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Germ Cell Dysfunction is Universal in Adolescent Male Patients with β-thalassemia Following Earlier Successful Hematopoietic Stem Cell Transplantation

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What is already known on this topic?

Preparative conditioning regimens for hematopoietic stem cell transplantation (HSCT) which compose of primarily alkylating agents have gonadotoxic effect, potentially causing primary testicular insufficiency and abnormal spermatogenesis. However, all the studies were performed in matched-related donor and matched-unrelated donor HSCT.

What this study adds?

There are no reports on male gonadal functions following haploidentical HSCT regimen. Male patients with β-thalassemia after HSCT experienced universal spermatogenesis impairment and frequent Sertoli cell dysfunction but their Leydig cell function appears to be preserved. Comparing with match donor HSCT, frequency of impaired spermatogenesis tended to be higher in haploidentical HSCT, albeit not significant. This is likely due to limited sample size.

Abstract

Objective: To assess gonadal function in adolescent male patients with β-thalassemia who underwent earlier successful hematopoietic stem cell transplantation (HSCT).

Methods: Fifty-two male patients with β-thalassemia, aged ≥10 years, who had undergone HSCT ≥2 years previously were included. Clinical data, such as age, genital Tanner (GT) stage at HSCT and enrollment, and serum ferritin levels, were collected. Gonadal function was evaluated through measurements of serum luteinizing hormone, follicle-stimulating hormone (FSH), testosterone, inhibin B levels, and semen analysis.

Results: Age at enrollment and HSCT were 17 (10-31) and 9 (1-19) years, respectively. The duration from HSCT to enrollment was 7.5 (2-20) years. Of 52 patients, 46 (88%) exhibited Sertoli cell dysfunction. Thirty-one patients had relatively small testes for their GT stage, 34 of 44 with GT V had elevated FSH of ≥5 IU/L, and 20 of 49 with GT stages 2-5 had low serum inhibin B levels. None of the patients with GT stage 5 showed Leydig cell dysfunction or gonadotropin deficiency. Serum FSH ≥8 IU/L showed the best diagnostic accuracy for detecting oligo- and azoo-spermia. All 39 patients who underwent semen analysis had > 1 abnormal parameters. Having relatively small testes for GT stage and serum FSH ≥8 IU/L were associated with oligospermia or azoospermia ($p < 0.01$).

Conclusion: Male patients with β-thalassemia after HSCT experienced universal impaired spermatogenesis and frequent Sertoli cell dysfunction but their Leydig cell function appeared to be preserved. Male patients and/or their guardians should be informed of the high likelihood of future subfertility before HSCT.

Keywords: Gonadal function, spermatogenesis, male fertility, gonadotropin, inhibin B

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Introduction

Transfusion-dependent thalassemia (TDT) is an inherited hemolytic anemia disease, frequently found in many parts of the world. Chronic anemia necessitates repetitive red blood cell transfusions, leading to tissue iron overload. Both chronic anemia and tissue iron overload contribute to morbidities, including dysfunction of endocrine organs. The pituitary gland and testis are vulnerable to iron deposition, causing tissue damage and consequently resulting in a high prevalence of hypogonadism. Hypogonadotropic hypogonadism is commonly found in patients with TDT (1). The current curative treatment for TDT is hematopoietic stem cell transplantation (HSCT). Preparative conditioning regimens for HSCT which are composed primarily of alkylating agents, such as busulfan and cyclophosphamide, have a gonadotoxic effect, potentially causing primary testicular insufficiency and abnormal spermatogenesis (2,3,4,5,6). Recently, HSCT in patients with TDT has become more common, particularly haploidentical HSCT. Various conditioning regimens for HSCT exist, employing different types and doses of alkylating agents, which may have varying effects on male gonadal function. The selection of these regimens is based on the type of stem cell donor (matched-related, matched-unrelated and haploidentical) and the patient's age at transplantation. In previous studies, the prevalence of primary testicular dysfunction based on hormonal data in post-HSCT patients with thalassemia was 20-50%. However, all these studies were performed in matched-related donor (MRD) and matched-unrelated donor (MUD) HSCT (2,3,4,5). To the best of our knowledge, there are no reports of male gonadal function following haploidentical HSCT. Age and pubertal status at HSCT also significantly correlate with gonadal dysfunction (7,8). However, there are limited data regarding gonadal function, especially spermatogenesis, and the prognostic factors in male patients with β-thalassemia, particularly β-thalassemia/HbE, following HSCT.

Methods

Study Design and Participants

This was a cross-sectional study. All surviving male patients with β-thalassemia, aged ≥10 years, who had undergone successful HSCT at least two years earlier at the Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, were eligible (n = 75). Patients currently using medications affecting gonadal function, such as tacrolimus and systemic glucocorticoids, or who had severe systemic illness at enrollment were excluded.

The enrolled participants received hematopoietic stem cell infusion from human leukocyte antigen- (HLA-) matched or HLA-haploidentical donors. According to the current clinical practice at our institute, HLA-MRDs are the first choice, followed by HLA-MUDs from national donor registries, and HLA-haploidentical donors from patient's family members. For the conditioning regimen before stem cell infusion, a combination of chemotherapy was administered, which comprised either busulfan and cyclophosphamide or busulfan and fludarabine. Later, the patients received immunosuppressive agents, a calcineurin inhibitor and either methotrexate or mycophenolate, to prevent graft-versus-host disease (GVHD). For HLA-haploidentical donors, patients would receive cyclophosphamide post-transplant to help control GVHD.

The clinical data, including age at HSCT, serum ferritin (SF) levels prior to HSCT, post-HSCT and at the time of enrollment, type of stem cell donor, type and dose of alkylating agents used in conditioning regimens during HSCT, and any complications of HSCT, were obtained by reviewing the medical records. This study was approved by Human Research Ethics Committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University (decision no: MURA2022/149, date: 01.03.2023). Written informed consent was obtained from all patients and their parents.

Cyclophosphamide Equivalent Dose

This study employed the cyclophosphamide equivalent dose (CED) to quantify the exposure to various alkylating agents commonly administered to cancer survivors, which have shown a negative correlation with spermatogenesis impairment (9,10). In the context of HSCT, the alkylating agents used were busulfan and cyclophosphamide. Therefore, the CED was calculated using the following equation: CED (g/m^2) = 1.0 [cumulative cyclophosphamide dose (g/m^2)] + 8.823 [cumulative busulfan dose (g/m^2)] (10).

Gonadal Function Assessment

All physical examinations and laboratory assessments were performed between March 2022 and February 2023. Genital Tanner (GT) stage was assessed and testicular volume was measured using a Prader orchidometer by experienced pediatric endocrinologists (N.P., P.M.). Serum luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and inhibin B levels were measured. Patients who were able to ejaculate underwent semen analysis. Semen specimens were obtained freshly and collected in sterile containers and analyzed after liquefying within 30-60 minutes. Reports of semen analysis results were

referenced using the World Health Organization (WHO) Reference Values of Human Semen Characteristics (6th edition) (11). Semen qualitative abnormalities were defined as follows: low volume (< 1.4 mL), oligozoospermia (sperm concentration < 16 million/mL), azoospermia (absence of spermatozoa), total motility < 42%, progressive motility < 30%, teratozoospermia (abnormal sperm morphology with normal forms < 4%).

Primary testicular insufficiency was defined as the presence of any dysfunction of Sertoli cells, Leydig cells or germ cells. Sertoli cell dysfunction was indicated by a relatively small testicular volume for GT stage, compared with normal testicular volume of matched healthy boys i.e. testicular volume < 4 mL for GT stage 2, < 8 mL for GT stage 3, < 15 mL for GT stage 4 or < 20 mL for GT stage 5 (12,13), or elevated serum FSH ≥5 IU/L (95th percentile of normal) for GT stage 5 (14) or low serum inhibin B ≤60 pg/mL (5th percentile of normal) for GT stages 2-5 patients (15). Normal ranges of serum inhibin B for adolescent boys with GT stages 2-5 were from 60-330 pg/mL (5th to 95th percentile). The ranges were similar between GT stages 2 to 5 (15). Leydig cell dysfunction for GT stage 5 was defined as elevated serum LH ≥6.3 IU/L (95th percentile of normal) (14) with low testosterone < 326 ng/dL (5th percentile of normal) for GT stage 5 (14). Compensated Leydig cell dysfunction was defined as serum LH ≥6.3 IU/L with normal testosterone > 326 ng/dL. Germ cell dysfunction was defined as presence of at least one abnormal parameter according to the WHO criteria for semen analysis (16).

Patients with low serum FSH, LH, and testosterone levels were suspected of having gonadotropin deficiency. To confirm this, a gonadotropin-releasing hormone analog test (2-hour test) was performed using a subcutaneous injection of 0.1 mg triptorelin and serum LH and FSH obtained every 30 minutes during the test (17). Gonadotropin deficiency was diagnosed if peak serum LH response during the 2-hour test was lower than 9.74 IU/L (18). This cut-off value was shown to distinguish hypogonadotropic hypogonadism from constitutional delayed growth and puberty with sensitivity and specificity at approximately 80%.

Serum LH, FSH and testosterone levels were analyzed by chemiluminescent microparticle immunoassay, using Alinity i analyzer (Abbott, IL, USA). The lower limits of detection were 0.04 IU/L for LH, 0.02 IU/L for FSH, and 1.44 ng/dL for testosterone. Intra-assay and inter-assay coefficients of variation (CV) were 2.0-4.3% and 2.8-4.7% for LH, 1.8-2.2% and 1.9-2.7% for FSH, and 2.3-3.5% and 2.6-8.7% for testosterone, respectively. Serum inhibin B levels were measured by in-house sandwich enzyme-linked immunosorbent assay using inhibin β_B polyclonal antibody (Invitrogen™, MA, USA). Recombinant human inhibin β_B protein (Abcam, Cambridge, UK) served as the standard, with concentrations ranging from 4.88 to 5,000 pg/mL. The lower limit of detection was 5 pg/mL. Intra-assay CV was 2.0% (based on a single run in this study).

Statistical Analysis

Continuous variables are summarized as median (range). For comparisons, chi-squared tests were used for dichotomous outcomes, while t-tests and Mann-Whitney U tests were used for continuous outcomes. Bivariate analysis was performed using regression analysis. Spearman's correlation analysis was used to assess correlations between spermatogenesis impairment and contributing factors. A receiver operating characteristic (ROC) curve was generated to determine the area under the curve (AUC) for serum FSH level as a predictor of impaired spermatogenesis. All statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA). A p value of less than 0.05 was considered statistically significant.

Results

Clinical Characteristics

A total of 52 patients were enrolled in the study. Their median (range) age at enrollment was 17 (10-31) years, and median (range) age at HSCT was 9 (1-19) years. The median (range) duration from HSCT to enrollment was 7.5 (2-20) years. Fifty of 52 patients (96%) had β-thalassemia/HbE, while the remaining two patients had ß-thalassemia major. Both patients with β-thalassemia major had clinical characteristics comparable with those patients with β-thalassemia/HbE. Nearly half (n = 25) of the patients underwent haploidentical HSCT. Three patients (6%) have had chronic cutaneous GVHD at enrollment but none required systemic treatment. Seven of 52 patients (13%) had SF levels ≥1,000 ng/mL at enrollment, indicating moderate to severe iron overload (19). All patients were pubertal; 44 of 52 (85%) patients had GT stage 5, 6 of 52 (11%) had GT stage 4, and only 1 patient was in each GT stage 2 and 3. None had received testosterone replacement therapy (Table 1).

Gonadal Function (Sertoli Cell and Leydig Cell Function)

Sertoli cell dysfunction was identified in 88% (46 of 52 patients), as indicated by either testicular volume smaller than expected for their GT stage, or elevated serum FSH levels (≥ 5 IU/L) for patients with GT 5, or low serum inhibin B levels (≤60 pg/mL) for patients with GT stages 2-5. Leydig cell dysfunction for patients with GT stage 5 was not

identified in any patients. However, compensated Leydig cell dysfunction was found in 4 of 44 (9%) patients with GT stage 5. Gonadotropin deficiency was not identified in any patients (Table 1).

Semen Quality Assessment

Of 39 semen analysis specimens, all exhibited at least one abnormality in semen parameters. The most commonly affected parameters were sperm concentration and abnormal morphology (teratozoospermia). About half (51%)

Table 1. Clinical and hormonal characteristics and semen characteristics of 52 enrolled patients

Characteristics of 52 enrolled patients	Median (range) or n (%)[a]
Age at enrollment, years	17 (10-31)
Age at HSCT, years	9 (1-19)
Pubertal	52 (100)[a]
- GT 2	1 (2)[a]
- GT 3	1 (2)[a]
- GT 4	6 (11)[a]
- GT 5	44 (85)[a]
Duration from HSCT to enrollment, years	7.5 (2-20)
Type of thalassemia	
- ß-thalassemia major	2 (4)[a]
- ß-thalassemia/hemoglobin E	50 (96)[a]
Donor type	
- Matched-related donor	15 (29)[a]
- Matched-unrelated donor	12 (23)[a]
- Haploidentical	25 (48)[a]
CED, g/m^2	
- Matched-related donor	4.0 (3.4-4.6)
- Matched-unrelated donor	3.9 (3.2-4.6)
- Haploidentical	4.6 (3.5-6.0)
Chronic GVHD at enrollment	3 (6)[a]
Pre-HSCT SF, ng/mL	1,500 (49-7,166)
Post-HSCT SF, ng/mL	809 (110-5,510)
SF at enrollment > 1,000 ng/mL	7 (13)
Clinical and hormonal characteristics of testicular dysfunction	**n (%)**
Sertoli cell dysfunction	46 of 52 (88)
- Relatively small testicular volume for genital Tanner stage	31 of 50 (62)
- Serum FSH > 5 IU/L (GT 5)	34 of 44 (77)
- Serum inhibin B < 60 pg/mL (GT stages 2-5)	20 of 49 (41)
Leydig cell dysfunction	
- Serum LH > 6.3 IU/L with serum testosterone < 326 ng/dL (GT stage 5)	0 of 44 (0)
Compensated Leydig cell dysfunction	
- Serum LH > 6.3 IU/L with serum testosterone > 326 ng/dL (GT stage 5)	4 of 44 (9)
Gonadotropin deficiency	0 of 51 (0)
- Low serum LH, FSH and testosterone with low LH and FSH response to GnRHa stimulation test	
Semen characteristics of 39 enrolled patients	**n (%)**
Germ cell dysfunction	
- ≥ 1 abnormal semen analysis parameter	39 of 39 (100)
Low volume (< 1.4 mL/ejaculate)	20 of 39 (51)
Sperm concentration	
- Azoospermia	7 of 39 (18)
- Oligozoospermia	17 of 39 (44)
- Oligo- and azoospermia	24 of 39 (62)
- Normal sperm concentration (≥ 16 million/mL)	15 of 39 (38)
Teratozoospermia (normal forms < 4%)	27 of 29 (93)
Abnormal sperm motility	
- Total motility < 42%	2 of 32 (4)
- Progressive motility < 30%	3 of 32 (6)

[a]: number (%).

HSCT: hematopoietic stem cell transplantation, CED: cyclophosphamide equivalent dose, GT: genital Tanner, GVHD: graft-versus-host disease, SF: serum ferritin, LH: luteinizing hormone, FSH: follicle stimulating hormone, GnRHa: gonadotropin releasing hormone analog

had low semen volume. Azoospermia was identified in 18%, oligozoospermia in 44% and normal sperm concentration in 38%. Of the specimens with detectable spermatozoa, 93% exhibited teratozoospermia (Table 1).

When comparing patients with normal sperm concentration to those with oligo- and azoospermia, no significant differences were observed in age at HSCT, CED, and pre-HSCT and post-HSCT SF levels. While there were more patients with SF levels ≥1,000 ng/mL at enrollment in the oligo- and azoospermia group compared to the normal sperm concentration group, this difference was not significant. The median (range) duration from HSCT to enrollment appeared longer in the normal sperm concentration group compared to the oligo- and azoospermia group, but this difference did not reach statistical significance (Table 2). However, significant differences were found in serum FSH and LH levels. Patients with oligo- and azoospermia had significantly higher FSH and slightly higher LH levels compared to those with normal sperm concentration [11.5 (3.2-22.9) vs. 4.8 (1.5-22) IU/L, $p < 0.001$ and 3.7 (1.9-11.8) vs. 3.2 (1.3-5.0) IU/L, $p = 0.04$, respectively]. There were no significant differences in serum testosterone and inhibin B levels between the two groups (Table 2). Interestingly, a high proportion of patients in these two groups had low serum inhibin B levels, 5 of 15 (33%) in normal sperm concentration and 9 of 24 (38%) in oligo- and azoospermia groups. Even among those with normal inhibin B levels, their values ranged within the lower quartile of the normal range (Figure 1).

Comparing the clinical, hormonal, and semen characteristics among different types of HSCT donors, no significant differences were found in patient age at enrollment among the three groups (Table 3). Patients who underwent haploidentical HSCT were the oldest compared to MRD and MUD. They also had had the shortest duration from HSCT to enrollment compared to MRD and MUD. Among the three groups, patients who underwent haploidentical HSCT received the highest CED compared to those who underwent MRD and MUD. There was no difference in median pre-HSCT SF levels among groups, but the haploidentical HSCT group had significantly higher post-HSCT SF levels. This group also had the highest number of patients with SF levels >1,000 ng/mL at enrollment compared to MRD and MUD. No significant differences were found in serum FSH, LH, testosterone, and inhibin B levels, even though the haploidentical HSCT group tended to have higher FSH and lower inhibin B levels (Table 3).

ROC curve analysis was used to determine the optimal serum FSH level for predicting oligo- and azoospermia. The AUC was 0.815 indicating good discriminatory power. A serum FSH level at 8 IU/L provides 73% sensitivity and 93% specificity for predicting oligospermia or azoospermia. Therefore, serum FSH 8 IU/L appears to be the optimal cut-off for identifying patients with these abnormalities (Figure 2).

Among patients with GT stage 5 who underwent semen analysis (n = 37), those with small testicular volume (<15 mL, 20 of 37 patients) had significantly higher frequency of oligo- and azoospermia, (80%, 16 of 20) compared to those with a testicular volume of >15-25 mL, (47%, 8 of 17), $p = 0.006$ (Figure 3).

Table 2. Characteristics of patients with normal sperm concentration and oligo- and azoospermia

Characteristics	Sperm concentration		p
	Normal (n = 15)	Oligo- and azoospermia (n = 24)	
Age at enrollment, years	21 (13-28)	17 (13-31)	0.07
Age at HSCT, years	9 (4-19)	9.5 (1-16)	0.63
Duration from HSCT to enrollment, years	10 (3-20)	7 (2-20)	0.07
CED, g/m^2	4.3 (3.8-5.2)	4.4 (3.3-6.0)	0.92
Pre-HSCT SF, ng/mL	1,432 (49-3,700)	1,656 (500-7,100)	0.50
Post-HSCT SF, ng/mL	660 (110-3,056)	943 (113-5,510)	0.71
Δ Pre-post HSCT ferritin, ng/mL	313 (64-2,426)	313 (-2,478-1,666)	0.95
SF >1,000 ng/mL at enrollment, n (%)	1 (6.6%)	5 (20.8%)	0.23
FSH, IU/L	4.8 (1.5-22)	11.5 (3.2-22.9)	<0.001
LH, IU/L	3.2 (1.3-5.0)	3.7 (1.9-11.8)	0.04
Testosterone, ng/dL	638 (291-1,406)	588 (350-1,369)	0.93
Inhibin B, pg/mL	99 (29-210)	77 (26-205)	0.52

Data were expressed as median (range).
HSCT: hematopoietic stem cell transplantation, CED: cyclophosphamide equivalent dose, SF: serum ferritin, FSH: follicle stimulating hormone, LH: luteinizing hormone

Table 3. Clinical, hormonal and semen characteristics among different donor-types HSCT

Characteristics	HSCT donor-types			
	MRD (n = 15)	MUD (n = 12)	Haploidentical (n = 25)	p
Clinical characteristics, median (range)				
Age at enrollment, years	18 (11-31)	18 (12-24)	17 (10-28)	0.08
Age at HSCT, years	6 (1-18)	6.5 (3-11)	11 (3-19)	0.003
Duration from HSCT to enrollment, years	10 (3-20)	9.5 (6-20)	5 (2-9)	< 0.001
CED, g/m^2	4.0 (3.4-4.6)	3.9 (3.2-4.6)	4.6 (3.5-6.0)	0.004
Pre-HSCT SF, ng/mL	1,374 (49-3,700)	2,053 (1,170-3,208)	1,500 (500-7,166)	0.44
Post-HSCT SF, ng/mL	630 (110-3,056)	557 (117-2,250)	1,176 (384-5,510)	0.02
Pre-post HSCT SF, ng/mL	314 (-91 to 2,210)	313 (43 to 1,968)	414 (-2,475 to 1,910)	0.77
SF at enrollment > 1,000 ng/mL, n (%)	1 (6.6)	0 (0)	7 (28)	0.04
Hormonal characteristics, median (range)				
Serum FSH, IU/L	7.4 (1.5-22.9)	5.0 (1.6-18.6)	10.7 (1.5-21.7)	0.20
Serum LH, IU/L	3.8 (1.3-9.8)	2.9 (1.4-5.8)	3.5 (0.4-11.8)	0.41
Serum testosterone, ng/dL	505 (267-1,406)	440 (118-1,140)	666 (39-1,369)	0.43
Serum inhibin B, pg/mL	90 (29-205)	84 (26-210)	61 (23-142)	0.07
Semen characteristics, n (%)				
Patients with semen analysis	14 (93)	8 (67)	17 (68)	-
Low volume (< 1.4 mL/ejaculate)	6 (43)	4 (50)	7 (41)	0.67
Sperm concentration				0.37
- Azoospermia	2 (14)	2 (25)	3 (18)	
- Oligozoospermia	4 (29)	3 (37.5)	10 (59)	
- Oligo- and azoospermia	6 (43)	5 (62.5)	13 (77)	
- Normal sperm concentration (≥ 16 M/mL)	8 (57)	3 (37.5)	4 (23)	
Sperm concentration, M/mL, median (range)	17.1 (0-136)	6 (0-63)	1.3 (0-149)	0.66
Teratozoospermia (normal forms < 4%)	12 of 12 (100)	3 of 4 (75)	12 of 13 (92)	0.23
Abnormal sperm motility				
- % motility, median (range)	59.5 (32-88)	69 (13-91)	65 (47-87)	0.66
- Total motility < 42%	1 of 12 (8)	1 of 6 (17)	0 of 14 (0)	0.34
- % progressive motility, median (range)	49 (27-85)	64 (13-89)	58 (43-75)	0.65
- Progressive motility < 30%	2 of 12 (17)	1 of 6 (17)	0 of 14 (0)	0.27

MRD: matched-related donor, MUD: matched-unrelated donor, HSCT: hematopoietic stem cell transplantation, CED: cyclophosphamide equivalent dose, SF: serum ferritin, FSH: follicle stimulating hormone, LH: luteinizing hormone, M: million

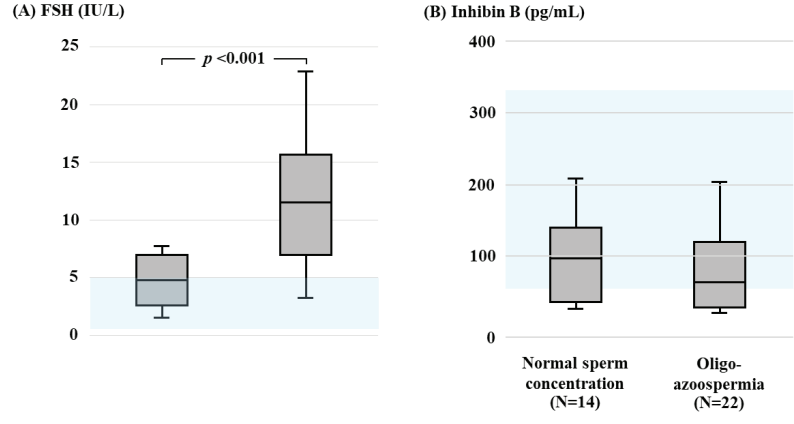

Figure 1. Comparison of serum FSH and inhibin B levels between GT stage 5 patients with normal sperm concentration and oligo- and azoospermia. In patients with oligo- and azoospermia, serum FSH levels were significantly higher than those with normal sperm concentration [11.5 (3.2-22.9) vs. 4.8 (1.5-22) IU/L, p < 0.001, respectively] (A). Serum inhibin B levels were not different between the two groups [77 (26-205) vs. 99 (29-210) pg/mL, p = 0.52, respectively]. In these 2 groups, about 35% of patients had low serum inhibin B levels (< 60 pg/mL) and those with normal serum inhibin B, their levels fell in the lower quartile of normal (B). Shaded areas represent normal ranges of serum FSH (0.6-5 IU/L) (A) and serum inhibin B levels (60-330 pg/mL) levels (B) in healthy males with GT stage 5

FSH: follicle stimulating hormone, GT: genital Tanner

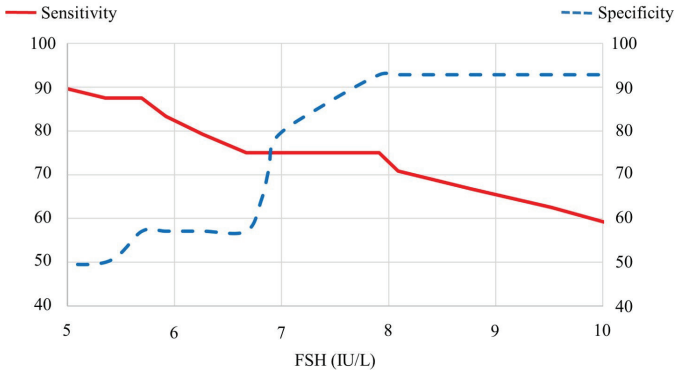

Figure 2. Sensitivity and specificity of serum FSH levels for predicting oligozoospermia and azoospermia. Serum FSH level at 8 IU/L gives the optimal cutoff for predicting oligozoospermia and azoospermia with sensitivity of 73% (solid line) and specificity of 93% (dash line)

FSH: follicle stimulating hormone

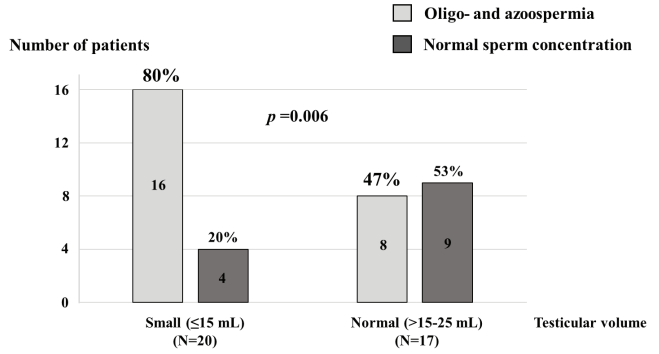

Figure 3. Compare spermatogenesis between patients with small and normal testicular size for genital Tanner stage 5. Patients with small testicular volume (≤15 mL) had a significantly higher frequency of oligo- and azoospermia than those with normal testicular volume (> 15-25 mL)

Discussion

This study provided comprehensive gonadal function assessment, including spermatogenesis, in a group of male patients with β-thalassemia/hemoglobin E who had undergone HSCT, and in particular, haploidentical HSCT. The main findings were that small testicular volume ≤15 mL among GT 5 patients and an FSH cut-off value (≥8 IU/L) were predictive factors for oligo- and azoospermia.

Germ cells are highly vulnerable to HSCT conditioning regimens as evidenced by all semen analysis specimens with at least one abnormal parameter and a high frequency of impaired spermatogenesis (62% oligo- and azoospermia) in 100% of the specimens. It is well-established that alkylating agents exhibit gonadotoxic effects and have the potential to disrupt normal spermatogenesis. In the present study, patients were administered a relatively low dose of alkylating agents (CED 3.3-6 g/m²), which contrasts with the higher doses typically used in cancer treatment (20). Consequently, the majority of patients in this study exhibited detectable sperm in their semen. However, it is noteworthy that almost all (93%) of the detectable sperm displayed abnormal morphology. This finding is consistent with a previous study in cancer survivors who underwent alkylating agent therapy without radiation exposure, where higher CED was linked to an elevated risk of azoospermia. Specifically, individuals who received CED > 10 g/m² were more likely to experience azoospermia, whereas those who received CED < 6 g/m² retained varying degrees of detectable sperm (19).

In recent years, haploidentical HSCT has been increasingly used due to limited availability of matched-donors. Haploidentical HSCT usually requires higher cumulative doses of busulfan than matched-donor HSCT (approximately 520 vs. 400-500 mg/m², respectively) whereas cumulative doses of cyclophosphamide are about 100 mg/kg in haploidentical HSCT and 0-200 mg/kg in matched-donor HSCT (21). Since there is greater gonadal toxicity when using busulfan than cyclophosphamide, CED in haploidentical HSCT was significantly higher than matched-donor HSCT. Thus, the frequency of impaired spermatogenesis tended to be higher in haploidentical HSCT, albeit not significantly. This is likely due to the small number of patients.

Impaired spermatogenesis has been documented in patients with TDT who exhibit intact hypothalamic-pituitary-gonadal (HPG) axis function (1,6). Chen et al. (1) highlighted that abnormal semen analysis findings in these patients were associated with the presence of iron deposits in the testes (22). In recent decades, T2-weighted magnetic resonance imaging (T2*-MRI) has become a key tool for diagnosing tissue iron overload in patients undergoing chronic blood transfusions (23). Despite its efficacy, SF, a conventional biomarker of iron deposit, is frequently used as a cost-efficient and readily available screening tool for assessing the likelihood of developing iron overload. A previous study demonstrated that SF levels ≥1,000 ng/mL displayed a high sensitivity (92%) and negative predictive value (91%) in discriminating between moderate to severe and mild iron overload as determined by T2*-MRI (2). In the present study, no significant association was found between oligo- and azoospermia and iron overload identified by SF levels ≥ 1,000 ng/mL. This absence of a significant association may be attributed to the limited number of patients, which may have constrained the statistical power of the study.

Previous studies have indicated that iron overload in β-thalassemia predisposes sperm to oxidative injury, leading to sperm DNA damage and subsequent subfertility (1,24). Interestingly, while pituitary function remains unaffected by iron loading, the testes are vulnerable and impacted by chronic iron overload. Rostami et al. (6) observed a rise in the frequency of oligo- and azoospermia in patients with TDT, increasing from 40% to 63% following HSCT. This finding aligns with our study, where the observed frequency was 62%. The higher occurrence of oligo- and azoospermia in patients with TDT following HSCT suggests an additional risk factor from exposure to alkylating agents during the transplantation procedure.

A greater frequency of normal sperm concentration was observed in post-HSCT patients following longer duration after HSCT (20). Our study also observed a trend towards a higher frequency of normal sperm concentration in post-HSCT patients with a longer duration since the procedure. This finding suggests the potential for spermatogenesis recovery, which aligns with a retrospective study reporting an 80% recovery rate in post-HSCT cancer survivors at seven years (25). However, our study was cross-sectional and cannot definitively assess the reversibility of spermatogenesis.

Spermatogenesis can be directly assessed by semen analysis. However, obtaining ejaculates from adolescents and young adults can sometimes be challenging. Patients frequently decline to masturbate in a private room during hospital visits. Therefore, predictive factors for oligo- and azoospermia obtained from physical examination and biochemical tests play a crucial role in clinical assessment. GT stage during pubertal progression relies primarily on testosterone effects, whereas testicular volume is mainly influenced by germ cell maturation. Consequently, patients with impaired spermatogenesis but relatively preserved Leydig cell function may exhibit smaller testes relative to their GT stage. This clinical observation is essential for predicting spermatogenesis impairment. A relatively small testicular volume for GT stage and an elevated FSH (≥8 IU/L) were identified as predictors for oligo- and azoospermia. Inhibin B, a hormone produced by Sertoli cells and post-pubertal germ cells (26), was not found to be associated with impaired spermatogenesis. This lack of association could be attributed to the low to low-normal serum inhibin B levels observed in the majority of patients.

Germ cells and Sertoli cells exhibited high vulnerability to HSCT treatment, while Leydig cell function remained comparatively preserved. Gonadotropin deficiency, a common occurrence in patients with TDT primarily caused by hypothalamic-pituitary hemochromatosis (27), was not observed in the present study. This finding could be attributed to either optimal iron chelation therapy or the possibility of reversible HPG function following HSCT. However, since we did not evaluate HPG function before HSCT, the reversibility of this function cannot be definitively proven by our results.

Study Limitations

We acknowledge several limitations in this study. First, a relatively small number of patients were recruited. Second, pre-HSCT data on puberty and serum gonadotropins to compare with post-HSCT data were unavailable. Third, healthy controls for comparison were not used. Fourth, the inability to evaluate semen analysis in adolescents who are uncomfortable or unable to masturbate significantly limited the study. Fifth, the absence of sequential semen analysis prevents us from demonstrating reversibility of spermatogenesis. Finally, there was a lack of longitudinal data on pubertal progression in patients. To enhance the robustness of future research in this area, a prospective study with a larger sample size would be beneficial. This design would provide more comprehensive information. A longitudinal study with sequential semen analysis would enable the assessment of spermatogenesis reversibility. In addition, collecting data on sequential pubertal progression and testicular maturation would improve our understanding of the impact of various factors on reproductive health outcomes.

Conclusion

Male patients with β-thalassemia/hemoglobin E who underwent HSCT during childhood or adolescence exhibited universal germ cell abnormalities and a high frequency of impaired spermatogenesis. Sertoli cell dysfunction was also frequent, while Leydig cell function remained preserved. Given these findings, patients or their guardians should be informed about the high likelihood of future subfertility and counseled on sperm cryopreservation prior to HSCT.

Ethics

Ethics Committee Approval: This study was approved by Human Research Ethics Committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University (decision no: MURA2022/149, date: 01.03.2023).

Informed Consent: Written informed consent was obtained from all patients and their parents.

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Footnotes

Authorship Contributions

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