hipoparatiroid, which reached 20% of cases. The mean serum ferritin level reached 3253,7□707,1 ng / ml. In the study, there was a significant negative correlation between parathyroid hormone levels and serum ferritin. (Habeb AM et al, 2013; Hagag AA et al, 2015)

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a**. Conclusion**

1. Calcium levels of children with thalassemia major significantly lower than healthy controls.
2. Phosphorus levels of children with thalassemia major did not differ significantly with healthy controls.
3. Vitamin D levels of children with thalassemia major did not differ significantly with healthy controls.
4. PTH levels of children with thalassemia major did not differ significantly with healthy controls.
5. Serum ferritin levels have a moderate negative correlation with calcium, a weak positive correlation with phosphorus, a weak negative correlation with vitamin D and a very weak correlation with PTH.

**b. Suggestion**

1. Routine calcium supplementation should be done in children with thalassemia major.
2. Provision of vitamin D supplementation should not be given routinely in children with thalassemia major.
3. Need to do further research related to the effectiveness of giving calcium supplementation in children with thalassemia major

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