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Metabolism Clinical and Experimental 54 (2005) 15–23

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Human chorionic gonadotropin therapy in adolescent boys with constitutional delayed puberty vs those with β-thalassemia major

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Received 16 July 2002; accepted 13 July 2004

Abstract

We studied 12 adolescent boys with β-thalassemia major and delayed puberty (age, 15.8 ± 1 years) with Tanner I sexual development treated with a long-term low-transfusion regimen. Ten nonthalassemic adolescents (>14 years) with constitutional delay of growth and puberty (CDGP) served as controls. Auxologic parameters and testicular size were measured, and bone age was determined. Measurement of basal gonadotropin (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]) and testosterone (T) levels taken at 8 AM revealed prepubertal levels in both groups of patients. Human chorionic gonadotropin (hCG, 2500 U/m^2) was injected intramuscularly twice weekly for 6 months, and anthropometric data, testicular diameter, and serum T concentrations were remeasured after 1 and 6 months. The testicular diameter after 6 month of hCG therapy was significantly correlated with the testicular diameter and T level after 1 month of therapy (r = 0.93 and 0.39, respectively, P < .01). After 6 months of hCG therapy, the mean growth velocity (GV) increased from 4.1 to 8.6 cm/y in thalassemic patients and from 4.6 to 10.3 cm/y in those with CDGP during hCG therapy. In thalassemic boys, the mean T concentration increased from 0.93 to 2.7 nmol/L (mean increase = 1.8 nmol/L) vs an increase from 0.47 to 4.81 nmol/L (mean increase = 4.32 nmol/L) in those with CDGP. All adolescents with CDGP, but only 7 the 12 thalassemic adolescents, had T secretion above 2 nmol/L after 6 months of hCG therapy and maintained their growth and pubertal development for a year after stopping hCG. The 5 thalassemic patients with defective T secretion after hCG therapy had significantly higher ferritin level (1985 ± 658 ng/mL) vs the other 7 patients (1100 ± 425 ng/mL). These findings denoted significant testicular dysfunction in those patients with higher iron overload (testicular siderosis). Statural GV was significantly correlated with insulin-like growth factor 1 (IGF-1) concentrations and testicular diameter after hCG therapy (r = 0.5 and 0.43 respectively, P < .001). In summary, hCG therapy was effective in treating 7 of 12 (58%) of thalassemic adolescents with delayed puberty. In the rest of patients (5/12, 46%) with significantly higher iron overload, hCG therapy failed to stimulate testicular growth and adequate T. Proper iron chelation appears to protect against testicular dysfunction. In the first group of patients, hCG therapy can be used for the treatment of their hypogonadism, whereas T replacement remains the therapy of choice for the second group.

1. Introduction

Generalized iron loading (siderosis) of the organs has been a recognized complication of β-thalassemia. The excess iron is derived both from transfusion and from intestinal absorption; in adequately transfused children, the former mechanism predominates. The degree of iron overload derived from blood transfusion obviously depends on the type of transfusion regime. Siderosis of the liver, pancreas, renal, and endocrine glands markedly contribute to the morbidity of these patients [1-3]. The proper use of deferoxamine has been shown to delay the development of iron-induced damage of cardiac and liver tissue, resulting in improved survival. The ability of deferoxamine to prevent endocrine damage is less clear [4,5].

Many investigators studied gonadotropin secretions in thalassemic patients. Lassman et al [6] found low luteinizing hormone (LH) levels in their adolescent subjects who

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0026-0495/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2004.07.006

also showed clinical hypogonadism and a low concentration of estrogen and testosterone (T) in the blood. Others reported reduced LH and follicle-stimulating hormones (FSH) rise in response to gonadotropin-releasing hormone (GnRH) [7,8]. Chatterjee et al [9] found low-normal GnRH-stimulated gonadotropin levels in 15 thalassemic girls who developed secondary amenorrhea. Studying the spontaneous pulsatile properties of LH and FSH in those thalassemic girls revealed progressive neurosecretory dysfunction of their gonadotrophins [10,11]. Magnetic resonance imaging studies revealed structural abnormalities of the pituitary gland and its stalk in some of these patients [12]. Wang et al [13] treated 3 of their hypogonadotropic thalassemic patients with pulsatile subcutaneous GnRH therapy in addition to chelation therapy for 6 months with no improvement [13].

However, pulsatile GnRH treatment and short-term human chorionic gonadotropin (hCG) therapy in boys with thalassemia resulted only in partial correction of the pituitary-gonadal function and induced and maintained secondary sexual characteristics in only some of them [14-17]. Testicular autopsies revealed varying degrees of testicular interstitial fibrosis with small heavily pigmented undifferentiated seminiferous tubules, hyalinized slings, and an absence of Leydig cells, suggesting end-organ fibrosis secondary to iron loading [18].

These studies collectively reported hypogonadotropic hypogonadism in the majority of patients with thalassemia major secondary to hypothalamic-pituitary siderosis. Proper gonadotrophin therapy improved some but not all patients. This is apparently because of concomitant involvement of the gonads as well by the generalized hemosiderosis process.

The question is, How many of those thalassemic patients with hypogonadotropic hypogonadism will respond to hCG therapy (their main pathology is central hypogonadism) and how many will not respond (having testicular dysfunction as well)?

Further studies are required to investigate the effect of short- and long-term therapy with hCG on T secretion and linear growth in adolescents boys with thalassemia major vs those with constitutional delay of growth and puberty (CDGP).

2. Patients and methods

Twelve adolescent boys with β-thalassemia major and delayed puberty were the subjects of this study. They were recruited from the thalassemia clinic of Alexandria University Children's Hospital. All were on a long-term low-transfusion regimen (to keep their hemoglobin above 9 g/dL), with intramuscular or subcutaneous iron chelation treatment (3-5 times a week), and were receiving 5 mg of folic acid per day. All patients were older than 14 years and with sexual maturity rating of Tanner stage I (ie, testicular volume < 4 mL and largest testicular diameter < 2 cm, no pubic hair, and no maturation of scrotum or penis) [19]. Ten nonthalassemic healthy adolescents above the age of 14 years with delayed onset of puberty (Tanner stage I, ie, CDGP) served as controls. None of the adolescents had intrauterine growth retardation, severe malnutrition, diabetes, or dysmorphic trait. None had exposure to irradiation or any other systemic disease.

Pubertal development was assessed according to the method of Tanner and Whitehouse [19], and the largest testicular diameter and testicular volume were measured by an accurate ruler and Prader orchidometer, respectively.

All the study subjects were subjected to the following:

1. Thorough history taking.
2. Anthropometric data, including weight, height, mid-arm circumference (MAC), and triceps skin-fold thickness, were measured. Height standard deviation scores (HtSDS) and body mass index (BMI) were calculated. Growth velocity (GV) was measured over a whole year for all the patients. Normal population data were according to Tanner and Whitehouse [19].
3. Skeletal age was determined by the method of Greulich and Pyle [20].
4. Routine laboratory investigations included measurement of serum creatinine, ferritin, and urea concentrations.
5. Hormonal studies and investigations included the following:
   (a) measurement of serum concentrations of T, LH, and FSH concentrations in a fasting venous sample taken at 8 AM;
   (b) hCG (2500 U/m^2) was injected intramuscularly daily for 3 days, and serum T concentration was measured on the fourth morning;
   (c) hCG (1500 U/m^2) were injected intramuscularly in the buttock (by the hematology nurse) twice weekly for 6 months, and anthropometric data, testicular diameter, and serum T concentrations were remeasured after 1 and 6 months of beginning therapy.

Informed consent was obtained from all adolescents and their parents before being included in the study. The protocol of the study was approved by the Ethics Committee of Alexandria University.

Serum concentrations of T were determined in duplicate by a radioimmunoassay using coated-tube technology (Spectria) from Orion Diagnostics (Espoo, Finland). All the samples collected were run in a single assay. The volume of the serum used was 50 μL instead of 25 μL to increase the sensitivity of the kit; otherwise, the radioimmunoassay was conducted according to the manufacturer's instructions. The detection limit was 30 pmol/L. The intraassay coefficient of variation was 10.6% for concentrations of 0.21 nmol/L and below 7% for concentrations of 0.42 nmol/L or higher. The interassay coefficient of

Table 1
Anthropometric and hormonal data of boys with thalassemia vs CDGP

	Thalassemia (n = 12)		CDGP (n = 10)	
	Before	After hCG (6 months)	Before	After hCG (6 months)
Age (y)	15.8 ± 1	16.3 ± 1	14.9 ± 0.7	15.5 ± 0.7
Height (cm)	152.1 ± 9.5	156.4 ± 8.7*	151.5 ± 6.1	158 ± 5.4*
HtSDS	2.64 ± 0.4	2.2 ± 0.45*	2.2 ± 0.4	1.65 ± 0.4*·**
Weight (kg)	44.1 ± 11	46.5 ± 8.1*	45.4 ± 4.2	50.6 ± 9.7*
GV (cm/y)	4.1 ± 0.24	8.6 ± 2.9*	4.6 ± 0.03	10.3 ± 2.1*·**
MAC (cm)	19.09 ± 2.8	20.1 ± 1.98*	22.4 ± 4.2**	24.3 ± 1.5*
Testicular diameter (cm)	1.6 ± 0.62	2.4 ± 0.9*	1.1 ± 0.3	2.6 ± 0.6*
Testicular increase (cm)	ND	0.8 ± 0.5	ND	1.5 ± 0.6**
Albumin (g/dL)	3.9 ± 0.1	4.08 ± 0.09	4.2 ± 0.2	4.3 ± 0.3
ALT (IU/L)	88.5 ± 15	82.5 ± 12	21.2 ± 4.2	20.4 ± 5.2
LH (IU/L)	1.2 ± 0.7	2.1 ± 1	1.8 ± 0.8	3.5 ± 1.2*
FSH (IU/L)	2.1 ± 0.9	1.9 ± 0.7	2.4 ± 0.7	3.2 ± 1.8
GH-P-clonidine (μg/L)	9.5 ± 2.5	ND	11.2 ± 3.5	ND
T (nmol/L)	0.93 ± 0.56	2.7 ± 1.7*	0.47 ± 0.35	4.81 ± 1.85*·**
T increment (nmol/L)	ND	1.8 ± 1.5	ND	4.3 ± 1.6
IGF-1 (ng/mL)	33 ± 9.6	67.2 ± 11.4*	90.6 ± 14.1**	148.3 ± 19.2*·**

GH-P-clonidine indicates GH peak response to clonidine after priming with T; ALT, alanine aminotransferase; ND, not done.
* $P < .05$ before vs after hCG therapy.
** $P < .05$ boys with thalassemia vs CDGP.

variation was 31% for concentrations of 0.19 nmol/L and below 7.4% for concentrations of 0.92 nmol/L or higher. Statistical analyses were performed using the unpaired *t* test to compare analyte concentrations among groups when the data were normally distributed and Wilcoxon rank sum test when they were not. Correlation among variables (anthro-

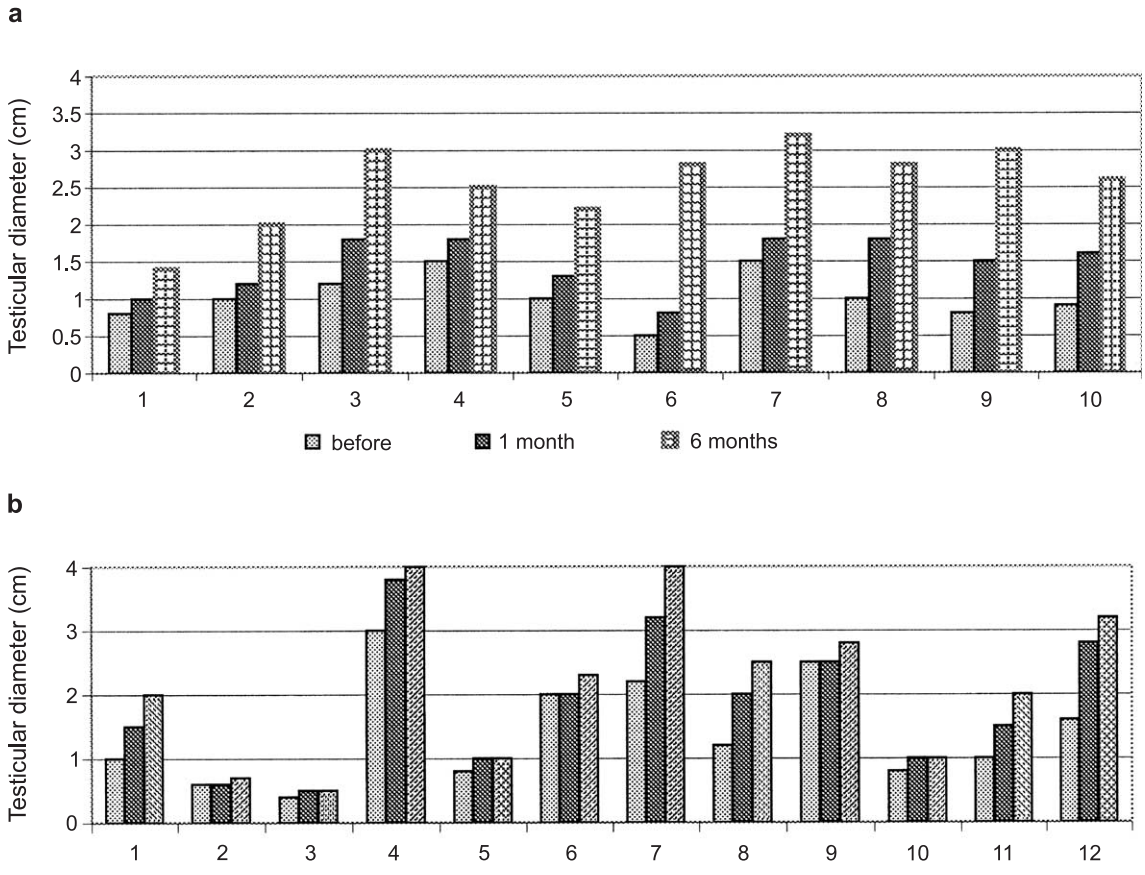

Fig. 1. a, Testicular diameter in boys with CDGP: before (basal) and 1 and 6 months after hCG therapy. b, Testicular diameter in boys with thalassemia before (basal) and 1 and 6 months after hCG therapy.

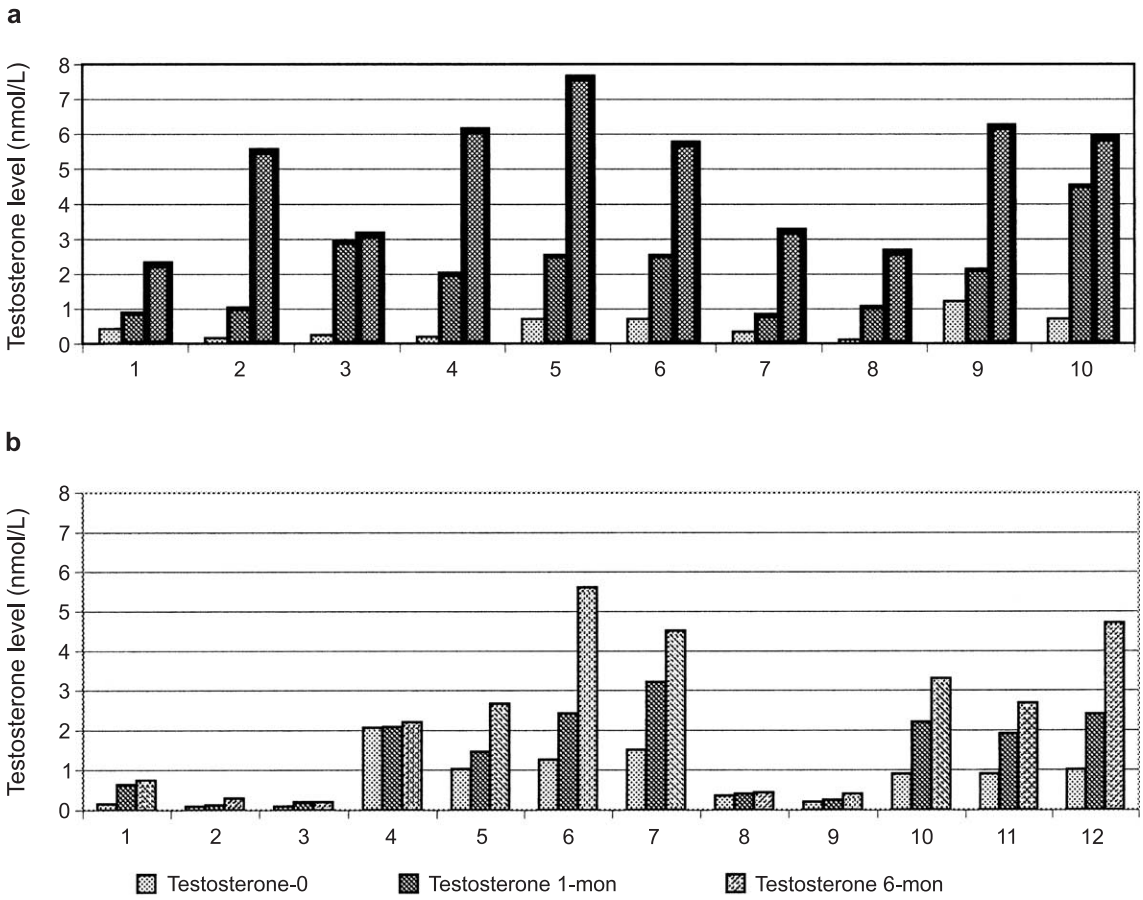

Fig. 2. a, T response to hCG in boys with CDGP before (basal) and 1 and 6 months after hCG therapy. b, T response to hCG in boys with thalassemia before (basal) and 1 and 6 months after hCG therapy.

pometric data, testicular size, and T data) were examined by linear regression analysis.

3. Results

Adolescents with thalassemia had significantly decreased HtSDS and smaller MAC compared with nonthalassemic boys with CDGP. Age, BMI, and subcutaneous fat thickness did not differ between the 2 groups. The bone age of the CDGP group (11.8 ± 1.5 years) was younger compared with the thalassemic group (12.9 ± 2.1 years). Peak GH response to provocation with clonidine did not differ between the 2 groups. However, the mean circulating insulin-like growth factor 1 (IGF-1) concentration was significantly lower in the thalassemic group. After 6 months of hCG therapy, the average height of thalassemic adolescents increased from 152.1 to 156.3 cm, with acceleration of their mean GV from 4.1 to 8.6 cm/y. In the CDGP group, the mean height increased from 151.5 to 158 cm, with an average increase in their mean GV from 4.6 to 10.3 cm/y. The HtSDS and MAC increased significantly in both groups of patients after hCG therapy but more in the CDGP group (Table 1).

Table 1 and Figs. 1 and 2 illustrate the changes in the testicular size and T secretion after 1 and 6 months of hCG therapy in adolescents with CDGP vs those with thalassemia. In the thalassemic group, the largest testicular diameter increased significantly from a mean of 1.61 cm to 2.21 and 2.4 cm after 1 and 6 months of hCG therapy, respectively. In the CDGP group, the mean testicular diameter increased significantly from 1.02 cm to 1.46 and 2.6 cm after 1 and 6 months of hCG therapy, respectively. Adolescents with CDGP had significantly bigger increase in testicular diameter (1.5 ± 0.5 cm) vs the thalassemic group (0.8 ± 0.5 cm) after 6 months of hCG therapy.

The 3-day hCG stimulation test increased T secretion significantly in boys with CDGP (from a basal of 0.47 to 1.25 nmol/L), but the T response was not significant in thalassemic patients (from 0.9 to 1.1 nmol/L).

In thalassemic adolescents, the mean T concentration increased from a mean of 0.93 nmol/L before therapy to 1.7 and 2.7 nmol/L after 1 and 6 months of hCG therapy, respectively. In adolescent boys with CDGP, the mean T concentration increased from a mean of 0.47 nmol/L before hCG therapy to 2.01 and 4.81 nmol/L 1 and 6 months after

Table 2
Correlations (r values) between different variables before and after hCG therapy in all adolescents (n = 22)

	GV	Test-D before	Test-D 1 month	Test-D 6 months	Test-D increment	T basal	T after 1 month	T after 6 months	T increments	IGF1 before	IGF1 6 months
GV	1										
Test-D before	0.04	1									
Test-D 1 month	0.32*	0.85*	1								
Test-D 6 months	0.43*	0.85*	0.93*	1							
Test-D increment	0.76*	0.077*	0.45*	0.58*	1						
T basal	0.09	0.49*	0.56*	0.42*	0.04	1					
T after 1 month	0.13	0.18	0.39*	0.18	0.07	0.79*	1				
T after 6 months	0.006	0.08	0.2	0.05	−0.03	0.61*	0.9*	1			
T increments	0.025	0.088	0.017	0.1	−0.06	0.34*	0.76*	0.95*	1		
IGF-1 before	0.35*	−0.2	−0.17	0.28	0.63*	−0.345*	0.27*	0.504*	0.599*	1	
IGF-1 after	0.5*	−0.17	−0.14	0.28	0.58*	−0.223	0.42*	0.68*	0.76*	0.9*	1

GV indicates growth velocity (cm/y); Test-D, testicular diameter; T, testosterone level; IGF1, insulin-like growth factor 1.
* $P < .01$.

hCG therapy, respectively. After hCG therapy for 6 months, the mean T concentration in the thalassemic group (1.8 nmol/L) was significantly lower vs those with CDGP (4.32 nmol/L). All adolescents with CDGP had T secretion above 2 nmol/L after 6 months of hCG therapy, whereas 5 of the 12 thalassemic children did not attain this level of T secretion after 6 months of hCG therapy, denoting defective Leydig cell response in these patients. Testosterone secretion after 1 and 6 months of hCG therapy was significantly correlated in all treated patients (n = 22, $r = 0.9$, $P < .001$; Table 2), including thalassemic (n = 12, $r = 0.87$, $P < .001$) and nonthalassemic boys with CDGP (n = 10, $r = 0.93$, $P < .001$). In thalassemic patients, both peak GH secretion after provocation and IGF-1 levels after hCG therapy were significantly lower in nonresponders vs responders (Table 3).

The 3-day hCG test was less predictive for T secretion when compared with the 6 months test both in boys with CDGP (n = 10, $r = 0.422$, p, 0.01) and those with thalassemia (n = 12, $r = 0.312$, $P < .01$).

Thalassemic adolescents who responded to hCG therapy, with increased T secretion of 2 nmol/L or more (n = 7), had significantly lower serum ferritin levels, bigger testicular diameter, faster GV, and higher IGF-1 concentrations compared with nonresponders (n = 5). Nonresponders had significantly higher ferritin concentrations (1985 ± 658 ng/mL), reflecting severe iron overload affecting their testicles and growth axis vs those who responded to hCG therapy (1100 ± 425 ng/mL; Table 3).

Serum ferritin concentration was correlated with T and IGF-1 concentrations after 6 months of hCG therapy ($r = 0.29$, $P < .04$, $r = 0.32$, $P < .1$ respectively), as well as with the testicular diameter ($r = 0.278$, $P = .02$) after hCG therapy.

All adolescents with CDGP continued their pubertal development (testicular enlargement) as well as growth spurt (GV = 10.8 ± 1.5 cm/y) during the year after stopping of hCG therapy. Six of the 7 thalassemic patients who responded to hCG stopped their pubertal growth (nonprogression, n = 4; regression of the testicular size, n = 2; slowing of linear growth, GV = 6.3 ± 1.4 cm/y) when followed up for 6 months without hCG therapy. This proved their permanent defect and their need to continue hCG therapy.

Table 3
Data of thalassemic patients: responders to hCG therapy vs nonresponders

	Responders		Nonresponders	
	Before	After hCG (6 months)	Before	After hCG (6 months)
Number	7	7	5	5
Age (y)	15.6 ± 0.8	16.2 ± 0.9	15.9 ± 1	16.5 ± 1
Height (cm)	153.2 ± 8.2	158.8 ± 7.9*	151.5 ± 9.7	154.3 ± 10.2*
GV (cm/y)	4.3 ± 0.29	10.2 ± 2.8*	4.05 ± 0.4	5.3 ± 0.74*·**
Testicular diameter (cm)	1.8 ± 0.8	2.8 ± 0.58	1.45 ± 0.55	1.78 ± 1**
GH-P-clonidine (μg/L)	10.8 ± 2.8	ND	7.9 ± 2.2*	ND
T (nmol/L)	0.98 ± 0.45	3.1 ± 0.5*	0.77 ± 0.4	1.5 ± 0.55*·**
T increment (nmol/L)	ND	2.2 ± 1.2*	ND	0.75 ± 0.6
LH (IU/L)	1.1 ± 0.5	2.8 ± 1	1.3 ± 0.6	1.8 ± 0.7
FSH (IU/L)	2.2 ± 1.1	2.1 ± 0.9	1.9 ± 0.9	2.3 ± 1.1
IGF-1 (ng/mL)	33 ± 9.6	78.5 ± 19.5*	39.5 ± 13.5	55.7 ± 18**
Ferritin (ng/mL)	1023 ± 356	1100 ± 425	1985 ± 658	2122 ± 684*·**

GH-P-clonidine indicates GH peak response to clonidine after priming with T; ND, not done.
* $P < .05$ before vs after hCG therapy.
** $P < .05$ responders vs nonresponders.

In all adolescents (n = 22) treated for 6 months, the linear GV was significantly correlated with circulating IGF-1 concentration ($r = 0.5$, $P < .001$) as well as with the increment of testicular diameter ($r = 0.76$, $P < .01$), but not with the T increments (Table 3).

4. Discussion

Hypogonadism, delayed puberty, and short stature are still frequent complications of thalassemia major, even after improvement of blood transfusion regimens and iron chelation techniques [16]. Thalassemic patients were shorter and had lower IGF-1 concentrations compared with those with CDGP. This could be explained by hepatic siderosis with defective IGF-1 production. Both hypogonadotropic hypogonadism and testicular dysfunction are described in thalassemic patients by different authors [6-13,16,21]. These findings can be explained by the variable involvement of the pituitary gonadotrophs and the gonads by hemosiderosis [11,16].

In boys between infancy and puberty, the evaluation of testicular function remains difficult because of the low activity of the hypothalamic-pituitary-testicular axis. The response of T to hCG has long been used successfully to evaluate the presence or absence of testicular tissue and to elucidate defects of T biosynthesis or action [22,23].

In this study, all adolescents with CDGP and those with thalassemia had normal (prepubertal) concentrations of basal FSH and LH. Levels of LH and FSH did not differ between the 2 groups. The presence of these prepubertal levels in boys past the age of 14 years suggested either delayed timing of the central activation of the hypothalamic-pituitary gonadal axis or defective gonadotropin secretion by their gonadotrophs [11,24,25].

Wang et al [13] and Soliman et al [11] showed that not only the basal levels of gonadotropins might be low but also LH response to GnRH stimulation, and the spontaneous secretion of their gonadotrophins overnight were defective in a considerable number of thalassemic boys with delayed puberty and high iron overload. Magnetic resonance imaging revealed pituitary infiltration with iron and different degrees of pituitary atrophy. Although patients with testicular atrophy are expected to have high FSH and LH levels (hypergonadotropic hypogonadism), the presence of concomitant defective gonadotropin secretion due to pituitary siderosis in thalassemic adolescents with defective Leydig cell response can explain the low level of their gonadotrophins.

In this study, hCG was injected to bypass the central delay of testicular activation of the testicles by the hypothalamic-pituitary unit and to test T synthesis and secretion in these adolescents. Administration of exogenous hCG in boys with normal testicular function elicits proper T secretion with the development of secondary sexual characteristics and the accompanying linear growth spurt in adolescent boys. This effect was demonstrated clearly in our adolescents with CDGP (n = 10). All showed significant increase of testicular size (> 4 mL) and T secretion and pubertal development during hCG therapy. They had T secretion comparable with those of normal adolescent boys at Tanner stage II ($\geq$ 1.9 nmol/L) [13,25-29].

After 6 months of hCG therapy, the changes in testicular size and T secretion were significantly smaller in the thalassemic group vs the CDGP group. In the thalassemic group, testicular volume increased to more than or equal to 4 mL (or largest diameter $\geq$ 2 cm) in only 7 of the 12 patients. The other 5 patients (nonresponders) who did not secrete adequate T ($\geq$ 2 nmol/L) after 6 months of hCG stimulation apparently had both hypogonadotropic hypogonadism and testicular failure. In these patients, the testicular pathology appears to contribute significantly to hypogonadism. These data supported previous reports of lower T response to hCG in pubertal patients with thalassemia [11,16].

The highly significant correlation between T response and hCG therapy after 1 and 6 months (n = 22, $r = 0.91$, $P < .001$) validated the predictive value of a month's trial of hCG in these adolescents with either thalassemia (n = 12, $r = 0.87$, $P < .001$) or CDGP (n = 10, $r = 0.93$, $P < .001$). The 3-day hCG test appeared to be a less predictive test.

Thalassemic patients who responded to hCG with increased T secretion and testicular size had significantly lower serum ferritin concentrations vs nonresponders. The mean ferritin level of nonresponders (1985 ng/mL) was double that of the responders (1023 ng/mL). In addition, both T secretion and testicular size after hCG therapy were significantly correlated with ferritin concentration. This indicted that testicular siderosis, and subsequently, testicular dysfunction, is more significant with increasing iron load. These findings clarified the importance of early and intensive iron chelation in these patients in avoiding the deleterious effect on gonads as well as pituitary functions. Although the correlation between serum ferritin level and T response to hCG was not highly significant, there might be a threshold level of iron overload (as measured by ferritin level), above which hCG treatment will not work.

Collectively, our findings proved that the presence of defective T secretion, in addition to hypogonadotropic hypogonadism, in a considerable number of adolescent boys with thalassemia (5/12) who had high iron overload. In these patients, hCG therapy failed to support genital development or stimulate adequate T secretion necessary for linear growth and bone mineral accretion. Testosterone therapy (replacement) is required at the proper time to support these functions. Human chorionic gonadotropin therapy was effective to induce pubertal development in all adolescents with CDGP without any significant side effects.

The increase in T secretion after 6 months of hCG therapy was significantly correlated with the change in their testicular diameter and T response after 1 month of hCG therapy in thalassemic patients (n = 12, $r = 0.26$ and 0.86, respectively, $P < .01$) as well as in all studied

patients (n = 22, $r = 0.21$ and 0.91, respectively, $P < .01$). These data validated the use of 1 month of hCG therapeutic test as a predictor of the long-term response therapy in boys with delayed puberty.

Dynamic interactions among growth hormone, IGF-1, and sex steroid hormones have a major role in the achievement of full height potential and the body composition changes in adolescence. Testosterone and estrogen affect the growth hormone's neuroendocrine rhythms, and growth hormone, in turn, potentiates many of the metabolic actions of the sex steroids. In this study, IGF-1 level was significantly lower in the thalassemic group compared with the CDGP group despite their normal GH response to clonidine stimulation. Hepatic siderosis, and consequently, impaired hepatic IGF-1 synthesis, partially explains this decreased IGF-1 concentration in thalassemia. The significant correlation between IGF-1 and ferritin levels and the fact that those with higher serum ferritin level (n = 5) were shorter that those with lower ferritin level (n = 7) suggested that growth axis is deleteriously affected in the poorly chelated patients.

Many studies showed evidence of a dysregulation in growth hormone secretory production in adolescents with CDGP, and subsequently, low IGF-1 concentration for age, which may be the cause of poor growth and short stature. This defect may be overcome by T, growth hormone treatment, or both, depending on the age of the patients [29-34].

During puberty, there is a well-established physiological association between the amount of growth hormone produced and the gonadal steroids produced. The observation of at least a doubling of growth hormone production in puberty in boys and girls has been confirmed in human studies. When prepubertal boys are given exogenous T therapy or are compared with fully pubertal adolescent boys of the same age, there is a significant increase in the amount of growth hormone that can be measured by radioimmunoassay during frequent sampling studies in the presence of T. It is clear that the impact of T on the ultradian rhythm of growth hormone is mostly the result of amplification of the growth hormone mass (pulse amplitude). Longitudinal studies of normal boys observed for many years throughout puberty demonstrate that peak growth hormone production rates coincide with the peak height velocity of males [29,35-38]. After 6 months of hCG therapy, T and IGF-1 concentrations increased significantly in the CDGP group and in some of the thalassemic patients (7/12). Acceleration of linear growth and increased HtSDS occurred only in these subjects but not in the thalassemic group with poor T response. IGF-1 concentrations were significantly correlated with increments of T concentrations and testicular diameter in thalassemic ($r = 0.65$ and 0.47, respectively, $P < .01$) and nonthalassemic adolescents ($r = 0.82$ and 0.59, respectively, $P < .001$). After 6 months of hCG therapy, the linear GV was significantly correlated with IGF1 concentrations as well as with increment of testicular size in thalassemic patients (n = 12, $r = 0.48$ and 0.65, respectively, $P < .001$) and in all treated patients (n = 22, $r = 0.52$ and 0.76, respectively, $P < .01$), but not with circulating T concentration.

These findings can be explained by the anabolic effects of the endogenously secreted T on linear growth through augmenting spontaneous GH secretion and IGF-1 production. However, the rise in T and IGF-1 concentrations and the acceleration of GV were significantly lower in thalassemic boys vs those with CDGP. Thalassemic boys who did not respond to hCG therapy had lower peak GH response to provocation as well as lower IGF-1 concentration after hCG, suggesting significant affection of the GH-IGF-1 axis as well. In support of this view, Bozzola et al [39] showed that treatment with hCG significantly increased GH response to L-dopa administration as well as sleep GH secretion and increased serum growth factors, evaluated as thymidine activity during deep sleep in thalassemic patients. However, the ability of T to stimulate pituitary GH secretion seems to be a transient phenomenon expressed only early in puberty, because GH and IGF-1 levels fall significantly during late puberty and adulthood despite continued high concentrations of gonadal steroid hormones [20,40].

The BMI was significantly correlated with the change in the testicular diameter after 6 months of hCG therapy (n = 22, $r = 0.45$, $P < .001$). Testosterone supplementation in other several human models, including aging men, healthy young men, men with muscular dystrophy, hypogonadal men, and prepubertal boys, has been shown to have significant anabolic effect with increases in fat-free mass [41-54]. In concert with our study, a longitudinal study that monitored the effects of low-dose T treatment in adolescents boys with delayed puberty showed increased fat-free mass and decreased postabsorptive proteolysis and protein oxidation, demonstrating sparing of protein breakdown and increased weight gain in these patients [50]. In addition, studies have suggested that androgens have many other important physiological actions, including effects on sexual function, muscle, body composition, bone, bone marrow, prostate, and the central nervous system [42,50-53].

Data are sparse regarding the effects of sex steroids on some of these target systems in thalassemic patients. However, recent data have shown that adolescents with thalassemia have decreased bone mineral density and muscle mass and have some degenerative effect on their nervous system evidenced in magnetic resonance imaging of their brain [54-58]. Hypogonadism seems to play an important role in the development of osteopenia-osteoporosis in thalassemia major; continuous hormone replacement therapy with transdermal estrogen for women or hCG for responding men can improve the bone density parameters [58].

Moreover, men with thalassemia had high incidence of azospermia and oligospermia [12]. In rodent testis, the demise of spermatogonia, spermatocytes, and spermatids was shown to occur through an apoptotic mechanism regulated by gonadotropins and androgens. In rat and human testes, hypophysectomy or treatment with a GnRH antagonist as well as gonadotropin ablation induces germ-

cell apoptosis. This effect is partially inhibited with T or hCG. A decrease in the T concentration induces a significant increase in the number of apoptotic germ cells in most stages of tdhe cycle of the seminiferous epithelium. These data suggest that androgens (stimulated by LH/hCG) are indispensable for the maintenance of spermatogenesis [26-28,59]. Replacement with hCG and/or T early in the process of sexual maturation might as well support the development of seminiferous epithelium and spermatogenesis. In this study and others [12], the enlargement of the testicles after hCG therapy (mainly due to growth of the seminiferous tubules and increased vascularity) in the presence of increased endogenous T secretion seems to be more physiological compared with exogenous T therapy. Testosterone replacement alone has been shown to decrease the testicular volume and sperm count in men with thalassemia compared with gonadotrophin therapy [12]. Again, failure of inducing proper spermatogenesis, even with long-term gonadotropin therapy, in some thalassemic men, suggested significant dysfunction of seminiferous epithelium, which might be secondary to siderosis or excessive apoptosis [12,60].

In summary, our adolescent boys with thalassemia have high incidence of delayed puberty and defective linear growth. Measurement of basal serum gonadotropin and T revealed prepubertal levels denoting hypogonadotropic hypogonadism and/or marked delay of hypothalamic-pituitary-gonadal axis. Treatment with hCG for 6 months or more proved that 7 (58%) of the 12 adolescents boys with thalassemia responded to this therapy by increasing their linear GV, accompanied with significant increase in their testicular size and T secretion. In these patients, the etiology of hypogonadism appeared to be mainly due to hypogonadotropic hypogonadism secondary to pituitary siderosis. In the other thalassemic patients (5/12, 46%), with poor iron chelation, hCG therapy was unsuccessful, denoting defective testicular function (siderosis). In the first subgroup of patients, hCG therapy can be used for the treatment of their hypogonadism, whereas T replacement is the treatment of choice for the second subgroup.

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