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Original Article

Serum IGF-1 and short stature in adolescents with ß-thalassemia major

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Abstract

Background The prevalence of short stature in thalassemia patients ranges from 39.3 to 65%. The cause of short stature is complex and still up for debate. In Indonesia, data on the prevalence and risk factors of short stature in adolescents with thalassemia have been limited.

Objective To assess for the prevalence and risk factors of short stature in adolescents with beta-thalassemia major.

Methods This cross-sectional study was done from February to March 2017 at the Thalassemia Clinic at Dr. Hasan Sadikin General Hospital, Bandung, West Java. The baseline characteristics data of 80 adolescents with thalassemia aged 10-14 years were recorded. Short stature was assessed by height-for-age, (Z-score <-2SD) based on the *2007 WHO Reference Growth Chart*. Mid-upper arm circumference was scored according to age and sex and serum IGF-1 was measured by ELISA method.

Results Subjects were 40 males and 40 females, 81.2% of whom had short stature. The mean serum IGF-1 level was 32.2 (SD 26.38) ng/mL. The IGF-1 cut-off point by ROC curve was ≤ 38.51 ng/mL, with sensitivity of 64.4% and specificity of 86.7%. The risk factors of short stature were IGF-1 level ≤ 38.51 ng/mL (PR 40.66; 95%CI 4.37 to 377.58) and low family income (PR 19.76; 95%CI: 1.152 to 256.08).

Conclusion IGF-1 level may be useful as a predictor of short stature in adolescent beta-thalassemia major patients. **[Paediatr Indones. 2018;58:151-8; doi: http://dx.doi.org/10.14238/pi58.4.2018.151-8]**.

Keywords: *adolescent; IGF-1; short stature; thalassemia*

About 39.3 to 65% of children with thalassemia have growth disorders during the fetal, neonatal, pre-pubertal, and pubertal periods.[1-5] About 20-30% might have growth hormone deficiency and 80% might have very low growth hormone that were lower than would be expected for constitutional short stature.[6] In Indonesian thalassemia patients, 65% had short stature, 20% delayed puberty, 41% hypoparathyroidism, and 29% delayed bone age.[5] A previous study at Dr. Hasan Sadikin Hospital found that 67% of 10 to14-year-old patients had delayed growth.[7] Another study reported that more than half of thalassemia patients aged 13.8 and >15 years had delayed growth. The delayed growth and puberty might also occur in 12 to 17-year-old thalassemia patients.[8]

The cause of growth disorders on thalassemia patients is complex and still being debated. The growth hormone (GH) and insulin-like growth factor-1 (IGF-1) axis was reported to have an

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important role in these patients. The most probable cause of growth disorders is the decrease in serum IGF-1 concentration in response to GH.[9] We aimed to identify the risk factors of short stature in thalassemia patients, in hopes that adolescents with thalassemia who have undergone proper treatment and management including routine transfusion, iron chelation, and monitoring of side effects, can achieve optimal growth according to age for a longer and better quality of life.

Methods

Eighty patients with thalassemia major were recruited at the Thalassemia Clinic, Dr. Hasan Sadikin General Hospital, West Java, Indonesia, during February-March 2017. Subjects' parents provided informed consent after having been briefed on the study protocol.

The inclusion criteria were children aged 10-14 years, diagnosed with beta-thalassema major, and who had undergone routine transfusions. The exclusion criteria were patients with other chronic diseases (malignancy, tuberculosis, chronic hepatitis, congenital heart disease, chronic kidney injury, epilepsy, or diabetes mellitus), another type of thalassemia, nutritional disorders, or congenital syndromes, other than family history of short stature.

For this cross-sectional study we collected subjects by consecutive sampling of thalassemic adolescents who fulfilled the inclusion criteria and routinely visited the Thalassemia Clinic at Dr. Hasan Sadikin Hospital. We recorded patient data and interviewed parents about the time of diagnosis, the use of iron chelation and number of transfusions, and subjects provided blood specimens for IGF-1 hormone examination. We performed physical and anthropometric examinations [body weight, height, and mid-upper arm circumference (MUAC)]. The hemoglobin (Hb) level before and after transfusion, and the ferritin level within the three months prior were collected from the medical records or the records from the Clinical Pathology Laboratory of Dr. Hasan Sadikin Hospital, Bandung.

Body weight was measured by *SECA sensa 804* scale, with 0.1 kg accuracy. Body height examination using microtoise *SECA 217*. Short stature was assessed by height-for-age, (Z-score <-2SD) and body weight-for-age basaed on *2007 WHO Reference Growth Chart*.[10] For nutritional assessment, the MUAC measurement was taken midway between the tip of the acromion and olecranon processes, and classified according to Frisancho as follows: below adequate: ≤ 5th percentile; adequate: between 5th and 95th percentile; and above adequate: ≥95th percentile.[11,12] Sexual maturation was assessed by Tanner criteria.[13] Testicular volume was evaluated by Prader orchidometer.[14]

Serum IGF-1 concentration was measured by enzyme-linked immunosorbent assay (ELISA)-*Mediagnost®*. The mean inter- and intra-assay coefficient of variation (CV) was determined to be 6.8% and 6.7%. The mean minimum detectable concentration of IGF-1 in this assay was 0.09 ng/mL. with classified: very low levels, i.e below the age-related 0.1th percentile, low levels, i.e. close to or below the age-related 5th percentile and normal level, i.e. above the age-related 5th percentile. The IGF-1 levels were measured at Department Clinical Pathology and Laboratory, Dr. Hasan Sadikin Hospital, Bandung.

Chi-square, Mann-Whitney, or Fisher's exact tests were used to assess for associations between characteristics and short stature. Logistic regression analysis was used to determine the most significant factors associated with short stature. A P value of <0.05 was considered to be statistically significant. The study was approved by the Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital, Bandung.

Results

This study on the risk factors of short stature in adolescents with beta-thalassemia major was performed at the Thalassemia Clinic, Dr. Hasan Sadikin Hospital, Bandung. During February-March 2017, 102 patients aged 10-14 years visited the Thalassemia Clinic. We interviewed parents and performed anthropometric examinations (weight, height, and mid-upper arm circumference), maturation assessment, and blood sampling on the patients. Eighty patients fulfilled the inclusion criteria and were analyzed, as shown in **Figure 1**.

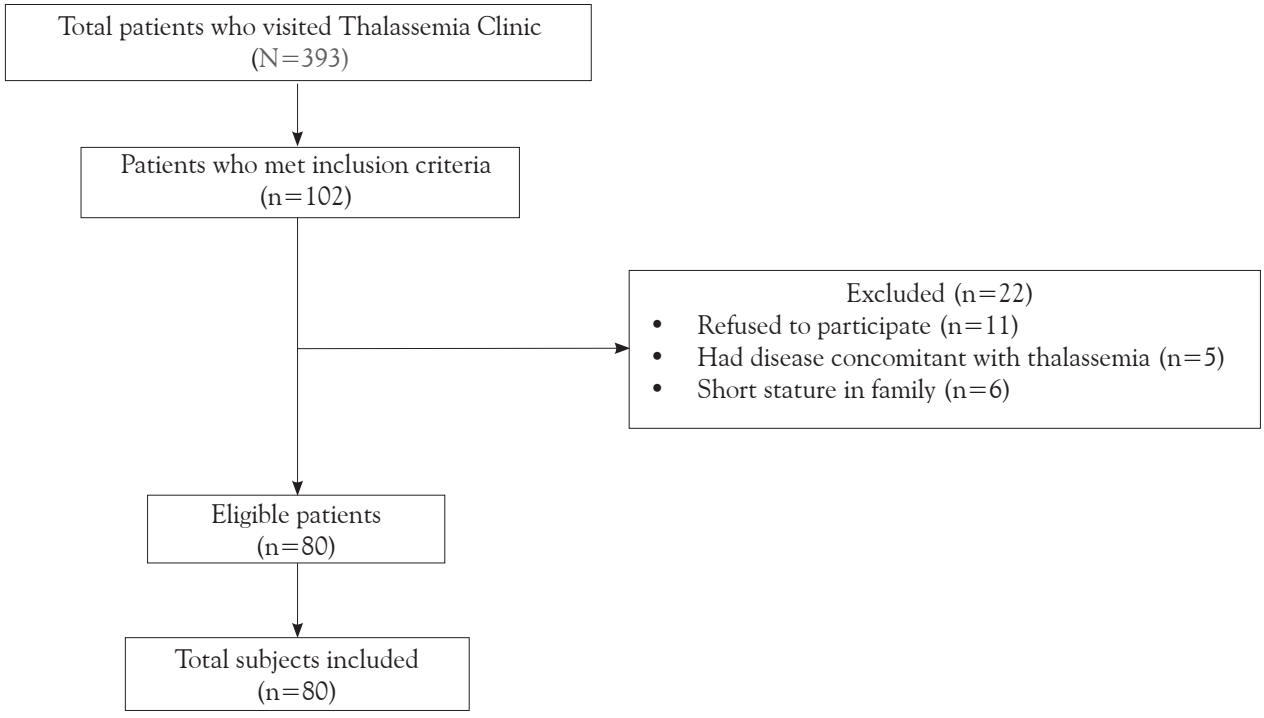

Figure 1. Study flow chart

Table 1 shows that the numbers of male and female subjects were the same. Based on the MUAC by age and gender, nutritional status of subjects was as follows: 47 (58.8%) subjects below adequate, 33 (41.2%) had adequate, and none had above adequate nutrition. Short stature (height-for-age Z-score <-2SD) was observed in 65 (81.2%) subjects. For IGF-1 concentrations, 76 (95%) subjects had very low IGF-1 levels and only 1 (1.3%) subject had a normal level.

Table 2 shows the comparison of various characteristics between the short stature and normal height groups. There was a significant difference in mean IGF-1 levels between short stature and normal subjects [32.22 (SD 26.38) *vs.* 68.58 (SD 51.46) ng/mL, respectively (P<0.001)]. The median IGF-1 level in the short stature group was also significantly lower than that of the normal group [30.94 (range 1.03-150.72) *vs.* 49.54 (range 14.87-187.68) ng/mL, respectively (P <0.001)].

Since IGF-1 levels were significantly lower in the short stature group than in the normal height group, a receiver-operator characteristic (ROC) curve analysis was done to define an IGF-1 cut-off level, as a predictor for short stature. The ROC curve revealed the IGF-1 cut-off point to be ≤38.51 ng/mL, as a predictor of short stature. Furthermore, using the IGF-1 cut-off ≤ 38.51 ng/mL, we analyzed for a correlation between serum IGF-1 and short stature, as shown in **Table 3**. The IGF-1 cut-off point of ≤ 38.51 ng/mL had 64.6% sensitivity, 86.7% specitivity, and 68.8% accuracy. Adolescents with beta-thalassemia major and IGF-1 level ≤ 38.51 ng/mL had 1.49 times higher risk of short stature compared to those with IGF-1 > 38.51 ng/mL. As such, IGF-1 may be useful to predict short stature in beta-thalassemia major patients of adolescent age.

Multiple logistic regression analysis was used to further analyze bivariate results with P<0.25, and clinical significance. The results are shown **Table 4**. Adolescents with beta-thalassemia and low family income had 19.8 times the risk of short stature (PR19.76; 95%CI 1.52 to 256.08; P=0.022) and IGF-1 level ≤38.51 ng/mL had 40.7 times the risk of short stature (PR40.66; 95%CI 4.37 to 377.58; P<0.001).

Table 1. Baseline characteristics of subjects

Characteristics	N=80
Median age (range), months	140 (120-200)
Sex, n(%)	
Male	40 (50)
Female	40 (50)
Family history of thalassemia, n(%)	
Yes	14 (17.5)
No	66 (82.5)
Family income, n(%)	
Low (< IDR 1,500,000)	32 (40)
High (>IDR 1,500,000)	48 (60)
Nutritional status (MUAC for age and sex), n(%)	
Below adequate	47 (58.8)
Adequate	33 (41.2)
Height-for-age (Z-score), n(%)	
Normal	15 (18.8)
Stunted	27 (33.8)
Severely stunted	38 (47.4)
Puberty, n(%)	
Appropriate	80 (100)
Age at the time of diagnosed, n(%)	
<6 years	75 (93.7)
≥6 years	5 (6.3)
Iron chelation therapy, n(%)	
Yes	77 (96.3)
No	3 (3.7)
Type of iron chelation therapy, n(%)	
Deferoxamine/DFO	1 (1.3)
Deferasirox/DFX	27 (33.7)
Deferiprone	49 (61.2)
None	3 (3.8)
Serum ferritin level, n(%)	
<2,000 ng/mL	18 (22.5)
≥2,000 ng/mL	62 (7.5)
Duration of time since the diagnosis, n(%)	
<8 years	10 (12.5)
≥8 years	70 (87.5)
Pre-transfusion hemoglobin level, n(%)	
<9 g/dL	80 (100)
Number of transfusions per month, n(%)	
<2x	78 (97.5)
3x	1 (1.3)
4x	1 (1.3)
IGF-1 levels, n(%)	
Very low	76 (95)
Low	3 (3.7)
Normal	1 (1.3)
History of chemical agent, n(%)	
Yes	9 (11.3)
No	71 (86.7)

Discussion

This study was conducted to assess for risk factors of short stature in adolescents with beta-thalassemia major. There were 80 subjects aged 10-14 years, consisting of 40 (50%) males and 40 (50%) females. Nutritional status was assessed by MUAC, according to age and sex. Below adequate nutritional status was seen in 47 (58.8%) of subjects, adequate in 33 (41.2%) subjects, and above adequate in none of the patients. About 65 (81.2%) of the subjects had short stature (stunted and severely stunted, based on height-for-age Z-score).

Hashemi *et al.* reported that 65.71% of thalassemia patients had short stature.[15] Similarly, another study at the Thalassemia Clinic of Dr. Hasan Sadikin Hospital, Bandung on thalassemia patients aged 10-14 years found that 62% had short stature.[7] Both studies assessed nutritional status using body mass index for age, with Hashemi *et al.* reporting 81.4% normal and 18.6% malnourished subjects.[15] Rachmat *et al.* assessed nutritional status of thalassemia patients by upper arm circumference for age, and found that 50.9% had normal nutritional status and 49.1% had malnutrition.[16] Many factors may contribute to short stature in thalassemia patients, such as nutritional deficiency, chronic anemia, hypersplenism, zinc deficiency, growth hormone deficiency, and disorders of the hypothalamus-hypophysis gonadal axis.[17] Of those with beta-thalassemia major, 57.6% had disorders of linear growth and 45.5% had pubertal disorders noted as adults.[18]

Thalassemia patients generally receive combined therapy of blood transfusion and chelation.[19-21] Routine blood transfusion might result in better prognosis, but can lead to iron accumulation that disturbs cellular processes.[22] In our study, there was no significant difference in Hb level before blood tranfusion between the short stature (6.79 g/dL) and normal (6.76 g/dL) groups (P=0.566). Similarly, Pemde e*t al.* found no correlation of body height Z-score and mean Hb pre-transfusion.[23] At present, patients with beta-thalassemia major typically have Hb levels of above 9 g/dL or 9.5-10 g/dL,1[9,22,24] to prevent bone abnormality and splenomegaly.[22] Hb levels of below 9 g/dL is caused by a lack of compliance to receive regular blood transfusions. Also, low family income may affect the compliance.

Table 2. A comparison of characteristics between the short stature and normal height groups

CharacteristicS	Short stature (n=65)	Normal height-for-age (n=15)	P value
Age, months			
Mean (SD)	144.12 (15.9)	140.53 (16.6)	0.378*
Median	140	135	
Range	120-200	123-170	
Sex			
Male	33 (50.8)	7	0.775**
Female	32 (49.2)	8	
Family income			
Low (<IDR 1,500,000)	29 (44.6)	3	0.229**
High(>IDR1,500,000)	36 (55.4)	12	
Time of diagnosed			
<8 years	9 (13.8)	1	0.678***
≥8 years	56 (86.2)	14	
Pre-transfusion hemoglobin level (g/dL)			
Mean (SD)	6.79 (1.05)	6.76 (1.13)	0.566*
Median	6.60	7.00	
Range	4.60-9.10	5.70-8.10	
Total transfusion per month			
<2 x	63 (83.1)	13 (16.9)	1.000***
>3 x	2 (33.3)	2 (66.7)	
Median	3,900	4,200	
Range	2,700-5,700	3,000-5,700	
Iron chelation therapy			
Yes	64 (98.5)	13 (16.9)	0.089***
No	1 (1.5)	2 (66.7)	
Serum ferritin level, ng/mL			
Mean (SD)	3,704.54 (1,998.48)	3,775.91 (1,993.40)	0.510*
Median	3,824	3,450	
Range	775-10,223	721-7,327	
Type of iron chelation therapy			
Deferoxamine/DFO	1 (1.5)	0	0.118**
Deferasirox/DFX	24 (36.9)	3	
Deferiprone	39 (60.0)	10	
None	1 (1.5)	2	
IGF-1 value, ng/mL			
Mean (SD)	32.22 (26.38)	68.58 (51.46)	<0.001*
Median	30.94	49.54	
Range	1.03-150.72	14.87-187.68	
Nutritional status			
Below adequate	40 (61.5)	7	0.292**
Adequate	25 (38.5)	8	

SD = standard deviation; *Mann-Whitney test; * *Chi-square test; *** Fisher's exact test

Table 3. Correlation between serum IGF-1 concentration and short stature

	IGF-1 level (ng/mL)	Short stature, n(%) (n=65)	Normal, n(%) (n=15)	PR (95%CI)	P value
Cut-off point	≤38.51	42 (64.6)	2	1.49 (1.16 to1.93)	<0.001
	>38.51	23 (35.4)	13		

PR: prevalence ratio (95% confidence interval); sensitivity: 42/65=64.6%; specificity: 13/15 = 86.7%; positive predictive value: 42/(42+2) = 95.5%; negative predictive value: 13/(23+13)=36.1%; accuracy: (42+13)/80=68.8%.

Table 4. Multivariate analysis between the variables and short stature

Variables	Koev (B)	SE (B)	PR (95%CI)	P value
Serum ferritin level	-8.178	3.908	0.64 (0.08 to 4.78)	0.660
Family income				
Low	2.984	1.307	19.76 (1.52 to 256.08)	0.022*
High	0.907	1.128	2.52 (0.28 to 23.02)	0.412
Pre-transfusion Hb	0.856	0.486	2.35 (0.91 to 6.10)	0.078
Iron chelation therapy	1.681	1.671	5.37 (0.20 to 141.85)	0.314
Nutritional status	0.491	0.756	1.63 (0.37 to 7.19)	0.516
IGF-1 value ≤38.51 ng/mL	3.704	1.138	40.66 (4.37 to 377.58)	0.001*

Note: *significant if P<0.05; logistic regression analysis

High levels of ferritin have been strongly correlated with growth disorders, endocrine disorders, or other complications.[20] As such, chelation therapy may prevent these complications.[24] Serum ferritin measured at routine intervals (at least every 3 months) and can be used as proxy measures, with recommended target levels of 1,000 ng/mL.[19,20] We found no significant difference in the range of serum ferritin level between the short stature (775-10,223 ng/mL) and normal height (721-7,327 ng/mL) groups (P=0.510). In contrast, Joshi *et al.* reported the range of serum ferritin level of 5,295 (SD 2,736) ng/mL and Hb value of 7.8 (SD 0.6) g/dL were not significantly different in the thalassemia group and normal group.[25] Another study found a correlation between serum ferritin level >2,000 ng/mL and height-for-age (Z-score) >10-15 years (P<0.001).[23] Study in Indonesia reported a correlation between serum ferritin level (OR=3.248; 95%CI 1.304 to 8.086) and growth disorder in thalassemia patients (OR=3.964; 95%CI 1.192 to 13.190).[26]

Iron chelation therapy is used to prevent increased ferritin level and subsequent organ injury.[27] Three kinds of chelation therapy generally used are deferoxamine (DFO), deferiprone (DFP), and deferasirox (DFX). Iron chelation is usually started after 10-20 blood transfusions or if the serum ferritin level is more than 1,000 ng/mL. Deferasirox is more commonly used because of its lower toxicity,[28] but several studies have found a high incidence of short stature in thalassemic children and adolescents who received DFX therapy.[24] We found no significant difference between the short stature and normal groups among the three chelation therapy types (P=0.118). A number of 96.2% got deferiprone as iron chelation therapy and there was no significant relation towards short stature on thalassemia patients (P=0.089 and P=0.118). More than 50% of our subjects had chelation therapy, but how optimal the use of iron chelation therapy is unknown.

In our study, subjects with low family income had 19.7 times increased risk of short stature (PR 19.76; 95%CI 1.52 to 256.08; P=0.022). In addition, subjects with serum IGF-1 ≤ 38.51 ng/mL had 40.7 times increased risk of short stature (PR 40.66; 95%CI 4.37 to 377.58; P=0.001). Other factors such as long transfusion duration, chelation therapy, ferritin level, duration of time since the diagnosis , Hb level, number of transfusions, and nutritional status were not significant risk factors for short stature. Another cross-sectional study on thalassemic patients revealed that patient age (OR 5.42; 95%CI 1.29 to 12.41; P=0.016) and low family income (OR 2.32; 95%CI 1.06 to 5.06; P=0.036) were risk factors of growth disorders.[18] Yet another study reported that age at diagnosis, number of transfusions, size of lymph, serum ferritin level, and Hb level were associated with growth disorders in thalassemic patients (P<0.05).[2] Al-Salehe *et al.* found that short stature in thalassemic patients was strongly associated with high serum ferritin (P=0.006), but was not associated with sex, history of splenectomy, more than one tranfusion per month, or the use of chelation therapy (deferasirox and desferoxamine).[29]

The growth orders of the IGF-1, the IGF binding protein-3 (IGFBP3), and acid-labile subunit (ALS), all these proteins are mentioned to be the biomarker of secretion, activities and physiologic role of GH.[30] The IGF-1 produced by the liver[31] is an important growth factor on most of the growth effects.[30,32] The serum

level of IGF-1 is affected by nutritional status and other hormones, such as insulin.[32] Deficiency of circulating IGF-1 can lead to malnutrition, hypothyreosis, as well as renal and liver insufficiency.[31] In thalassemia major, IGF-1 deficiency has been attributed to chronic anemia, hypoxia, chronic liver disease, iron overload, and other associated endocrinopathies, e.g., GH deficiency.[33] Other studies have noted decreased IGF-1 levels in thalassemia patients,[18,31,32] similar to our results in that all subjects had decreased IGF-1 levels, except for one subject with normal serum IGF-1 level.

Using IGF-1 as an indicator of GH deficiency is still debatable, as it has low specificity. However, while low IGF-1 could strengthen a diagnosis of GH deficiency, GH stimulation still needs to be performed as the gold standard of GH deficiency, especially when the IGF-1 level is normal ($r=0.56$ and $P>0.05$).[31] The correlation of IGF-1, IGFBP-3, and body height-for-age remains unclear in thalassemic patients, indicating that growth disorders may be related, not only to the GH-IGF-1 axis.[24] Ali *et al.* reported an IGF-1 diagnostic sensitivity of 83.87% and specificity of 76.2%.[34] However, Alawneh *et al.* found a 47% sensitivity and 65% specificity in detecting GH deficiency.[31]

A limitation of our study was not performing the growth hormone stimulation test, nor assessing for a correlation between IGF-1 and GH. So far, there is no study which reveal about the sensitivity and specificity of IGF-1 level based on short stature on thalassemia patients. In our study, we found that patients with IGF-1 value ≤38.51 ng/mL had 1.49 times higher risk of short stature compared to those with IGF-1 value >38.51 ng/mL, with sensitivity of 64.6%, specificity of 86.7%, and accuracy of 68.8%. Based on these results, IGF-1 level could be useful in identifying children with beta-thalassemia major who are at risk of short stature. Another limitation of this study was analyzing serum ferritin levels instead of the actual serum ferritin levels at the exact assessment time of growth status, we used serum ferritin levels in the 3 months prior and need for growth monitoring, nutrition and medication adherence from early diagnosis of beta thalassemia major. In conclusion, serum IGF-1 level of ≤38.51 ng/mL is a risk factor for short stature in adolescents with beta-thalassemia major.

Conflict of interest

None declared.

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