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Abstract

Patients with thalassemia major who received allogeneic hematopoietic cell transplantation are at increased risk of gonadal insufficiency and reduced fertility due to the toxicity of both the transfusional iron overload and the gonadotoxic effects of drugs used in the conditioning regimen. We present a case of an ex-thalassemic patient with spontaneous recovery of spermatogenesis that fathered a healthy, term male neonate. Maternal hemoglobin electrophoresis was within normal limits. At the age of 9.5 years the patient underwent hematopoietic cell transplantation. The conditioning therapy included busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg). No irradiation was administered. Thirty-two days after the hematopoietic cell transplantation the patient developed acute graft-versus-host disease needing long-term with methylprednisolone, treatment and cyclosporine immunoglobulin. Although consecutive semen analyses after the hematopoietic cell transplantation revealed azoospermia, the last semen analysis before conception, at the age of 33 years, was improved and normal follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (Te) levels were detected. The current pregnancy was the result of physical conception. In this case, it seems that thalassemia major along with the respective treatment prior to- and posthematopoietic cell transplantation did not irreparably impair spermatogenesis, probably due to the pre-pubertal time frame they were implemented.

Introduction

Regular transfusions and intensive chelation therapy for patients with thalassemia major have increased their life span well into late adulthood and improved their quality of life.¹ As a result, their expectation for reproduction has also increased. However, endocrine complications due to hemosiderosis and especially hypogonadotrophic hypogonadism are still present leading to dysfunction of the reproductive system in a considerable number of patients. Although reports of successful pregnancies in female patients, either spontaneous or after induction of ovulation, have been adequately presented, fatherhood in male patients is considered relatively rare.² Etiology of impaired male fertility is multifactorial including hypogonadism, sperm abnormalities, and complications of additional medical therapies.

Pregnancies in female patients with thalassemia major, as well as fatherhood in male patients who had allogeneic hematopoietic cell transplantation have been reported only occasionally.3 These patients are at increased risk of gonadal insufficiency and reduced fertility, not only because of the effects of transfusional iron overload, but also because of the gonadotoxic effects of drugs used in the conditioning regimen.4 We report a case of a Greek 33-year-old male with history of thalassemia major and a successful allogeneic hematopoietic cell transplantation that has fathered a healthy offspring, following physical conception.

Case Report

The 33-year-old father had a history of thalassemia major (genotype: IVSI-110/IVSI-110) diagnosed at the age of 15 months. Subsequently to diagnosis, he was treated with regular transfusions every 14-17 days maintaining hematocrit levels between 28-30%. Additionally, he received chelation with subcutaneous infusions of desferrioxamine (DFO) at a dose of 1.5-2 g/day, 5 days per week. His ferritin levels ranged around 2000 ng/ml. No signs of extramedular hemopoiesis, skeletal malformations, or other clinical pathology were detected. His growth was otherwise normal.

At the age of 9.5 years the patient underwent matched related hematopoietic cell transplantation, with his younger sister being the donor. The conditioning therapy included busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg). Low dose methylprednisolone and Cyclosporine were Correspondence: Vassilis Ladis, Thalassemia Unit, Division of Pediatric Hematology-Oncology, First Department of Pediatrics, National and Kapodistrian University of Athens, Agia Sofia Children's Hospital, Athens Greece, Papadiamantopoulou and Levadias Str., Goudi 11527, Athens, Greece. E-mail: vassilisladis@gmail.com

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given for graft-versus-host disease prophylaxis. No irradiation was administered. Thirty-two days after the hematopoietic cell transplantation the patient developed acute graft-versus-host disease with prominent symptoms from the gastrointestinal tract (diarrhea), respiratory system (emphysema) and skin. The patient required long term treatment, nearly for four years, with methylprednisolone, cyclosporine, immunoglobulin and co-trimoxazole. During that period, he developed insulin dependent hyperglycemia, which was restored soon after drug discontinuation. Mild skin chronic graft-versus-host disease activity resolved ten years after the hematopoietic cell transplantation. Today patient preserves a complete allogeneic engraftment, maintaining a hematocrit >30% and hemoglobin around 13 g/dL. His ferritin dropped gradually to normal levels. Low bone density was detected at the age of 27 years.

Consecutive semen analyses post hematopoietic cell transplantation at 17 and 18 years of age revealed azoospermia, a finding attributed to the toxicity related to his previous medical history. The development of external genitalia and basal gonadotropin and testosterone levels were normal, but the luteinizing hormone-releasing hormone (LH-RH) test revealed a poor response of follicle stimulating hormone (Tables 1 and 2).







Hormone profile		Age (y	vears)		8 months after conception	Normal values
	17	18	20	33		
FSH	3.23	2.66	3.00	2.17	3.03	0.7-11.1 mU/mL
LH	2.45	1.80	4.2	2.3	2.47	0.8-7.6 mU/mL
Testosterone	6.00	5.90	9.00	4.64	4.61	2.86-15.1 ng/mL
SHBG	-	-	-	29.2	30.2	13-71 mU/mL
TSH	1.03	-	0.98	0.966	0.58	0.25-4.5 IU/mL
Т3	1.2	-	1.02	1.8	1.43	0.8-2.2 nmol/L
FT4	-	-	-	1.65	1.27	0.8-1.76 ng/dL
PRL	35	36	30	45	-	1.5-16 ng/mL
Semen analysis						
Total volume	8	5	-	2.5	5 mL	≥2.5 mL
Sperm count	0	0	-	6.25x10 ⁶	22.5x10 ⁶	40-300x10 ⁶
Sperm count/mL	0	0	-	2.5x10 ⁶	$4.5 x 10^{6}$	≥20x10 ⁶ /mL
рН	7.5	7.7	-	8	7.8	7.1-8.0
Motility	-	-	-	45%	60%	≥50%
Morphology-normal	-	-	-	70%	65%	≥30%
Liquefaction	30	30	-	30	40	≤20 min

FSH, follicle stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin; TSH, thyroid-stimulating hormone; T3, triiodothyronine; FT4, free thyroxine; PRL, prolactin.

Twenty-three years after the hematopoietic cell transplantation the patient became father with physical methods. A few months prior to conception the pituitary hormones (FSH, LH) and the testosterone basal levels were normal (Table 1). Semen analysis showed severe oligospermia (2.5 million sperm/mL) but improved when compared with prior tests; motility at 2h was sufficient, and morphology was normal. Pituitary MRI was performed due to hyperprolactinemia, demonstrating no enhancement of pituitary adenoma or any other pathology. Mother a primigravida primipara 24-year-old healthy woman had an uneventful pregnancy. Maternal hemoglobin electrophoresis, as part of her antenatal care revealed HbA₂ 2.9% and HbF <0.1% (within normal limits). Because of the paternal medical history, prenatal diagnosis was suggested (for possible mutations of hemoglobin's genes), which was not accepted due to religious reasons.

The offspring, a term male neonate (gestational age of 39+1 w), was born by lower segment cesarean section due to failure to progress. Apgar score was 9 at 1 min and 10 at 5 min. Birth weight was 3350 g corresponding to the 70th customized centile (WHO Child Growth Standards). Clinical examination was normal and no stigmata of congenital malformations were present.

Discussion

Thalassemia major is a severe transfu-

sion-dependent anemia requiring treatment with regular red blood cells transfusions, iron chelation and supplementary treatment of secondary complications, most commonly attributed to iron overload.⁵ Hematopoietic cell transplantation from a related or unrelated HLA identical donor is the only curative treatment currently available for these patients.³

Endocrine abnormalities are among the most common complications of thalassemia major. In a multicenter study, hypogonadism. being the most frequent 40.5%endocrinopathy, affected of patients.⁶ Several other studies show that fertility is more compromised in male transfusion-dependent thalassemia major patients, with oligospermia and asthenospermia being present in 53%.2,7

Infertility in thalassemia major is mainly attributed to iron accumulation in the pituitary gland, which is toxic for the gonadotropin secreting cells leading to hypogonadotrophic hypogonadism.8-10 The anterior pituitary gland is particularly sensitive to free radicals produced by oxidative stress, which further contributes to male infertility through increased production of reactive oxygen species (ROS) that may further damage the sperm membrane, nucleus, and proteins.9,11 Moreover, hypoxemia, present in transfusion dependent thalassemia major patients, further damages germinal epithelium of testes; adverse effects on testes and sperm can last for several weeks or even months, mainly depending on the degree and duration of hypoxemia.12

Table 2. Poor response of FSH during LH-RH test.

Time (sec)	LH (mIU/mL)	FSH (mIU/mL)
0´	0.50	0.90
20	6.10	0.60
40´	7.00	0.80
60´	6.40	0.80
120	4.00	0.90

FSH, follicle stimulating hormone; LH, luteinizing hormone; LH-RH, luteinizing hormone-releasing hormone.

Even though our patient received regular transfusions and effective chelation treatment, the relative hypoxemia and oxidative stress due to free iron, are considered the initial contributors to his infertility. Additionally, fertility was further challenged with hematopoietic cell transplantation conditioning therapy, acting on germ cells during spermatogenesis and leading to prolonged or even permanent azoospermia. The extent of damage is dependent on the age of the patient, the agent administered, the dose delivered, the combination of cytotoxic drugs and the potential synergic interaction of radiotherapy if used.¹³

Since both alkylating agents and irradiation are mutagenic with the potential of injury to germ cell chromosomes, children born to patients who recover gonadal function may be at increased risk for development of genetic diseases and congenital anomalies.¹⁴ In this respect, close pediatric evaluation and possibly genetic consultation for the offspring are required.

Spermatogenesis recovers in approxi-

mately 80% of male patients within a minimum of 5 years after busulfan and cyclophosphamide treatment.15 In one study, patients given cyclophosphamide combined with busulfan presented initial sperm recovery after 3 years and 50% of them had sperm in the ejaculate after 7 years. Therefore, recovery after hematopoietic cell transplantation may be underestimated if sperm samples are evaluated too early.16 Borgmann-Staudt et al. reported that at a median of 6 years after hematopoietic cell transplantation, 31% of males seemed to be fertile. Three male participants in this group had thalassemia major. Analyzing fertility separately for malignant and nonmalignant diseases, they found that fertile patients were 21% and 42% in these groups respectively.17 Normal serum levels of follicle stimulating hormone and spermatozoa were detected in semen samples, suggesting that progressive recovery of spermatogenesis is possible after busulfan-based conditioning for hematopoietic cell transplantation in childhood. Younger age was reported to be associated with an increased likelihood of fertility recovery.18-21 Only one study reported that pre-pubertal therapy in males increased the risk for infertility, which is inconsistent with findings of other recent studies.¹⁷ Furthermore, recipients with prolonged chronic graft-versus-host disease had a significant trend for lower sperm counts thus enhancing infertility.22 On the contrary, the absence of chronic graft-versus-host disease was associated with a higher incidence of spermatogenesis.21

Our thalassemia major patient was subjected to hematopoietic cell transplantation at the age of 9.5 years and suffered from acute graft-versus-host disease, followed by mild chronic graft-versus-host disease. Luteinizing hormone levels were normal suggesting that Leydig cells were not damaged in such extend to cause testosterone deficiency. In addition, semen analyses at the age of 17 and 18 years showed azoospermia with normal follicle stimulating hormone levels. However, follicle stimulating hormone response after luteinizing hormone-releasing hormone stimulation was not normal suggesting some degree of secondary hypogonadism. More recent semen analysis that has been done soon before and after conception indicates gradual improvement of spermatogenesis.

Conclusions

We present a case of physical conception and delivery of an apparently healthy baby, from a couple where the father was an ex- thalassemia major patient. He had been properly treated with regular transfusions and chelation therapy, followed by hematopoietic cell transplantation early in childhood. The nature of his disease, and the respective treatments prior to- and posthematopoietic cell transplantation, seemed to be the contributors to his transient infertility. Although there was no DNA test to confirm the paternity of the neonate, a more recent normal semen analysis, eight months after conception, definitively confirms recovery of fertility. Nevertheless, it seems that the above medications and treatment complications did not impair spermatogenesis permanently, probably due to the prepubertal time frame they were implemented, a finding that is consistent with the current literature. Finally, for the ex-thalassemic, post-hematopoietic cell transplantation patient, the need for appropriate genetic counseling and genotyping should be emphasized for thalassemia prevention strategy. It should be noted that hematopoietic cell transplantation does not reverse the genetic defect but it is a treatment option for thalassemia major that offers effective control of clinical symptoms.

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