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Original Article

Factors associated with insulin-like growth factor-1 in children with thalassemia major

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Abstract

Background Septic shock remains a major cause of mortality Background Insulin-like growth factor-1 (IGF-1) deficiency is the major cause of growth disorders and delayed puberty in children with thalassemia. Hence, identifying factors contributing to IGF-1 deficiency in thalassemia is of importance.

Objectives To evaluate the correlation between IGF-1 level and nutritional status, ferritin, pre-transfusion hemoglobin, thyroid, as well as alanine transaminase levels.

Methods We conducted a study in children aged 2 to 18 years with thalassemia major who visited outpatient clinics at two hospitals in Indonesia, Dr. Sardjito Hospital, Yogyakarta and Dr. Moewardi Hospital, Surakarta, Central Java, from July to December 2015. Clinical, laboratory, and demographic data were reviewed from medical records. IGF-1 levels were measured using an immunochemiluminiscent method.

Results A total of 48 children were recruited into the study. Subjects mean IGF-1 level was 109.28 (SD 90.26) ng/mL. Seventy-five percent of the children had IGF-1 level < -2SD. Subjects mean ferritin, pre-transfusion hemoglobin and ALT levels were 3.568 (SD 2131.31) ng/mL; 7.97 (SD 0.85) g/dL and 49.7 (SD 43.1), respectively. Most of the children (91.7%) was eutyroid, with a mean of TSH and FT4 level was 2.7 (SD 1.5) nmol/L and 12.3 (SD 7.1) μIU/ml, respectively. Ferritin level had no significant correlation with IGF-1 level (r=-0.794; P=0.431). However, a strong, positive correlation was documented between pre-transfusion hemoglobin level and IGF-1 level (r=2.380; P=0.022). Multivariate linear regression analysis revealed that factors with significant correlations to IGF-1 level were pre-transfusion hemoglobin level <8 g/dL (β=-0.090; 95%CI -0.002 to 0.182; P=0.056) and undernutrition (β=0.077; 95%CI 0.045 to 0.109; P<0.001).

Conclusion Low pre-transfusion hemoglobin level and undernutrition are significantly correlated to low IGF-1 level in children with thalassemia major. **[Paediatr Indones. 2019;59:72-8; doi: http://dx.doi.org/10.14238/pi59.2.2019.72-8]**.

Keywords: *thalassemia major; insulin-like growth factor-1; undernutrition*

Thalassemia is a critical health problem in children since it leads to growth and developmental disorders.[1] In 1994, The *World Health Organization* (WHO) reported that 5.2% of the world's population had the thalassemia trait and that increased to 7% in 2001.[2] Each year, there are about 300,000 infants born with thalassemia major worldwide. In Indonesia, the incidence of β-thalassemia, α-thalassemia, and hemoglobin (Hb) E carriers are 10%, 1.2 to 11%, and 1.5 to 36%, respectively.[3]

Due to medical advances in thalassemia management, most patients achieve normal growth in their first decade. However, significant growth disorders and delayed puberty may occur in adolescence,[4] because of insulin-like growth factor-1 (IGF-1) deficiency. A multicenter study conducted in Italy and Qatar reported that 67% of thalassemic adults had IGF-1 deficiency (IGFD).[5] In addition, an Iranian study showed lower mean IGF-1 levels in children with thalassemia [61.33

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(SD 67.64) ng/mL] compared to healthy children [126.93 (SD 156.7) ng/mL].[6] Several factors contributing to the reduction of IGF-1 synthesis include transfusion-induced iron overload, chronic anemia, undernutrition, impaired thyroid function, and high levels of alanine transaminase (ALT).[7] Hence, we aimed to assess such factors for associations with low IGF-1 levels in children with thalassemia major.

Methods

We conducted a retrospective study in outpatient clinics of two hospitals in Indonesia, namely, Dr. Sarjito Hospital, Yogyakarta and Dr. Moewardi Hospital, Surakarta, Central Java, from July to December 2015. We recruited children with thalassemia major aged 2 to 18 years, who were diagnosed based on Hb electrophoresis results. Those who had complete medical records, including ferritin and pre-transfusion hemoglobin levels over the past year, thyroid-stimulating hormone (TSH), free thyroxine (FT4), and ALT levels, were included in the study. We obtained written informed consent from subjects' parents or guardians.

We performed history-taking and medical record reviews to collect the following data: age at diagnosis of thalassemia, Hb electrophoresis results, as well as mean ferritin, pre-transfusion hemoglobin, TSH, FT4, and ALT levels over the past year. The nutritional status were defined by mid upper arm circumference (MUAC), which was measured at the midpoint between the tip of the shoulder and the tip of the elbow (olecranon process and the acromium), and classified according to the classification of Frisanchoas as followed: below adequate ($\leq 5^{th}$ percentile), adequate (between 5^{th} and 95^{th} percentile), and above adequate ($\geq 95^{th}$ percentile). The IGF-1 levels were measured at *Prodia Laboratory* Surakarta, by an automated chemiluminescence immunoassay (*Immulite®2000*), using a solid-phase, enzyme-labeled chemiluminescent immunometric assay. A reference range study performed with *DPC's IMMULITE IGF-*I kit (**Table 1**).[8] The blood was collected at least a week before blood transfusions to avoid elevation of IGF-1 levels due to the transfusion.

Data on the characteristics of subjects were summarized as proportion, mean, or median, where appropriate. We performed a simple linear regression analysis to evaluate for correlations between IGF-1 level and nutritional status, mean ferritin level, mean pre-transfusion hemoglobin level, thyroid function, and ALT level. Pearson's/Spearman's correlation coefficient was used to analyze the strength of linear relationships between variables. The decision in hypothesis testing was based on the significance level of $P<0.05$.[9] The data were analysed using *SPSS 15* software. This study was approved by the Health Research Ethics Committee of the Universitas Gadjah Mada Faculty of Medicine, Public Health and Nursing, Yogyakarta, Indonesia.

Table 1. IGF-I pediatric reference ranges (ng/mL)[8]

Age, year	Median	Central 95% range	0.1 percentile
1	134	55-327	33
2	125	51-303	31
3	119	49-289	30
4	118	49-283	29
5	119	50-286	30
6	124	52-297	31
7	134	57-316	34
8	148	64-345	39
9	169	74-388	46
10	200	88-452	55
11	247	111-551	70
12	315	143-693	91
13	395	183-850	118
14	462	220-972	143
15	486	237-996	157
16	452	226-903	152
17	376	193-731	132
18	308	163-584	112

Results

A total of 48 patients were recruited into the study, 12 children from Dr. Sarjito Hospital and 36 children from Dr. Moewardi Hospital. Subjects' characteristics are presented in **Table 2**. The number of males and females was similar and subjects had a mean of age was 11 (SD 3.6) years. Their mean duration of thalassemia diagnosis in this study was 7.65 (SD 3.60, range 1.2-14.5) years. Subjects mean ferritin level in the past year was 3,568 (SD 2131.3)

ng/mL, with a range of 566ng/mL to 8,453 ng/mL. The highest pre-transfusion Hb level was 9.8 g/dL while the lowest was 5.7 g/dL, with a mean of 8.0 (SD 0.9) g/dL. Based on MUAC measurements, the majority (64.6%) of children were adequate nutrition and none had severely depleted nutrition. Using the 2007 WHO height-to-age *z*-score charts, 75% of subjects fell into the short stature category and the rest were normal.[10] Most of the children (91.7%) were euthyroid, with mean TSH and FT4 levels of 2.7 (SD 1.5) nmol/L and 12.2 (SD 7.1) μIU/mL, respectively.

Table 2. Characteristics of subjects with thalassemia major

Characteristics	(N = 48)
Sex, n (%)	
Male	26 (54.2)
Female	22 (45.8)
Mean age (SD), years	11.0 (3.6)
Mean age at diagnosis (SD), years	3.5 (3.1)
Mean time since diagnosis (SD), years	7.7 (3.6)
Mean ferritin level (SD), ng/mL	3,568 (2,131.3)
Ferritin level, n (%)	
<2,000 ng/mL	15 (31.3)
≥2,000 ng/mL	33 (68.7)
Mean pre-transfusion Hb level (SD), g/dL	8.0 (0.9)
Hb level, n (%)	
<8 g/dL	23 (47.9)
≥8 g/dL	25 (52.1)
Nutritional status (based on MUAC), n (%)	
Adequate	31 (64.6)
Moderately depleted	17 (35.4)
Severely depleted	0 (0)
Height (age-adjusted), n (%)	
Average	12 (25)
Short	7 (14.6)
Extremely short	29 (60.4)
Mean serum TSH (SD), nmol/L	2.7 (1.5)
Mean FT4 (SD), μIU/mL	12.2 (7.1)
Thyroid function, n (%)	
Euthyroid	44 (91.7)
Hypothyroid	4 (8.3)
Mean ALT (SD), U/L	49.7 (43.1)

Hb=hemoglobin; MUAC=mid-upper arm circumference; TSH=thyroid-stimulating hormone; FT4=free thyroxine; ALT=alanine transaminase

Subjects' mean IGF-1 level was 109.3 (SD 90.3) μg/L, with a range of 25 to 491 ng/mL. The majority of the children (75%) had IGF-1 level of less than -2SD, and the rest ranged between -1SD and -2SD. None of the children had IGF-1 levels above the reference value.

Table 3 shows the simple linear regression analysis. Pre-transfusion Hb levels (r=2.380; 95%CI -21.486 to 37.155; P=0.02) and nutritional status (r=5.031; 95%CI 15.409 to 34.503; P=0.000) had significant positive correlations with IGF-1 level. Increased Hb level strongly and significantly correlated with elevated IGF-1 level (**Figure 1**). The equation

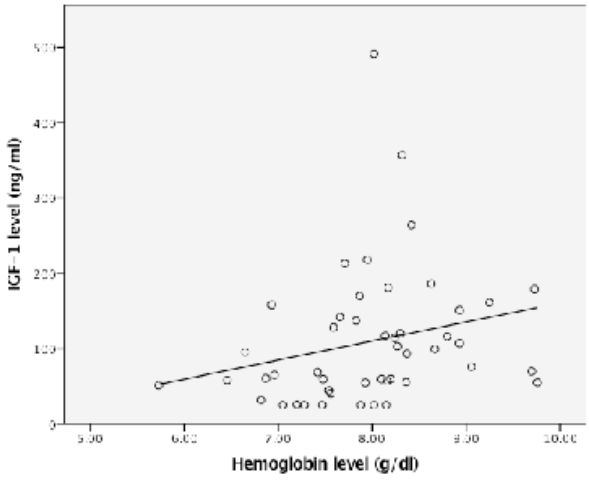

Figure 1. Analysis of IGF- 1 and hemoglobin levels

Table 3. Factors analyzed for correlations with IGF level

Model	Unstandardized coefficients		Standardized coefficients	r	95% CI for B	P value
	B	SE	Beta			
(Constant)	-0.028	0.533		-0.053	-646.924 to -97.408	0.958
Ferritin level	-2.26E-005	0.000	-0.148	-1.048	-0.022 to 0.003	0.301
Hb level	0.898	0.431	0.160	2.380	-21.486 to 37.155	0.022
Nutritional status	0.085	0.017	0.617	5.031	15.409 to 34.503	0.000
Thyroid function	-0.176	0.143	-0.151	-1.231	-116.844 to 46.913	0.226
ALT	0.023	0.117	0.025	0.196	-0.362 to 0.801	0.846

Hb=hemoglobin; ALT=alanine transaminase

from the analysis showed that a decrease of 1 g/dL Hb level would lead to a 0.128 ng/mL decrease in IGF-1 level. Nutritional status had stronger correlation with IGF-1 level, with lower nutritional status significantly associated with lower IGF-1 level (**Figure 2**). The equation indicated that increased nutritional status correlated with elevated IGF-1 level.

The correlation between ferritin and IGF-1 level was negative, but weak and not statistically significant (r=-1.048; 95%CI to 0.22 to 0.003; P=0.301) (**Figure 3**).

Multivariate analyses revealed similar results, with pre-transfusion Hb level (β=0.090; 95%CI -0.002 to 0.182; P=0.056) and nutritional status

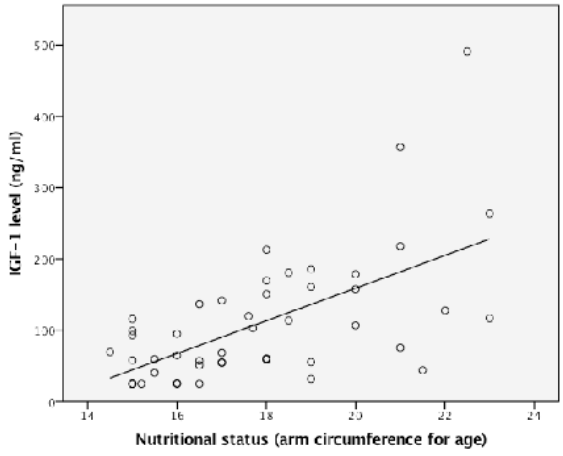

Figure 2. Analysis of IGF- 1 level and nutritional status (by MUAC for age)

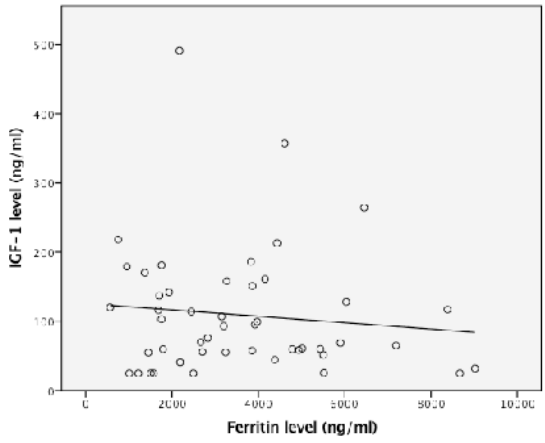

Figure 3. Analysis of IGF- 1 and ferritin levels

Table 4. Multivariate linear regression of independent variables and IGF-1 level

	Model	Unstandardized coefficients		Standardized coefficients	T	95% CI for B	P value
		B	SE	Beta			
1	(Constant)	-0.028	0.533		-0.053	-1.105 to 1.049	0.958
	Ferritin level	-2.26E-005	0.000	-0.148	-1.048	0.000 to 0.000	0.301
	Hb level	0.062	0.051	0.160	1.216	-0.041 to 0.166	0.231
	Nutritional status	0.085	0.017	0.617	5.031	0.051 to 0.118	0.000
	Thyroid function	-0.176	0.143	-0.151	-1.231	-0.466 to 0.113	0.226
	ALT	0.023	0.117	0.025	0.196	-0.214 to 0.260	0.846
2	(Constant)	0.028	0.445		0.062	-0.871 to 0.927	0.951
	Ferritin level	-2.15E-005	0.000	-0.141	-1.044	0.000 to 0.000	0.303
	Hb level	0.060	0.049	0.154	1.216	-0.040 to 0.160	0.231
	Nutritional status	0.084	0.016	0.614	5.102	0.051 to 0.117	0.000
	Thyroid function	-0.173	0.141	-0.149	-1.231	-0.458 to 0.111	0.225
3	(Constant)	-0.127	0.420		0.303	-0.975 to 0.721	0.763
	Hb level	0.081	0.045	0.209	1.802	-0.010 to 0.172	0.079
	Nutritional status	0.079	0.016	0.579	5.005	0.047 to 0.111	0.000
	Thyroid function	-0.219	0.134	-0.188	-1.639	-0.489 to 0.051	0.109
4	(Constant)	-0.171	0.428	-	0.400	-1.033 to 0.691	0.691
	Hb level	0.090	0.046	0.230	1.963	-0.002 to 0.182	0.056
	Nutritional status	0.077	0.016	0.562	4.787	0.045 to 0.109	0.000

Hb=hemoglobin; ALT=alanine transaminase

(β=0.077; 95%CI -0.045 to 0.109; P=0.000) positively correlated with IGF-1 level (**Table 4**). The resulting equation indicated that pre-transfusion hemoglobin level of less than 8 g/dL would decreasing the IGF-1 level of 0.09 μg/L. Meanwhile, for nutritional status, severly depleted in a child with thalassemia major would decreasing the IGF-1 level of 0.077 μg/L.

Discussion

Most thalassemia patients (75%) in our study had low IGF-1 levels [mean 109.3 (SD 90.3) μg/L]. Similarly, a previous study reported that 67% of patients with thalassemia had IGF-1 levels < -2SD.[5] Chronic anemia in thalassemia can cause hypoxia in hepatocytes. This hypoxic state can inhibit protein synthesis in the liver, which leads to increased IGF-binding proteins specific (IGFBPs), especially IGFBP-1. The increased IGFBP-1 may inhibit IGF-1 function by binding to to it and preventing IGF-1 from binding to the IGF-1 receptor (IGF-1R).[9,11]

Repeated blood transfusions in thalassemia major patients may cause elevated levels of free iron in blood serum, which then converts hydrogen peroxide into hydroxide ion (OH-), leading to increased reactive oxygen species (ROS) and oxidative stress.[12] Increased ROS decreases mRNA expression of IGF-1, leading to muscle atrophy, sarcomopenia, wasting, and myopathy. On the other hand, decreased ROS will increase IGF-1 levels, have a positive effect on skeletal muscle protein balance, as well as prevent oxidative damage and other chronic diseases.[13] Our subjects' mean ferritin level was 3,568 (SD 2,131.31) ng/mL, which was higher than reported in an Iranian study [2,962 (SD 1,606) ng/mL].[14] Another study in Malaysia documented that optimal growth in thalassemia major patients can be achieved if their mean ferritin level is less than 2,271 (SD 1804) ng/mL.[15] Several factors can affect ferritin levels in thalassemia patients, including blood transfusion frequency and regularity of iron chelation therapy.[16] We did not analyze factors affecting high ferritin levels in this study because there was no medical record data on the blood transfusion frequency and adherence to iron chelation therapy.

According to *Standards of Care Guidelines for Thalassemia 2009*, Hb levels in children with thalassemia major are ideally maintained at 9-10 g/dL in order to achieve optimal growth.[17] In our study, mean pre-transfusion hemoglobin level was 8.0 (SD 0.9). We noted that Hb level was positively and significantly correlated with IGF-1 level in thalassemia major patients in the univariate analysis (r=2.380; P=0.022), but not in the multivariate analysis.

Micronutrients play a role in growth, protein and DNA synthesis, neurosensory function, immunity, thyroid function, and bone metabolism. Some micronutrients are known to affect IGF-1 level, including magnesium, selenium and zinc. There are two mechanisms involved in the relationship between these nutrients and IGF-1: oxidative stress and inflammation. Micronutrient deficiency increases oxidative stress, free radicals, and oxygen peroxidase production, but decreases antioxidant enzyme expression, which then leads to down-regulated IGF-1 secretion.[18,19]

In our study, nutritional status was assessed by measuring MUAC, due to organomegaly in hepatocytes and/or hemodynamics in thalassemic children. The proportion of subjects in nutritional status categories (64.6% adequate and 35.4% severly depleted) was different from a study conducted in Iran (39.3% adequate and 60.7% severly depleted).[6] Other studies reported the prevalences of severly depleted in thalassemic children to be 64% and 49%, respectively.[6,20] As such, we seemed to have a higher proportion of well-nourished children in our study population. Nonetheless, poor nournishment was significantly correlated to decreased IGF-1 levels. Decreased IGF-1 level in undernourished thalassemic children is caused by elevated interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) secretion in micronutrient deficient conditions. The IL-6 is a major proinflammatory cytokine with a negative effect on muscle function and IGF-1 synthesis.[21]

We noted the prevalence of hypothyroidism in thalassemic children was 8.3%, but this prevalence can vary by country. Previous studies conducted in Indonesia, Iran, and Turkey reported hypothyroidism prevalences of 20%, 6%, and 12.8%, respectively.[22-24]

A previous study found that ALT levels were significantly correlated with IGF-I levels (r=0.26; P=0.05) in thalassemia major patients,[7] contrary

to the findings by De Sanctis *et al.* who found no significant relationship between ALT and IGF-1 levels ($r=0.01$; $P>0.05$).[24]

A limitation of our study that should be considered was that we did not examine IGFBPs levels which can affect IGF-1 levels in the circulation. Moreover, we did not assess factors affecting ferritin levels and nutritional status, such as dietary intake and nutritional interventions.

In conclusion, univariate analysis shows that pre-transfusion hemoglobin levels and nutritional status significantly correlate with IGF-1 levels in children with thalassemia major. Those whose mean pre-transfusion Hb level is less than 8 g/dL and with undernutrition typically have lower IGF-1 levels.

Conflict of Interest

None declared.

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