

Serum ferritin levels and endocrine disorders in children with thalassemia major

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Abstract

Background Endocrine disorders in thalassemia major children occur due to iron overload and hemosiderosis in endocrine organs. Early detection is needed to prevent complications and improve the quality of life. An association between serum ferritin and endocrine disorders in thalassemia patients has been inconclusive to date.

Objective To analyze for possible associations between serum ferritin and endocrine disorders (short stature, delayed puberty, delayed bone age, hypothyroidism, impaired glucose tolerance, and diabetes mellitus) and the incidence of each disorder in thalassemia major.

Methods There were 115 thalassemia major patients aged 10-18 years involved in our cross-sectional study from June 2019 - June 2020 in the Pediatrics Department, DR. M. Hosein Hospital, Palembang, South Sumatera. Anthropometry and socioeconomic status informations were collected from physical examination and interview. Ferritin, FT4, TSH, Hb and glucose levels measured by using standard methods for each item in the laboratory, mean while the skeletal age assessment was determined by using FELS method.

Results This study included 83 (72.2%) girls and 32 (27.8%) boys. There were 89 (77.4%) subjects with short stature, 74 (64.4%) with delayed bone age, 30 (26.1%) with impaired glucose tolerance, 25 (21.7%) with delayed puberty, 4 (3.5%) with diabetes mellitus (DM), and none with hypothyroidism. Bivariate and multivariate analyses revealed no associations between serum ferritin and short stature, delayed bone age, impaired glucose tolerance, delayed puberty, and DM.

Conclusion There is a high prevalence of endocrine disorders in pediatric thalassemia patients, especially short stature and delayed bone age. However, there are no associations between serum ferritin and endocrine disorders in these patients. [Paediatr Indones. 2021;61:125-32 ; DOI: 10.14238/pi61.3.2021.125-32].

Keywords: association; thalassemia major; children; ferritin; complications; endocrine disorders

Thalassemia major is an inherited autosomal recessive disorder that causes chronic anemia. According to the *World Health Organization* (WHO), there were 250 million (4.5%) thalassemia carriers in the world's population in 2009, of whom 80-90 million were β -thalassemia carriers. A high prevalence of thalassemia was found at the thalassemia belt region, including Indonesia. Most β -thalassemia carriers were located in Sumatera, with nearly 10% in Palembang. In April 2018, 287 thalassemia patients were registered at DR. M. Hosein Hospital (RSMH), Palembang, South Sumatera, and 50.5% of them were aged 7- 18 years.¹⁻³

Thalassemia major patients suffer from ineffective erythropoiesis of the bone marrow, so continuous transfusions are required to maintain hemoglobin levels above 9-10.5 g/dL. These transfusions cause iron overload and hemosiderosis, which are toxic to the body. Free iron catalyzes reactive oxygen species (ROS) and damages cells. These patients take chelating agents

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Submitted November 30, 2020. Accepted May 28, 2021.

in order to excrete the iron through the urine or feces.⁴⁻⁷

Some common endocrine complications of thalassemia are short stature, gonadotropin hypogonadism, delayed puberty, impaired glucose tolerance, hypothyroidism, hypoparathyroidism, and diabetes mellitus. Complications may also occur in non-endocrine organs, such as the heart, kidneys, bones, eyes, and other organs.⁷⁻¹⁰

The gold standard to determine tissue iron level is a tissue biopsy. Serum ferritin is considered to be representative of iron overload and hemosiderosis, and has been used in past studies of endocrine disorders. The prevalence of thalassemic endocrine disorders was reported to range from 34% to 92%.¹¹⁻¹⁵ Early detection of endocrine complications in thalassemic patients is needed to improve their quality of life. The aim of this study was to analyze for possible associations between serum ferritin and endocrine disorders (short stature, delayed puberty, delayed bone age, hypothyroidism, impaired glucose tolerance, and diabetes mellitus) and the incidence of each disorder in thalassemia major.

Methods

This cross-sectional study was conducted from June 2019-June 2020 at the Pediatrics Department, Dr. Moh. Hosein Hospital, Palembang, South Sumatera, in thalassemia major patients aged 10-18 years who underwent routine blood transfusions and received iron chelation treatments. Patients taking high-dose steroids for two weeks or more were excluded from this study. The subjects were included by consecutive sampling. Patients and families provided written informed consent for study inclusion. Data were obtained from interviews, as well as physical, laboratory, and radiology support examinations.

Short stature was defined as a height more than two standard deviations below the mean for age (less than the 3rd percentile) CDC curve. Subject was categorized as having a delayed puberty if she/he showed the lack of any pubertal signs by the age of 13 years in girls and 14 years in boys. A decreased rate of skeletal maturation was classified as retarded bone and can be diagnosed on the basis of an estimation of the bone age from radiographs of specific bones in the human body. Diabetes mellitus and impaired glucose intolerance was diagnosed based on *International Society*

*of Pediatric and Adolescent Diabetes (ISPAD) 2018.*¹⁶ Hypothyroid was defined as having FT4 level < 0.7 ng/dL.

Serum ferritin levels from venous blood was measured using a turbidimetric method by the hospital laboratory staff in units of ug/L. The nutritional status was determined based on percentage of weight for height (WFH) in CDC criteria; severely undernourished (if WFH <70%) , undernourished (if WFH 70-90%), well nourished (if WFH 90-110%) and overnourished (if WFH >110%). Endocrine disorders were reported as a percentage, while numerical data were reported as

Table 1. Characteristics and hematological profiles of subjects

Characteristics	(N=115)
Gender, n (%)	
Male	32 (27.8)
Female	83 (72.2)
Median age, months (range)	168 (120-216)
Nutritional status, n (%)	
Severely undernourished	2 (1.7)
Undernourished	28 (24.3)
Well-nourished	82 (71.3)
Overnourished	3 (2.6)
Median age at diagnosis (range), months	54 (3-188)
Median duration of illness (range), months	108 (4-213)
Median pre-transfused Hb (range), gr/dL	7.4 (4.1-11.7)
Pre-transfused Hb, n (%)	
<9 g/dL	102 (88.7)
≥9 g/dL	13 (11.3)
Frequency of transfusion, n (%)	
≥ 2 times/month	19 (16.6)
< 2 times/month	96 (83.4)
Type of chelation agent, n (%)	
Deferiprone	102 (88.7)
Deferasirox	13 (11.3)
Median duration of chelating therapy (range), months	84 (3-192)
Duration of chelating therapy, n (%)	
≤ 84 months	64 (55.5)
> 84 months	51 (44.3)
Median ferritin level (range), ng/dL	3,761 (102-78760)
Ferritin levels, n (%)	
<1,000 ng/dL	7 (6.1)
1,001-5,000 ng/dL	70 (60.9)
5,001-10,000 ng/dL	31 (27)
>10,000 ng/dL	7 (6.1)

median (abnormally distributed).

This study was approved by the Ethics Committee of Universitas Sriwijaya Medical School/ Dr. Moh. Hoesin Hospital, Palembang, South Sumatera. Data analysis was performed by using SPSS version 22 and Microsoft Excel 2007. The results were considered significant for P values <0.05, with a confidence interval of 95%.

Results

There were 115 thalassemia patients included in our study, 83 girls and 32 boys. Most subjects had good nutritional status and received deferiprone as a chelating agent. Subjects' median age at diagnosis was 54 months, duration of illness was 108 months, pre-transfusion Hb was < 9 g/dL (Table 1). Eighty-nine (77.4%) subjects had short stature, 25 (21.7%) had delayed puberty, 74 (64.4%) had retarded bone age, 30 (26.1%) had impaired glucose tolerance, 4 (3.5%) had DM, and

none had thyroid hormone disorders (Table 2).

All study subjects suffered any of endocrine disorders, and most had ferritin levels of 1,001 to 5,000 ng/dL and 5,001-10,000 ng/dL. Seven (6%) subjects had ferritin level <1000 ng/dL, but still had endocrine disorders (Table 3).

Bivariate and multivariate analyses revealed no associations between serum ferritin level and all endocrine disorders. For bivariate analysis, we categorized ferritin levels 1000 ng/dL (as a cutoff point for iron overload) and also cut-off point ferritin level of each endocrine disorder in this study as comparison. The ferritin cut-off points were 2,998 ng/dL for delayed puberty, 3,721 ng/dL for impaired glucose tolerance, 4,985 ng/dL for DM, 2,956 ng/dL for short stature, and 2,956 ng/dL for delayed bone age (Table 4, Table 5).

Discussion

In our study, most subjects were girls (72.2%), similar

Table 2. Characteristics of endocrine disorders in subjects (N = 115)

Parameter	Short stature (n=89)	Late puberty (n=25)	Retarded bone age (n=74)	DM (n=4)	Impaired glucose tolerance (n=30)
Gender, n					
Males (n=32)	29	10	19	2	10
Females (n=83)	60	15	55	2	20
Transfusion frequency, n					
< 2 times/mo	75	22	60	4	25
≥ 2 times/mo	14	3	14	0	5
Nutritional status, n					
Severely undernourished	1	0	2	0	0
Undernourished	24	8	21	2	8
Well-nourished	62	17	49	2	22
Overnourished	2	0	2	0	0
Type of iron chelation, n					
Deferiprone	78	24	64	3	28
Deferasirox	11	1	10	1	1
Median age (range), mo	168 (120-216)	192 (132-216)	168 (120-216)	174 (138-216)	168 (120-216)
Median age at diagnosis (range), mo	48 (3-188)	36 (3-180)	48 (3-180)	24 (8-42)	39 (3-168)
Median duration of illness (range), mo	108 (4-213)	161(12-213)	51.85 (12 -213)	149 (114-192)	115 (24-213)
Median duration of chelating therapy (range), mo	84 (3-192)	120 (12-192)	84 (12-192)	168 (132-180)	96 (12-192)
Median pre-transfusion Hb (range), gr/dL	7.5 (4.1-11.7)	7.2 (5.1-9.5)	7.4 (4.1-11.7)	6.7 (6.1-7.2)	6.83 (4.2-10)
Median serum ferritin (range), ng/dL	3,761 (102-78,760)	3,200 (102-34,270)	4,087 (207- 78,760)	5,724 (3500-7588)	4,276 (102-78,760)

Table 3. Distribution of endocrine disorders according to serum ferritin level (N = 115)

Parameter	Ferritin levels, ng/dL				P value
	<1,000 (n=7)	1,001-5,000 (n=70)	5,001-10,000 (n=31)	> 10,000 (n=7)	
Glucose tolerance, n (%)					0.888
Normal	6 (7.3)	48 (59.3)	22 (27.2)	5 (6.2)	
Disturbed	1 (3.3)	20 (66.7)	7 (23.3)	2 (6.7)	
DM	0 (0)	2 (50)	2 (50)	0 (0)	
Stature, n (%)					0.455
Short	6 (6.7)	53 (59.6)	23 (25.8)	7 (7.9)	
Normal	1 (3.8)	17 (65.4)	8 (30.8)	0 (0)	
Puberty, n (%)					0.047
Delayed	4 (16)	12 (48)	6 (24)	3 (12)	
Normal	3 (33.3)	58 (64.4)	25 (27.8)	4 (4.4)	
Bone age, n (%)					0.196
Delayed	3 (4.1)	42 (56.8)	23 (31.1)	6 (8.1)	
Normal	4 (9.8)	28 (68.3)	8 (19.5)	1 (2.4)	

to other epidemiological reports. However, some studies had greater proportion of boys. Thalassemia is an inherited autosomal recessive disorder, so males and females have similar risk of inheritance from their carrier parents.^{17,18}

Parents had received much information about taking care of thalassemic children, both through direct education and social media. As such, parents had increased awareness of their children's nutritional requirements, so that despite their low income, most subjects (82%) were well-nourished. Similarly, a study also found that most thalassemic children at Dr. Moh. Hoesin Hospital, Palembang, South Sumatera, had good nutrition.¹⁹ In contrast, another study reported higher proportions of malnutrition in their thalassemic subjects.²⁰ Many factors can influence children's nutritional status, such as individual treatments and nutritional intake.^{7,21,22}

Deferiprone and deferasirox were the iron chelators prescribed at RSMH at this time. Irregular patient visit, mainly due to cost and time factors, affected patient adherence to consuming iron chelation and led to low pre-transfusion hemoglobin (Hb) levels and increased serum ferritin. In our study, most subjects (88.7%) had pre-transfused Hb under 9 g/dL, and 93.9% had serum ferritin >1,000 ng/dL. The prevalences of the different endocrinopathies in this study ranged from 3.5 to 77.4%. Endocrine disorders in thalassemia mostly begin at 10 years of age due to iron overload.¹⁵⁻²³

There were 7 subjects with ferritin levels <1,000

ng/dL, but who had endocrine disorders. Serum ferritin only represents for about 1% of the human total iron reserves and the level influenced by several factors, such as viral and bacterial infections, malignancy, inflammation, and vitamin C deficiency.^{11,23,24}

Theoretically, ferritin interferes in bone metabolism through direct toxicity of osteoblasts. The high serum ferritin level may not have been due to only the frequency of transfusions, but to other factors not analyzed, such as viral or bacterial infections or vitamin C deficiency. The causes of growth disorders in thalassemia are multifactorial, including gonadotropin secretion abnormalities, chronic anemia, hypoxia, liver disease, folic acid and zinc deficiency, emotional factors, growth hormone-insulin-like factor-1 (GH-IGF1) axis disorder, and bone dysplasia induced by iron chelation therapy. As zinc is required for growth, some studies suggested that zinc supplementation may improve growth.^{11,24-27}

In our study, short stature occurred in 59.6% of subjects, and most of them (59.6%) had ferritin levels of 1,001-5,000 ng/dL. We found no association between serum ferritin levels and short stature, similar to a study from Padang, West Sumatera, concluding that ferritin levels did not affect endocrine disorders in β -thalassemia major patients. Several studies also concluded that levels of ferritin did not affect bone growth and age in thalassemia patients.¹⁹⁻²¹

A previous study reported on growth disorders and bone age in thalassemic children. Bone histomorphometry showed osteoid thickening

Table 4. Bivariate analyses of serum ferritin level and endocrine disorders

Ferritin levels	Endocrine disorders		N	OR 95% CI	P value
	With	Without			
Delayed puberty					
n(%)	25 (21.7)	90 (78.3)			
Median (range)	3200 (102-34,270)	3800 (413-78,760)			0.780*
n(%)					
<1,000 ng/dL	3 (42.9)	4 (57.1)	7	0.48 (0.19 to 1.21)	0.173**
≥1,000 ng/dL	22 (20.4)	86 (79.6)	108		
n(%)					
<2,998 ng/dL	11 (24.4)	34 (75.6)	45	0.82 (0.41 to 1.64)	0.573**
≥2,998 ng/dL	14 (20.0)	56 (80.0)	70		
Impaired glucose tolerance					
n(%)	34 (29.6)	81 (70.4)			
Median (range)	4276 (102-78760)	3420 (207-46806)			
n(%)					
<1,000 ng/dL	2 (28.6)	5 (71.4)	7		
≥1,000 ng/dL	32 (29.6)	76 (70.4)	108		
n(%)					
<2,998 ng/dL	13 (22.8)	44 (77.2)	57		
≥2,998 ng/dL	21 (36.2)	37 (63.8)	58		
Diabetes mellitus					
n(%)	4 (3.5)	111 (96.5)			
Median (range)	3681 (102-78760)	5764 (3500-7588)			0.309 *
n(%)					
<1,000 ng/dL	0 (0)	7 (100)	7	1.04 (0.31 to 3.47)	0.659**
≥1,000 ng/dL	4 (3.7)	104 (96.3)	108		
n(%)					
<2,998 ng/dL	1 (1.3)	75 (98.7)	76	1.58 (1.88 to 2.86)	0.115***
≥2,998 ng/dL	3 (7.7)	36 (92.3)	39		
Short stature					
n(%)	89 (77.4)	26 (22.6)			
Median (range)	3761 (102-78,760)	3670 (649-9,719)			0.718 *
n(%)					
<1,000 ng/dL	6 (85.7)	1 (14.3)	7	0.93 (0.89 to 0.98)	0.587 **
≥1,000 ng/dL	83 (93.3)	25 (23.1)	108		
n(%)					
<2,998 ng/dL	33 (75.0)	11 (25.0)	44	1.05 (0.85 to 1.29)	0.629 ***
≥2,998 ng/dL	56 (78.9)	15 (21.1)	71		
Retarded bone age					
n (%)	74 (64.4)	41 (35.6)			
Median (range)	3761 (102-78760)	3670 (649-9719)			0.718 *
n(%)					
<1,000 ng/dL	6 (85.7)	1 (14.3)	7		
≥1,000 ng/dL	83 (93.3)	25 (23.1)	108		
n(%)					
<2,998 ng/dL	33 (75.0)	11 (25.0)	44		
≥2,998 ng/dL	56 (78.9)	15 (21.1)	71		

*Mann-Whitney test; **Fisher's exact test; *** Chi-square test

and delayed maturation and mineralization of osteoids caused the matrix maturation disruption and mineralization seen along with iron accumulation. This condition could lead to delayed bone maturation

and focal osteomalacia, leading to pathogenesis of bone disorders by suboptimal blood transfusions and iron overload.¹³ In this study, bivariate or multivariate analyses revealed no associations between serum ferritin

Table 5. Multivariate analysis of serum ferritin level and endocrine disorders

Variables	95%CI	P value
Delayed puberty	0.301 (0.069 to 1.319)	0.111
Impaired glucose tolerance	1.657 (0.299 to 9.183)	0.563
Diabetes mellitus	0.378 (0.000 to 0.000)	0.999
Short stature	0.270 (0.030 to 2.446)	0.244
Retarded bone age	1.548 (0.376 to 6.381)	0.545

and bone age. These results were similar with a study that showed no associations between serum ferritin and bone age.²⁹

Most subjects with impaired glucose tolerance had ferritin levels between 1,001 and 5,000 ng/dL. Subjects with DM were distributed among the 1,001 -10,000 ng/dL ferritin groups. Pancreatic and hepatic iron overload and hemosiderosis were suggested to be responsible for insulin deficiency and resistance. Excess iron and oxidative stress lead to apoptosis and loss of pancreatic beta cells, so that the ability to secrete insulin progressively decreases, leading to glucose intolerance and diabetes. Oxidative stress alone through the Fenton reaction can cause direct damage and fibrosis of insulin receptors in the liver and muscles, which also leads to hyperinsulinemia and insulin resistance.^{25,30} Suvarna *et al.*²⁶ concluded that fasting plasma insulin and insulin resistance index were significantly increased in thalassemia patients, even with no impaired glucose tolerance or DM. They speculated that insulin resistance had occurred before the impaired glucose tolerance and DM. A high insulin level is compensation of insulin resistance to maintain euglycemia.³¹ A previous study stated that ferritin affected glucose tolerance disorders.³⁰ In contrast, we found no association between serum ferritin and impaired glucose tolerance or DM, though with a higher ferritin cut-off point than for other endocrine disorders.

Although the exact mechanism of iron overload causing tissue damage is not completely clear, some evidence suggests that free radicals and lipid peroxidases induced damage to mitochondria, lysosomes, and sarcoplasmic membranes. Iron deposits and oxidative damage caused by free radicals resulted in pituitary (especially the anterior) damage, which is very sensitive to oxidative stress produced by free radicals and follicles in the ovaries. As a consequence, disturbances in hypothalamus-pituitary axis and gonad

function eventually leads to delayed puberty, despite intensive iron chelation therapy.^{25,32-34}

In our study, the ferritin cut-off of 2,998 ng/dL was assumed to cause delayed puberty. However, 2 previous studies used ferritin cut-off points for delayed puberty at 3,000 ng/dL and 2,100 ng/dL, respectively.^{31,35} A study concluded that every 100-unit increase in ferritin level increased the risk of delayed puberty by 2.5%.³⁵ We found no association between ferritin and delayed puberty. Another study also found no significant association between hypogonadism and serum ferritin levels.¹⁵

In conclusion, short stature is the most common endocrine disorder (77.4%) in thalassemic children, followed by delayed bone age (64.4%), impaired glucose tolerance (26.1%), delayed puberty (21.7%), and diabetes mellitus (3.5%). There are no thyroid hormone (hypothyroid) disorders in this study. Serum ferritin is not associated with any endocrine disorders in children with thalassemia major.

Conflict of Interest

None declared.

Funding Acknowledgment

Hematology and bone age examinations are routine examinations for thalassemia patients and covered by government health insurance (BPJS).

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