Assessment of Puberty in Relation to L-carnitine and Hormonal Replacement Therapy in β-thalassemic Patients


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Summary
Objective: To investigate puberty in a group of thalassemic patients with delayed or arrested pubertal development and to compare the effects of hormonal and L-carnitine therapy on puberty in those patients.
Patients: Thirty-two β-thalassemic patients with arrested or failure of puberty were enrolled for 1 year in this study.
Method: Clinical pubertal assessment and laboratory investigations were done for all patients at the beginning, 6 months later clinical pubertal assessment was done. Patients were divided into two groups (16 each): first group received L-carnitine therapy, while the second group received hormonal therapy. Pubertal and laboratory assessment were done 6 months after hormonal and L-carnitine therapy.
Results: Failure of puberty was confirmed in 71.4% of boys and 33.3% of girls, while arrested puberty was observed in 28.6% of boys and 66.7% of girls. All girls had amenorrhea, primary amenorrhea in 88.9% and secondary amenorrhea in 11.1%. Menses occurred in 20% of female patients after L-carnitine therapy and in 37.5% of them after hormonal therapy. Improvement of pubertal staging was observed in 50% of males after L-carnitine therapy compared to 75% of them after hormonal therapy. While improvement of pubertal staging was seen in 90% of females after L-carnitine therapy compared to 100% of females after hormonal treatment. However, these results showed no significant difference between both groups.
Conclusion: Delayed puberty in β-thalassemia major is either due to failure of gonads or failure of the whole hypothalamic pituitary gonadal axis. L-carnitine as well as hormonal replacement therapy had a positive effect on puberty in the thalassemic patients. Further studies are needed to clarify the role of L-carnitine on puberty in these patients.

Key words: puberty, L-carnitine, hormonal therapy, β-thalassemic.

Introduction
Present transfusional regimen protocols have increased the life expectancy of patients with β-thalassemia major but caused a progressive iron overload that can be prevented or limited only by appropriate iron chelation [1]. As a result of iron overload those patients develop hepatic cirrhosis [2], endocrine abnormalities [3, 4] and cardiac failure [5].

Short stature and hypogonadism are extremely frequent in patients with thalassemia [6]. Controversy still exists regarding the etiology of gonadal failure; primary gonadal failure due to iron deposits in the ovaries and testes and secondary gonadal failure due to siderosis of the pituitary gland [7].

Carnitine is a natural substance (3-hydroxy-4-N-trimethyl aminobutyric acid) synthesized in the liver, brain and kidney from protein bound lysine and methionine. Many authors reported that failure to thrive and developmental delay are associated with secondary carnitine deficiency and growth significantly improved after L-carnitine supplementation [8].

Genazzani et al. [9] reported that acetyl L-carnitine (ALC) has been used to modulate the inhibitory mechanisms responsible for the hypothalamic blockade of gonadotrophin releasing hormone (GnRH) activity. ALC is effective in increasing leutinizing hormone (LH) plasma levels and LH response to standard GnRH test.
Aim
The present study was designed to investigate puberty in a group of thalassemic patients with delayed or arrested pubertal development and also to compare the effects of hormonal and L-carnitine therapy on puberty in those patients.

Patients and Methods

Patients
Thirty-two β-thalassemic patients were enrolled in this study. They were recruited from the Hematology Clinics of Ain Shams and Cairo University Children hospitals. They were 14 males and 18 females. Not all patients were on regular transfusion therapy, while subcutaneous desferroxamine was given (40 mg kg⁻¹ day⁻¹) 5 days per week (it was received irregularly) and folic acid (5 mg) every 2 days orally.

Inclusion criteria.
(i) Age ≥14 years for both male and female β-thalassemic patients.
(ii) Delayed puberty defined as pubarche and gonadarche Tanner stage II or less at the age of 14 years or slow progression of puberty.

Exclusion criteria.
(i) Age <14 years for males and females.
(ii) Hepatic or cardiac decompensation.
(iii) Associated anaemia due to different etiology.
(iv) Severe malnutrition, diabetes and dysmorphic trait.

Methods
A written informed consent was obtained from all studied patients or their legal guardians before enrollment in the study. All patients were subjected to the following.

Thorough history and clinical examination.

Pubertal assessment. Rating of breast development in girls, genital development in boys, pubic and axillary hairs in both sexes were assessed according to Tanner’s classification [10]. Testicular volume in boys was also assessed by Prader’s Orchidometer. Patients enrolled either had:

(a) Lack of pubertal development: which was indicated by absence of breast development in girls by 13 years of age and lack of testicular enlargement in boys (<4 ml) as measured by Prader’s Orchidometer by 14 years of age [11].
(b) Arrested puberty: this was defined by lack of pubertal development for 12 months or longer, with reduced or absent growth velocity [12].
(c) Primary amenorrhea: any female who had not yet started her menarche by the age of 16 years [13].
(d) Secondary amenorrhea: this was defined as absent menses for a 6 months period at any time after menarche [12].

Laboratory investigations. An indwelling catheter was inserted in a forearm vein of all studied participants. A basal venous blood is taken for the following laboratory investigations: complete blood picture, liver transaminases (alanine transaminase and aspartate transaminase), serum ferritin, free T3, free T4, thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), LH, estradiol (in females) and testosterone (in males).

Investigations of hypothalamic-pituitary gonadal (HP) axis: (i) GnRH stimulation test: after taking a basal venous blood sample, GnRH (gonadorelin 100 µg; Ferring Gmbh) was administered intravenously. Blood samples were obtained 20, 40, 60, 90 and 120 min later to estimate FSH and LH in all these samples. (ii) Human chorionic gonadotrophin (HCG) stimulation test (in males): HCG 2500 µm⁻² was injected intramuscularly daily for 3 days. Serum testosterone concentrations were measured before injection and on the fourth day after injection.

Follow-up of the patients. Follow-up of the patients (6 months later, before starting treatment) for clinical pubertal assessment was done.

Therapy. To assess the impact of different therapeutic regimens on puberty, the studied patients were divided into two groups: the first group received L-carnitine therapy, the second group received hormonal therapy.
(a) L-carnitine therapy: Sixteen patients (6 males and 10 females) received L-carnitine orally at a dose of 50 mg kg\(^{-1}\) day\(^{-1}\) after meals divided into three doses for a period of 6 months (L-carnitine 350 mg capsules; MEPACO, Egypt).

(b) Hormonal therapy: Sixteen patients (8 males and 8 females) received hormonal therapy.

(1) Females: Conjugated equine estrogen (premarin, 0.625 mg day\(^{-1}\)) for 25 days per month was given orally for females who had not yet reached Tanner stage II at the start of the study. In the last 10 days of the month, Medroxy progesterone (provera, 5 mg day\(^{-1}\)) was added to conjugated estrogen for female patients who were either in Tanner stage II from the beginning of the study or reached that stage or above after estrogen therapy. Hormonal therapy was continued for 6 months.

(2) Males: Long acting testosterone (testosterone propionate 100–150 mg, IM every 4 weeks) was given to all male patients for 6 months.

Two female patients developed liver dysfunction 1 month after oral estradiol therapy and were put instead on estradiol transdermal therapeutic system (TTS). Patches with daily delivery of 100\(\mu\)g of 17\(\beta\) estradiol (Evorel\textsuperscript{a}, Estradiol Patch of Janssen-Cilag Ltd, England) were applied to the skin below the waist. They were changed twice per week on fixed days for 3 weeks. Patches were associated with complementary treatment of medroxy progesterone 5 mg daily for the last 10 days of each 4 weeks course.

In males Anapliotou et al. [14] had given human chorionic gonadotrophin therapy for patients with still functioning leydig cells as evidenced by HCG stimulation test. In the present study two males received continuous treatment of IM HCG 1500 IU (Pregnyl of Organon) twice per week for 6 months.

Follow up after 6 months. Follow up after 6 months period of therapy for clinical pubertal assessment was done. Laboratory assessment of basal LH, FSH, testosterone (in males) and estradiol (in females) was also done.

**Statistical analysis**

Statistical Science for Social Package (SPSS) software computer program version 9 was also used for data analysis. Data were presented as mean ± SE and range. For comparison of two groups the non-parametric test for dependent and independent variables was used. Linear Pearson’s correlation was done.

**Results**

Thirty-two patients with \(\beta\)-thalassemia major 18 females (56\%) and 14 males (44\%) were included in this study. Table 1 demonstrates the demographic characteristics of \(\beta\)-thalassemic patients. Table 2 shows the prevalence of pubertal disorders in male and female patients with \(\beta\)-thalassemia major. Failure of puberty was seen in 71.4\% of males and 33.3\% of females. Arrested puberty was observed in 28.6\% of males and 66.7\% of females. Hypogonadism was demonstrated in all studied

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td><strong>Descriptive statistics of demographic data of patients with (\beta)-thalassemia major</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>All patients ( (n = 32) )</th>
<th>L-carnitine group ( (n = 16) )</th>
<th>Hormonal group ( (n = 16) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) Mean ± SE\textsuperscript{a} (range)</td>
<td>16.7 ± 0.3 (14.0–22.0)</td>
<td>16.5 ± 0.6 (14.0–22.0)</td>
<td>16.7 ± 0.5 (14.0–20.2)</td>
</tr>
<tr>
<td>Age of onset of disease (years)</td>
<td>1.3 ± 0.2 (0.3–4.0)</td>
<td>1.1 ± 0.2 (0.5–2.1)</td>
<td>1.5 ± 0.3 (0.3–4.0)</td>
</tr>
<tr>
<td>Age of start of desferal (years)</td>
<td>9.0 ± 0.7 (4.0–17.0)</td>
<td>7.7 ± 0.8 (0.5–14.1)</td>
<td>10.4 ± 1.0 (4.0–17.0)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}SE: Standard error of the mean.

**TABLE 2**

<table>
<thead>
<tr>
<th>Variables ( (n = 32) )</th>
<th>No.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failure of puberty ( (n = 16; 50%) )</td>
<td>Male 10</td>
<td>71.4</td>
</tr>
<tr>
<td>Female 6</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td>Arrested puberty ( (n = 16; 50%) )</td>
<td>Male 4</td>
<td>28.6</td>
</tr>
<tr>
<td>Female 12</td>
<td>66.7</td>
<td></td>
</tr>
<tr>
<td>Gonadal hypogonadism ( (n = 10; 31.3%) )</td>
<td>Male 4</td>
<td>28.6</td>
</tr>
<tr>
<td>Female 6</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td>Pituitary and gonadal hypogonadism ( (n = 22; 68.7%) )</td>
<td>Male 10</td>
<td>71.4</td>
</tr>
<tr>
<td>Female 12</td>
<td>66.7</td>
<td></td>
</tr>
<tr>
<td>Amenorrhea</td>
<td>Primary 16</td>
<td>88.9</td>
</tr>
<tr>
<td>Secondary 2</td>
<td>11.1</td>
<td></td>
</tr>
</tbody>
</table>
group of patients, whether gonadal hypogonadism or both pituitary and gonadal hypogonadism in 28.6 and 71.4% of males and 33.3 and 66.7% of females, respectively. Primary amenorrhea was seen in 16 (88.9%) of females while 2 (11.1%) of them had secondary amenorrhea. Seven (21.9%) patients had subclinical hypothyroidism. No statistically significant correlation was found between both peak LH/FSH ratio and peak LH (after GnRH stimulation) and all demographic and laboratory data in males and females with β-thalassemia major except that peak LH/FSH ratio showed a statistically significant positive correlation with mean hemoglobin level ($r = 0.8$, $P = 0.0001^*$) in male patients only. Basal testosterone in male β-thalassemic patients was significantly increased after hormonal treatment ($p$-value $= 0.03^*$); whereas the increase demonstrated following l-carnitine therapy did not reach statistical significance ($p$-value $= 0.1$) (Table 3). Basal LH in female β-thalassemic patients was significantly decreased after hormonal treatment ($p$-value $= 0.02^*$). In addition, basal estradiol significantly increased after l-carnitine therapy ($p$-value $= 0.05^*$) but the increase in its level after hormonal therapy was not significant ($p$-value $= 0.1$) (Table 4). Menses occurred in 20% of female patients after l-carnitine therapy and in 37.5% of them after hormonal therapy. However this finding was not statistically significant (Table 5). Also Table 5 showed the comparison between clinical pubertal response both in male and female β-thalassemic patients after l-carnitine and hormonal treatment. Improvement of pubertal staging was observed in 50% of males after l-carnitine therapy compared to 75% of them after hormonal therapy. While improvement of pubertal staging was seen in 90% of females after l-carnitine therapy compared to 100% of females after hormonal treatment. However, these results showed no significant difference between both

### Table 3
Comparison between LH, FSH and testosterone in male β-thalassemic patients before and after treatment (l-carnitine or hormonal)

<table>
<thead>
<tr>
<th>Variables</th>
<th>l-carnitine treatment</th>
<th>Hormonal treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td></td>
<td>Mean ± SE (N=6)</td>
<td>Mean ± SE (N=6)</td>
</tr>
<tr>
<td>LH basal (IU^{-1})</td>
<td>1.1 ± 0.4</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>FSH basal (IU^{-1})</td>
<td>1.4 ± 0.4</td>
<td>2.2 ± 1.0</td>
</tr>
<tr>
<td>Testosterone basal</td>
<td>0.2 ± 0.03</td>
<td>1.8 ± 1.6</td>
</tr>
</tbody>
</table>

* $p$-value is significant if $\leq 0.05$.

### Table 4
Comparison between LH, FSH and estradiol in female β-thalassemic patients before and after treatment (l-carnitine or hormonal treatment)

<table>
<thead>
<tr>
<th>Variables</th>
<th>l-carnitine treatment</th>
<th>Hormonal treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td></td>
<td>Mean ± SE (N=10)</td>
<td>Mean ± SE (N=10)</td>
</tr>
<tr>
<td>LH basal (IU^{-1})</td>
<td>5.8 ± 1.6</td>
<td>4.3 ± 0.9</td>
</tr>
<tr>
<td>FSH basal (IU^{-1})</td>
<td>5.4 ± 1.1</td>
<td>5.6 ± 1.2</td>
</tr>
<tr>
<td>Estradiol basal</td>
<td>0.4 ± 0.1</td>
<td>4.4 ± 1.5</td>
</tr>
</tbody>
</table>

* $p$-value is significant if $\leq 0.05$.

### Table 5
Comparison between LH, FSH and estradiol in male and female β-thalassemic patients after l-carnitine and hormonal treatment

<table>
<thead>
<tr>
<th>Variables</th>
<th>l-carnitine treatment</th>
<th>Hormonal treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Percentage (%)</td>
</tr>
<tr>
<td>Pubertal response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3/6</td>
<td>50</td>
</tr>
<tr>
<td>Female</td>
<td>9/10</td>
<td>90</td>
</tr>
<tr>
<td>Menses</td>
<td>2/10</td>
<td>20</td>
</tr>
</tbody>
</table>

* $p$-value is significant if $\leq 0.05$. 
groups. The interval between consecutive blood transfusion in weeks had a significant increase after l-carnitine treatment ($p$-value $= 0.05^\circ$).

**Discussion**

In our study, clinical pubertal assessment of the studied thalassemic patients was concerned. Failure of puberty was found among 71.4% of males and 33.3% of females while arrested puberty occurred in 28.6% of males and 66.7% of females. In the majority of patients testicular volume was arrested between 4 and 6 ml by Prader's Orchidometer and breast size was arrested at Tanner stage II or III. All females had amenorrhea either primary in 88.9% or secondary in 11.1%.

An Italian working group [12], reported that failure of puberty was the commonest endocrine complication in β-thalassemic patients. It was observed in 51% of males and 47% of females in their studied group of patients. Arrested puberty was present in 15.7% of males and 12.6% of females; where as secondary amenorrhea was present in 23% of post-menarchal patients.

In the present study laboratory assessment of sex hormones and pituitary hormones before and after stimulation was done in order to determine the cause of delayed puberty whether pituitary or gonadal. In females gonadal hypogonadism was diagnosed in 33.3% and both gonadal and pituitary hypogonadism were diagnosed in 66.7% considering the fact that diagnosis of gonadal hypogonadism in females is not conclusive as low estradiol level may be the result of pituitary hypogonadism. In the studied male group of thalassemics, delayed puberty was due to gonadal hypogonadism in 28.6% as evidenced by low basal testosterone level and no response after HCG stimulation test (no increase in basal testosterone by 10-folds after HCG). On the other hand, 71.4% had both gonadal and pituitary hypogonadism. These patients had, in addition to absence of response after HCG stimulation test, no response after GnRH bolus test (i.e. peak LH below 8 IU l$^{-1}$ and peak LH/FSH ratio below 1). In these conditions gonadal hypogonadism could be primary or secondary to prolonged inhibition by pituitary hypofunction [15].

This agreed with the results of Soliman et al. [16], who reported that the primary abnormality in thalassemic patients is defective gonadotrophin secretion secondary to siderosis of the pituitary gland. However, low testosterone level in those patients even with normal nocturnal LH secretion supports the concept of testicular failure and atrophy due to siderosis. As a result HCG therapy might fail in a considerable number of these thalassemic patients [16].

In the current study the correlation between pituitary and gonadal hormones and other demographic and laboratory data was evaluated, no significant correlation was found between peak LH, peak FSH, peak LH/peak FSH, testosterone or estradiol and other demographic and laboratory parameters e.g. age of onset of the hemolytic disease, age of start of desferrioxamine therapy, bone age, serum ferritin and liver enzymes in both male and female β-thalassemic patients. Nevertheless, the mean hemoglobin level showed a highly significant positive correlation with peak LH/FSH ratio in male thalassemics.

This coincide with the result of Valenti et al. [17], who reported that, there was no significant correlation between serum ferritin concentration, liver enzymes (ALT, AST) and peak gonadotrophin values (FSH, LH) after GnRH or estradiol concentration. They stated that the magnitude of hemosiderosis does not always parallel the degree of functional disturbance. This discrepancy is particularly evident in the thyroid gland which often has heavy iron deposits with little or no functional damage. Primary and secondary hypothyroidism, reported in thalassemic patients, may add to short stature and delayed puberty [18].

In the current study all thalassemic patients were tested for thyroid functions but none of them had overt primary hypothyroidism. This agreed with some studies which reported a low prevalence of hypothyroidism ranging from 0 [19] to 6.2% [20]. Contrary to others, who reported a higher prevalence ranging from 11 [21] to 17–18% [22, 23].

Seven of our studied patients (21.9%), on the other hand, demonstrated a picture of subclinical hypothyroidism which was diagnosed by normal free thyroxine level (FT$_4$) with elevated TSH above 5.5 mIU ml$^{-1}$.

Similarly, Landau et al. [24] reported subclinical hypothyroidism in 43% of thalassemic patients and explained this by the occurrence of thyroid damage by iron overload. Therefore, patients with subclinical hypothyroidism should have further follow up for detection of the occurrence of overt hypothyroidism and intensive chelation therapy should be commenced early together with early thyroid replacement therapy.

Pubertal development was also affected by l-carnitine in this study. Ten thalassemic female patients received l-carnitine for 6 months. Eight of whom had exaggerated response to GnRH test indicating the presence of gonadal failure and two had secondary amenorrhea. Nine patients (90%) improved in pubertal (Tanner) staging, but menstruation occurred in only two girls with gonadal failure and primary amenorrhea.

The six studied males on the other hand, who received l-carnitine for 6 months showed improved Tanner staging in three (50%), with gonadal failure in one and both gonadal and pituitary hypogonadism in two.
Genazzani et al. [9] reported a significant increase in basal LH levels and in spontaneous and GnRH induced LH discharge after ALC treatment only in hypogonadotrophic women and absence of significant hormonal changes in normogonadotropinemic amenorrheic subjects. This could suggest that the low LH group responsive to ALC was characterized by a hypothalamic impairment of GnRH secretion. Alternatively, the normo-LH amenorrheic subjects appeared to have had an impaired sensitivity to gonadal steroid feedback regulation of pituitary gonadotrope activity. The authors also reported that ALC was effective in increasing LH pulse amplitude rather than pulse frequency in hypogonadotropinemic women following 6 months treatment. ALC restored a normal ovulatory cycle in 60% of patients between the 3rd and 6th months of therapy. These data support the hypothesis that ALC positively affects neuro-endocrine activity modulating hypothalamic hypogonadotropinemic amenorrhea.

In the current study, the mean hemoglobin level showed a highly significant positive correlation with peak LH/FSH ratio in the male subjects. Consequently improvement of mean hemoglobin level before transfusion in this group of patients might cause improvement in pubertal staging.

In thalassemic patients hypoxemia, hemosiderosis and increased energy expenditure can cause secondary carnitine deficiency [25]. Therefore, L-carnitine therapy is expected to regulate HP axis through correction of anaemia as well as replacing secondary carnitine deficiency and regulation of energy metabolism in addition to its effect on the liver functions.

The second group of studied patients included eight males and eight females who received hormonal therapy (testosterone propionate for males and ethinyl estradiol for females) for 6 months.

In the current study, pubertal assessment was done for β-thalassemic patients who received hormonal treatment for 6 months. Six boys (75%) and eight girls (100%) improved in pubertal staging but only three females (37.5%) got menses.

Since most thalassemic patients have impaired liver function and/or cholelithiasis and/or heart disease, we investigated the efficacy of newer schemes of replacement therapy which minimize the side effects such as those of conjugated oestrogens [26] or depot testosterone esters. Such schemes included the use of transdermal oestrogens for females. They had been used in two of the studied patients. Oestrogen level increased after treatment and both of them improved in pubertal staging as evidenced by Tanner staging. One of them had menstruation and no liver dysfunction was reported. It was reported that their absorption in thalassemic patients was found to be similar to that observed in normal female population despite the skin hemochromatosis [14].

Chorionic gonadotrophin was given to two males in the present study and a good response was found in both patients; one showed marked increase in testosterone level and increase in testicular size from 2 to 6 ml by Prader’s Orchidometer which indicates that Leydig cells were still normally functioning despite gonadal hemochromatosis observed in those patients.

So the previous findings illustrated that the clinical pubertal response (Tanner staging) in male and female β-thalassemic patients was found to be improved after both hormonal and l-carnitine treatment. However, these findings showed no significant difference between both groups.

Conclusion

Delayed puberty in β-thalassemia major is either due to failure of gonads or failure of the whole HP axis. L-carnitine as well as hormonal replacement therapy had a similar positive effect on puberty in the thalassaemic patients. Continuous schemes of hormonal replacement therapy with both oral and transdermal oestrogens for females, or testosterone and HCG for males are effective therapies in pubertal thalassemics in addition to their positive effect on bones. L-carnitine also had a positive effect in decreasing transfusion requirements in the studied β-thalassemic patients. In addition, L-carnitine has the advantages of being orally administered, safe, inexpensive, easily available drug in treatment of transfusion dependent thalassemic patients.

We recommend: regular follow up of thalassemic patients for early detection of endocrine complications in order to improve their quality of life. Careful follow up of thalassemic girls who have already had menarche is essential since they are vulnerable to develop secondary amenorrhea even if they have intensive transfusion and chelation therapy. Routine L-carnitine treatment in all thalassemic patients is necessary to avoid occurrence of delayed puberty in addition to the benefit of decreasing transfusion requirement in these patients. Continuous schemes of sex hormone replacement therapy in pre-adolescent β-thalassemic patients are important to improve pubertal growth and to avoid the occurrence of osteoporosis as it improves bone density.

References