ISSN: 13412051
Volume 25, Issue 09, September, 2020

Growth Hormone and Insulin-Like Growth Factor-1 Status in Pediatric Patients with β- Thalassemia Major

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ABSTRACT— Patients with β-thalassemia major (β-TM) often present with endocrine abnormalities, mainly due to dysfunction in their hypothalamic-pituitary axis, such as delayed growth and puberty. We aimed to assess the growth parameters of patients with β-TM and to evaluate the growth hormone (GH)-insulin like growth factor-1 (IGF-1) axis. This case-control study included 50 patients, 8-19 years old, with β-TM registered at Basra Center for Hereditary Blood Diseases, Southern Iraq, and 75 apparently healthy subjects. Anthropometric data were evaluated using the WHO Child Growth Standards. Growth hormone provocation test, serum IGF-1, ferritin, thyroid and gonadotropin hormones were also measured. Twenty-six (52%) β-TM patients had short stature. Patients with β-TM had significantly lower peak GH levels (after induction) and IGF-1 levels compared to the control group, ($P < 0.01$ and $P < 0.001$, respectively). Growth hormone deficiency and impaired IGF-1 were found in 65% and 92% of patients with short stature, respectively. The GH deficiency was observed after a provocation test with a cut- off peak less than 7 or 10 ng/ml. Moreover, significant negative associations were reported between serum ferritin and peak GH (r - 0.239), IGF-1 (r - 0.386), thyroxine (r - 0.423), and hemoglobin (r - 0.612) levels. IGF-1 can be considered as a useful and sensitive test in assessing growth retardation among pediatric patients with β-TM. In addition, more than one-third of patients were GH-sufficient, suggesting a multifactorial origin rather than GH deficiency alone.

KEYWORDS: Beta-thalassemia major, growth, growth hormone, insulin-like growth factor- 1 (IGF-1),

1. INTRODUCTION

The inherited hemoglobin disorders, mainly sickle cell disease and thalassemia, present a significant health problem [1]. Globally, 1.5% of the world's population carries genes for β-thalassemia, with approximately 60,000 symptomatic individuals born annually, the great majority in the developing world [2]. In Basra, Southern Iraq, β-thalassemia major (β-TM) is an important health problem. A previous study showed that the overall carrier frequency of β-thalassemia was 4.6%, but with a diversity in carrier rate in different areas of Basra ranging from 3.3% to 7.9%, mainly due to high rates of relative marriages [3]. Patients with β-TM need regular lifelong transfusions and chelation therapy. However, multiple red cell transfusions over a long period of time with poor compliance to chelation therapy result in iron overload (IOL), which causes increased morbidity and mortality in these patients, including damage to the heart, endocrine glands, pancreas and liver [4-7]. Despite the improved survival of β-TM patients, especially with optimum transfusion therapy and iron chelation [8,9], complications are still common [10,11]. Growth retardation is a common problem and has multifactorial etiology. Causes include inadequate transfusion, hypoxia, chronic liver disease, IOL, hormonal changes (abnormalities in growth hormone (GH) secretion or in its receptors, GH-IGF-1 axis dysregulation, reduced secretion of adrenal androgen and hypothyroidism), zinc and folic acid deficiencies and deferoxamine (DFO) toxicity [12-14]. Beta-Thalassaemia is a hereditary disorder that is highly prevalent in our locality [3]. The pattern of hormonal profiles that might affect the growth and development of our β-TM children and have interplay in their metabolic maturity, intervention and

managements have not been explored well in our area, and knowledge is limited. Therefore, the aim of this study was to assess growth parameters and the hormonal profiles of mainly GH (baseline and peak levels using provocation test with a cut- off peak of less than 7 or 10 ng/ml) and insulin-like growth factor-1 (IGF-1) among patients with β- TM and to observe the correlation of these parameters with serum ferritin level (as an indicator of IOL).

2. Subjects and Methods

2.1 Subjects

This case-control study was carried out on 50 patients with β-TM who are registered at Basra Center for Hereditary Blood Diseases (CHBD), which is the only center caring for these patients. Their age ranged from 8 to 19 years. Seventy-five apparently healthy children and adolescents matched for age were included as a control group. They had normal haemoglobin patterns and no previous history of relevant medical illnesses. Patients with heart failure, diagnosed by using echocardiogram when the ejection fraction was < 45% (the left ventricular function was moderately to severely reduced) [15], or on growth hormone therapy were excluded. For patients, history included date of birth, sex, age of diagnosis of the disease, frequency of blood transfusion, age of starting iron chelation therapy, deferoxamine treatment, its daily dose, and frequency of DFO intake per week. At the time of the study, this drug was the only available iron chelator.

2.2 Anthropometric data

The height, weight, and body mass index (BMI) were assessed for all children and adolescents and plotted on age- and sex-appropriate growth charts (WHO Child Growth Standards) [16]. The individual was considered as having short stature when the length or stature was height less than −2 standard deviations (SD) of the age- and gender-matched population [16]. An X-ray of the non-dominant hand was taken to determine the bone age of β-TM patients according to the Tanner-Whitehouse 2 (TW2) method.

2.3 Laboratory analysis

Growth hormone (GH) provocation test (L-dopa provocation test): An L-dopa tablet was given orally at a dose of 500 mg/1.73 m2 body surface area, with a maximum dose of 500 mg [17,18]. After overnight fasting, a blood sample was taken at 8:30-9:30 am (basal sample). Then, L- dopa was given, and after 2 hours, another blood sample was taken (provocative sample). The sera were stored at -18ºc until the time of assay. Serum GH and insulin-like growth factor-1 (IGF-1) were estimated in duplicate by an enzyme-linked immunosorbent assay (ELISA) technique according to the methods described by the manufacturer leaflets enclosed with the kits (Monobind, BioCheck Inc., USA), with sensitivity of 0.5 ng/ml, and DRG (Germany), with a sensitivity of 1.29 (range 9.75-600) ng/ml, respectively. The inter- and intra-assay coefficients of variation for GH were 5.3% and 3.9%, respectively, while the inter- and intra- assay coefficients of variation for IGF-1 were 8.3% and 6.9%, respectively. Serum GH levels are reported as basal and provocative values, and the difference between them was referred to as the peak response level. The GH secretion was considered sufficient if the peak stimulation level from baseline after the provocation test was more than 7 or 10 ng/ml [19,20]. The normal range for IGF-1 was calculated from our normal control group, and a low serum IGF- 1 level was defined as a value below the 95% confidence intervals (CIs) of the mean values of the control group according to the sex and age. Mean pre-transfusion hemoglobin (Hb) was estimated by an Automated Hematology Analyzer (Mindray BC 6800 Shenzhen, China). Serum ferritin, thyrotropin (TSH), thyroxine (T4), free thyroxine (fT4), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured using an ELISA technique. The procedures were followed as instructed by the manufacturer (Human, Germany). The College Council and the Ethical Committee of the College of Medicine, University of Basra, approved the protocol of the study. The importance of the study and the

possible side effects of the L-dopa tablet were explained, and a written consent was obtained from the patients' and controls' parents.

2.4 Statistical analysis

Data were analysed using Statistical Package for the Social Sciences (SPSS) software version 22 (IBM, Chicago, Illinois, USA), and the results are presented as tables or figures. The results are expressed as mean ± SD or percentage whenever appropriate. Using independent t-Test, the differences in the parameter means under study from two different groups were identified. The Chi Square or Fisher's exact test was used as appropriate to assess the significance between groups. A bivariate correlation analysis (Pearson's) was used to assess the correlation between the variables. A P value <0.05 was considered the lowest limit of significance.

3. Results

The study revealed that 26 (52%) of β-TM patients had short stature, Table 1. The prevalence of short stature increased from 41% in pre-pubertal age to 72% in pubertal age, P<0.001. The mean age of diagnosis of patients as having β-TM was 12.1±7.9 months; the patients received blood transfusion every 22± 9 days, with a mean frequency of blood transfusion of 17±7 / year. At the time of the study, DFO was the only available iron chelator. The mean age of starting DFO was
4.6 ± 2.1 years.

Table 1: Selected demographic and growth parameters among studied individuals

Variable	**Cases (**N = 50) N (%)	**Controls (**N = 75) N (%)	**P value**
Mean Age (Y)	13 ± 3.37	13.19 ± 3.12	>0.05 *
Gender			
Males	27 (54)	41 (54.7)	0.238†
Females	23 (46)	34 (45.3)	
Height for age			
Short stature	26 (52)	0 (0)	<0.001‡
Normal stature	24 (48)	75 (100)	
BMI			
Underweight	15 (30)	6 (8)	<0.001‡
Normal weight	34 (68)	46 (61.4)	
Overweight	1 (2)	13 (17.3)	
Obese	0 (0)	10 (13.3)	

*t – test was used to measure P value, † Chi-square was used to measure P value, ‡ Fisher's exact test was used to measure P value

Mean height, weight, and BMI were significantly lower among patients with β-TM compared to the control group, P<0.001. The study revealed that β-TM is associated with significantly lower peak GH (after induction) and IGF-1 levels compared to the control group, P<0.01 and P<0.001, respectively, Table 2. In addition, statistically significantly lower levels of T4 and fT4 among β- TM patients compared to controls (P<0.001) were also found. In only 4 patients (8%), all the tests indicative for hypothyroidism were abnormal. Furthermore, the levels of LH and Hb were significantly lower among patients compared to healthy subjects, P<0.05 and P<0.001, respectively, Table 2.

Table 2: Selected clinical, biochemical and hormonal levels among β-TM patients and the control group

Variable		Cases (N = 50)	Controls (N = 75)	P value
Height (cm)		137.41±14.95	152.83±15.93	<0.001
Weight (kg)		32.84±10.69	49.77±18.51	<0.001
BMI (kg/m^2)		16.94±2.35	20.59±4.68	<0.001
GH (ng/ml)	**Baseline Level**	10.77±8.78	11.34±7.82	0.704
	Peak Level	17.14±22.24	29.83±25.15	0.005
IGF-1 (ng/ml)		102.9±65.7	207.1±104.3	<0.001
TSH (mIU/l)		2.95±2.47	2.43±1.67	0.165
T_4 (µg/dl)		5.57±1.67	7.48±1.84	<0.001
fT_4 (ng/dl)		0.94±0.2	1.14±0.32	<0.001
FSH (IU/l)		2.74±3.67	3.92±3.58	0.075
LH (IU/l)		2.04±2.15	3.51±3.45	0.009
S. Ferritin (ng/ml)		5192.8±3207.1	93.09±57.12	<0.001
Hemoglobin (gm/l)		66.2±11.1	117.7±11.1	<0.001

Values are expressed as mean ± SD

Several diagnostic cut-off values for GH deficiency were used and we tried to compare 2 used values: the provocation test with peak levels response from baseline of less than 7 and 10 ng/ml. Growth hormone deficiency was found in 20 (40%) and 22 (44%) out of 50 β-TM patients when the GH peak stimulation used was < 7 or < 10 ng/ml, respectively. The rest of the β-TM patients were GH- sufficient and had good response to the stimulation test, Table 3. In the control group, GH deficiency was found in 9 (12%) out of 75 control individuals whether the GH peak stimulation used was < 7 or < 10 ng/ml, and the rest were GH-sufficient. The results for IGF-1 assessment revealed low levels of IGF-1 hormone (less than 95% CI stratified according to age and sex) in 32 out of 50 (64%) β- TM patients and 6 (8%) out of 75 control subjects, Table 3.

Table 3: Growth hormone status (Peak level < 7 and < 10 µg/l) and IGF-1 (µg/l) in β-TM patients and the control group

Hormonal Status		**β-TM patients** (N = 50)		**Controls** (N = 75)		**Total N = 125** (%)
Growth Hormone (ng/ml)						
Status	Peak	N (%)	Mean ± SD	N. (%)	Mean ± SD	
Deficient	< 7	20 (40)	2.08 ± 1.07	9 (12)	3.4 ± 2.4	29 (23)
	< 10	22 (44)	2.67 ± 2.16	9 (12)	3.4 ± 2.4	31 (25)
Sufficient	> 7	30 (60)	28.5 ± 20.7	66 (88)	33.15 ±22.8	96 (77)
	> 10	28 (56)	30.6 ± 20.7	66 (88)	33.15 ±22.8	94 (75)
IGF – 1 (ng/ml)						
Low (deficiency) * (< 95 % CI)		32 (64)	78.9 ± 27.5	6 (8)	110 ± 45.1	38 (30)
Adequate (>95 % CI)		18 (36)	145 ± 89.2	69 (92)	222.1 ± 102.7	87 (70)

* IGF-1 deficiency is considered as less than 95% CI of the mean, stratified according to age and sex

Of the 26 β-TM patients with short stature, 16 (62%) did not respond to the GH provocation test (peak level of less than 7 ng/ml) compared to 17 (65%) who did not respond to the GH provocation test (peak level of less than 10 ng/ml), with peak GH levels of 2.14 ± 1.06 ng/ml and 2.51 ± 1.83 ng/ml, respectively, P>0.05,

ISSN: 13412051
Volume 25, Issue 09, September, 2020

indicating GH deficiency. The remaining 10 or 9 (38% or 35%) patients had good response to the stimulation test, and peak GH levels of more than 7 and 10 ng/ml was obtained, at 19.45 ± 9.46 ng/ml and 20.68 ± 9.15 ng/ml, respectively. On the whole, the mean GH level in the 26 β-TM patients with short stature was statistically significantly lower, at 8.8± 10.3 ng/ml versus 27.8± 24.34 ng/ml in the 24 patients with normal stature, P=0.003. Moreover, IGF-1 estimation showed that 24 out of 26 β-TM patients with short stature (92%) and 7 out of 24 patients with normal stature (29%) had significantly low IGF-1 levels (77.4 ± 25.76 ng/ml and 74.57 ± 30.10 ng/ml, respectively) compared to 6 out of 75 (8%) in the control group (110.0 ± 45.1 ng/ml), P<0.05. The prevalence rates of low levels of GH and IGF-1 were significantly higher in β-TM patients with short stature as compared to those with normal stature and the controls, P<0.001, Figure 1. The bone age SDS for chronological age in all patients with β-TM was -1.77± 1.82 years; - 2.7±1.5 years for those with short stature compared to - 0.13± 0.83 years in patients with normal stature, P<0.05.

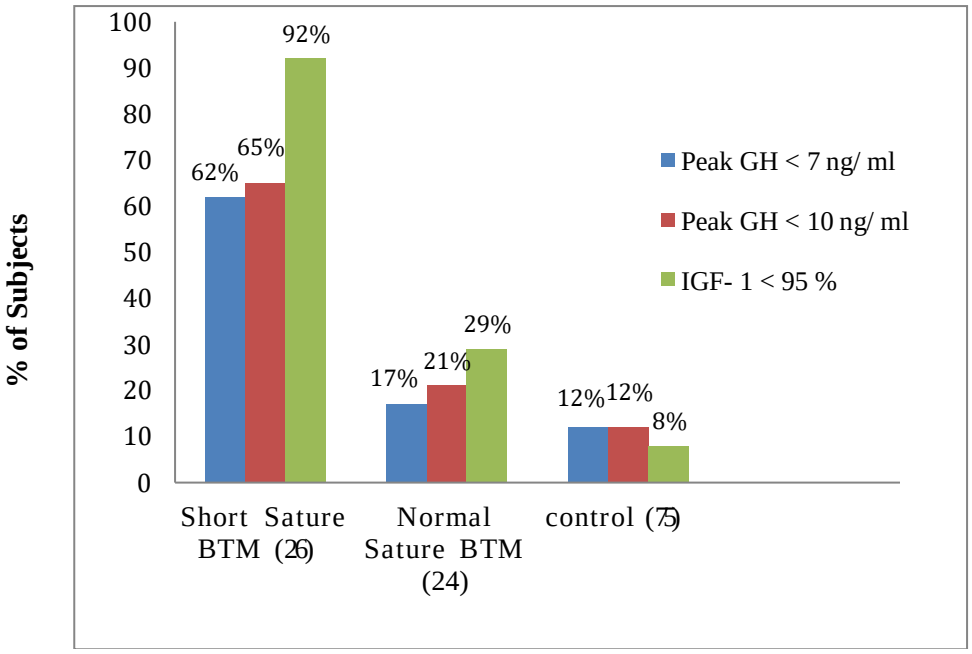

Figure 1: Prevalence rates of GH and IGF-1 deficiency in BTM patients with short or normal stature and the control group

The bivariate analysis using Pearson's coefficient correlation (r) of different factors and biochemical tests that could be associated with serum ferritin as an indicator of IOL, peak GH and IGF-1 among studied individuals revealed that serum ferritin had significant negative associations with Hb, peak GH, IGF-1 and T4. Furthermore, IGF-1 had significant associations with each of Hb, T4, LH, FSH, and peak GH, P<0.05, Table 4. Receiver operating characteristic (ROC) analysis was used to test the validity of the GH provocation test and IGF estimation as indicators for short stature in thalassemic patients. The curve analyses showed that serum IGF-1 estimation had a significantly higher diagnostic accuracy than the GH induction test in detecting short stature in our patients with β-TM,
Figure 2.

Table 4: Pearson correlation (r) between serum ferritin, peak GH, IGF-1 and other parameters among studied subjects

Variable	Ferritin (r)	Peak GH (r)	IGF-1 (r)

Age	0.064	- 0.188*	0.266**
Sex	- 0.012	- 0.171	- 0.113
Height	- 0.331**	0.118	0.546**
Weight	- 0.389**	0.055	0.530**
BMI	- 0.337**	0.067	0.407**
BMI Z	- 0.429**	0.089	0.245*
FT4	- 0.174	0.014	0.11
T4	- 0.423**	0.220*	0.256*
TSH	- 0.028	0.115	0.072
LH	- 0.161	0.047	0.265**
FSH	- 0.021	- 0.073	0.265**
Hb	- 0.612**	0.315**	0.471**
Peak GH	- 0.239**	1	0.233**
IGF-1	- 0.386**	0.233**	1
Ferritin	1	- 0.239**	- 0.386**

* P <0.05, ** P <0.01

With a cut-off value less than 10 ng/ml increment from baseline after the provocation test for growth hormone, the AUC (area under the curve) was 0.681, with a sensitivity of 65.4% and a specificity of 70.8% (P=0.028). For IGF-1, the threshold value was set at less than 95 % CI of the mean after adjustment for age stratification, and the AUC was 0.878 with a sensitivity of 92.3% and a specificity of 83.3%, P<0.001, Figure 2.

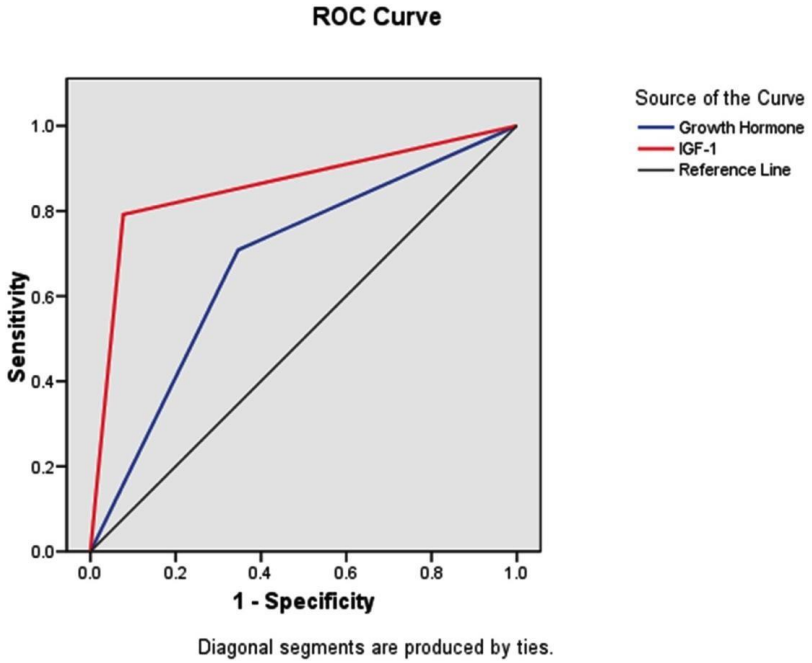

Figure 2: Receiver operating characteristic (ROC) curves of serum IGF-1 and peak GH in targeting short stature in patients with β-thalassemia major

The red and blue lines indicate the levels of IGF-1 and peak GH in β-TM patients, respectively. The AUCs (± SE) and P-values for serum IGF-1 and peak GH in β-TM patients are 0.857 ± 0.058, P = 0.0001, and 0.681 ± 0.077, P = 0.028, respectively.

4. Discussion

In the present study, short stature was found in 52% of patients with β-TM, with higher frequency among females. Approximately 2/3 of our patients with short stature were growth hormone- deficient, assessed using the L-dopa provocation test, with no significant difference in whether the cut-off growth hormone peak response above baseline was less than 7 or 10 ng/ml. Furthermore, IGF-1 was more sensitive and specific than the GH assay. The frequency of short stature in the current study is consistent with that reported by Badfar et al. in Iran (52.3%) [21], but lower than that reported by Moiz et al in Pakistan (65%) [22]. However, our results are higher than that of Tan et al. in Malaysia (40.2%) [23], and Thuret et al. in France (20%) [24]. The lower prevalence of short stature among our prepubertal age patients is consistent with that reported by Fica et al. in Romania, where they found that pathologic short stature occurred to a lesser extent in prepubertal patients (23%) compared to patients at pubertal and adult age (61%) [25]. The variations in the frequency of short stature among different countries can be attributed to the greater adherences to the management plan, including blood transfusion, regular and optimum use of iron-chelating agents, early detection and treatment of complications through close follow-up and monitoring since diagnosis. Body mass index, which is one of the most preferred methods to assess underweight and obesity, was found to be low in 30% of cases. Asadi-Pooya and Karamifar reported that 31% of β-TM patients were underweight (26% males and 35% females) [26], while Moiz et al reported low BMI in 42% pediatric β-TM patients in Pakistan [22]. Growth retardation in β-TM appears to be multifactorial. During early life, hypoxia, caused by anemia, may be the main factor affecting growth. Moreover, DFO can produce marked stunted growth if used prior to the age of 3 years or before the development of iron overload [27]. Subsequently, other factors are added, including iron overload, IGF-1 deficiency, and hormonal causes, such as GH deficiency or hypothyroidism, hypogonadism, zinc deficiency or DFO toxicity [12,25]. In the present work, in addition to the clinical and auxological assessment, combined with a bone age X-ray, biochemical tests of the GH-IGF axis were also performed. Provocation tests are used for the detection of GH deficiency with different stimulators and cut-off values. In our work, the L-dopa provocation method was used, as it seems to be safer and easier to control than the insulin tolerance test (reference method). Several diagnostic cut-off values for GH deficiency have been employed among different researchers [18,20,28]. We tried to compare a provocation test with 2 cut-off values with peak levels less than 7 and 10 ng/ml for the detection of GH deficiency.

No significant difference assessed was found using the L-dopa provocation test whether the cut- off GH peak response less than 7 or 10 ng/ml. Published reports have generally accepted that a cut-off growth hormone peak response of 7.5-10 ng/ml is a reasonable value for determining whether an individual is growth hormone-sufficient or insufficient [19,28]. IGF-1 was determined to be low in more than half of the β-TM patients. This result was significantly lower than in the control group. Among patients with short stature, 92% of them had low IGF-1. The present study showed that even in patients with normal stature, the mean IGF-1 level was significantly lower compared to normal individuals. These results are similar to what has been reported by other researchers [29-32]. In our study, the sensitivity and specificity of serum GH and IGF-1 levels as predictors of short stature in β-TM patients were assessed using receiver operating characteristic (ROC) analysis, which is considered to be a useful tool for evaluating the performance of diagnostic tests. The results showed that IGF-1 is more sensitive and specific than the GH assay. Therefore, the concentration of serum IGF-1 would be a more useful predictor of short stature in β-TM patients, a finding that has been observed by other researchers [33,34]. The significantly lower levels of T4 and fT4 reported among thalassemic patients are in agreement with those reported by Gathwala et al. in India [35] and Mohey El-Deena et al. in Egypt [36]. The frequency of decompensated hypothyroidism (8%) is comparable with other studies carried out by Shamshirsaz et al. (7.7%) in Iran [37] and by Tan et al. in Malaysia (10%) [23]. Other researchers have reported conflicting results; for example, Moayeri et al. in Iran

reported a lower percentage (4.4%) [38], while Fica et al. [25] and Najafipour [39] observed higher percentages (17.18% and 16%, respectively). These discrepancies cannot be attributed to differences in patients' ages, but rather to different treatment protocols, including different transfusion rates and chelating therapies [37]. Most complications in patients with β-TM result from iron overload, which results from red blood cell transfusions and increased absorption of iron through the gastrointestinal tract. In the present work, the serum ferritin level showed negative correlations with all studied parameters except age, indicating the toxic effect of IOL in the studied individual. Iron overload causes transferrin saturation and circulation of non-transferrin-bound iron (NTBI) species in the plasma with excess generation of reactive oxygen species (ROS), leading to lipid peroxidation. Lipid peroxidation leads to the generation of both unsaturated and saturated aldehydes, which are implicated in cellular dysfunction, cytotoxicity, and cell death [4,40]. Our study has the following limitations. First, the sample size was small, which could affect proper analysis and conclusions. Second, one provocative test was used; two separate provocation tests would be more efficient in detecting GH deficiency; failure to respond to them would indicate GH deficiency. Third, it is well known that the measurement of serum IGF-1 is of recognized value in children with growth disorders. However, IGF-1 level is influenced by age, gender, pubertal stages, nutritional status, liver function and physical activity. Therefore, it is suggested that along with GH and IGF-1; IGF-1-binding protein-3 (IGFBP-3) is used in the diagnosis of GH deficiency. The advantages of measuring IGFBP-3 over IGF-1 are that IGFBP- 3 is present in higher concentrations and with less age dependence. Unfortunately, an IGFBP-3 assay was not available at that time.

5. Conclusion

From this study, it can be concluded that GH deficiency can be detected by L-dopa provocation test whether its peak response is less than 7 or 10 ng/ml. IGF-1 is the more sensitive test in assessing growth retardation among patients with β-TM, and more than one-third of them are growth hormone-sufficient, suggesting that the growth retardation is of multifactorial origin rather than due to GH deficiency alone. In addition, iron overload was significantly associated with GH and IGF-1 status. These data support the need for watchful follow-up of patients with β-TM to detect and treat endocrine dysfunctions at an appropriate age.

6. Acknowledgment

We would like to thank Dr. Asaad Yahya, Department of Animal Production, College of Agriculture, University of Basra, for his indispensable help in the statistical effort of this work.

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