Assessment of the ovarian reserve in patients with beta-thalassemia major: a prospective longitudinal study

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Abstract

Objective: Repeated blood transfusions in women with beta-thalassemia major (BTM) may lead to iron overload and increase oxidative stress, consequently resulting in ovarian damage. The aim was to evaluate alterations in ovarian reserve in transfusion-dependent BTM patients over a time period of one year and to compare levels of anti-Mullerian hormone (AMH) in women with BTM and their healthy peers.

Material and Methods: This longitudinal prospective study was conducted in women with transfusion-dependent BTM at a tertiary level hospital. The hospital database was interogated for women diagnosed with BTM between 1996 and 2021. AMH levels were assessed at baseline and one year later.

Results: Forty-one women with BTM were identified, of whom 25 (60.9%) had amenorrhea and 16 (39.1%) had normal cycles. The mean AMH level of all women was 2.7±1.8 ng/mL at baseline, significantly lower than the age-matched nomogram value of 4.0±0.4 ng/mL for a healthy population (p=0.001). The baseline AMH level of patients with amenorrhea were significantly lower than patients with normal menstrual cycles (2.1±1.8 vs. 3.6±1.5 ng/mL, p=0.009). After one-year follow-up, there was a trend towards a decrease in the AMH levels of patients with normal menstrual cycles.

Conclusion: Serum AMH values are decreased in patients with transfusion-dependent BTM. BTM patients should be educated about the possible effects of repeated blood transfusions on fertility. (J Turk Ger Gynecol Assoc 2023; 24: 159-64)

Keywords: Ovarian reserve, beta thalassemia, anti-mullerian hormone

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Introduction

Beta-thalassemia is the most common major monogenic hemoglobin disorder worldwide (1). Deficiency of β-globin chain synthesis is a condition that causes decreased production of red blood cells and hemoglobin, resulting in severe anemia (1,2). This disease, which was previously life-threatening, is now treated with repeated blood transfusions and iron chelation therapy, providing a decrease in morbidity and mortality. However, repeated blood transfusions may lead to an increase in reactive oxygen species over time due to excess production of free radicals and pro-oxidant/antioxidant imbalance with iron overload in multi-transfusion patients (3,4).

The accumulation of hemosiderosis in the hypothalamus and pituitary gland, also as a result of repeated blood transfusions, may cause hypogonadotropic hypogonadism (HH) in women

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(2,5) and is the most common endocrinopathy in thalassemia patients, affecting between 40-91%, and leads to sexual dysfunction and subfertility (6-8). Hypothalamo-pituitary damage is usually irreversible (2) and HH still appears to be common, despite advances in chelation regimens (6,9).

Anti-Mullerian hormone (AMH), secreted by granulosa cells of preantral and antral follicles, is a member of the transforming growth factor-β family (5,10) and its levels do not change throughout the menstrual period. It is the most reliable biochemical marker showing ovarian reserve. AMH levels are widely used in studies to show the extent of ovarian damage after the use of gonadotoxic agents in women, particularly in women who received chemotherapy after cancer diagnosis.

It is still unclear whether ovarian reserve is intact in patients with beta-thalassemia major (BTM), despite chronic blood transfusion and HH secondary to hemosiderosis. In this prospectively designed study, the aim was to evaluate the alterations in the ovarian reserve of BTM patients over a time period of one year and to compare the AMH levels between women with BTM and a healthy population. In addition, patients with BTM were divided into two groups, those with normal menstrual cycles and those who were amenorrheic, and the aim was to compare the ovarian reserve between these groups.

Material and Methods

Patient population

This longitudinal prospective study was conducted in women with transfusion-dependent BTM at a tertiary level hospital. The hospital database was interogated for women diagnosed with BTM between 1996 and 2021. In women with BTM, the clinical aim of the transfusion regimen was to maintain hemoglobin levels between 9.5 and 14.0 g/dL. Women were transfused at 14-28 day intervals. Chelation was initiated after two years of transfusion or when the serum ferritin level was consistently higher than 1000 mg/L. Clinical management aims included reducing ferritin levels below 1000 mg/L. All patients received chelation therapy. Deferasirox (maximum: 40 mg/kg/d) and deferiprone (40 mg/kg/d) were administered orally, alone or in combination. Deferoxamine was administered at a dose of 25-40 mg/kg over 5-7 days in combination with deferasirox or deferiprone. After verbal and written information about the study, all eligible and voluntary participants gave informed consent. Exclusion criteria were the presence of any comorbid systemic condition, prior chemotherapy, hormonal therapy or immunotherapy and previous ovarian surgery. This study was approved by the İzmir Katip Çelebi University Local Ethics Committee (IRB: 0044, date: 24.02.2021) and conducted according to the principles of the Declaration of Helsinki.

Serum iron and ferritin levels were tested. AMH levels were measured at admission to the study and one year later. The volume of the ovaries and the number of antral follicles were evaluated using ultrasound by a single gynecologist. Antral follicle count measurements were performed using high resolution transvaginal ultrasonography during the early follicular phase. The results of follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E_2) hormone, used to assess the pituitary and gonadal functions of the patients were noted. HH was defined as FSH and LH levels <2 IU/L with accompanying E_2 levels <20 pg/mL (11). Menstrual regularity was assessed by self-report. Blood samples taken for pre-transfusion biochemistry monitoring of all cases were collected from the biochemistry laboratory after the examination, and two blood serum samples collected from female patients at the baseline and 12 months were stored at -80 °C until evaluation.

AMH measurement

Serum AMH levels were analyzed using the Elecsys AMH Plus test with AMH assay on the Cobas-E electrochemiluminescence immunoassay platform (Roche Diagnostics GmbH, Mannheim, Germany). Units for serum AMH were ng/mL and the assay range was 0.01-23 ng/mL. The intra and inter-assay coefficients of variation were <8% and <12%, respectively.

Outcome measures

The primary outcome was to assess AMH levels after a one-year follow-up and to compare AMH levels of patients with BTM to a healthy population. The secondary aim was to compare the characteristics and AMH values of the patients based on menstrual status. Although pregnancy outcomes were not an aim of the study, information about patients who continued to be followed up and who became pregnant over time or who received assisted reproductive therapy were also included in the study.

Statistical analysis

Mean and standard deviation values of measurable variables were calculated. The normality of data distribution in data sets were evaluated using Smirnov-Kolmogorov analysis. Subsequently, a paired t-test was used to compare normally distributed data while the Wilcoxon test was used for non-normally distributed data. Categorical variables were compared using the χ^2 test. A one-sample t-test was used to determine whether AMH levels of women with BTM differs from the AMH levels of a healthy Turkish population. The correlation between AMH and ferritin levels were calculated using Pearson's correlation analysis. All statistical analyses were performed

with SPSS, version 18.0 (IBM Inc., Armonk, NY, USA). A p-value of <0.05 was accepted as statistically significant.

Results

Forty-one patients with BTM were included, with a mean age of 23.0±5.7 years. Twenty-five (60.9%) had amenorrhea while 16 (39.1%) had normal cycles. The mean age (24.4±5.9 years vs. 20.8±4.9 years; p=0.061) and body mass index (21.8±1.8 vs. 21.9±1.9 kg/m^2; p=0.781) were similar in the amenorrheic and normal cycle groups, respectively. The clinical characteristics of all patients included in the study are given in Table 1.

The mean baseline AMH value of all women was 2.7±1.8 ng/mL. This was significantly lower than the expected healthy population mean of 4.0±0.4 ng/mL, published elsewhere (p=0.001) (12). The mean AMH levels of all women was evaluated after one year to find out whether there was a significant decline in AMH levels over this short time period. The mean AMH levels tended to be lower after one year compared to baseline but this was not significant (baseline 2.7±1.8 ng/mL vs. 2.5±1.8 ng/mL at one year; p=0.207).

Ovarian reserve of women with BTM was evaluated in subgroups, stratified by menstrual status. The mean number of antral follicles in the amenorrhea group was 8.6±5.7 while this was 12.0±2.3 in patients with normal cycles (p=0.048). Mean ovarian volume was 5.5±4.4 cm^3 and 10.9±5.7 cm^3 in patients with amenorrhea and normal cycle, respectively (p=0.003). Baseline AMH values in patients with amenorrhea were significantly lower than in patients with normal menstrual cycles (2.1±1.8 vs. 3.6±1.5 ng/mL, respectively, p=0.009). FSH and LH values of amenorrheic patients were also lower than in patients with normal cycles (Table 2). After a one-year follow-up, there was a trend towards a decrease in AMH levels in the normal cyle group, while a significant difference persisted between patients with amenorrhea and normal cycle (2.1±1.8 vs. 3.3±1.5, respectively; p=0.031).

In the correlation analysis, a significantly inverse correlation was found between AMH levels and ferritin levels, both at baseline (r=-0.486, p=0.004, Figure 1A) and one year later (r=-0.488, p=0.003, Figure 1B). Ferritin levels were higher in amenorrheic patients than in patients with normal menstrual cycle (p=0.041).

More than half of the cohort (22/41, 53.7%) was married. Twenty were married to healthy partners, whereas two were married with partners with BTM. Of these 22, 10 women were amenorrheic and 12 had normal menstrual cycle. Six of the 12 patients with normal menstrual cycle (50%) gave birth to seven healthy babies without receiving any treatment. Out of 10 women with amenorrhea, four underwent in vitro fertilization procedure. Only one (25%) achieved pregnancy and had a live birth. The remaining six women did not wish to become pregnant at the time of the study and had not started any infertility treatments.

Table 1. The demographic and clinical characteristics of all patients with beta-thalassemia major

	Patients (n=41)
Age, years	23.0±5.7
BMI, kg/m^2	21.8±1.8
FSH, mIU/mL	3.1±2.4
LH, mIU/mL	2.9±2.9
E_2, pg/mL	58.9±129.4
Initial AMH levels, ng/mL	2.7±1.8
AMH levels at 12 month, ng/mL	2.5±1.8
AFC	9.9±4.9
Mean ovarian volume, mm^3	7.6±5.5
Mean uterine volume, cm^3	29.2±23.5
Initial ferritin levels, ng/mL	2,208±1,811
Ferritin levels after one year, mL/ng	2,280±2,040

BMI: Body mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E_2: Estradiol, AMH: Anti-Mullerian hormone, AFC: Antral follicle count

Table 2. Comparison between the clinical features of patients with amenorrhea and normal cycle

	Amenorrhea, (n=25)	Normal cycle, (n=16)	p-value
Age, years	24.4±5.9	20.8±4.9	0.061
Years receiving transfusions	20±5	18±5	0.005
BMI, kg/m^2	21.8±1.8	21.9±1.9	0.781
FSH, mIU/mL	2.5±2.3	4.2±2.1	0.040
LH, mIU/mL	1.7±1.5	4.7±3.6	0.001
E_2, pg/mL	16.4±14.2	125.7±121.5	0.011
Initial AMH levels, ng/mL	2.1±1.8	3.6±1.5	0.009
AMH levels at 12 month, ng/mL	2.1±1.8	3.3±1.5	0.031
AFC	8.6±5.7	12.0±2.3	0.048
Mean ovarian volume, mm^3	5.5±4.4	10.9±5.7	0.003
Mean uterine volume, cm^3	19.1±19.0	45.0±21.5	0.001
Initial ferritin levels, mL/ng	2,742±2,019	1,475±1,155	0.041
Ferritin levels after one year, mL/ng	2,862±2,285	1,400±1,066	0.032

BMI: Body mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E_2: Estradiol, AMH: Anti-Mullerian hormone, AFC: Antral follicle count

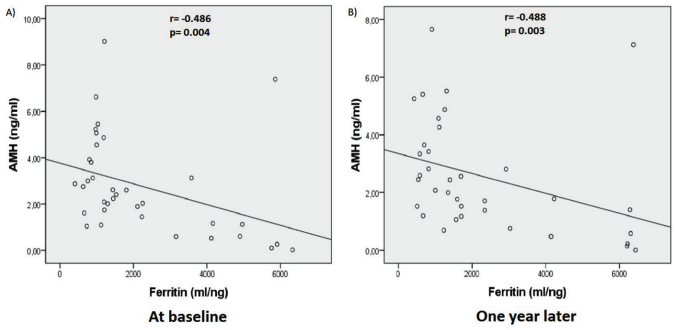

Figure 1. A) Correlation curve of initial anti-Mullerian hormone (AMH) and ferritin values at baseline, B) correlation curve of AMH and ferritin values one year later

Discussion

In this prospectively designed study, the effects of repeated blood transfusions on AMH levels at baseline and after a one-year follow-up in women with BTM were investigated and it was found that women with BTM had lower AMH levels compared to age-matched population norms. Furthermore, there was a trend towards a decrease in AMH levels even over such a short follow-up period. The presence of amenorrhea seemed to be mainly associated with hypothalamic dysfunction, rather than ovarian reserve. Hovewer, it appears that longer duration of blood transfusions may lead to hypogonadotrophic hypogonadism, resulting in lower AMH levels and decreased ovarian volume compared to women with BTM and with regular cycles. To the best of our knowledge, this is the first prospective longitudinal study demonstrating an alteration in ovarian reserve of women with BTM after only a one-year follow-up.

HH (70-80%) and amenorrhea due to hemosiderosis of the hypothalamus and pituitary gland are common in BTM patients (2,11,13). However, the effect of iron load on the ovaries is unclear (14-16). There are only limited data available in concerning ovarian reserve in patients with BTM (2,5,9,10), In a study conducted with 17 BTM patients (9 amenorrheic, 8 with normal menstrual cycles), the mean age of the patients was 33.8±5.6 years (2). AMH values of all 17 BTM patients were significantly lower than the control group and AMH levels in the amenorrheic group were lower than in patients with normal menstrual cycles. In our cohort, the frequency of amenorrhea was 60.9% in BTM and the same pattern was seen when comparing AMH levels between amenorrheic group and women with normal cycles. In another study, 29 BTM patients with a mean age of 21.4±5.8 years and 29 control patients with a mean age of 21.5±6.2 years were evaluated in terms of ovarian reserve. Although AMH values were lower in the BTM group compared to the control group, the result was not significant (5). Singer et al. (9) evaluated 26 patients with BTM and compared AMH levels between amenorrheic women (29±5 years) and women with normal menstrual cycles (27±8 years) and they concluded that there was no significant difference between the two groups in terms of AMH values and antral follicle numbers. All these studies were retrospective and study populations were small (2,5,9). Uysal et al. (10) compared AMH values between BTM patients (n=43) and healthy controls (n=44) in a larger, prospectively designed, case-control study. The mean age of patients with BTM was 23.4±5.1 years. When they compared the BTM group with the control group, the median AMH values (1.7 vs. 3.5 ng/mL; p=0.002, respectively) and median antral follicle numbers (3 vs. 11; p<0.001, respectively) were found to be lower in the BTM group. When subgroup analysis was performed among BTM patients, amenorrheic patients had lower AMH and antral follicle counts than patients with normal menstrual cycles. Since women with normal menstrual cycles were younger than the amenorrheic group and they had a shorter duration of transfusion therapy, it seems reasonable that they would have better ovarian reserves (10). Similar to the study by Uysal et al. (10), in the present study the amenorrheic group had lower AMH levels, antral follicle numbers and mean ovarian volumes. Given that there are studies reporting that AMH was not influenced by hypothalamic amenorrhea (17,18), significantly decreased AMH levels in women with amenorrhea may be due to the direct gonadotoxic effects of iron overload.

In a study that investigated patients with BTM who received chelation therapy for 5 years, high serum ferritin level, poor compliance with chelation therapy and early-onset transfusion therapy were found to be the main risk factors associated with endocrine complications (8). The same study also reported that serum ferritin levels of 2000 ng/mL were related with hypogonadism. Chang et al. (5) reported ferritin levels to be significantly higher in patients with HH than in those without HH. In addition, a negative correlation was found between ferritin and AMH levels (5,10). These authors concluded that high ferritin levels may lead to ovarian damage as well as the dysfunction in hypothalamo-pituitary axis. Although women with HH may have lower AMH levels due to long-term ovarian suppression (11,17,18), AMH level is still one of the best predictors of controlled ovarian stimulation in these women (17,18). In the present study, a significant inverse correlation was found between AMH and ferritin levels, tested both at baseline and one-year follow-up. Consistent with other studies (10), ferritin levels were demonstrated to be higher in amenorrheic patients than patients with normal menstrual cycles.

It is a matter of debate whether pregnancy and fetal outcomes are comparable between the healthy population and patients with BTM. Cesarean section is frequently preferred in women with beta-thalassemia, and infant weight at birth is usually

lower than the general population, despite term delivery (19). Spontaneous abortion, fetal loss, preterm delivery, fetal growth restriction and low birth weight are more common in pregnant women with BTM compared to healthy women (20). In the present study, we reported a small number of pregnancy outcomes. All went to term without any fetal or obstetric complications.

Study Limitations

One of the limitations of this study was the lack of control group. However, the availability of an age-specific AMH nomogram for the Turkish population was used to compare AMH levels. Furthermore, when we limited the study to the patients with regular menstruation, there was no difference in AMH levels with the healthy population. This may have been due to the inclusion of a relatively younger population and shorter follow-up period. Therefore, further research with a longer follow-up period is warranted to draw more definite conclusions regarding the possible time period in which iron accumulation initiates ovarian damage and to distinguish HH-related low AMH levels from low AMH levels due solely to direct ovarian damage.

Conclusion

The AMH values of transfusion-dependent BTM patients decreased significantly over time and the frequency of amenorrhea increased with advance in age. It would be beneficial to inform all patients regarding possible effects of repeated blood transfusions on fertility. Further research is required to understand the extent of ovarian damage in older BTM women with HH.

Ethics Committee Approval: *This study was approved by the İzmir Katip Çelebi University Local Ethics Committee (IRB: 0044, date: 24.02.2021) and conducted according to the principles of the Declaration of Helsinki.*

Informed Consent: *After verbal and written information about the study, all eligible and voluntary participants gave informed consent.*

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