R e v i e w

Hypogonadism in male thalassemia major patients: pathophysiology, diagnosis and treatment

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Summary. Failure of pubertal growth, delay or absence of sexual development, infertility and sexual dysfunction due to hypogonadism and defective spermatogenesis are frequent and well recognized disturbances among male patients with transfusion dependent (TD) thalassaemia major (β-thal). These problems are attributed mainly to the damage caused by chronic anaemia and the deposition of excess iron in the pituitary gland and testicles. This is a short review of male pubertal disorders in patients with β-thal written by pediatric endocrinologists and haematologists with an interest and active involvement, in the diagnosis and management of these complications in this group of patients. A vigilant clinical evaluation of growth and puberty, as well as an appropriate hormonal evaluation in poly-transfused (TD β-thal) patients is strongly recommended for early detection and treatment of endocrine dysfunction. Of crucial importance also, is the implementation of an efficient chelation regime from early life, to prevent severe iron load and permanent damage to the endocrine glands, particularly those responsible for gonadal function. (www.actabiomedica.it)

Key words: thalassaemia, hypogonadism, iron overload, chelation therapy, spermatogenesis, fertility in males

Introduction

Patients receiving frequent blood transfusions as supportive therapy to treat chronic anemias, such as thalassemia and sickle-cell disease, are at risk of severe iron accumulation. A typical regular transfusion regimen leads to an average daily iron accumulation of approximately 0.3-0.5 mg/kg/day with a considerable interpatient variability in iron loading (1, 2). Chelation therapy is necessary to remove the excess of iron in order to prevent or treat iron toxicity. The greater fraction of iron overload is stored in the liver; however, iron overload does occur in other organs, such as the heart and endocrine glands, leading to specific organ dysfunction. Several studies have reported that as many as 51% to 80% of transfusion dependent thalassemia (TD) patients may have pubertal failure, sexual dysfunction and/or infertility, due to hypogonadism (3-5).

This is a short review written by pediatric endocrinologists and haematologists who are interested, and are actively involved, in the diagnosis and management of pubertal disorders in male TD β-thalassemia (β-thal) patients.

Endocrine control of pubertal development in males

Puberty is a complex process reflecting adrenal maturation, sexual development, and accelerated linear growth. The adrenal component of pubertal maturation in healthy subjects begins approximately 2 years before the increase in gonadotropins and gonadal steroids secretion. The progressive increase in the secretion of adrenal androgen and its precursors is known as adrenarche. Plasma levels of Δ5-steroids, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS) increase in both boys and girls, beginning before age 8 with skeletal age of 6 to 8 years and continuing through early adulthood (6).

The hypothalamic-pituitary-gonadal axis (H-P-G) lies dormant and is activated during puberty. Programmed increases in KiSS-1 mRNA and G protein coupled receptor 54 (GPR54) sensitivity to kisspeptin, possibly due to an increased number of receptors at the cell surface, activate the pulsatile release of GnRH, 'awakening' the reproductive axis and starting pubertal maturation (7). Gn-RH is secreted by neurons of the hypothalamus into the portal system and acts on the anterior pituitary controlling the release of both follicle stimulating hormone (FSH) and luteinising hormone (LH), which in turn stimulate the secretion of gonadal steroids. Gonadal steroids modulate the pattern of the gonadotropin pulse and Gn-RH secretions (Figure 1). FSH stimulates the seminiferous tubules to produce sperm, while LH stimulates specialized cells in the testes called Leydig cells to secrete the male hormone, testosterone.

The rate of LH secretion is influenced by the amount of testosterone circulating in the blood, whereas FSH secretion is controlled by inhibin. The rate of inhibin secretion is governed by the amount of sperm being made by the seminiferous tubules (8). Besides producing secondary male characteristics, testosterone enhances the production of sperm. Two important negative feedback loops exist to regulate the secretion of gonadotropins.

The testosterone negative feedback loop is established in fetal life and inhibits hypothalamic and pituitary production of Gn-RH and LH. Inhibin-B, produced by the Sertoli cells, exerts inhibitory effects on FSH secretion from the pituitary gland.
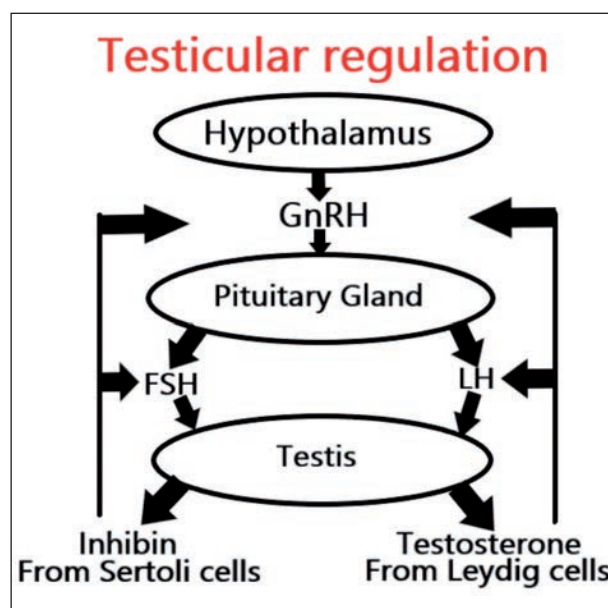

Figure 1. Gonadotropins secretion and feedback control in males

This negative feedback loop is only established at around puberty (9). Any disruption of this system or dysfunction of its components may lead to infertility.

The physical changes of puberty

The timing of puberty is the result of both genetic constitution and environmental influences. Chronic systemic diseases are often associated with delayed puberty.

At birth the volume of the testes is 1 ml; during the following 2-3 months this increases to 2 ml, and then decreases again around 6 months of life. These changes are due to a surge of gonadotropins secretion, known as minipuberty, causing a testosterone increase with a peak at about 3 months, followed by a decrease of androgens in the second decade of life.

Few changes occur until age 11-12 years, when the first signs of puberty develop with an increase in testes volume, with 99 % showing a testicular volume of 4 ml or greater by the age of 14 years (3, 10). The maximum increase, about 4 ml per year, occurs at a bone age of about 13-14 years. Some children reach complete sexual development in less than 2 years, while others require more than 4 years (3, 10).

Testicular enlargement is mainly due to the increase in volume and tortuosity of seminiferous tubules. Spermatogenesis commences during puberty and continues throughout life until old age (3).

The assessment of testicular volume is done using Prader's orchidometer. This is a series of plastic ellipsoids ranging from a volume of 1 ml to 25 ml. The testis, held longitudinally between thumb and index finger, is compared with the ellipsoid with the same volume (10).

Interestingly, in thalassemic boys, a delayed adrenarche was reported, during prepubertal and peripubertal years (11, 12). Adrenal androgen production declines further with advancing puberty and might explain the poor development of pubic and axillary hair observed in this condition.

Physiology of testicular function

The testes fulfil two tasks: steroidogenesis and spermatogenesis. Steroidogenesis takes place in the Leydig (interstitial) cells, situated between the seminiferous tubules, and spermatogenesis in the germinal epithelium of these tubules. The germ cells undergo various stages of development from spermatogonia before spermatozoa (mature sperm) reach maturation. The process of sperm maturation takes about 60 days and then another 10-14 days to pass through the epididymis and vas deferens (3). Analysis of repeated semen samples provides greater specificity in identifying semen abnormalities; a single-sample analysis will falsely identify about 10% of men as abnormal, but repeating the test reduces this to 2% (13).

The minimum number of specimens to define good or poor quality of semen is three samples over a 6-8 week interval with a consistent period of abstinence of 2-3 days. A repeated semen analysis should be requested after two to three months, bearing in mind that a complete spermatogenesis cycle lasts for 74 days (3, 14).

Disorders of pubertal development and iron overload

Substantially, there are 2 types of male hypogonadism, regardless of age of onset.

Primary hypogonadism involves testicular failure that results in low testosterone levels, impairment of spermatogenesis, and elevated gonadotropin levels. *Secondary hypogonadism* results from central defects of the hypothalamus or pituitary, and is associated with low to normal LH and FSH levels and low testosterone level (14, 15).

Delayed puberty is defined as the complete lack of pubertal development in boys by the age of 14 and hypogonadism as the absence of testicular enlargement (less than 4 ml) by the age of 16 years. In addition, pubertal arrest after some spontaneous development may result in hypogonadotropic hypogonadism (HH) (16).

HH still remains the commonest endocrinopathy in patients with TD β-thal; it has been proved to be the result of hemosiderosis of the gonadotroph cells (Figure 2) (3-5, 17-20). Gonadal damage may occur especially in patients with severe iron overload (Figure 3), but this damage rarely is reversible (20).

An emerging endocrine disorder in young adult TD β-thal subjects is acquired hypogonadotropic hypogonadism (AHH) (21). AHH is a disorder caused by the inability of the testes to produce physiologic levels of testosterone and normal numbers of spermatozoa as a result of a disruption of the H-P-G axis (18).

We studied 11 adult men with β-thal, aged between 26 to 54 years (mean±SD: 34.3±8.8 years) with AHH. The presenting symptoms were a loss of libido (7 patients) or infertility (2 married patients);
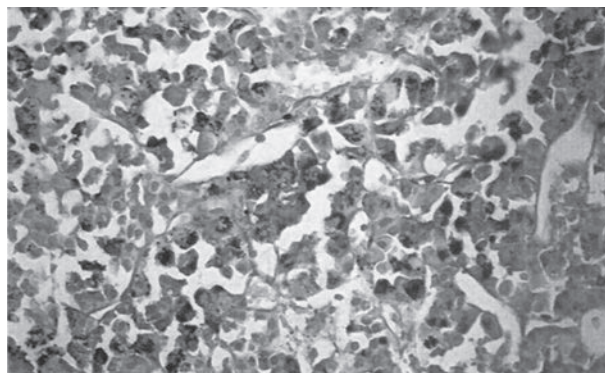

Figure 2. Iron deposition in anterior pituitary in a young adult β-thal patient with severe iron overload. (From: Cianciulli P, Del Poeta G. Reperti istopatologici gonadici ed ipofisari nell'anemia di Cooley. In: La maturazione sessuale nella β-Talassemia major. De Sanctis V Ed. Tipografia Artigiana F.lli Dasi, Ferrara, 1986; pp 107-116, with the Editor's permission)
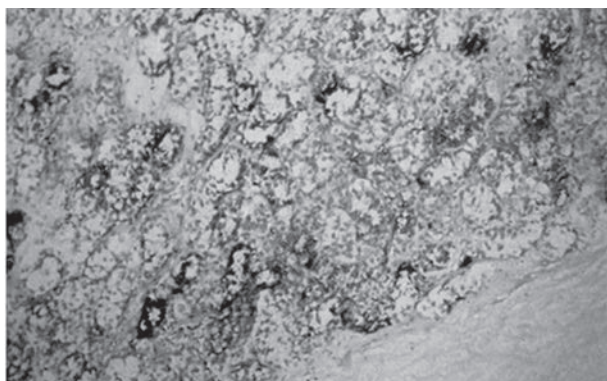
Figure 3. Histology of testicular tissue in a young adult β-thal patient, with severe iron overload, showing testicular interstitial fibrosis with small, heavily pigmented, undifferentiated seminiferous tubules and absence of Leydig cells (From: Cianciulli P, Del Poeta G. Reperti istopatologici gonadici ed ipofisari nell'anemia di Cooley. In: La maturazione sessuale nella β- Talassemia major. De Sanctis V Ed. Tipografia Artigiana F.lli Dasi, Ferrara, 1986; pp 107-116, with the Editor's permission)

incomplete and/or non-persistent erection (9 patients); fatigue (1 patient) and ejaculatory dysfunction (decreased or watery semen production: 11 patients). These symptoms had been present for a mean of 6±2 months (range 2-9 months) before the first endocrine

evaluation. They were previously attributed to the chronic disease itself, iron overload, anemia, liver dysfunction, and associated endocrine complications (21). These patients had significantly elevated levels of γ GT and higher prevalence of HCV-RNA seropositivity and associated endocrinopathies compared to controls without AHH. Serum ferritin level >2000 ng/ml (severe iron overload) was present in 5 patients (45.4%) with AHH versus 1 patient (8.3%) without AHH.

These findings suggest that liver disease and associated endocrine disorders are important contributing factors in the etiology of AHH. Early identification and management of AHH are crucial to avoid subsequent long-term morbidity, including infertility, sexual dysfunction, osteoporosis, weakness and disturbed quality of life.

Prevalence of endocrine complications in thalassemia in countries with high incidence of β-thalassemia

The number of publications on the spectrum and prevalence of endocrine complications in TD β-thal patients in populations with high incidence of TD β- thalassemia is considerable. As example, Table 1

Table 1. Short stature, IGF-1 levels endocrine and non endocrine complications observed in TD β-thal patients (>6 years of age) followed in Doha in 2011 (Soliman AT and Yassin MA, personal observations). Hypogonadotropic hypogonadism was diagnosed in 11/24 (45.8%) female and 26 /57(48.1%) male patients

Number	81
Age (years)	17 1 ± 10.5 years
Sex	24 F /57 M
Short stature HtSDS < -2	62%
Low IGF-I (IGF-I < -2 SD)	85%
GHD (Peak GH < 7 ng/ml) (n = 42)	45%
Impaired Glucose tolerance (OGTT)	22,2%
Diabetes mellitus (FBG > 7.2 mmol/l or 2h BG > 11.1 mmol/l)	6,2%
Hypothyroidism (low free T4 and/or high TSH)	5%
Hypocalcemia (Ca < 2 mmol/L)	7,5%
Hyperphosphatemia (> 1.9 mmol/L)	8,6%
Hypoparathyroidism	7,5%
Vitamin D Deficiency (25 OHD < 20 ng/ml)	52%
Impaired liver function (elevated ALT)	22,2%
Cardiomyopathy (clinical and echocardiography)	31%
Hypogonadotropic hypogonadism	46%
Osteoporosis/Osteopenia	25%
Serum Ferritin Concentration (ng/mL)	2,500 ±1,550

summarizes the spectrum of endocrine and non-endocrine complications in 81 patients above 16 years in Quatar. In contrast, publications distinguishing the prevalence of HH in males and females are scanty. Table 2 summarizes the publications of the last 10 years reporting prevalence on male and female hypogonadism. In general, the majority of patients with endocrinopathies were iron overloaded. This could be the result of poor compliance with treatment, late start and or inadequate dose of chelator.

Toxicity starts when the iron load in a particular tissue exceeds the tissue or blood-binding capacity of iron, and free non-transferrin iron appears. The 'free iron' is a catalyst of the production of oxygen species that damage cells and peroxidize membrane lipids leading to cell destruction. Other possible causes of hypogonadism in TD β-thal include: liver disorders, chronic hypoxia and associated endocrine complications, such as diabetes (16). Thalassemia genotype differences may influence the patient's susceptibility to HH, possibly as a result of differences in the amounts

of blood transfused and/or their vulnerability to free radical damage.

Diagnostic assessment in male subjects with delayed puberty or hypogonadism

Physical examination should assess the following:
1. Vital signs, body height and weight (BMI), arm-span, secondary sexual characters, and examination of thyroid gland;
2. Features of hypogonadism: complete or partial development of secondary sexual characteristics (penis length, distribution of body hair, including beard growth, axillary hair and pubic hair);
3. Testicular size: failure of one or both of the testes to descend into the scrotum or damage to the testicles, such as by injury or after a mumps infection, may reduce sperm production. As approximately 85% of testicular mass consists of germinal tissue, a reduced germinal cell mass

Table 2. Prevalence of hypogonadism in male and female TD and NTD in β-thal patients, in different countries, published within the last 10 years

Authors - references and countries	Patients relevant data	HH Prevalence	
		Males (%)	Females (%)
Ong CK et al. Med J Malaysia. 2008; 2 :109-112. **(Malaysia)**	25 TD β-thal patients (10 males). Mean age at the time of study was 23.1 ± 5.9 years.	**40%**	**46.7%**
Vogiatzi MG et al. Br J Haematol. 2009; 146: 546–556. **(USA)**	361 subjects (176 males and 185 females). Mean age 23.2 years (range 6.1 to 75.4 years), 236 h TD β-thal, 43 with β - thalassemia intermedia (TI), 43 with E- β, 19 with HbH, and 20 with HbH/CS or other non-deletional mutations.	Hypogonadism was the most frequent endocrinopathy in TD and NTD patients. **Age < 20 years =** 25.5%	4.3%
		Age > 20 years= 60%	52.4
Al-Akhras A et al. Biomed Rep. 2016; 4:728-736. **(Egypt)**	100 TD β-thal patients >10 years	Hypogonadism was identified in **82.4%** with no significant differences between males females. **59.1% in patients <14 years**	
De Sanctis V et al. Indian J Endocr Metab 2016; 20:451-459. **(Italy)**	43 TD β-thal. Male 20; female 23. Age range: 45.8-59.5 years.	**70%**	**39.1%**

would be associated with a reduced testicular size and a soft consistency;

4. Presence of gynaecomastia: this may be the result of medications, genetic disorders, hyperprolactinemia, or chronic liver disorders.

Assessment of iron overload

Although serum ferritin is considered not very indicator of body iron burden as its level may be influenced by other factors such as inflammation, liver damage, and vitamin C deficiency, it has been shown that it correlates with total liver iron load, although not with iron load in the heart, pancreas, and hypophysis (22-24).

A group of researchers has earlier shown that a high serum ferritin level during puberty (> 2,500 ng/mL) is a risk factor for hypogonadism and a serum ferritin level of >3,000 ng/mL, during the first decade of life is a predictor of short adult stature (25).

Preclinical iron deposition in the hypophysis can be detected using magnetic resonance imaging (MRI). Se-

vere iron deposition is associated with decreased or absent gonadotropins response to Gn-RH stimulation test.

Magnetic resonance imaging (MRI) measures tissue iron concentration indirectly by detecting the paramagnetic influences of storage iron (ferritin and hemosiderin) on proton resonance behaviour. Preclinical iron deposition in the pituitary can be demonstrated even in the first decade of life, but clinical manifestations are usually not evident until the onset of puberty (18,21). Shrinkage of the pituitary gland is associated with more significant, irreversible loss of gonadotrophic production (Figure 4). However, further clinical validation and technical standardization is necessary before pituitary MRI can be incorporated into routine clinical monitoring, and this is an active area of research (18).

Hormonal assessment

In the early stages, only a diminished gonadotropin reserve with intact gonadotropin pulse is observed (26). Later, the gonadotropin reserve significantly di-

Stage I: Iron overload in the anterior pituitary (focal [heterogeneous] decrease in signal intensity) is associated with:
- **Decreased GH pulse (frequency and amplitude)**
- **Decreased LH pulse (frequency and amplitude)**

Stage II: More iron overload in the anterior pituitary with decreased size(atrophic changes) is associated with:
- **More attenuation of GH pulses**
- **More attenuation of LH pulses**

Stage III: More iron overload in the anterior pituitary (homogeneous decrease in signal intensity) is associated with:
- **More disturbance of GH pulse (frequency and amplitude)**
- **Apulsatile pattern of LH secretion**

Stage IV: More iron overload in the anterior pituitary (homogeneous decrease in signal intensity with decreased volume) is associated with:
- **Lack of GH spontaneous nocturnal pulses**
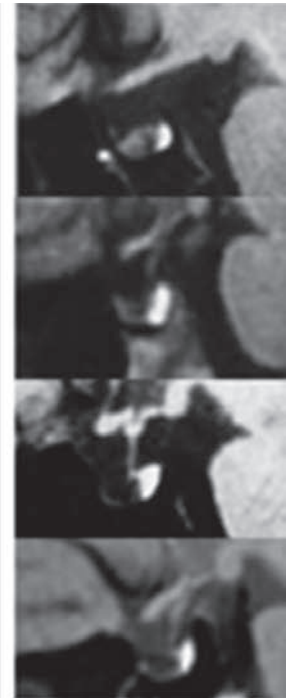- **Lack of LH spontaneous pulses**

Figure 4. Spectrum of MRI appearances of pituitary gland in TD β-thal patients with pituitary siderosis (AT Soliman et al.)

minishes, with markedly reduced spontaneous pulsatile gonadotropin activity which may lead to irreversible damage of the H-P-G axis (Figures 5 and 6). The selective damage of gonadotroph cells in the pituitary is explained by the expression of transferrin receptors in these structures.
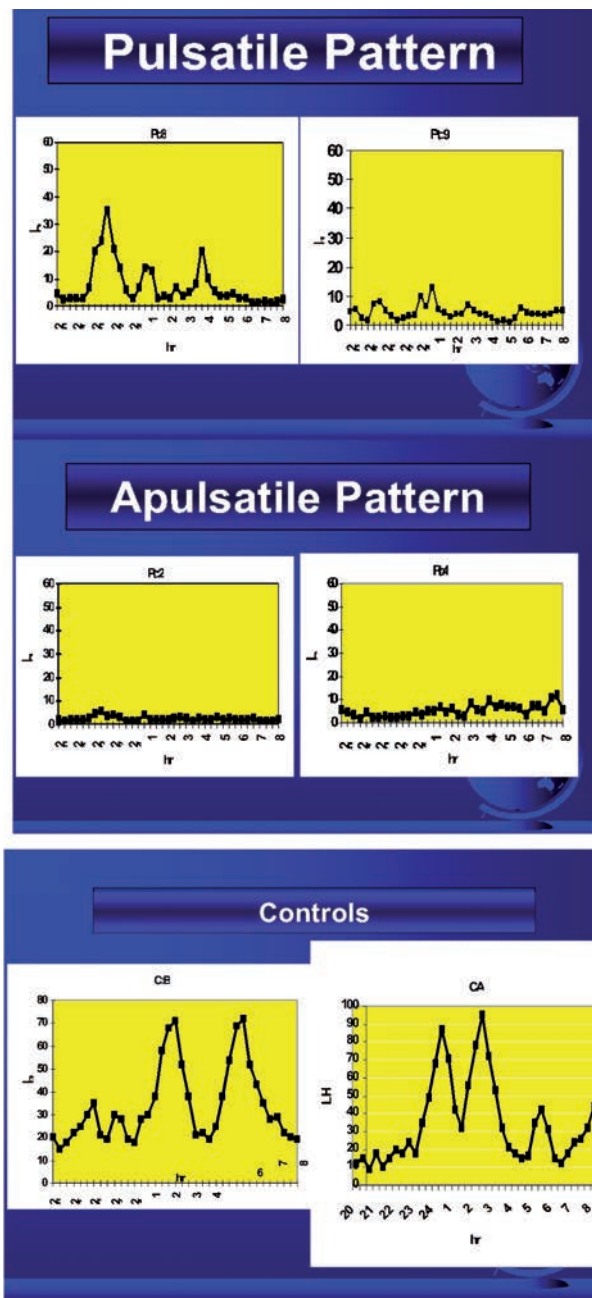

Figure 5. Pulsatile and apulsatile LH (mU/ml) pattern in four TD β-thal male patients, in pubertal age, compared to two control subjects (Soliman A, personal observations)
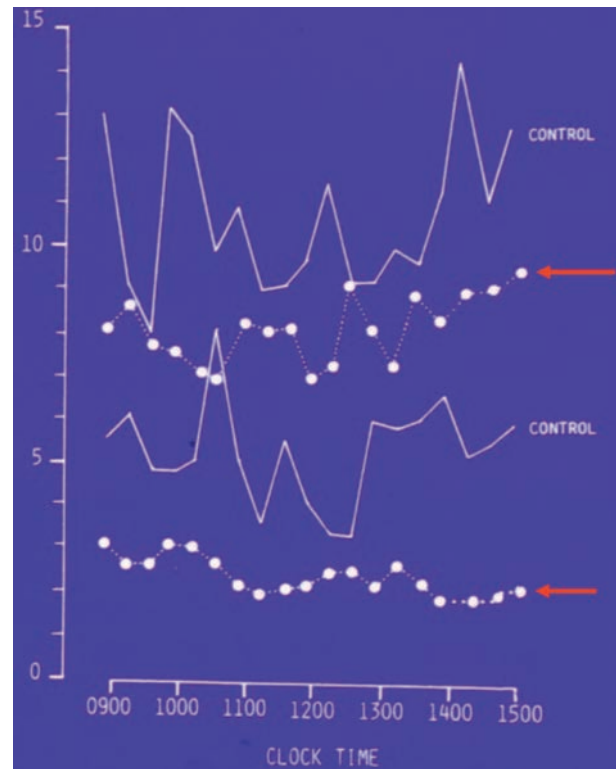

Figure 6. Spontaneous circadian LH secretion (mU/ml) in a 26 yr old TD β-thal patient who developed a progressive apulsatility (dotted line) of gonadotropins (De Sanctis V, personal observation)

Initial hormonal screening in β-thal patients with delayed or arrested puberty, or hypogonadism, can be limited to the determination of serum concentrations of FSH, LH, testosterone, thyroid stimulating hormone (TSH) and prolactin (PRL). A low testosterone level at pubertal age is one of the best indicators of HH of pituitary origin. Elevated FSH and LH values help to distinguish primary testicular failure (hypergonadotropic hypogonadism) from secondary testicular failure, due to HH (27).

Blood to check serum testosterone should be drawn between 7:00 am and 11:00 am, as as the levels are highest at this time of day. The American Association of Clinical Endocrinologists identifies, in adult subjects, a total testosterone level below 200 ng/dL as low, while The Endocrine Society identifies 300 ng/dL as the threshold (28).

The diagnostic value of PRL measurement is extremely low in men with semen abnormalities unless these are associated with decreased libido, erec-

tile dysfunction, and evidence of hypogonadism. PRL measurement is warranted in patients with low serum testosterone levels without an associated increase in serum LH levels (28).

Treatment

a. Iron chelation therapy

Combined chelation therapy (use of deferiprone and desferrioxamine), may improve puberty of β-thal adolescent males with preserved pituitary and testicular function (29) and may reverse hypogonadism and endocrine complications in severe iron overloaded TD β-thal subjects (30). Iron chelation therapy with deferasirox also has a role in the prevention of endocrinopathies, in reversing the existing disease, and in decreasing the prevalence of osteoporosis (31,32).

b. Hormonal treatment

Treatment of hypogonadism may be initiated for 2 purposes: androgenization and/or induction of fertility. Hormonal treatment of pubertal disorders in thalassaemia is a complex issue due to the many associated complications. Therefore, each patient has to be assessed individually. Collaboration between endocrinologists and other doctors is critical.

a. Androgenization

The treatment of delayed or arrested puberty, and HH depends on factors such as age, severity of iron overload, damage to the H-P-G axis, chronic liver disease, and the presence of psychological problems resulting from hypogonadism (33).

For delayed puberty in males, low doses of intramuscular depot-testosterone esters (25 mg) are given monthly, at a bone age of about 12 years, for 6 months, to stimulate growth velocity. This may induce pubertal development. In patients with hypogonadism, therapy continues and the dose increased to 100 mg/monthly until the growth rate begins to wane. The full virilising dose is 75-100 mg of depot-testosterone esters every 10 days administered intramuscularly or 100 mg/m²

twice a month (34). The same effects can be achieved with topical testosterone gel.

Testosterone has a clear direct effect on bone health. It stimulates osteoblasts to form trabecular bone and helps osteocytes in prevention of trabecular bone loss (which may lead to the decreased bone mineral density (BMD) and fracture risk seen in men with both primary and secondary hypogonadism). Testosterone also has indirect effects on bone through its aromatization to estrogen via aromatase. Bisphosphonates should be first-line therapy in the treatment of male hypogonadism-related osteoporosis, with consideration for the addition of testosterone replacement therapy. Other pharmacological therapies specifically for male osteoporosis secondary to hypogonadism have yet to be studied (35).

Long term surveillance should be carried out in TD β-thal patients who have undergone bone marrow transplantation. In some patients absence of pubertal development is due to gonadotropin insufficiency, probably secondary to previous iron overload and/or gonadal damage, secondary to the cytotoxic effects of the preparative transplant regime with alkylating agents. Only in this way can those requiring hormone replacement be identified and treated avoiding both the impairment of growth and sexual development that would affect their social life (36).

b. Induction of fertility

TD β-thal patients who fail to enter puberty or whose puberty is arrested before complete sexual maturation, have been considered to be sterile for life; however this does not seem necessarily true as gonadotropin treatment (hCG and hMG) can achieve spermatogenesis (37). The initial regimen of hCG is usually 1,000 to 2,000 IU administered intramuscularly two times a week. The clinical response is monitored, and testosterone levels are measured every 2 to 3 months. Dosage adjustments of hCG may be needed to determine the optimal schedule. The disadvantages of hCG include the need for more frequent injections and the higher cost. Because of advances in fertilization and sperm banking technologies, all individuals, even those with extremely low sperm counts and motility, should be considered candidates for sperm cyropreservation (38).

In the presence of infertility, the male and female partners are evaluated to determine the cause and best treatment options. Infertility is defined by the World Health Organization as the "inability of a sexually active, non-contracepting couple to achieve pregnancy in one year." Although no universally accepted consensus exists between specialties on the management of infertility, several algorithms have been devised to provide an initial assessment of the infertile male (39).

International guidelines are required to assist these patients because it is widely accepted that infertility and involuntary childlessness, and the decision to engage with assisted reproduction technology services as a patient, donor or surrogate can entail wide-ranging psychosocial issues (40).

Conclusions

Failure of pubertal growth, delay or absence of sexual development, infertility and sexual dysfunction due to hypogonadism and defective spermatogenesis are well recognized disturbances among male patients with TDT β-thal. These problems are attributed mainly to the damage caused by chronic anaemia and iron deposition in the pituitary gland and testicles. These findings support the need for vigilant clinical evaluation of growth and puberty, as well as appropriate hormonal evaluation in TD β-thal patients in order to detect and treat endocrine dysfunction early. The authors also recommend efficient chelation regimen from early life for prevention of permanent damage to the endocrine glands. Aggressive and combined forms of chelation can be used in a trial to reverse hypogonadism when diagnosed.

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