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The experience of a tertiary unit on the clinical phenotype and management of hypogonadism in female adolescents and young adults with transfusion dependent thalassemia

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Summary. *Background:* Transfusion-dependent β-thalassemia (TDT) is associated with several complications necessitating a multidisciplinary approach for diagnosis, treatment and follow-up. Hypogonadism in female TDT patients is one of the most common endocrine complications, requiring hormone replacement therapy (HRT) throughout reproductive life. Little is known about the balance of benefits versus risks of treatment with sex steroids. *Aim:* The aim of this manuscript is to review the action and the associated adverse effects of HRT in hypogonadal TDT females. *Design:* Retrospective medical database records from a single centre, over a period of 38 years (January 1980 to June 2018), were reviewed. *Study population:* Forty-two cases of hypogonadism in TDT females followed in a pediatric and adolescent outpatient clinics, were in included in the study. *Methods:* Auxological, clinical, laboratory, hormonal and imaging investigations were reviewed, as well as all adverse events registered during HRT. *Main results:* In general, HRT was safe for most patients. There were few minor side effects and a couple of rare but serious adverse events. *Conclusions:* The study provides a representative clinical profile of long-term effects of HRT in hypogonadal adolescents and young adult TDT women. Our results highlight also the need for further research in other areas for which HRT may have a role. We hope this will contribute to a wider understanding, and potential improvement, of patient safety and quality of life. (www.actabiomedica.it)

Key words: transfusion-dependent β-thalassemia, hypogonadism, primary and secondary amenorrhea, hormone replacement therapy, stroke, thin endometrium, adverse events, ICET-A

Introduction

Hypogonadism is one of the most common endocrine complications in transfusion dependent β-thalassemia patients (TDT). It is mainly caused by iron overload of the pituitary gland; in females it is clinically characterized by the absence of pubertal development or by menstrual cycle disturbances. Careful history, physical examination and selected laboratory testing can often detect the site of the defect. Hypogonadotropic hypogonadism (HH) is biochemically characterized by low serum concentrations of lutein-

izing hormone (LH) and follicle-stimulating hormone (FSH), and of sex steroids (1, 2).

Hormone replacement therapy (HRT) in patients with hypogonadism aims to alleviate symptoms of estrogen deficiency and prevent long-term complications, such as osteoporosis. Hormones are also important in female sexual functioning; low serum estrogen causes vaginal atrophy and higher vaginal pH, which predispose to infections, incontinence, and sexual dysfunction (3). Multiple formulations of estrogens are available for treatment: oral, micronized, vaginal, transdermal patches, and gel. Progesterone therapy is needed to avoid an unopposed estrogen effect and maintain endometrial health (3).

The dosage and route of administration of HRT is extremely complex because of the prolonged period of treatment which can run for decades and the changes in physical and psychological status of the individual over this period. Furthermore, HRT treatment is extremely complex in TDT patients because of associated co-morbidities, such as iron overload, thrombophilic status, chronic liver disease, impaired glucose tolerance or diabetes, and cardiomyopathy (4).

Despite the large numbers of TDT patients for whom HRT is prescribed, few data exist to aid clinicians in making decisions on optimal treatment regimes. Furthermore, no reliable experiences or clinical studies about the potential risk factors and side effects of continuous hormonal therapy in adolescents and young adult TDT women with hypogonadism have been reported in the literature. Therefore the co-ordinator (VDS) of the International network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine (ICET-A) promoted a retrospective study on the effects of HRT in hypogonadal women with TDT.

In this report the findings of the study are presented and discussed. We hope that our findings will promote further understanding of hypogonadism and will facilitate the management of young female TDT patients with HH.

Patients and Methods

Forthy-two TDT patients were selected for the study: 26/42 (61.9%) patients regularly followed in Ferrara and 16/42 (38.1%) patients referred to the tertiary Ferrara clinic for endocrine investigations or second opinion. All patients were following the national (www.site-italia.org) and international guidelines of Thalassemia International Federation (TIF) (5).

In TDT patients regularly followed in Ferrara, transfusional management was changed over time. Before 1972, blood transfusions were given when anemia was severe enough to cause symptoms. Thereafter, patients were regularly transfused every 2-3 weeks to maintain the mean hemoglobin (Hb) level around 9.5 g/dl. Treatment with intramuscular desferrioxamine mesylate (DFO) at a dose of 20 mg/kg body weight (BW) was available for most patients since 1969. Regular subcutaneous (SC) DFO infusion was started in 1978 in patients older than 2 years. Initially, the recommended DFO dose was 20 mg/kg body weight administered daily at night, by infusion pump over 10 hours. Based on transfusional iron input the dose increased to 40 mg/kg BW in 1982 and up to 60 mg/kg BW in 1984. Ascorbic acid was added orally at a dose of 2-5 mg/kg (maximum dose 200 mg) in a selected group of patients. Since 1995, the oral chelator deferiprone (DFP) has been available; it was given at a dose of 75 mg/kg BW to some patients over the age of 11 years, as monotherapy or combined with DFO. In 2007, the new oral chelating agent deferasirox (DFX) was introduced at a dose of 25-30 mg/kg BW for patients in whom treatment with DFO was contraindicated or inadequate.

A basic examination and a re-examination at 3-6-12 month intervals were carried out. Patients' information were abstracted from medical database records, including: country of origin, demographic, clinical and pubertal characteristics, chelation therapy, diagnosis and treatment of hypogonadism, associated endocrine complications, bone metabolism and mineral density (BMD), pelvic ultrasonography, and associated adverse events (AE) registered during HRT.

Inclusion study criteria were: 1) diagnosis of TDT, based on universally accepted hematological criteria and 2) duration of follow-up not less than 4 years. Exclusion criteria were: 1) TDT patients with delayed puberty; 2) non-transfusion-dependent thalassemia patients (NTDT); 3) eating disorders; 4) renal insufficiency; 5) bone marrow transplanted patients; 6)

patients positive for HIV and 7) TDT patients with incomplete data.

Heights were routinely measured on a wall mounted stadiometer. Short stature was defined as height below the third percentile using the Italian growth charts of Cacciari et al. (6). Height velocity was calculated as the difference in height, divided by the difference in age between consecutive annual study visits. Body mass index (BMI) was assessed using the formula: kg/height in m^2 (7). A subject was considered overweight when the BMI was between 25 and 30 and obese above 30 (8).

Sexual maturation was determined by physical examination. Tanner stage 2 breast (B2) development was used to define pubertal onset. Delayed puberty (DP) was diagnosed clinically as the absence of the first signs of pubertal development beyond the normal range for the population. In Italy, this means the absence of breast development (B2) by age of 13 years. Pubertal arrest was defined as the lack of pubertal progression for >2 years after spontaneous breast bud onset. Primary amenorrhoea (PA) was defined as the absence of menarche at the age of 16 years, and secondary amenorrhea (SA) as the cessation of menses for at least 6 months in an already cycling woman. HH was diagnosed in the presence of low serum concentrations of gonadotropins, in the setting of normal serum prolactin and low concentrations of 17 β-estradiol (E_2 <20 pg/ml), in combination with signs and symptoms of estrogen deficiency.

The presence of associated endocrine complications was defined according to the I-CET guidelines, published in 2013 (8).

Serum FSH, LH, prolactin, 17 β- estradiol, FT4, TSH, insulin, cortisol and ferritin were measured by radioimmunoassay, immunoradiometric assay or chemiluminescent assay.

To evaluate liver function, serum concentrations of alanine aminotransferase (ALT), gamma glutamyl transferase (γ GT), total and direct bilirubin, total proteins, and albumin were measured at 1-3 months intervals.Urea, creatinine, electrolytes, lipids [cholesterol, HDL, low-density lipoprotein (LDL), triglycerides], and coagulation tests (platelets count, prothrombin activity, activated thromboplastin time, fibrinogen, antithrombin III, protein C and S) were also assessed at 3-6 month intervals using routine laboratory methods.

Uterine development was evaluated by transabdominal ultrasound (US); size (uterine length and uterine volume), shape (by calculation of the fundus-to-cervix ratio) and maximum endometrial thickness (the highest value of endometrial thickness in the plane through the central longitudinal axis of the uterine body) were recorded. To characterize uterine maturity the following parameters were used: mature uterus: a length of ≥6.5 cm; transitional uterus: length 5.0-6.4 cm, and immature uterus: length <5.0 cm (9). The ovarian volume was calculated using the approximate formula for an ellipsoid: length × breath × width × 0.523.

Bone mineral densitometry (BMD) was measured at lumbar spine, from L2 to L4, and at the femoral neck using a DXA-scan (dual energy x-ray absorptiometry) device (Hologic). The diagnosis of osteopenia/osteoporosis was based on the definitions of the National Bone Health Alliance Working Group (10).

Iron overload was classified at the time of first examination, as mild (serum ferritin <1,000 ng/ml), moderate (serum ferritin >1,000 ng/ml to <2,000 ng/ml) or severe (serum ferritin >2,000 ng/ml). In females the manufacturer's normal reference serum ferritin range values was 15-150 μg/l (11).

HRT adverse event was defined as an unfavourable medical event that in coincidence may present during treatment with a pharmaceutical product, which does not necessarily have a causal relationship with the product. Attribution was also made if a major side effect resulted in discontinuation of the drug, even without re-challenge.

All procedures were carried out with the adequate understanding and consent of parents or patients. As the survey was a retrospective collection of data, research ethics committee approval was not required.

Statistical analysis was carried by Student's "t test"; a p value less than 0.05 was considered as the limit of significance. Linear regression analysis was employed for evaluating correlations between parameters.

Results

Forty-two cases of hypogonadism in TM females of Italian ethnic origin were registered over a period

of 38 years (January 1980 to June 2018). Sixteen out of 42 patients (38%) were examined for absence or arrested puberty, and 26 out of 42 patients (61.9%) for secondary amenorrhea which started from 6 months to 17.5 years (mean: 6.2±5.2 years) after menarche. Three patients (7.1%) reported a single menstrual cycle followed by a prolonged period of amenorrhea (6-9 months). At the first evaluation their median age was 21 years (range: 15-32 years), and their mean serum ferritin level was 2,470 ng/ml (range: 760-6,260 ng/ml).

At the last observation 32 patients were on treatment with subcutaneous DFO (76.1%), 8 with DFP (19%), and 2 with DFX (4.7%).

The diagnosis of HH was made based on the available medical records (Basal E_2: 11.6±4.1 pg/mL - controls: 31.6±4.1 pg/mL, range: 28.0–40.0 pg/mL, p<0.01; basal FSH: 3.3±0.5 IU/L- controls: 10.2±3.8 IU/L, range: 7.3-13.2 IU/L, p<0.001; and basal LH: 3.6±1.0 IU/L- controls: 6.2±1.4 IU/L; range: 5.3-9.5 IU/L, p<0.001). After an acute Gn-RH stimulation test, a poor or lack of gonadotropins response was observed in all patients followed in Ferrara. Twenty-two healthy young adult women served as controls.

Four patients were on treatment with L-thyroxine, (3 for primary and 1 for central hypothyroidism), 4 had insulin dependent diabetes, 5 an impaired glucose tolerance, and 4 were on treatment with calcitriol for hypoparathyroidism. Hypocortisolism (basal cortisol: 3.5 μg/dl=98 nmol/L or less) was not reported in the records of patients. None of them was on treatment with recombinant growth hormone.

All TDT patients received 6 different HRT regimes (Table 1). The majority (29/42, 69.0%) were taking either oral conjugated estrogens (CE) or oral contraceptive pills (CO). Relatively few were using transdermal or vaginal ring (Table 1). The latter treatment was mainly recommended by gynecologists. The patients were followed, regularly or occasionally, by the same clinician for a period of 4-24 years (median: 15 years).

In patients with PA, estrogen replacement was initiated at a low dose (ethinyl estradiol, 5 μg/d) and gradually increased, every 4-6 months, to a maximum dose of 20-30 μg/d. Medroxyprogesterone acetate (MPA 5 mg/d) was added when breakthrough bleeding occurred or after 24-28 months, on days 12-21 of each month, to induce menstrual flow. At this stage, HRT with oral conjugated equine estrogen (0.625 mg/d) combined with MPA, CO, 17 β-estradiol or by transdermal patch combined with MPA or ethinyl estradiol/ norelgestromin transdermal patch were advised and discussed (pros and cons) with the patient. CO was the most accepted method (49.9%) of HRT by the patients.

The compliance to HRT treatment was, in general, fair. Ten out of 42 TDT patients (23.8%) stopped HRT, after 6 months to 2 years, because of experience of discomfort, appearance of adverse events, or financial constraints.

Imaging of the uterus by transabdominal high-resolution US documented that only 50% of TM patients with PA had a mature, heart-shaped uterine configuration and endometrial thickness after 2-3 years of treatment; 19/42 patients (45.2%) presented a smaller sized uterus (transitional) with normal endometrial thickness and 2/42 patients (4.7%) a transitional uterus associated with thin endometrial lining (2-3 mm) with failure of endometrium to respond to long-term treatment with cyclic transdermal estrogen (TE: 37.5 μg and 50 μg, respectively; three weeks on and one week off) and MPA (5 mg in the last 12 days of TE). The same US finding was found in a patient with SA with failure of endometrium to respond to oral HRT with ethinyl estradiol 30 μg + gestodene 0.075 mg. In two patients with PA and transitional uterus, associated with thin endometrial lining, the serum ferritin levels were 3,420 ng/ml and 2,210 ng/ml, respectively, and 910 ng/ml in the patients with SA and failure of endometrium to respond to oral HRT. In spite of intensive iron chelation therapy, amenorrhea persisted. In general, ovarian US showed ovaries with a reduced volume, containing few small follicles.

A reduction of BMD (osteopenia: Z score ≥-1.0 to -2.5 and osteoporosis: Z score ≤-2.5) at spine and/or femoral neck sites occurred in 81% of patients with PA and SA. The degree of low BMD was less evident in patients with a greater BMI and with recent development of SA [r (s)=0.239; p< and 0.172, respectively; p<0.01]. The BMD values increased during the first 2-3 years of treatment by an average of 7.7% at lumbar spine and of 8.9% at left femoral neck. Two patients

Table 1. Adverse events (AE) registered during hormone replacement therapy (HRT) in hypogonadal female TDT patients

HRT treatments	Number of patients treated- (percentage)
Cyclic oral conjugated estrogen (**CE**: 0.3-0.625 mg daily, administered cyclically - e.g. three weeks on and one week off) combined with **medroxyprogesterone acetate** (**MPA** 5 mg in the last 12 days of CE)	8/42 (19%)
Ethinyl estradiol (30 **µ**g) **+ gestodene** (0,075 mg) **(EE+GSD)**	13/42 (30.9%)
Ethinyl estradiol (20 **µ**g) **+ desogestrel** (0,15 mg) **(EE+DSG)**	8/42 (19%)
Transdermal estrogen (**TE**) patch (25-50 **µ**g, administered cyclically - e.g. three weeks on and one week off) combined with **medroxyprogesterone acetate** (5 mg in the last 12 days of TE)	9/42 (21.4%)
Vaginal ring (ethinyl estradiol/ etonogestrel)	4/42 (9.5%)
AE and HRT	
Skin irritation at the application site or discomfort on patch removal (TE)	4/9 (44.4%)
Partial patch detachment under conditions of heat, humidity, and exercise or partial peeling of the patch corner (TE)	3/9 (33.3%)
Mild breast tenderness (EE+GSD)	4/42 (9.5%)
Shorter duration of bleeding in patients with secondary amenorrhea (CE+ MPA and EE+DSG)	5/42 (11.9%)
Failure of endometrium to respond to estrogen (see results)	3/42 (7.1%)
Deterioration of glucose tolerance from normal to impaired (3 patients) from impaired to diabetes (1 patient) (EE+DSG and EE+DSG)	4/42 (9.5%)
Melasma of face (EE+DSG and EE+DSG)	3/42 (7.1%)
Acne (EE+DSG and EE+DSG)	3/42 (7.1%)
Fluid retention (EE+GSD)	2/42 (4.6%)
Mild elevation of liver enzymes (EE+DSG and EE+DSG)	3/42 (7.1%)
Mild elevation of total bilirubin (EE+DSG and EE+DSG)	3/42 (7.1%)
Mild elevation of lipids (EE+DSG and EE+DSG)	3/42 (7.1%)
Headache (EE+GSD)	2/42 (4.6%)
Unusual weight gain (>3 kg) (EE+GSD)	1/42 (2.3%)
Stroke (HRT: see results)	1/42 (2.3%)
Retinal artery spasm (HRT: see results)	1/42 (2.3%)

with SA, during the HRT, presented fractures of ribs, secondary to a mild trauma.

Two severe adverse events (AE) were observed during the long-term HRT treatment: a stroke with right hemiparesis in a 31-year-old splenectomized TDT patient who had been taking sequential ethinyl estradiol (20 µg/d) combined with MPA for the last 3 years, and an episode of transient monocular visual loss in a 29-year-old splenectomised patient treated for the last 4 years, with a CO (ethinyl estradiol: 30 µg/d combined with a third-generation progestin). In both patients a family history of thromboembolism was negative. An immediate discontinuation of HRT was recommended in both patients.

In the first patient with stroke a systematic clinical and laboratory evaluation revealed normal blood

pressure and BMI, negative history of migraine, severe iron overload (serum ferritin 3,215 ng/ml), diabetic curve after oral glucose tolerance test, and a hypercoagulable state condition (deficit protein C and S, and platelets count of 570×10^9). In the second patient, the vascular accident was due to central retinal artery spasm. It was not associated with migraine, smoking, obesity, hypertension, abnormal lipids or impaired glucose metabolism. Her serum ferritin level was 1,755 g/ml. Thrombocytosis post-splenectomy (platelets count: 480 $x10^9$), and a reduction of plasminogen <60% were documented. Both patients were on treatment with low dosage of acetylsalicylic acid (ASA:100 mg/d), as a thromboprophylaxis, but the compliance to treatment was inconsistent.

Seven out of 9 patients (77.7%) complained of skin irritation at the patch application site, or patch detachment under conditions of heat, humidity, and exercise.

Short stature (<2 SD below the mean height for age and sex) was reported in 10/16 patients (62.5%) with PA and in 6/26 patients (23.6%) with SA. A BMI between 25 and 30 kg/m^2 was reported in 3 patients (32.1, 25.7 and 27.4 kg/m^2, respectively). Reduced pubertal height gain (peak height velocity 5.8±2.0 cm, and pubertal growth gain to final height 13.7±3.0 cm) was observed in TDT patients with PA, while in European and British female population the average peak height velocity is 8.0±1.4 cm (12) and the contribution of pubertal growth to final height is ~25 cm, accounting for 17% of the final height.

A deterioration of glucose tolerance was observed in 4 patients (2 with moderate and 2 with severe iron overload); in 3 patients from normal tolerance to impaired glucose tolerance (IGT) and 1 (with HCV related liver disease) from IGT to diabetes. The impact of HRT on blood pressure was negligible. The other AE registered in the database are reported in table 1.

Discussion

Thalassemic disorders have a spectrum of severity with different clinical phenotypes, complications, and strategies for treatment. The grade of this severity depends on the β-globin gene mutation and coinheritance of other genetic determinants. The degree of transfusion dependence is one of the elements considered in a recent classification of thalassemic disorders into TDT and NTDT. In TDT patients, iron accumulation in organ tissues is highly evident and leads to organ toxicity and dysfunction (1, 2).

Patients with TDT have a variety of medical needs throughout their lives. Absent pubertal development and secondary amenorrhea due to HH are the most common complications in TDT patients. For the management of these patients no evidence-based guidelines exist, and recommendations are based on the theoretical knowledge of physiology and endocrinology, and extrapolated from the evidences of HRT in patients with estrogen deficiency, such as premature ovarian insufficiency.

The goals of therapy for hypogonadal adolescents or young adult females are the induction and maintenance of normal puberty, induction of fertility when the patient desires, psychosocial support, annual screening to assess metabolic and endocrine functions, and routine preventive health care. Young women with HH are also at risk for bone loss and fractures.

Multiple formulations of estrogen are available and include oral estradiol, oral conjugated estrogens, transdermal estrogen patches, and gel. Transdermal estradiol may provide a more physiological mechanism for estrogen replacement than oral administration because of delivering estrogen into the systemic circulation and avoiding exposure of the liver to supraphysiological estrogen concentrations (13, 14).

At present, patients who have not yet started pubertal development, induction of puberty is initiated and carried out in a manner that simulates the normal growth and development of secondary sex characteristics as closely as possible. The inability to make an accurate differential diagnosis between delayed puberty and HH at initial presentation presents difficulties in providing appropriate counselling for prognosis; this may generate anxiety among adolescents and families, and can affect treatment decisions. Our policy is to treat these patients with a short low dose course of estrogens (3-4 months after the age of 13-14 years), and to monitor the following at regular intervals (3-4 months): sexual maturation (by Tanner staging on physical examination), gonadotropins and sex hor-

mone levels, and bone age. In patients presenting with lack of spontaneous pubertal progression, estrogen therapy is restarted at low dose increased gradually (at intervals of 3-6 months).

Transdermal E2 application avoids liver exposure to increased estrogen concentrations and provides a more physiologic mechanism for hormone delivery. Doses are adjusted to the response (Tanner stage, bone age or uterine growth), with the aim of completing feminization gradually over a period of 2-3 years, after which time cyclic progesterone is added (or after the first menstrual bleed) to maximize breast development.

In post-menarcheal TDT women who ceased menstruating due to acquired HH secondary to iron overload, estradiol is given orally (at a dose of 1-2 mg) or transdermally (50 μg daily by patch) as a maintenance dose with a cyclic progestin regimen (5 mg of MPA for 12-14 days of the month) to avoid endometrial hyperplasia. Oral contraceptives provide a variety of estrogen and progesterone forms and dosing options. Although there is no clinical trial to support an optimal length of time, HRT should continue at least until the average age of natural menopause (5).

If a risk of unwanted pregnancy cannot be ruled out, there is a need to consider contraception. Although no case of pregnancy was reported in our retrospective study, two patients (not included in the present study) complained of "irregular menstrual bleeding" during HRT; in both patients pregnancy test was positive. The first TDT patient had a spontaneous abortion after 3 weeks. The second patient, with successful bone-marrow transplantation, had a normal pregnancy and delivered a healthy boy (De Sanctis V, personal observations).

Due to the long-term of treatment and the many physical and psychological changes that occur during this period, the treatment options should be carefully discussed with patient, considering the pros and cons of therapy, the patients' age, the duration of hypogonadism, the medical history, and personal preferences (5).

CO, containing ethinyl estradiol, or transdermal HRT are usually recommended by doctors and preferred by the patients. We assessed the attitudes and practices of doctors taking care of 2,326 females and males with TDT. Twelve different formulations and three routes of administration for HRT were used. In 33.3% of cases CO pill: ethinyl estradiol 30 μg/drospirenone 3 mg as first-line treatment choice followed by ethinyl estradiol 20 μg/drospirenone 3 (25%). Ethinyl estradiol 35 μg/cyproterone acetate 2 mg and ethinyl estradiol 20 μg/drospirenone 3 mg were reported, as second-line treatment choice, in 41.6% and 29.1%, respectively. Transdermal estrogen patch, ethinyl estradiol/ norelgestromin transdermal patch, and etonogestrel/ethinyl estradiol vaginal ring were used and recommended by 16.6%, 4.1%, and 4.1%, respectively (15).

Similar results emerged from an international survey conducted online, in July 2016. Oral HRT with different progestin contents were the first treatment choice in 11 centers (68.7%) (5).

Oral HRT has an increased risk for venous thromboembolic events (pulmonary embolism or deep vein thrombosis), and arterial thrombotic events (acute myocardial infarction, ischemic stroke), cardio metabolic changes (increase in blood pressure, unfavourable lipid profiles), and adverse liver dysfunction (16-18). In our patients, two severe AE have been observed during long-term HRT.

In a retrospective multicentre study, Taher et al. (19) reported that thromboembolic events (TE) occurred in a clinically relevant proportion (1.65%) of 8,860 thalassemia patients (75.3% with TDT). A survey, done in 9 Italian thalassemia Centres, disclosed that 32 patients out of a total of 735 (683 with TDT and 52 with NTDT), had venous thromboembolic events (VTEs) corresponding to an incidence of 3.95% and 9.61%, respectively. Localization of TE varied; the main one (16/32) involved central nervous system (20).

Several factors have been implicated in the pathogenesis of the hypercoagulable state in patients with thalassemia, such as the specific changes in the lipid membrane composition of the abnormal red blood cells with increased expression of negatively charged phosphatidylserine at the outer surface, post splenectomy thrombocytosis, cardiac dysfunction, and liver dysfunction leading to protein C and protein S reduction. Furthermore, it has been suggested that absence of the spleen in a variety of hemolytic diseases may contribute to an increased propensity to thromboembolic complications, related to extreme thrombocytosis (21).

This potential risk was confirmed by Haghpanah and Karimi (22), The authors conducted an electronic search on PUBMED (MEDLINE), SCOPUS, and Google Scholar databases up to January 2011. Out of 152 thalassemic patients with cerebral thromboembolic events; 48% were splenectomised, Nine TDT patients had diabetes and activated protein C resistance, decreased protein C or protein S or plasminogen level were detected in 8 patients. In brief, cerebral involvement was associated with age (older patients), inadequate transfusion, splenectomy, thrombocytosis, and decreased protein C level (22).

Before prescribing any kind of HRT, physicians should be aware of the potential associated side effects of therapy and how best way to address them. Attention should be made before administration of HRT in TDT patients with splenectomy, impaired glucose tolerance/diabetes, hypercoagulable state (5), migraine with aura (23), and family history of thrombophilic defect. In TDT patients with a known hypercoagulable state (such as deficiency of antithrombin, protein C or protein S), that has been identified through screening, the pros and cons of HRT treatment should be discussed with a specialist. Patients should be informed that there is an increase in the risk of blood clots with HRT use and that there are symptoms that would prompt immediate medical attention, such as warning signs of VTEs (leg swelling or pain), visual disturbances, sensory or motor impairment, chest pain, and new headache.

In such patients, the application of transdermal estrogen can be considered after careful individual evaluation (24). Splenectomised TDT patients with hypogonadism on HRT should receive anti-platelet therapy with aspirin. Young women should be counselled as to alcohol and tobacco avoidance, daily exercise for obesity prevention, and an appropriate diet to achieve optimal cardiovascular health (5).

Patients with a personal history of deep vein thrombosis, or pulmonary embolism are assigned to risk category 4, according to the World Health Organization Medical Eligibility Criteria (WHO MEC), "a condition which represents an unacceptable health risk if the contraceptive method is used" (25).

If HRT is contraindicated or declined, weight-bearing exercises, increased calcium and vitamin D intake, and avoidance of tobacco and alcohol should be recommended. It must be emphasized that these latter strategies have been shown to be inadequate at maintaining bone density in the reproductive-aged population (26). Clinicians should maintain serum 25-hydroxy-vitamin D levels in the normal range (30-80 ng/mL). Women with HH should take 1,000 to 2,000 IU of vitamin D3 (cholecalciferol) daily, along with 1200 mg of elemental calcium, either through dietary sources or supplements to optimize bone health. Bone density should be monitored every 12-24 months and follow-up visits should be scheduled at 6-12-month intervals with periodic laboratory testing.

Failure of the endometrium to respond to oral or transdermal HRT was reported in 3 patients. A thin endometrium is mostly defined as an endometrial thickness of <7 mm and can result from various factors. The most common are inflammatory (acute or chronic infection can lead to the destruction of the basal layer of the endometrium) and iatrogenic (surgical, due to repeated or vigorous curettage damaging the basal layer of endometrium or indiscriminate use of drugs such as clomiphene citrate) (27). Thin endometrium may not necessarily be secondary to a disease process, because it can result from individual uterine architecture (28), intrinsic properties of endometrium that affect its growth (29), and inadequate estrogen stimulation for endometrial proliferation (30). In the general population, numerous treatments have been tried to improve refractory endometrium, but success has been limited (27).

An additional factor could be the increased iron content (hemosiderosis) of endometrium as documented by Birkenfeld et al. in 3 patients with TDT (31). Iron deposition was mainly evident in the apical part of endometrial glandular epithelium cells above the nuclei, and should be taken into consideration as a contributing factor to the infertility in these patients by altering endometrial receptivity for implantation (26). Iron chelating treatment with desferrioxamine induced a fully or significant regression of the endometrial hemosiderin (31).

A deterioration of glucose tolerance was observed in 4 patients, from normal tolerance to impaired in 3 and from impaired to diabetes in 1 (with chronic HCV liver disease). Available data in a healthy population do not support a significant influence of OC on glucose and insulin homeostasis (32). It is highly likely

that the risk of diabetes development depends on individual patient characteristics, such as family history of diabetes, age, ethnicity, BMI, severity of iron overload and presence of a chronic liver disease. Therefore, in patients with TDT a cardio- metabolic risk assessment needs to be performed, including a 75-g standard 2-hour OGTT and lipid profile at baseline and during follow-up at regular intervals. An annual visit is required to control for further compliance, side effects, and evaluation of glucose tolerance and lipids. Future studies evaluating the long-term effects and safety of oral HRT in the treatment of TDT patients with hypogonadism are needed.

Despite an adequate HRT and general measures including control of anemia, adequate chelation therapy, healthy nutrition and lifestyle, two insufficiency fractures of ribs were registered in our patients with SA. Therefore, since the origin of bone disease in TDT is multifactorial and some of the underlying pathogenic mechanisms are still unclear, further research in this field is needed, to design the optimal therapeutic measures (33).

Melasma of face was reported in 3 patients. It is a common dermatosis that involves changes in normal skin pigmentation, resulting from the hyperactivity of epidermal melanocytes, predominantly affecting women of childbearing age. The pathogenesis is not yet fully understood, but there is relation with a genetic component, sun exposure, OC, HRT, cosmetics, photosensitising medication, pregnancy, and psychological stress (34, 35).

In summary, TDT is a chronic disease associated with a number of complications and conditions which need the attention of specialised multidisciplinary teams for diagnosis, treatment and follow-up. TDT patients with complications need optimum surveillance strategies. Despite the wide agreement that hormonal substitution remains the most effective option for treating the signs and symptoms of hypogonadism, a careful evaluation of the benefit-risk balance is essential prior to prescribing a HRT regimen. Our results highlight also the need for further research in other areas for which HRT may have a role. These areas include: growth, skeletal development and mineralization, glucose tolerance, safety, social interactions, and sexuality. Therefore, research consortia should be established to allow investigation of these important questions, and to allow clinicians to make judicious analysis for the best possible health care decisions.

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