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RESEARCH LETTER

Gonadotropin replacement in male thalassemia major patients with arrested puberty and acquired hypogonadotropic hypogonadism (AAH): preliminary results and potential factors affecting induction of spermatogenesis

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Although spontaneous fertility can occur in well-transfused and well-chelated patients, with β-thalassemia major (β-TM), the majority are sub fertile due to hypogonadotropic hypogonadism (HH), secondary to transfusional haemosiderosis [1]. In several studies, 40 to 80% of β-TM patients reported to have arrested puberty, pubertal failure, sexual dysfunction, and infertility [1, 2].

In many non-thalassemic patients with HH, human chorionic gonadotropin (hCG) alone or in combination with human menopausal gonadotropin (hMG) induced spermatogenesis [3–5]. Very little is known about the induction of spermatogenesis in adolescents and young adult β-TM patients [6, 7]. In this study, we report the preliminary results of gonadotropin replacement for induction of spermatogenesis in five young adult β-TM patients with arrested puberty and acquired hypogonadotropic hypogonadism (AHH), complaining sexual dysfunction and fertility problems.

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Patients and methods

Five young adult β-TM patients (aged 30.8 to 39.3 years) with a clinical history of arrested puberty (1 patient) and AHH (4 patients) were prospectively studied. All had been treated, in the previous 5–11 years, with depot testosterone, testosterone undecanoate or testosterone gel, with variable compliance.

They had normal secondary sexual features, and their testicular volume, varied from 8 to 15 ml (Table 1). The body mass index (BMI) ranged between 21 and 26 kg/m^2.

The following hormones were measured in a fasting AM venous sample: follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (TT), estradiol (E2), free thyroxine (FT4), thyrotropin (TSH), basal cortisol, serum prolactin, insulin-like growth factor-1 (IGF-1), and serum vitamin D level (25OHD) using chemiluminescence immunoassays (CLIA). All other biochemical and hematological measurements were performed using commercial kits for standard methods.

Analysis of the quality of semen findings were based on The World Health Organization (WHO) recommendations [8].

Pituitary MRI was performed in 3 patients with AHH and very low IGF-1 levels, using a 1.5T scanner (Sonata, Siemens Medical, Erlanger, Germany).

Gonadotropins replacement therapy for induction of spermatogenesis

After baseline investigations, treatment was initiated by subcutaneous (SC) administration of hCG alone (induction phase), 2000 I.U. applied twice weekly for 8–12 weeks. Adjustments were made to achieve testosterone levels within the normal adult range. Following the induction

Table 1 Summary of clinical and laboratory data in five thalassemia major patients with acquired hypogonadism before and after gonadotropins therapy

Patients (no.)	1	2	3	4	5
Age at starting gonadotropins treatment (years/months)	38.7	31.9	35.1	39.3	30.8
Testis volume (mL), assessed, before and after hCG+hMG treatment	Before: 10 ml After: 12 ml	12 ml 12 ml	8 ml 10 ml	12 ml 15 ml	10 ml 12 ml
Pre-transfusion hemoglobin levels (g/L)	90	90–95	90–95	90	90
Iron chelation therapy during HRT:	DFO 40 mg/kg/day; 5–6 times/ week	DFO 35 mg/kg/day 5–6 times/ week	DFP 75 mg/kg/daily	DFX 30–35 mg/kg/ daily	DFX 30–35 mg/kg/ daily
Serum ferritin level (ng/mL) (a) On start of treatment[a] (b) At peak[a] (c) Age at peak (years)	960 3.965 11	740 5.400 14	780 4.780 16	456 4.900 20	670 3.770 19
Splenectomized	Yes	Yes	Yes	Yes	Yes
Previous hormone replacement therapy (HRT) with testosterone	Irregularly for 10 years	Irregularly for 5 years	Irregularly for 11 years	Irregularly for 7 years	Irregularly for 5 years
Basal LH levels (normal: 1.7–8.6 U/L)	0.9	1.7	1.2	0.9	0.5
Basal FSH levels (normal: 1.5–12.4 U/L)	2.5	0.9	1.0	1.6	0.4
Serum testosterone (normal values: 300–900 ng/dL) before and after hCG +hMG treatment	Before: 11After: 464	128 470	73 371	105 422	59 495
Concominant complications	CLD; O	O; IFG	GHD; O	GHD; O	O
ALT (U/L) (normal values: < 40 U/L)γ-GT (U/L) (normal values: 8–35 U/L)	21 22	46 24	13 17	29 41	32 16
Insulin-like growth factor 1 (IGF-1; ng/mL) during the treatment[b]	30.8	78.8	23.1	57.5	97.0
Vitamin D (ng/mL)	23.0	24.2	27.0	18.9	24.1
HRT:hCG (twice weekly)hMG (thrice weekly)	2000 I.U. 75 I.U.	2000 I.U. 75 I.U. followed by 150 I.U.	2000 I.U. 75 I.U. followed by 150 I.U.	2000 I.U. 75 I.U.	2000 I.U. 75 I.U. followed by 150 I.U.
Duration of treatment hCG+hMG (months)	12	14	10	18	16
First sperm appearance during hCG+hMG treatment and semen analysis	– AZ	6 months OAZS	– AZ	– AZ	9 months OAZS

ALT alanine aminotransferase, *AZ* azoospermia, *CLD* chronic liver disease HCV related, *DFO* deferoxamine, *DFP* deferiprone, *DFX* deferasirox, *GHD* growth hormone deficiency, *γ-GT* gamma-glutamyltransferase, *IFG* impaired fasting glucose, *O* osteoporosis at the lumbar spine (L1-L4), *OAZS* oligoasthenozoospermia

[a]Normal serum ferritin levels: 20–200 ng/mL. The serum ferritin levels was arbitrarily categorized as mild (<1.000 ng/mL), moderate (1.000–2.000 ng/mL), or severe (>2.000 ng/mL)

[b]Normal range IGF-1 levels in adult males (aged 30–40 years): 169–178 ng/mL

phase, hMG was given S.C. at a dose of 75–150 I.U. thrice weekly. During combined treatment, FSH levels were between 4–6 IU/L prior to hMG injection avoiding levels >9 IU/L [9]. Patients were followed every 3–4 months for evidence of spermatogenesis, hormonal response to treatment and possible side effects (FSH, testosterone, E2, prostate-specific antigen, liver enzymes, serum glucose, electrolytes, serum ferritin, and semen analysis).

Oral supplementation of vitamin D3 (cholecalciferol) was given to all patients due to the presence of serum 25 (OH)D < 30 ng/mL.

Results

Table 1 shows the pre- and post-treatment clinical and laboratory characteristics of the patients. MRI in 3 patients (no. 1, 3, and 4) revealed a low signal intensity of the pituitary gland with reduced gland size (Table 1). All β-TM patients had at baseline TT level below 300 ng/dL (<10.4 nmol/L), indicating androgen deficiency according to the Endocrine Society guidelines [10].

TT levels normalised 3–4 months after starting hCG therapy, and spermatogenesis was restored, during the

combined treatment, at 8 and 12 months in two patients (no. 2 and 5; oligoasthenozoospermia: total sperm count: 1 million/ml and 1.6 million/ml, and total sperm motility: 25% and 30%, respectively). In both patients the semen samples were cryopreserved. Centrifuged semen samples confirmed azoospermia in patients no. 1, 3, and 4.

Gonadotropins were well tolerated, with mild gynecomastia and acne observed in 3 patients.

After 12, 10, and 18 months of combined treatment, the three azoospermic patients stopped gonadotropins replacement, because they were tired from the number of injections and disappointed for the absence of induced spermatogenesis, and drugs cost. Testosterone levels declined soon after stopping gonadotropins therapy.

In summary, serum testosterone level above 300 ng/ml was attained after 3 months of hCG treatment but azoospermia persisted in 3 out of 5 (60%) β-TM patients treated with hCG+hMG.

Discussion

Significant hemosiderosis and iron-induced oxidative stress (OS) in various endocrine glands [1, 2] are still present in a significant, though reducing, number of transfusion-dependent thalassemia patients worldwide, obviating their desire for parenthood [11].

Hypogonadism refers to decreased function of the reproductive organs in both male and female, irrespective of the cause. The treatment of male infertility in patients with pituitary insufficiency is based on the use of gonadotropins [12]. hCG stimulates Leydig cells in the testis to produce testosterone and leads to intratesticular production of IGF-1, which plays an integral role in Leydig cell LH receptor upregulation, steroidogenesis, and maturation. FSH stimulates Sertoli cells in the testis, which supports spermatogonial differentiation and maturation [12].

Many studies on non-thalassemic patients with hypogonadism showed that gonadotropin replacement therapy induced a significant positive effect on the quantity and quality of sperms in 50% or more patients [13]. In 80% of cases, spermatogenesis appeared within 9–18 months after hCG+hMG therapy [3–5].

Our study showed that hCG treatment in β-TM patients with arrested puberty and AHH successfully increased serum testosterone level above 300 ng/mL, after 3 months, However, azoospermia persisted in 3 out of 5 (60%) treated patients.

In the general population, several factors appear to be important for predicting the success of spermatogenesis induction, including large baseline testis volume, previous spontaneous puberty, and repeated treatment cycles. On the other hand, previous exposure to exogenous testosterone, elevated BMI, and the presence of previous cryptorchidism predict a slow or poor response to treatment [3, 14, 15].

None of our patients had a history of anosmia, cryptorchidism, brain injury, exposure to chemicals or drug abuse, varicocele or prior gonadotropin therapy.

The three azoospermic β-TM patients (no. 1, 3, and 4) presented a medical history of irregular but prolonged hormone replacement therapy (HRT) with testosterone for 10, 11, and 7 years, respectively. Peritubular fibrosis has been reported in the literature following androgen treatment [16]; however, histologic testicular changes have been also documented in patients receiving hCG treatment [17] and in Gn-RH deficient patients [18]. Therefore, the potential negative effects of HRT still remain an intriguing puzzle.

In patients with β-TM, other potential negative prognostic indicators for treatment with gonadotropins should be considered, as the advanced age of patients at the induction of spermatogenesis, the severity of iron overload, the potential negative effects of chelators, the alteration of trace elements and antioxidant enzymes [11], the short duration of gonadotropin replacement therapy and the concomitant presence of growth hormone deficiency (GHD) [1, 2, 19].

The reported prevalence of adult GHD and/or IGF-1 deficiency in β-TM patients varies from 8% to 44% in different centers [19]. All β-TM patients enrolled in the study had a low level of IGF-1 with normal liver enzymes. GHD was found in two out of three β-TM patients tested for GH secretion (Table 1: no. 3 and 4).

Although GH, used as an adjuvant therapy, induced spermatogenesis in non-responder patients with HH [20], its role in promoting spermatogenesis in patients with thalassemia remains unexplored.

Conclusions

Full spermatogenesis seems to occur less often in hypogonadal β-thal patients compared to non-thalassemic patients with HH. Therefore, more extensive prospective multicentre studies are required to investigate the effects of prolonged and earlier onset of gonadotropin replacement therapy to achieve satisfactory spermatogenesis. Assisted reproductive technologies (ART) can be helpful in men with severely compromised sperm count and/or quality. Although questions have been raised regarding the potential increased risk of iron overload in predisposing sperm to OS injury and DNA damage this possibility remains controversial and there is no clear consensus on this issue.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study were in accordance with the ethical standards of Quisisana Hospital and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants, complaining sexual dysfunction and fertility problems, included in the study.

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