

**Research paper** **$\beta$ -thalassemia and gonadal axis: a cross-sectional, clinical study in a Greek population**

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**ABSTRACT**

$\beta$ -thalassemia ( $\beta$ -thal) is characterized by disturbances of the reproductive system. The aim of the present study was: 1) to assess the hypothalamic – pituitary - gonadal axis in patients with  $\beta$ -thal in relation to their phenotype and 2) to determine prognostic features of current gonadal status. We studied 135 patients (67 males and 68 females) with  $\beta$ -thal through history, physical examination, spermiograms and GnRH test. These patients were divided into  $\beta$ -thal major (51 males and 62 females) and  $\beta$ -thal intermedia phenotypes (16 males and 6 females). Male patients with  $\beta$ -thal major were subdivided into three groups a) eugonadal (35%, Tanner's stage V, normal testicular volume, normal spermiograms, normal basal and stimulated hormone values), b) patients with hypogonadotrophic hypogonadism (HH) of late onset (24%, Tanner's stage II-V, low-normal testicular volume, abnormal spermiograms, normal basal gonadotrophin values and abnormal response to GnRH test) and c) patients with HH of early onset (41%, Tanner's stage I, small testicular volume, abnormal spermiograms, abnormal basal and stimulated hormone values). Female patients with  $\beta$ -thal major were subdivided into: a) eugonadal (32%, Tanner's stage V, regular menstruation, normal basal and stimulated hormone values), b) patients with hypogonadotrophic hypogonadism (HH) of late onset (34%, Tanner's stage II-V, secondary amenorrhea, subnormal basal and stimulated gonadotrophin values) and c) patients with HH of early onset (34%, Tanner's stage I, primary amenorrhea, subnormal basal and stimulated hormone values). Patients with  $\beta$ -thal intermedia were subdivided into eugonadal (75% of males - 33% of females) and hypogonadal (25% of males - 67% of females). Current gonadal status could not be predicted by means of transfusion or chelation parameters. In conclusion,  $\beta$ -thal patients could be eugonadal or develop early or late onset HH.  $\beta$ -thal intermedia patients have a more favorable profile than  $\beta$ -thal major individuals. Current gonadal status of  $\beta$ -thal patients cannot be predicted by means of history, clinical or laboratory parameters.

**Key Words:**  $\beta$ -thalassemia major,  $\beta$ -thalassemia intermedia, gonadal axis, GnRH test, spermiogram

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**INTRODUCTION**

Homozygous beta-thalassemia ( $\beta$ -thal), an autosomal recessive disorder, is characterized by a reduction or absence of normal  $\beta$ -globin synthesis<sup>1</sup>. The

genes for  $\beta$ -thal are prevalent in several ethnic groups distributed in a broad geographic belt from the Mediterranean basin through the Middle East and the Indian subcontinent and into southeast Asia<sup>2</sup>.  $\beta$ -thal is a serious genetic disorder which results in a considerable increase in both acute and chronic morbidity and mortality<sup>3</sup>. Failure of pubertal growth, delay or absence of sexual development, amenorrhea, sexual dysfunction and infertility due to hypogonadism are well-recognized disturbances of the hypothalamic – pituitary – gonadal axis in  $\beta$ -thal patients<sup>4</sup>. Aberrant gonadotrophin response to GnRH administered acutely and in a pulsatile fashion strongly indicates failure of gonadotroph cells, which seem to be extremely vulnerable to iron damage<sup>5</sup>.

$\beta$ -thal has extremely variable clinical phenotypes. Extensive investigations carried out in the last two decades have to a great extent defined the molecular basis of the phenotypic heterogeneity of  $\beta$ -thal syndromes. Differentiation between the main clinical phenotypes of  $\beta$ -thal major and  $\beta$ -thal intermediate is critical for appropriate treatment.

In the past, children with  $\beta$ -thal rarely survived beyond adolescence<sup>6</sup>. The improved expectancy and quality of life in  $\beta$ -thal patients due to introduction in the late 1970s of regular optimum red blood cell transfusions and almost daily subcutaneous iron chelation therapy made the necessity for normal reproductive and sexual life more pressing<sup>7</sup>.

Although thousands of people worldwide have  $\beta$ -thal and therefore are at increased risk for reproductive health problems, the literature on these issues is scarce, particularly the relation between clinical phenotype and gonadal axis status.

The aim of the present study was: 1) to assess the hypothalamic – pituitary - gonadal axis in patients with  $\beta$ -thal according to their phenotype and 2) to relate history, clinical and laboratory parameters with current gonadal status in an attempt to define prognostic factors of current gonadal status.

## SUBJECTS & METHODS

**Patients.** The study group included 135 patients (67 males and 68 females) with  $\beta$ -thal. The patients were divided into two groups: 113 patients with  $\beta$ -thal major (51 males and 62 females) and 22 patients with  $\beta$ -thal intermedia (16 males and 6 females). Inclusion

criterion for the  $\beta$ -thal intermedia subgroup was the ability to maintain a hemoglobin level compatible with comfortable survival in the absence of regular transfusions. The 135 patients constituted the total number of referrals to the Unit of Reproductive Endocrinology from five district Thalassemia Units, throughout Northern Greece, during a decade (1991-2000). Patients' characteristics are given in table 1.

**Study characteristics.** Cross-sectional, clinical - laboratory study.

**Methods.** A uniform questionnaire was used in order to collect history information. Sexual maturation was assessed using the criteria proposed by Tanner<sup>8</sup>. Testicular volume was evaluated by the Prader orchidometer. All patients were clinically evaluated by a single experienced physician (J.P.).

The Unit of Reproductive Endocrinology where the study was conducted is the referral infertility center for the whole of Northern Greece. Although every possible effort has been made for all  $\beta$ -thal patients to be referred to the Unit for gonadal axis evaluation regardless of their gonadal status, there was no way of confirming that this had actually happened. Thus, our population can be described most accurately as a selective cohort of Greek patients with  $\beta$ -thal.

Ferritin was measured in a random blood sample at the time of referral to our Unit. Therefore it is most probable that the ferritin values are not representative of the mean ferritin levels of the patient throughout the previous years.

A 100  $\mu$ g gonadotrophin-releasing hormone (GnRH) test (Relefact, Hoechst) was performed in order to evaluate pituitary reserve for gonadotrophins. Samples were obtained at 0, 30 and 60 minutes. Gonadotrophins were measured by ELISA (IMX Abbott Labs), reference range of basal values FSH: 1-15 mIU/ml (males), 2-15 mIU/ml (females, follicular phase), expected peak FSH: 10-35 mIU/ml (males and females), basal values LH: 3-25 mIU/ml (males), 2-20 mIU/ml (females, follicular phase), expected peak LH: 10-35 mIU/ml (males and females). Basal levels for free testosterone measured by ELISA: 16-39 ng/dl (males, 20 to 40 years old), estradiol measured by ELISA: 30-300 pg/ml (females, follicular phase) and sex-hormone binding globulin (SHBG) measured by ELISA: 20-130 nmol/l (females).

Sperm samples were collected by masturbation after 3-4 days of abstinence. All samples were evaluated according to World Health Organization (WHO) criteria<sup>9</sup>. A minimum of two spermograms was obtained at 3- month intervals.

**Statistics.** Data are expressed as mean ± standard error of the mean (SEM). Chi-square test was used in order to search for differences regarding the means in nominal parameters whereas Mann-Whitney U (for comparisons between 2 groups) and Kruskal-Wallis (for comparisons in more than 2 groups) were used for interval data. A stepwise discriminant analysis was performed using the Wilks' Lambda statistic in order to weigh the addition or removal of variables from the procedure. Statistical analysis was performed using the SPSS 10 statistical package (SPSS Inc, Chicago, Ill.).

**RESULTS**

**Clinical features and laboratory profiles.** On the basis of clinical and laboratory parameters, male β-thal major patients were divided into three subgroups

(Table 2a). Group A (eugonadal): eighteen patients (35%) who had normal sexual development (Tanner's stage V), normal testicular volume, normal spermograms and normal basal and stimulated gonadotrophin values. Group B: (hypogonadotropic hypogonadism of late onset): twelve patients (24%) who had a variable degree of sexual development (Tanner's stage II-V), low-normal testicular volume, abnormal spermograms, normal basal gonadotrophin values and abnormal response to GnRH test (Figure 1). These patients had either arrested puberty (Tanner's stage II-IV) or full development of secondary sexual characteristics (Tanner's stage V) and subsequently developed HH. Group C: (HH of early onset): twenty-one patients (41%) who were prepubertal (Tanner's stage I) with small testes, abnormal spermograms and low basal and stimulated hormone values. On the basis of the same clinical and laboratory parameters, male β-thal intermedia patients were subdivided into eugonadal (n=12 - 75%) and hypogonadal (n=4 - 25%) (Table 2b) subjects.

Similarly, women with β-thal major were divided into three subgroups (Table 3a). Group A: (eugonad-

**Table 1.** Patients characteristics.

	males		females	
	β-thal major n=51 (76%)	β-thal intermedia n=16 (24%)	β-thal major n=62 (91%)	β-thal intermedia n=6 (9%)
age (years)	21.3±0.8	26.7±2.6	20.7±0.5	28.3±3.6
marriage	3 (6%)	1 (6%)	3 (5%)	3 (50%)
endocrinopathy				
hypothyroidism	2 (4%)	0 (0%)	1 (2%)	0 (0%)
diabetes mellitus	2 (4%)	0 (0%)	1 (2%)	0 (0%)
smoking	9 (18%)	3 (19%)	7 (11%)	1 (16%)
transfusions				
starting age (years)	2.3±0.4	6.6±1.7	1.6±0.2	6.0±1.0
hemoglobin (g/dl)	9.8±0.2	10.2±0.3	9.9±0.1	9.3±1.1
chelation therapy				
starting age (years)	9.9±0.9	20.0±3.4	7.4±0.6	-
ferritin (ng/ml)	3472±390	2487±1167	2742±211	2416±369
antibodies				
HBsAg positive	3 (6%)	0 (0%)	2 (3%)	0 (0%)
anti-HCV positive	7 (14%)	0 (0%)	11 (18%)	0 (0%)
surgery				
splenectomy	18 (35%)	11 (69%)	21 (34%)	4 (67%)
cholecystectomy	3 (6%)	3 (19%)	6 (10%)	2 (33%)

Data are given as mean±SEM of absolute value (percentage). β-thal: β-thalassemia, HBsAg: hepatitis B surface antigen, HCV: hepatitis C virus.

**Table 2a.** Studied parameters in male patients with  $\beta$ -thal major.

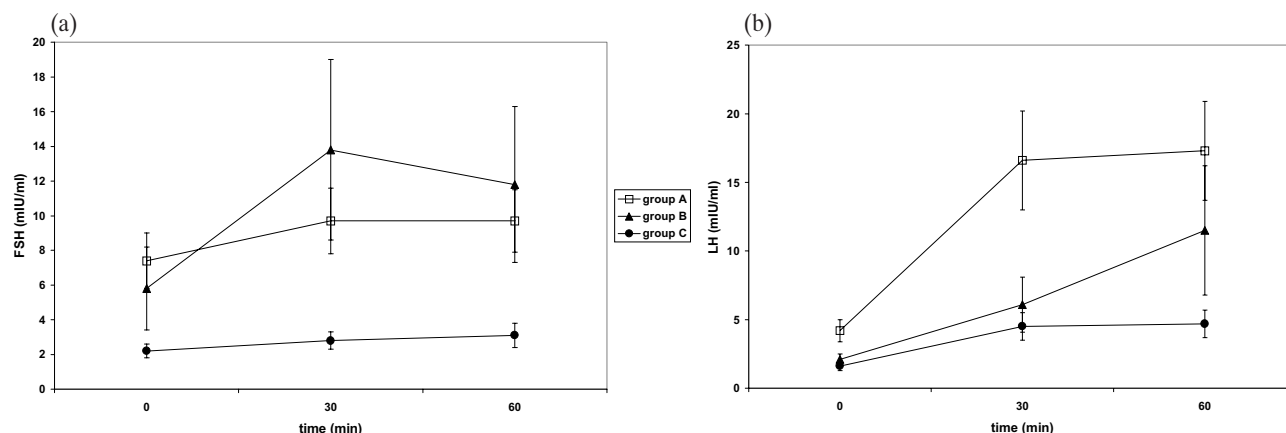
	group A	group B	group C	p value
	eugonadal n=18 (35%)	HH-late onset n=12 (24%)	HH-early onset n=21 (41%)	
age	22.4±1.2	22.5±2.1	19.6±0.9	NS
<b>transfusion</b>				
starting age (years)	2.7±1.0	3.0±0.6	1.6±0.4	NS
sessions per month	2.2±0.2	1.4±0.3 (*)	2.9±0.8	0.05
hemoglobin (g/dl)	9.7±0.6	9.5±0.6	9.9±0.2	NS
<b>chelation therapy</b>				
starting age (years)	11.4±1.3	11.8±1.8	7.7±1.3 (*)	0.05
sessions per week	2.5±0.6	3.2±0.9	3.7±0.7	NS
ferritin (ng/ml)	4331±927	2088±550	3574±469	NS
time gap (years)	9.3±1.5	8.8±1.9	5.8±1.4 (*)	NS
<b>clinical characteristics</b>				
Tanner stage	V	II-V	I	
mean testicular volume (ml)	21.3±1.0	12.4±1.5 (*)	5.1±0.8 (*)	0.001
<b>semen parameters</b>				
volume (ml)	2.2±0.3	1.3±0.4	1.3±0.3	NS
sperm count (x 10 <sup>6</sup> /ml)	65.0±14.3	6.7±4.1 (*)	10.0±7.3 (*)	0.05
motility at 1 h (%)	54.2±4.2	46.7±12.0	22.0±12.0 (*)	0.05
morphology (% normal)	40.2±6.8	9.7±6.8 (*)	5.3±3.7 (*)	0.001
<b>hormonal profile</b>				
<b>GnRH test</b>				
FSH at baseline (mIU/ml)	7.4±1.6	5.8±2.4	2.2±0.4 (*)	0.01
FSH at 30 min (mIU/ml)	9.7±1.9	13.8±5.2	2.8±0.5 (*)	0.01
FSH at 60 min (mIU/ml)	9.7±1.8	11.8±4.5	3.1±0.7 (*)	0.01
LH at baseline (mIU/ml)	4.2±0.8	2.1±0.4	1.6±0.3 (*)	0.01
LH at 30 min (mIU/ml)	16.6±3.6	6.1±2.0 (*)	4.5±1.0 (*)	0.001
LH at 60 min (mIU/ml)	17.3±3.6	11.5±4.7	4.7±1.0 (*)	0.001
free T at baseline (ng/dl)	16.1±3.6	9.8±5.8 (*)	9.2±4.3 (*)	0.05

Data are given as mean±SEM. p value (Kruskal-Wallis test) refers to comparison among all study groups. (\*) denotes statistically significant differences (p<0.05) vs. group A (Mann-Whitney U test). Time gap refers to interval between transfusion start and chelation start. In order to convert free T from ng/dl to nmol/l multiply by 0.03467.

al): twenty patients (32%) who had normal sexual development (Tanner's stage V), regular menstruation and normal basal and stimulated gonadotrophin values. Group B (HH of late onset): twenty-one patients (34%) who had a broad spectrum of sexual development (Tanner's stage II-V) with secondary amenorrhea and subnormal basal and stimulated gonadotrophin values (Figure 2). These patients had either arrested puberty (Tanner's stage II-IV) or full development of secondary sexual characteristics (Tanner's stage V) followed by the development of HH at a later age. Group C (HH of early onset): twenty-one pa-

tients (34%) who were prepubertal (Tanner's stage I) and had subnormal basal and stimulated gonadotrophin values. On the basis of the same clinical and laboratory parameters, female  $\beta$ -thal intermedia patients were subdivided into eugonadal (n=2 - 33%) and hypogonadal (n=4 - 67%) (Table 3b).

Males with  $\beta$ -thal intermedia had higher age at referral, starting age of transfusions, starting age of chelation therapy and lower risk of hepatitis B and hepatitis C antibody positivity as compared to males with  $\beta$ -thal major (Mann-Whitney U, p<0.05, Table 1). In



**Figure 1.** GnRH test results in male patients with β-thal major - (a) FSH and (b) LH values. Group A: eugonadal, Group B: hypogonadotropic hypogonadism - late onset, Group C: hypogonadotropic hypogonadism - early onset.

addition, males with β-thal intermedia as compared to β-thal major males had greater mean testicular volumes, higher basal and stimulated LH levels, higher basal free T levels and reduced risk of being hypogonadal (Tables 2a and 2b). There was no statistically significant difference in sperm parameters.

Similarly, females with β-thal intermedia were older at referral, at first transfusion and at start of chelation therapy as compared to females with β-thal major (Mann-Whitney U,  $p < 0.05$ , table 1). They were less frequently transfused and they presented lower risk for hepatitis B and hepatitis C as predicted by antibody positivity. On the other hand, no statistically significant differences in clinical or laboratory parameters were demonstrated in females with β-thal intermedia as compared to β-thal major females (tables 3a and 3b).

**Prognostic factors for gonadal status.** By means of a discriminant analysis, there was no parameter from patients' history that could predict current gonadal status of β-thal patients. Moreover, there was no statistically significant correlation between gonadal status and starting age of transfusion therapy, transfusion pattern, serum hemoglobin, starting age of chelation therapy, chelation therapy pattern or serum ferritin. Likewise, there was no significant correlation between current gonadal status and time interval between starting age of transfusion and starting age of chelation therapy.

On the other hand, there was statistically significant correlation between testicular volume and semen parameters (sperm count, motility at 1 hour and mor-

**Table 2b.** Studied parameters in male patients with β-thal intermedia

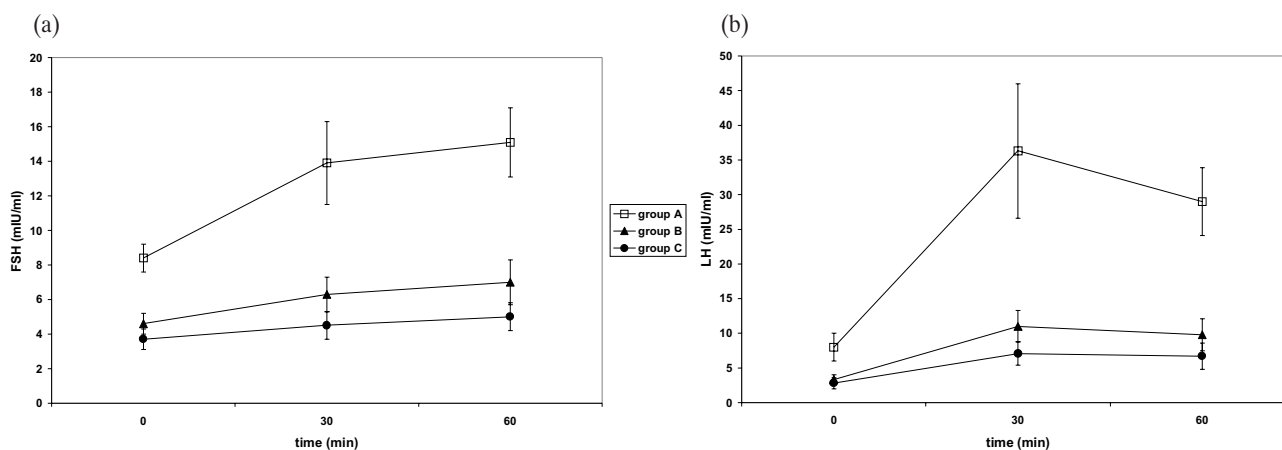
	group A	group B	p value
	eugonadal n=12 (75%)	hypogonadal n=4 (25%)	
age	26.5±3.1	27.3±4.9	NS
<b>transfusion</b>			
starting age (years)	7.0±2.3	5.5±1.7	NS
sessions per month	1.8±0.2	-	-
hemoglobin (g/dl)	10.2±0.3	-	-
<b>chelation therapy</b>			
starting age (years)	19.4±4.3	21.8±6.1	NS
sessions per week	3.2±0.8	-	-
ferritin (ng/ml)	3166±1342	-	-
time gap (years)	11.4±3.3	15.5±4.8	NS
<b>clinical characteristics</b>			
Tanner stage	V	I-V	
mean testicular volume (ml)	24.2±0.3	6.8±2.8	0.001
<b>semen parameters</b>			
volume (ml)	2.4±0.5	1.2±0.4	NS
sperm count (x 10 <sup>6</sup> /ml)	59.8±18.4	-	-
motility at 1 h (%)	38.9±6.6	-	-
morphology (% normal)	40.2±6.8	-	-
<b>hormonal profile</b>			
<b>GnRH test</b>			
FSH at baseline (mIU/ml)	7.0±4.1	6.1±3.8	NS
FSH at 30 min (mIU/ml)	19.5±10.9	8.6±6.0	NS
FSH at 60 min (mIU/ml)	9.3±3.5	12.0±8.7	NS
LH at baseline (mIU/ml)	4.9±1.2	13.3±11.7	NS
LH at 30 min (mIU/ml)	20.3±5.5	11.8±6.5	NS
LH at 60 min (mIU/ml)	17.1±5.6	17.6±11.1	NS
free T at baseline (ng/dl)	30.3±7.1	13±3.5	0.01

Data are given as mean±SEM. Time gap refers to interval between transfusion start and chelation start. In order to convert free T from ng/dl to nmol/l multiply by 0.03467.

**Table 3a.** Studied parameters in female patients with  $\beta$ -thal major.

	group A	group B	group C	p value
	eugonadal n=20 (32%)	HH-late onset n=21 (34%)	HH-early onset n=21 (34%)	
age	21.2 $\pm$ 1.2	21.9 $\pm$ 0.8	19.2 $\pm$ 0.4	NS
<b>transfusion</b>				
starting age (years)	2.3 $\pm$ 0.4	1.6 $\pm$ 0.4	0.9 $\pm$ 0.2 (*)	0.01
sessions per month	1.5 $\pm$ 0.1	1.7 $\pm$ 0.2	1.5 $\pm$ 0.2	NS
hemoglobin (g/dl)	9.6 $\pm$ 0.2	10.2 $\pm$ 0.2 (*)	10.1 $\pm$ 0.2 (*)	0.05
<b>chelation therapy</b>				
starting age (years)	9.0 $\pm$ 1.1	7.1 $\pm$ 1.0	6.3 $\pm$ 0.8	NS
sessions per week	5.5 $\pm$ 2.9	1.7 $\pm$ 0.4	3.7 $\pm$ 2.3	NS
ferritin (ng/ml)	2769 $\pm$ 320	2694 $\pm$ 428	2762 $\pm$ 370	NS
time gap (years)	6.7 $\pm$ 0.9	5.5 $\pm$ 1.0	5.3 $\pm$ 0.9	NS
<b>history</b>				
menarche (years)	14.4 $\pm$ 0.4	13.6 $\pm$ 0.3 (*)	16.5 $\pm$ 0.7 (*)	0.01
length of cycle (days)	36.0 $\pm$ 3.7	50.6 $\pm$ 7.1	-	NS
duration of menses (days)	5.3 $\pm$ 0.2	5.0 $\pm$ 0.4	-	NS
<b>clinical characteristics</b>				
Tanner stage	V	II-V	I	
<b>hormonal profile</b>				
<b>GnRH test</b>				
FSH at baseline (mIU/ml)	8.4 $\pm$ 0.8	4.6 $\pm$ 0.6 (*)	3.7 $\pm$ 0.6 (*)	0.001
FSH at 30 min (mIU/ml)	13.9 $\pm$ 2.4	6.3 $\pm$ 1.0 (*)	4.5 $\pm$ 0.8 (*)	0.001
FSH at 60 min (mIU/ml)	15.1 $\pm$ 2.0	7.0 $\pm$ 1.3 (*)	5.0 $\pm$ 0.8 (*)	0.001
LH at baseline (mIU/ml)	8.0 $\pm$ 2.0	3.3 $\pm$ 0.7 (*)	2.8 $\pm$ 0.8 (*)	0.01
LH at 30 min (mIU/ml)	36.3 $\pm$ 9.7	11.0 $\pm$ 2.3 (*)	7.1 $\pm$ 1.7 (*)	0.001
LH at 60 min (mIU/ml)	29.0 $\pm$ 4.9	9.8 $\pm$ 2.3 (*)	6.7 $\pm$ 1.9 (*)	0.001
E <sub>2</sub> at baseline (pg/ml)	196.0 $\pm$ 42.7	40.5 $\pm$ 8.7 (*)	46.6 $\pm$ 20.7 (*)	0.001
SHBG at baseline (nmol/l)	75.0 $\pm$ 21.1	96.1 $\pm$ 52.7	63.5 $\pm$ 30.0	NS

Data are given as mean $\pm$ SEM. p value (Kruskal-Wallis test) refers to comparison among all study groups. (\*) denotes statistically significant differences (p<0.05) vs. group A (Mann-Whitney U test). Time gap refers to interval between transfusion start and chelation start. In order to convert E2 from pg/ml to pmol/l multiply by 3.671.



**Figure 2.** GnRH test results in female patients with  $\beta$ -thal major - (a) FSH and (b) LH values. Group A: eugonadal, Group B: hypogonadotropic hypogonadism - late onset, Group C: hypogonadotropic hypogonadism - early onset.

**Table 3b.** Studied parameters in female patients with β-thal intermedia.

	group A	group B	p value
	eugonadal n=2 (33%)	hypogonadal n=4 (67%)	
age	28.5±0.5	28.3±5.7	NS
<b>transfusion</b>			
starting age (years)	-	-	-
sessions per month	-	1.2±0.8	-
hemoglobin (g/dl)	8.3±0.3	-	-
<b>chelation therapy</b>			
starting age (years)	20.0±7.0	28.3±6.8	NS
sessions per week	-	-	-
ferritin (ng/ml)	2131±131	2700±800	NS
time gap (years)	-	-	-
<b>history</b>			
menarche (years)	15.0±1.0	14.8±0.4	NS
length of cycle (days)	-	-	-
duration of menses (days)	-	4.5±0.5	-
<b>clinical characteristics</b>			
Tanner stage	V	I-V	
<b>hormonal profile</b>			
<b>GnRH test</b>			
FSH at baseline (mIU/ml)	8.3±1.4	1.4±0.6	NS
FSH at 30 min (mIU/ml)	-	1.8±0.6	-
FSH at 60 min (mIU/ml)	-	2.0±0.8	-
LH at baseline (mIU/ml)	3.2±0.6	0.9±0.5	NS
LH at 30 min (mIU/ml)	-	3.1±1.4	-
LH at 60 min (mIU/ml)	-	2.9±1.4	-
E <sub>2</sub> at baseline (pg/ml)	-	77.0±64.1	-
SHBG at baseline (nmol/l)	-	118.5±11.5	-

Data are given as mean±SEM. Time gap refers to interval between transfusion start and chelation start. In order to convert E2 from pg/ml to pmol/l multiply by 3.671.

phology - Kendall's tau-b, p<0.05), as well as testicular volume and hormone values (FSH / LH at 0, 30 and 60 minutes and free testosterone at 0 minutes - Kendall's tau-b, p<0.01).

**Other results.** Four of the β-thal male patients were married (one β-thal major eugonadal, one β-thal intermedia eugonadal and two β-thal major HH-late onset) but none had children. On the other hand, six of the β-thal female patients were married (four eugonadal and two HH-late onset) and four of them have given birth to five children (two β-thal major eugo-

nadal patients and one β-thal intermedia eugonadal patient with one child each and one β-thal intermedia eugonadal patient with two children).

**DISCUSSION**

Approximately 10% of patients with homozygous β-thal exhibit a phenotype characterized by intermediate hematologic severity due to a less severe defect in β-globin synthesis than in typical β-thal major. The patients with β-thal intermedia have a milder clinical phenotype as they co-inherit a form of α-thalassemia or they carry β-thal chromogenes with a higher than usual potential for high levels of γ-globin expression. Some of these patients can survive well into adult life, usually with normal onset and progression of puberty. As patients with β-thal intermedia pose therapeutic dilemmas and constitute a group of special interest among physicians involved with thalassemia, we have studied them as a separate subgroup.

In our study, 65% of male and 68% of female patients with β-thal major developed HH in contrast to 25% of males and 67% of females with β-thal intermedia. Tolis et al. have reported a higher incidence of hypogonadism in Greek subjects. In their series of 410 female β-thal patients, gonadal dysfunction, manifested as menstrual irregularities and hypoenstrogonemia was demonstrated in 95% of cases. However, only 74% of patients developed primary or secondary amenorrhea<sup>10</sup>. In a previous study of our group, 40% of male patients with β-thal were found to be hypogonadal<sup>11</sup>.

A direct comparison with non-Greek studies is not straightforward due to the great variety of β-thal genotypes and phenotypes in different parts of the world. In any case, Kwan et al. have reported similar results; 62% of boys and 75% of girls over the age of 12 years had HH, despite regular transfusion and chelation therapy<sup>12</sup>. Borgna-Pignatti et al. found in their β-thal group a complete lack of pubertal changes in 38% of females and 67% of males aged 12 to 18 years. Only 19% of females had experienced menarche but a third of them developed secondary amenorrhea.

The majority of researchers agree that in patients with β-thal intermedia, gonadal function is preserved and pubertal timing is not disturbed<sup>14</sup>. We have found a statistically significant lower incidence of hypogonadism in β-thal intermedia male patients as compared

to  $\beta$ -thal major individuals. We were not able to demonstrate the same effect in female patients, possibly due to small sample size.

The damage to the hypothalamic – pituitary – gonadal axis is most likely localized at a central level. The classic knowledge is that in transfusion-dependent  $\beta$ -thal patients, increased iron deposition in the pituitary gland has a cytotoxic effect, leading mainly to HH due to pituitary hyporesponsiveness to GnRH<sup>15</sup>. HH does not respond to chelation therapy given late in the course of the disease<sup>16</sup>. In our study, neither male nor female patients were found to have hypergonadotrophic hypogonadism. In general, hypogonadal male patients with  $\beta$ -thal intermedia had higher basal and stimulated LH levels than hypogonadal males with  $\beta$ -thal major (tables 2a and 2b) suggesting higher pituitary reserve. On the other hand, higher LH plasma levels do not necessarily denote better pituitary gonadotroph function as  $\alpha$ -subunit abnormalities have been suggested in previous studies<sup>17</sup>.

Hyperprolactinemia seems not to be implicated in the pathogenesis of HH in  $\beta$ -thal patients<sup>15</sup>. In all male and female hypogonadal patients in whom plasma prolactin levels were measured, they were found to be within the normal range (data not shown).

The progressive nature of the disease makes all eugonadal male  $\beta$ -thal patients candidates for developing premature forms of partial androgen deficiency of the aging male (PADAM). Therefore, these patients must enter a follow-up program in order for hypogonadism to be diagnosed early and treated appropriately.

The study was not designed to detect fertility rate in  $\beta$ -thal patients. Due to small sample size, no useful conclusions can be drawn regarding the clinical and laboratory characteristics of  $\beta$ -thal patients who became parents. However, the low marriage rate (5-6%) in  $\beta$ -thal patients cannot be attributed exclusively to hypogonadism. Previous studies have shown that  $\beta$ -thal patients experience substantially more emotional problems, social isolation and stigmatization than their peers, suggesting that these people have limited opportunities for normal sexual behavior and social development<sup>18,19</sup>.

In our study, a discriminant analysis failed to determine parameters that could predict current gonadal status. A literature review revealed that other re-

searchers experienced similar difficulties. Weintrob et al. suggested that beginning chelation treatment with desferrioxamine before the onset of puberty can help children with transfusion-dependent  $\beta$ -thal to attain normal sexual maturation<sup>20</sup>. Conversely, Soliman et al. stated that despite regular blood transfusions and desferrioxamine treatment, growth impairment and pubertal delay are commonly seen in children and adolescents with transfusion-dependent  $\beta$ -thal<sup>21</sup>. A multiple regression analysis of indicators of pubertal development with age, age at first transfusion, age at splenectomy, number of transfusions, serum transaminase and ferritin, and duration and intensity of chelation therapy failed to identify the factors responsible for the variation observed in sexual maturation among patients with  $\beta$ -thal<sup>13</sup>. To the best of our knowledge, there are no studies that identified prognostic parameters for gonadal status in patients with  $\beta$ -thal.

In conclusion, we conducted a cross-sectional study in 135 Greek patients with  $\beta$ -thal major and  $\beta$ -thal intermedia. The former can be classified by means of clinical and laboratory criteria into three groups: eugonadal patients and patients with early or late onset HH, whereas the latter can be classified into eugonadal and hypogonadal.  $\beta$ -thal intermedia patients have a more favorable profile than  $\beta$ -thal major individuals regarding gonadal function. No parameter from patients' histories can predict current gonadal status. Nevertheless, modern state-of-the-art management has contributed towards an improvement in quality of life in patients with  $\beta$ -thal thus making the desire for normal reproductive and sexual life more pressing.

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