Fertility assessment in thalassemic men

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Abstract

Male infertility in β -thalassemia patients is typically considered to be the consequence of iron deposition in the endocrine glands. Adult male patients with β -thalassemia, on regular blood transfusions, are prone to developing acquired hypogonadism. The aim of this study was to evaluate the fertility indicators in male patients with β -thalassemia major and intermedia. In this study we evaluated testicular volume, semen parameters and serum FSH, LH, and Testosterone concentrations in 62 male patients in reproductive age, with major and intermedia thalassemia, at a tertiary care hospital in Tehran, Iran. The range of serum ferritin level in our patients was from 182 to 11053 ng/mL (mean 2067 ng/mL). The mean concentration of sperm was 61.04 million per milliliter. The mean volume of right and left testes was 11.4 cc and 11.7 cc, respectively. Those patients who had lower testicular volumes significantly had lower sperm concentration, lower percents of motile and also lower percents of normal morphologic sperms ($P=0.04$). The frequency of hypogonadism was significantly higher in patients whose testicular volume was lower ($P=0.02$). Hypogonadism and hypothyroidism were seen in 22.6% and 17.7% of patients, respectively. Patients with hypogonadism had significantly lower ejaculate volume, lower sperm concentration, lower percents of motile and progressively motile sperms and also lower percents of normal morphologic sperms ($P=0.001$). This study suggests that in thalassemic men, concentrations of serum testosterone, LH, FSH has significant correlation with sperm parameters and testicular volume.

Introduction

β thalassemia is a genetic hemoglobinopathy that can result in severe anemia. The life expectancy of these patients has considerably extended by the combination use of transfusion and chelation therapy.¹ Despite the advances in treatment, endocrinopathies still harms the health of thalassemic patients. In fact, studies have stated that as many as 51% to 66% of these patients could have pubertal failure, sexual dysfunction and infertility, due to hypogonadism.²⁻⁸ The causes of male infertility in general population are multiple, while infertility in β -thalassemic patients is classically considered to be the result of iron deposition in the endocrine glands.⁴ Adult male patients with β -thalassemia, on regular blood transfusions, are prone to developing acquired hypogonadism. The frequency of acquired hypogonadism in β -thalassemia depends mostly on the degree of compliance with blood transfusion and chelation programs.^{9,10} Delay of pubertal sexual maturation in adolescents and decreased libido, erectile dysfunction, and lower quality of life in adults could all be part of clinical manifestations of hypogonadism. In very low levels of gonadotropins and testosterone, spermatogenesis would be impaired, and the volume of ejaculate is decreased. The diagnosis of acquired hypogonadism is confirmed by Low serum concentrations of testosterone and gonadotropins.¹¹⁻¹⁶

The aim of this study was to evaluate the fertility indicators including sexual hormone levels, sperm parameters and testicular volume in male patients with β -thalassemia major and intermedia.

Materials and Methods

This study was conducted between January 2011 and January 2013 at a teaching hospital in Tehran, Iran. The study included 62 males with β -thalassemia major and intermedia, whose ages ranged between 18 and 41 years. Among the patients, 52 had been regularly transfused since early childhood and underwent different chelation therapies using subcutaneous desferrioxamine and/or oral deferasirox and/or deferiprone. No participants were on medications known to cause hyperprolactinemia or steroids.

Puberty was evaluated in the patients according to Tanner's classification of testicular development.¹⁷ Testicular size <4 mL (long axis of ≤ 2.5 cm) was considered stage I (prepubertal genitalia), and size >25 mL (≥ 5 cm in length) was considered adult gen-

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italia. Delayed puberty was defined as absence of secondary sexual characteristics with a testicular volume of <4 mL at the age of 14 years.^{18,19} By scrotal ultrasound, transverse and longitudinal images of each testis were obtained, and length, width and height measurements were made using electronic calipers. Testicular volume was calculated using the empirical formula of Lambert, $\text{length} \times \text{width} \times \text{height} \times 0.71$.²⁰

Blood samples were collected from patients for evaluation of their basal LH, FSH, and Testosterone by immunoassay method. In addition, patients were evaluated for thyroid function. Normal serum levels for hormones in our central lab are: Testosterone 2.4-12 ng/mL; LH 1.5-9.3 mIU/mL; and FSH 1.6-8.0 mIU/mL). Hypogonadism was defined as lower than normal testosterone level.

Semen specimens were collected after an abstinence interval of 3-4 days and they were analyzed according to the World Health Organization guidelines. The following criteria were used to define the semen quality:²¹⁻²³

- The volume of ejaculate less than 1.5 mL was considered low. Azoospermia was defined as the absence of spermatozoa in patient's ejaculate. Oligospermia was defined as a total number of spermatozoa below the lower reference limit (5th percentile: 15 million/mL).

- Total motile sperm count (class A+B+C) fewer than 40% is considered abnormal. The percent of sperms with progressive motility (class A+B) less than 32% is considered abnormal. The percent of sperms with normal morphology less than 4% is considered abnormal.

- Iron overload was assessed by measuring serum ferritin level. Iron status was classified as mild (ferritin <1000 ng/mL), moderate (ferritin >1000 ng/mL and <2500 ng/mL) or severe (ferritin >2500 ng/mL).²⁴ T2* MRI of heart and liver was assessed for iron overload.

Statistical analysis

Standard computer program SPSS for Windows, release 16.0 was used for data entry and analysis. All numeric variables were expressed as mean±standard deviation. Results were analyzed by ANOVA, followed by the independent t test. $P \leq 0.05$ was considered statistically significant.

The study was approved by the University's Ethical Committee. Patient informed consent was obtained, before beginning the study.

Results

The patients' age range was between 18 to 41 years. Their mean age was 27.2 years. Considering the thalassemia type, 75.8% were major and 24.2% were reported as being intermedia, but at the time of the study 83.9% were transfusion dependent. Age, type of thalassemia and transfusion dependency did not have significant correlation with hormonal levels, sperm determinants and testicular volumes ($P > 0.05$).

The mean volume of patients' ejaculate was 2.3 cc. Five patients (8.1%) had dry ejaculate and 24.2% of patients had unac-

ceptable ejaculate volume (<1.5 mL). The mean concentration of sperm was 61.04 million per milliliter. Acceptable sperm concentration (≥ 15 M/mL) was observed in 61.3% of patients but 21% had azospermia and 22.4% had oligospermia.

The number of motile sperms and progressively motile sperms were less than normal in 22.4% and 34.6% of patients, respectively. The number of sperms with normal morphology were less than normal in 35.5% of patients. Normal ejaculate viscosity and PH was seen in 94.7% and 91.9% of patients, respectively. Agglutination in ejaculate sample was only seen in 3.6% of patients. The characteristics of patients' sperm parameters are summarized in Table 1. The mean volume of right testis was 11.4 cc and the mean volume of left testis was 11.7 cc. Only 3.2% of patients had testicular volume less than 4 ml, which is indicative of the puberty process not being started.

However, those patients who had lower testicular volumes significantly had lower ejaculate volume, lower sperm concentration, lower percents of motile and progressively motile sperms and also lower percents of normal morphologic sperms ($P = 0.04$). The frequency of hypogonadism was significantly higher in patients whose testicular volume was lower ($P = 0.02$). The relationship between testicular volume, hypogonadism and sperm parameters are summarized in Table 2.

Varicocele, hydrocele and microlithiasis were seen in 11.3%, 1.6% and 4.8% of patients, respectively. There was no correlation between having any of the mentioned abnormalities and sperm parameters or with hypogonadism.

Although 17.7% of patients had hypothyroidism, no significant correlation was found between having hypothyroidism and other evaluated determinants. The mean

Table 1. The characteristics of patients' sperm parameters.

Number of patients	Sperm parameter
47 (75.8%)	Volume ≥ 1.5 cc
56 (90.3%)	Viscosity ≥ 3.0
57 (91.9%)	PH ≥ 7.2
55 (96.4%)	No agglutination
38 (61.2%)	Sperm concentration > 15 M/mL
38 (61.2%)	Sperm motility $> 40\%$
32 (51.6%)	Sperm progressive motility $> 32\%$
40 (64.5%)	Sperm normal morphology $> 4\%$

Table 2. The relationship between testicular volume and hypogonadism and sperm parameters.

Testicular volume (Tanner stage)	<4 cc (1)	4-9 cc (2)	10-15 cc (3)	16-25 cc (4)	>25 cc (5)	P-value
Hypogonadism	2	10	2	0	0	0.000
Dry ejaculate	1	4	0	0	0	0.010
Low ejaculate volume	2	9	4	0	0	0.001
Azospermia	2	9	2	0	0	0.000
Oligospermia	2	13	7	1	1	0.003
Abnormal sperm motility	2	11	8	2	0	0.018
Abnormal sperm morphology	2	10	6	3	1	0.035

Table 3. The relationship between hypogonadism and sperm parameters.

Gonadal status	Normal	Hypogonadotropic hypogonadism	Hypergonadotropic hypogonadism	P-value
Dry ejaculate	2	2	1	0.1
Low ejaculate volume	7	5	3	0.003
Azospermia	5	6	2	0.001
Oligospermia	13	8	3	0.011
Abnormal sperm motility	11	8	4	0.001
Abnormal sperm morphology	12	7	3	0.006

level of FSH was 3.7 mIU/mL, LH was 4.6 mIU/mL, and testosterone was 4.8 ng/dL. The frequency of hypogonadotropic hypogonadism and hypergonadotropic hypogonadism were 16.1% and 6.5%, respectively. Patients with hypogonadism had significantly lower ejaculate volume, lower sperm concentration, lower percents of motile and progressively motile sperms and also lower percents of normal morphologic sperms ($P=0.001$). The relationship between hypogonadism and sperm parameters is summarized in Table 3.

The mean level of serum ferritin was 2067 ng/dL. In 74.2% of patients cardiac MRI was normal. In 21% of patients hepatic MRI was normal. Nevertheless, no significant correlation was found between the iron overload determinants and sperm parameters or having hypogonadism.

According to iron chelators, 50% of patients were using deferoxamine, 50% of patients were using deferasirox and 21% of patients were using deferiperrone. Using deferasirox did not have any impact on sperm parameters. On the other hand, in patients who used deferiperrone, oligospermia was significantly more frequent ($P=0.04$) and in patients who used deferoxamine, ejaculate volume was significantly lower ($P=0.006$). History of using hydroxyurea was documented in 32.3% of patients, yet using this drug did not have any significant impact on other evaluated parameters.

Finally, 12.9% of patients had a history of HCV RNA positivity and 48.4% of patients were splenectomized. Interestingly, HCV infection did not correlate with sperm parameters but patients who were splenectomized had significantly lower percents of motile and progressively motile sperms and also lower percents of normal morphologic sperms ($P=0.02$).

Discussion and Conclusions

Patients with β -thalassemia major experience severe anemia and so are dependent on regular blood transfusions, which in turn could lead to iron overload in different organ, particularly the cardiac, hepatic, and endocrine systems. Nevertheless, iron chelation therapy has noticeably improved the prognosis of these patients.^{25,26}

The exposure of anterior pituitary to free hydroxyl radicals, especially in early childhood leads to its damage.²⁷ Investigators have confirmed by magnetic resonance imaging that pituitary gland in β -thalassemic patients with hemochromatosis becomes atrophied²⁸ and that serum ferritin level correlates with the severity of pituitary dysfunction.^{29,30} Testicular function is regu-

lated by the pulsatile release of GnRH from the hypothalamus which stimulates the secretion of pituitary gonadotropins and then in the testis, LH mainly stimulates testosterone production and FSH promotes spermatogenesis.³¹

In our study, the correlation between pituitary hormones, testosterone, testicular volume presenting the degree of pubertal development and sperms' count and quality was confirmed. The total prevalence of hypogonadism in our patients was 22.6%, which was near what it was reported by another study in Iran (22.9%).³²

In the study by Soliman *et al.* testosterone levels were correlated significantly with all the sperm parameters and with LH levels.²⁸ This was also true in our study and patients who had lower levels of serum FSH, LH, and testosterone, had significantly lower ejaculate volume, lower sperm concentration, lower percents of motile and progressively motile sperms and also lower percents of normal morphologic sperms.

De Sanctis *et al.* showed that FSH, LH and testosterone levels were significantly lower in major thalassemia patients with acquired hypogonadism.¹⁰ This statement was also true in our study. However, they documented that the percentage of patients with serum ferritin level >2000 ng/mL (severe iron load) was significantly higher in patients with acquired hypogonadism. In our study, level of serum ferritin at the time of study did not correlate with FSH, LH, testosterone and sperm parameters. This is not a reliable conclusion because unfortunately we could not track their ferritin levels in the past. However, the severity of iron overload in heart and liver MRI of our patients, which are a more chronic representatives of iron burden, did not correlate with FSH, LH, testosterone and sperm parameters either. It has been demonstrated in different studies that the damage caused by iron overload to the gonads is an irreversible process, even if the iron level is corrected at a later stage.^{27,30,33} This affirms the significance of early and regular usage of iron chelation therapy in order to prevent the onset of damage to the pituitary gland and the gonads. ElAlfy *et al.* documented that combination chelation using deferiprone and desferrioxamine in transfusion dependant thalassemic males with good pituitary-testicular function led to progression of pubertal development but it did not improve their semen quality.³⁴ In our study, interestingly, iron chelator usage did not correlate with better sperm quality but in fact, the patients who used deferiprone and/or desferrioxamine had significantly lower ejaculate volume and also utilization of deferiprone was associated with having

oligospermia.⁵ Karimi *et al.* declared that long-term use of hydroxyurea has no effect on gonad function in β -thalassemia patients.³⁵ In our study as well, the history of hydroxyurea usage did not affect hormonal status or the sperm parameters.

The limitations of this study were as follows: Albumin and SHBG which could change the bioavailability of testosterone were not measured. Patients who had varicocele were not excluded from the study. For azoospermic patients, a karyotype or Y microdeletion assay were not performed to rule out alternative causes for poor semen parameters and smaller testicular size. Only one semen analysis was done for each subject, which is not adequate to overcome the significant variability of this test. The duration of each chelator's usage and the duration of hydroxyurea usage were not documented.

In conclusion, we found that there was a significant relationship between hypogonadism and sperm parameters, which could both affect our patients' fertility potential. As fatherhood is indubitably one of the main factors, which could improve the thalassemic patients' quality of life, therefore, the issue of male fertility and its affectors should be considered in mind.

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