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Characteristics of markers of ovarian reserve in women with transfusion-dependent beta thalassaemia major with a successful pregnancy outcome

Abstract

Objective: Spontaneous fertility and successful pregnancies have been reported in well-chelated and transfused women with beta thalassaemia major (BTM) however majority of women are sub fertile due to hypogonadotropic hypogonadism (HH) and lack of ovulation. Little is known about the effect of iron overload on ovarian follicles and whether ovarian reserve is affected by the condition or treatment. The predictive value of markers of ovarian reserve in relation to live birth in this group of women remains unclear. The aim of this study was to characterize the markers of ovarian reserve in women with transfusion-dependent beta thalassaemia major who had at least one successful live birth.

Study design: This is a retrospective study in which we collected data from 12 women with transfusion-dependent BTM and at least one successful live birth from our thalassaemia clinic between July 2007 to June 2022. Patient demographics, medical history, menstrual history, hormonal parameters (serum levels of FSH, oestradiol, TSH and AMH) and antral follicle count were recorded. Serum levels of ferritin, cardiac T2*, liver iron concentration and thyroid function results were recorded from clinic visit prior to either natural conception or assisted conception treatments.

Results: There was a wide variation in serum levels of AMH and antral follicle counts amongst women with BTM who had a successful live birth. Low serum AMH levels were noted in 4 women with HH with a background of BTM (33.3%) as compared to the established normal ranges for women of similar age. Also, low AFC counts were noted in 7 women (out of which 6 had HH) with BTM (58.3%) as compared to the established normal ranges for women of similar age.

Conclusion: Levels of serum AMH and antral follicle counts appeared lower in up to half the women with BTM and hypogonadotropic hypogonadism with a successful live birth as compared to established normal ranges for women of similar age suggesting that these markers may not accurately reflect the ovarian reserve for all in this group of women. There are limited data about the predictive value of contemporary markers ovarian reserve such as serum AMH and AFC levels in predicting successful fertility or pregnancy outcomes in women with transfusion dependent BTM and larger studies are needed.

Keywords: beta thalassaemia major, ovarian reserve, fertility, live birth

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Introduction

Beta-thalassaemia major (BTM) is the most common monogenic haemoglobin disorder in the world and its treatment consists of long-term blood transfusion therapy for correction of anaemia and iron chelation therapy.[1–3] Advances in the primary care of women with BTM have improved their quality of life as well as survival rates into adulthood, and fertility is a major consideration for many of these women.[1,4] Although spontaneous fertility and successful pregnancies have been reported in well-chelated and transfused women with spontaneous puberty, majority of women are subfertile due to hypogonadotropic hypogonadism (HH) because of damage to the hypothalamo-pituitary (HP) axis because of transfusional haemosiderosis.[4–8] There are multiple reports of successful pregnancies in women with BTM (with optimal chelation) with use of gonadotropins for ovulation induction and/or in vitro fertilization (IVF), suggesting that the ovaries may be spared from the damage caused by iron overload in earlier years of life.[4,5,8] However, little is known about the effect of iron overload on ovarian reserve, and it is unclear whether the iron accumulation and oxidative stress causes ovarian damage leading to low ovarian reserve and poor reproductive outcomes in women with BTM. Although it is known that ovarian reserve parameter like serum anti-Mullerian hormone (AMH) level has a poor prediction of fecundity in healthy women without background medical problems, there are limited data on the predictive value of contemporary markers of ovarian reserve in predicting successful fertility outcomes or live birth in women with BTM. In this retrospective analysis, we collected data about markers of ovarian reserve from 12 women with transfusion-dependent BTM who went on to have a successful live birth to assess the variation in such markers and compare their levels to the normal ranges accepted for healthy women without BTM.

Materials and methods

This was retrospective analysis involving 12 women with transfusion-dependent BTM from thalassaemia clinic who reported at least one live birth at the Reproductive Medicine Unit in University College London Hospital. We collected data from medical case records between July 2007 to June 2022. Opinion was sought from the Joint Research Office of the hospital and formal ethics approval was not

required as the project only involved non-identifiable data collection and no change in routine clinical practice. The study group consisted of 12 women diagnosed with BTM who required regular transfusion and iron chelation therapy for at least 10 years before inclusion. The transfusion regimen for these women allowed haemoglobin levels to be maintained between 9.5 and 14.0 g/dL. Women were transfused at intervals of 14–28 days; after two years of transfusions or when the serum ferritin level was consistently greater than 1000 µg/L, chelation was started. An effort was made to decrease ferritin levels below 1000 µg/L. The transfusion and chelation regime was followed according to our previously published protocol.[9] In cases of women who had more than one live birth, only data from clinic visit prior to the first live birth were collected. The exclusion criteria were any prior gynaecological surgery or any other past medications which could be considered toxic to ovaries. Patient demographics, medical history, menstrual history, hormonal parameters (serum levels of follicle stimulating hormone (FSH), oestradiol, thyroid stimulating hormone (TSH) and anti-Mullerian hormone (AMH – Elecsys) and antral follicle count (AFC) were recorded. Serum levels of ferritin, cardiac T2*, liver iron concentration and thyroid function results were recorded from clinic visit just prior to either starting to try for natural conception or embarking on assisted conception treatments such as ovulation induction (OI) or In vitro fertilisation (IVF). Women were excluded from the study if there was no information about any one of the three important ovarian reserve markers – FSH, AMH and AFC.

Blood samples in our unit are routinely obtained during the early follicular phase (between the 2nd to 5th days of the menstrual cycle) in women with spontaneous menstrual cycles, and one month after cessation of hormone replacement therapy in those with amenorrhoea. AFC measurements are performed using high-resolution transvaginal ultrasonography during early follicular phase. Results of serum AMH and AFC levels in women with BTM were compared to the established normal ranges for women of similar age from our unit laboratory. Following normal ranges were used for comparison of results of serum AMH levels - 20–24 years - 8.7–83.6; 25–29 years - 6.4–70.3; 30–34 years - 4.1–58; 35–39 years - 1.1–53.5; 40–44 years - 0.2–39.1; 45–50 years - 0.1–19.3. Normal ranges used for AFC were – 25-34 years – 11-19; 35-39 years – 8-10; 40-45 years – 5-7.

Results

The clinical and ovarian reserve markers data collected from 12 women with BTM with a successful live birth are presented in Table 1. Nine of the 12 women with BTM had HH and suffered from amenorrhoea and received hormone replacement therapy until natural conception or initiation of assisted conception treatment. There was a wide variation in serum levels of AMH (0.9 to 32.8 pmol/L) and antral follicle counts (2 to 22) amongst women with BTM who had a successful live birth. Low serum AMH levels were noted in 4 women with HH with a background of BTM (33.3%) as compared to the established normal ranges for women of similar age. Two women with BTM between 30-34 years and another two in the age group of 35-39 years had AMH levels below the expected normal range and conceived following ovulation induction treatment and had live birth. All four were diagnosed with hypogonadotropic hypogonadism and used hormone replacement therapy for many years prior to conception. Low AFC counts were noted in 7 women (out of which 6 had HH) with BTM (58.3%) as compared to the established normal ranges for women of similar age. One out of these seven women conceived naturally while two needed IVF treatment and other four had successful ovulation induction treatment.

Table 1 Clinical data collected from 12 women with BTM with at least one successful live birth

AMH (pmol/L)	Oestradiol (pmol/L)	TSH (mIU/L)	AFC	Ferritin (µg/L)	Liver iron (mg/g dry wt.)	Cardia T MRI (ms)	HbA1C	Total live births	Menstrualcycle pattern	HRT use	Age at pregnancy (years)	BMI (kg/m2)	Age at menarche (natural or HRT induced)	Menstrual cycle length in days	Mode of conception	Comorbidities
22	<44	0.21	19	1837	4.3	22	35	2	Amenorrhoea	Yes	31	20.5	15	28	OI	Hypothyroidism, Diabetes, Osteoporosis
2.9	<44	1.19	2	2384	15.1	28	41	1	Amenorrhoea	Yes	34	19.2	14	28	OI	
11.2	122	4.1	9	900	4.8	35	37	1	Oligomenorrhoea	No	39	32.3	13	45-60	OI	Hypothyroidism,
8.8	349	3.16	21	1482	6.9	50	31	1	Normal (32 days)	No	34	27	14	32	Natural conception	
32.8	<44	1.24	22	1870	15.2	31	29	2	Amenorrhoea	Yes	32	21.5	15	28	OI	
1	<44	5.2	5	3000	16.1	40	36	1	Amenorrhoea	Yes	38	29.2	14	28	OI	Hypothyroidism
6.3	68	1.61	4	500	3.8	18.7	34	1	Amenorrhoea	Yes	30	18	13	28	IVF	Hypertension
0.9	<44	2.14	2	1371	4.8	20	28	1	Amenorrhoea	Yes	36	29.3	14	28	OI	Diabetes
7.3	78	1.9	11	1626	6.5	23.2	39	1	Amenorrhoea	Yes	31	20.1	15	28	OI	
4.6	<44	2.39	5	1741	1.3	16	31	1	Amenorrhoea	Yes	35	24.9	18	28	IVF	Hypothyroidism, Diabetes, Hypertension
8.9	681	3.43	9	1497	7.1	NA	37	1	Oligomenorrhoea	No	28	23	16	35-40	Natural conception	
3.8	100	4.11	4	2775	2	19.1	25	1	Amenorrhoea	Yes	30	19	14	28	OI	Hypothyroidism

Discussion

Medical advances in the primary care of women with BTM (optimal blood transfusion and chelation therapies) have significantly improved their quality of life and long-term survival.[1–3] Many of these women are keen to start their own family and fertility has become a major consideration in adult life.[4] Although natural pregnancies have been reported in well-chelated and transfused women with spontaneous puberty, the commonest abnormality from a reproductive perspective for most women is HH.[4–8] HH is invariably irreversible and, in women, can present as primary amenorrhoea, delayed puberty or secondary amenorrhoea with consequent subfertility. HH due to transfusional iron overload affects 70 to 80% of thalassaemic patients worldwide.[6–8] There remains significant uncertainty about the effects of iron overload on ovarian function and endometrial health.[4,10–12] Studies have suggested that iron-induced damage impairs oocyte function, with increased levels of redox-active iron in follicular fluid contributing to chronic oxidative stress altered cellular function/protein synthesis, damage to DNA and subfertility.[5,10–13] Reproductive outcomes in BTM women cannot be well predicted by age, menstrual status or transfusion and chelation parameters.[14,15] The data form studies which have assessed ovarian reserve and fertility in women with BTM are heterogeneous as there is variation in study types, population under study, ethnicity, degree of iron load, extent of multi-organ involvement, types of investigations/interventions used and inclusion criteria for participants.

Several markers of ovarian reserve have been investigated with the aim of evaluating ovarian health and reproductive status. Tests for ovarian reserve have included – 1. Biochemical tests such as early follicular phase hormone levels (FSH, LH, oestradiol, Inhibin A and B, and AMH), 2. Ovarian stimulation tests and 3. Biophysical tests such as ultrasound techniques for assessing the number of antral follicles in the ovary in early follicular phase or ovarian volume. The FSH levels in the early follicular phase are not reliable in women with HH as markers of gonadal function.[12] AMH, a member of the transforming growth factor-b superfamily, is primarily secreted by the granulosa cells of growing follicles and may indirectly reflect the

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size of the primordial follicle pool in the ovary which constitutes the ovarian reserve.[12,16–18] AMH concentrations are constant during the menstrual cycle, have an excellent correlation with the antral follicle count and decline with age thus making it a useful marker of ovarian reserve.[12,16–21] Singer et al.[22] studied fertility markers in 26 women with thalassaemia major.[22] They found that low gonadotropin secretion resulted in reduced ovarian antral follicle counts and ovarian volume in such women. The levels of AMH were mostly normal. Chang et al.[12] demonstrated that serum AMH levels were lower in women with transfusion-dependent BTM when compared with healthy women of a similar age.[12] In addition, serum ferritin levels in women with BTM were also noted to be significantly and inversely related to the AMH concentrations. Other iron overload-related morbidities or risk factors such as advanced age, haematological phenotypes, diabetes, and the presence of HH were not related to the AMH levels in this study.[12] Another recent study which investigated serum AMH levels in 43 women with transfusion-dependent BTM in comparison to 44 age-matched healthy controls found that levels of FSH, LH, oestradiol, prolactin, AMH, antral follicle count and ovarian volume were significantly lower in women with BTM compared with controls.[23] A significant correlation was found between ferritin concentrations and amenorrhea.

Our previous 10-year longitudinal study in 17 women with transfusion-dependent BTM compared the results of ovarian reserve markers with 52 age-matched healthy women. We found significantly lower levels of AMH, early follicular phase oestradiol and antral follicle counts in women with BTM in comparison with healthy controls.[24] There was a positive correlation between AMH levels and AFC in women with BTM. It seemed unlikely that the low levels of AMH were purely because of HH as the levels of these markers were low even in women who had normal gonadotropins and spontaneous menstrual cycles. Although comparison of AMH levels between controls and women with menstrual activity and BTM did not result in a statistically significant result, the serum levels of AMH appeared low to what would be considered a normal ovarian reserve for women of this age group. This therefore suggested a direct effect of iron overload due to disease or treatment as an independent cause of ovarian damage apart from its indirect effect of suppression of ovarian activity due to HH. In our current study, we found a wide variation in serum levels of AMH (0.9 to 32.8 pmol/L) and antral follicle counts (2 to 22) amongst women with BTM who had a successful live birth. Low serum AMH levels were noted in 4 women with HH with a background of BTM as compared to the established normal ranges for women of similar age. Low AFC counts were noted in 7 women (out of which 6 had HH) with BTM as compared to the established normal ranges for women of similar age. There is little doubt that suppression of ovarian activity secondary to HH would be a significant factor causing low levels of AMH and AFC however direct damage to the ovary from iron overload also could be a contributing factor.

Our analysis shows that gold standard markers of ovarian reserve such as levels of AMH and AFC can be lower than the expected normal ranges in women with BTM especially with associated HH and may not accurately predict the ovarian reserve. Decisions regarding fertility treatment, therefore, should be individualized based on age, menstrual pattern, background medical history and combination of biochemical and biophysical parameters. Despite low AMH, ovulation induction using gonadotropins could be a reasonable first choice treatment for many of these women before resorting to complex and more intrusive treatments such as IVF as the AMH and AFC tests may underestimate ovarian reserve in this group of women. It is important to highlight the strengths and limitations of our study. We obtained data from a homogeneous group of women with BTM who were managed with uniform standardized protocols for chelation and fertility treatment in our hospital (preventing variability in management of BTM and subfertility), so it is a single center experience. However, serum levels of AMH do not accurately predict natural conception and live birth, and the rate of decline in ovarian reserve (and AMH) can vary considerably between individual women.[25,26] AMH levels are also shown to be less reliable as a marker of ovarian reserve in individuals with HH due to various causes.[27] Other limitations of our analysis include small cohort of patients given the background medical complexities and retrospective data analysis. Also, the data were collected over a long period of time and standards of clinical care can vary over time. We could not stratify patients according to the type of chelators and HRT they were receiving prior to conception. We also did not have detailed information about the time to conceive or type of medications and regimen used during fertility treatment for these women. However, the aim of this paper is to provide the health professionals who discuss tests of ovarian reserve with women with BTM, practical information about their relevance and characteristics in their unique situation. Not many similar reports exist in literature and data about ORT test results are scarce in settings of BTM. Further prospective studies with bigger participant numbers are therefore required to elucidate the mechanisms by which iron overload can adversely affect the ovarian reserve besides its impact through HH and to better characterize existing or novel markers of ovarian reserve in women with BTM.

Conclusion

Levels of serum AMH and antral follicle counts appeared lower in up to half of the women with BTM and hypogonadotropic hypogonadism with a successful live birth as compared to established normal ranges for women of similar age suggesting that these markers may not accurately reflect the ovarian reserve for all in this group of women. There are limited data about the predictive value of contemporary markers ovarian reserve such as serum AMH and AFC levels in predicting successful fertility or pregnancy outcomes in women with transfusion dependent BTM and larger studies are needed.

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Conflicts of interest

The author declares that there is no conflict of interest.

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