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A 10-year longitudinal study of evaluation of ovarian reserve in women with transfusion-dependent beta thalassaemia major

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ABSTRACT

Objective: Although spontaneous fertility and successful pregnancies have been reported in well-chelated and transfused women with beta thalassaemia major (BTM), majority of women are subfertile due to hypogonadotropic hypogonadism (HH). Little is known about the effect of iron overload on ovarian follicles and whether ovarian reserve is affected by the disease or treatment status. This study compares the markers of ovarian reserve in women with transfusion-dependent BTM over a period of ten years with healthy women from a control population.

Study design: We performed a 10-year mixed (retrospective and prospective) longitudinal study in 17 women with transfusion-dependent BTM from our thalassaemia clinic between July 2007 to June 2017. The results were compared with 52 age-matched healthy women without any medical conditions (control population) attending our fertility clinic. Patient demographics, medical history, menstrual history, hormonal parameters (serum levels of FSH, estradiol, TSH and AMH) and antral follicle count were recorded in all women from both groups. Serum levels of ferritin, cardiac T2*, liver iron concentration, thyroid function (TSH) and liver function test results were also recorded at three different time points.

Results: Serum AMH levels, estradiol levels and antral follicle count were significantly lower in women with BTM compared with the control group ($p < 0.05$ for all). Low AMH levels were noted in both groups of women (with and without HH) with a background of BTM. Serum AMH levels positively correlated with AFC in women with BTM.

Conclusion: Serum AMH level and AFC were significantly lower in women with transfusion dependent BTM as compared to age-matched healthy controls suggesting a direct impact of the disease activity or iron overload on the ovary.

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 as a result of transfusional haemosiderosis [4–6]. Almost invariably, such HP axis damage is irreversible although a recent case report demonstrated spontaneous recovery of menstruation and natural conception in a woman who was diagnosed with HH with evidence of iron overload in heart and anterior pituitary gland [4–8]. Recent literature also contains 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, there are limited data available on 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. Following factors may contribute to the scarcity of data available on this topic –

1. Women diagnosed with BTM who suffer from damage to HP axis, fail to enter spontaneous puberty and are commenced on hormone replacement therapy (HRT - for bone and cardiovascular

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benefits) which continues until natural age of menopause. Many women may not like to come off the medication only for the purpose of periodically testing their ovarian reserve, and an accurate assessment of ovarian reserve is not feasible in women while they are on HRT.

2. Iron overload with BTM is dynamic and will vary in the same woman from time to time depending on severity of the disease, compliance with chelation and other organ involvement (such as pancreas, heart, liver). Results of assessment ovarian reserve may therefore fluctuate depending on the timing of testing.

3. Iron overload, which causes organ dysfunction, can start very early in life (before puberty) and cumulative damage caused by iron deposition in various organs will increase with age.

4. 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.

Given the limitations of current available data, there is a need for long-term longitudinal study to assess the effect of iron overload on ovarian reserve in women with BTM. In this paper, we present the results of a mixed longitudinal study to investigate the markers of ovarian reserve - follicle stimulating hormone (FSH), anti-Mullerian hormone (AMH) and antral follicle count (AFC) in women with transfusion-dependent BTM over a period of ten years and compared results with healthy women from a control population. To the best of our knowledge, this is the first study which links longitudinal haematological data from long-term follow-up of women with BTM with their ovarian reserve markers and compares the results with suitable control population.

Table 1
Serial longitudinal data collected from 17 women with BTM at three different time points at least one year apart (T1 – 2007 to 2010, T2 – 2011 to 2014 and T3 – 2015 to 2017).

Case no.	Time point (T)	Menstrual cycle length (days)	TSH (mIU/L)	Liver Iron Concentration (mg/g dry wt.)	Ferritin (μg/L)	Cardiac T2* ms	Time from diagnosis of BTM to ovarian reserve testing (completed years)	Co-morbidity
1	T1	28-30	1.56	7.4	673	25	18	Hypertension
	T2	28-30	3.7	3.4	485	41		
	T3	28-30	2.21	3.8	700	35		
2	T1	Amenorrhoea	5.6	10.8	6122	21.4	23	Hypothyroidism
	T2		2.1	8	3001	33		Osteoporosis
	T3		3.7	5.2	1896	40		
3	T1	Amenorrhoea	2.56	2.2	2251	11	21	Recurrent gastrointestinal bleeding
	T2		0.76	4.3	1910	17		
	T3		1.13	3.1	1545	26		
4	T1	Amenorrhoea	1.11	14	1003	11	18	–
	T2		2.98	–	971	22		
	T3		–	18.3	2419	–		
5	T1	30-32	3.54	2.1	1999	10	23	Past history of elevated liver enzymes (abnormal liver function tests)
	T2	30-32	3.01	2.63	765	45		
	T3	30-32	3.78	4.1	1235	35		
6	T1	28	3.44	5.6	1452	12	27	Diabetes
	T2	27-28	1.56	3.24	976	32		
	T3	28	1.69	5.1	1222	31		
7	T1	Amenorrhoea	9.82	6.64	2531	34.8	19	Diabetes
	T2		4.45	4.45	–	34.5		Hypothyroidism
	T3		3.16	5.6	1897	29		Hepatitis C
8	T1	Amenorrhoea	3.87	3.7	8117	33.6	15	–
	T2		2.43	15.1	4904	32		
	T3		3.15	–	3191	28.3		
9	T1	30-32	2.13	4.51	1432	28	24	–
	T2	30	2.88	5.7	1011	35		
	T3	30	3.12	4.2	991	37.4		
10	T1	28-30	1.56	8.7	750	31	22	Osteoporosis
	T2	28-30	1.42	11.3	2059	25.6		Abnormal liver function tests
	T3	28-30	2.14	14	3641	21		
11	T1	Amenorrhoea	1.79	5.4	1623	11.8	10	–
	T2		3.24	3.9	1837	13.1		
	T3		3.67	4.9	3641	15		
12	T1	Amenorrhoea	2.15	3.2	3385	19	27	Past history of cardiac failure
	T2		4.01	4.5	2117	31		
	T3		3.15	4.67	1854	35		Pancreatitis
13	T1	30-32	1.87	10.4	1584	39.8	18	–
	T2	30	–	1.95	1492	46.6		
	T3	30-32	1.26	6.4	1834	38		
14	T1	28-30	1.93	5.2	1600	32.2	24	Osteoporosis
	T2	28-30	2.98	2.76	965	31		
	T3	28-30	4.09	4.44	1030	29		
15	T1	30	1.23	3.87	1187	42.1	23	Past history of deep vein thrombosis
	T2	30	2.53	6.54	1843	37.8		
	T3	30-32	1.68	4.6	1798	38.2		
16	T1	Amenorrhoea	1.09	3.2	978	36.6	18	Diabetes
	T2		1.09	–	1223	41.5		
	T3		0.98	4.67	1328	35		
17	T1	Amenorrhoea	1.11	8.23	1342	28.9	24	Arrhythmia
	T2		0.87	5.67	876	34.6		Hypertension
	T3		1.85	4.21	978	33.2		

Materials and methods

This was a 10-year mixed (retrospective and prospective) longitudinal study conducted in 17 women with transfusion-dependent BTM from thalassaemia clinic at the Reproductive Medicine Unit in University College London Hospital. We performed a retrospective analysis of medical case records of all 17 women and prospectively followed 9 women from 2014 to obtain data relevant to the study between July 2007 to June 2017. The results were compared with 52 age-matched healthy women without any medical conditions (control population) attending our fertility clinic.

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 17 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]. As a control group, case records of 52 age matched healthy women with regular menstrual cycles (21 to 35 days duration) were analysed from the fertility unit of our institution. These women were due to start fertility treatment due to male factor subfertility. None of the controls had a history of haematological disease. The exclusion criteria for both groups were prior gynaecological surgery and the existence of any co-morbid systemic conditions (such as thyroid disease or diabetes mellitus) for the control group.

To assess the effect of HH on the ovarian reserve (AMH and AFC) and distinguish it from the impact of iron overload on the ovary, we obtained data from 21 women with HH due to any cause other than thalassaemia or haematological disease and compared the markers of ovarian reserve in these women with BTM women.

Patient demographics, medical history, menstrual history, hormonal parameters (serum levels of FSH, estradiol, TSH and AMH) and antral follicle count were recorded in all women from both groups. Serum levels of ferritin, cardiac T2* (cardiac magnetic resonance relaxation parameter T2*), liver iron concentration, thyroid function (TSH) and liver function test results were also recorded in BTM women at three different time points at least one year apart (T1 – 2007 to 2010, T2 – 2011 to 2014 and T3 – 2015 to 2017). 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. Most women had ovarian reserve assessment at T3 (time point 3) however, some had their assessments at T2 (time point two). For analysis, we chose the serum ferritin level which was present at the time each woman had their ovarian reserve testing as that would be the most important or relevant level for this study.

The data were compared between cases and controls, and the normality of data distributions was assessed using the Kolmogorov–Smirnov test. Continuous variables are presented as mean (+/− standard deviation) if normally distributed or as median (interquartile range) if not normally distributed. Between-group differences were detected using Student *t*-test for parametric data and the Mann–Whitney test for non-parametric data. Correlations between AMH levels and other parameters were calculated using Pearson's correlation analysis (normally distributed data) or Spearman's rank test (data not normally distributed). Two-sided p-values <0.05 were considered significant.

Results

The serial longitudinal clinical data collected from 17 women with BTM is presented in Table 1 while the clinical and laboratory characteristics of women with BTM, healthy women (controls) and women with HH due to non-haematological disease are presented in Table 2. Nine of the 17 women with BTM had HH and suffered from amenorrhoea. Serum AMH levels, estradiol levels and antral follicle count were significantly lower in women with BTM compared with the control group ($p < 0.05$ for all). Low AMH levels were noted in both groups of women (with and without HH) with a background of BTM, suggesting that iron overload might have a direct effect on the ovary. The difference in serum levels of AMH was statistically significant in BTM women versus women

Table 2
The clinical and laboratory characteristics of women with BTM and controls (BMI – body mass index; FSH – follicle stimulating hormone; TSH – thyroid stimulating hormone; AMH – anti-Mullerian hormone; AFC – antral follicle count; HH – hypogonadotropic hypogonadism; [a] denotes significant difference). The values for serum levels of ferritin, cardiac T2*, liver iron concentration used for analysis were as collected at the time of ovarian reserve testing.

	BTM (n = 17)	Controls (n = 52)	p value	BTM women A – amenorrhoea B - menstruating A (n = 9) B (n = 8)		p value	BTM (n = 17)	HH (other causes) (n = 21)	p value
Age (years)	33.8 (+5.6)	35.3 (+3.9)	0.22	34.2 (+4.8)	33.3 (+6.7)	0.76	33.8 (+5.6)	32.3 (+5.7)	0.42
BMI	22 (+2.3)	21.7 (+1.7)	0.33	21.5 (+1.8)	22.3 (+−2.1)	0.81	22 (+2.3)	22.9 (+3.5)	0.47
Ferritin (μg/L)	2433.1 (+1866.7)	–	–	2244.5 (+1448.4)	1645.2 (+2338.8)	0.67	–	–	–
Liver iron concentration (mg/g dry wt.)	5.96 (+3.5)	–	–	6.95 (+4.36)	4.86 (+1.89)	0.22	–	–	–
Cardiac T2* ms	29.71 (+8.75)	–	–	30.62 (+7.79)	28.68 (+10.16)	0.66	–	–	–
FSH (IU/L)	4.79 (+5.47)	6.83 (+4.28)	0.11	0.99 (+1.13)	9.06 (+5.25)	0.0004[a]	–	–	–
Estradiol (pg/mL)	103.59 (+80.27)	226.06 (+88.69)	<0.00001[a]	57.78 (+28.94)	155.12 (+89.65)	0.007[a]	–	–	–
TSH (mIU/L)	1.56 (+1.13)	1.11 (+1.11)	0.06	1.57 (+1.07)	1.55 (+1.18)	0.75	–	–	–
AMH (pmol/L)	4.29 (+3.52)	17.39 (+16.1)	0.001[a]	2.55 (+1.87)	6.24 (+4.01)	0.0256[a]	4.29 (+3.52)	15.9 (+23.2)	0.047[a]
AFC	9.9 (+8.92)	15.1 (+8.3)	0.326[a]	6.5 (+6.8)	13.7 (+9.8)	0.09	9.9 (+8.92)	11.3 (+8.5)	0.62

with HH due to other conditions. Serum AMH levels positively correlated with AFC in women with BTM (Table 3 and Fig. 1).

When results for women with spontaneous menstrual cycles were compared with amenorrhoeic women with BTM – significantly lower levels of estradiol, AMH and FSH were noted. There was no correlation between serum levels of ferritin, cardiac T2*, liver iron concentration and ovarian reserve markers.

Comment

Women with transfusion-dependent BTM generally need to receive regular red blood cell transfusions from their first year of life as a life-saving measure [1–3]. However, repeated transfusions can lead to iron deposition in various organs and tissues such as the liver, heart and most endocrine glands causing tissue damage and organ dysfunction [1–3]. 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]. Many of these women are therefore keen to start their own family and fertility becomes a major consideration in adult life [4].

Although spontaneous fertility has 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 resulting from transfusional iron overload affects 70 to 80% of thalassaemic patients worldwide [6,5–8].

Although HH arising from hypothalamic-pituitary damage by iron overload has been well characterised, there is lack of clarity about the effect of iron overload on ovarian function [4,10–12]. It has been suggested that iron-induced damage also impairs oocyte function, with demonstrated increased levels of redox-active iron in follicular fluid, thus contributing to subfertility [5,13]. However, there are insufficient data on the direct effect of iron on the gonads [5,13]. Reports of successful ovulation induction and pregnancies indicate that ovarian function is preserved even in women with amenorrhea [4]. Ovarian health is central to success with fertility, and several studies have attempted to establish a relation between iron overload in BTM and ovarian function. But so far, most studies on ovarian reserve in women with BTM are limited by small sample size and cross-sectional data. To the best of our knowledge this is the first long-term longitudinal study on assessment of ovarian reserve in women with transfusion dependent BTM. Since fluctuations in ovarian function and iron load are both dynamic processes, longitudinal data are crucial for accurate evaluation of the effects of disease activity or iron load over time.

Moreover, reproductive capacity in thalassaemia major women cannot be well predicted by means of age, menstrual status, or transfusion and chelation parameters [14,15]. 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, estradiol, 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]. Studies which have measured the gonadal response to gonadotropin releasing hormone (GnRH) and gonadotropins stimulation to evaluate gonadal function have produced conflicting results [4,10–12]. 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 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. 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. AMH levels correlated with non– transferrin-bound iron (NTBI), suggesting a role of labile iron in the pathogenesis of decreased reproductive capacity. Chang et al. 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, estradiol, 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 study results also demonstrated significantly lower levels of AMH, early follicular phase estradiol and antral follicle count in women with BTM in comparison with controls. There was a positive correlation between AMH levels and AFC in women with BTM. However, it seems unlikely that the low levels of AMH are 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 ($p = 0.0590$), the serum levels of AMH appear low (6.24 ± 4.01 pmol/L) to what would be compared a normal ovarian reserve for women of this age group. This therefore suggests a direct effect of background diagnosis and/or treatment on ovarian tissue and follicle reserve. Due to retrospective nature of our study, we could not verify how many of the BTM women with amenorrhoea needed induction of puberty with certainty. Within women with BTM – AMH, FSH and estradiol levels were significantly lower in women with HH/amenorrhoea as compared to women having menstrual cycles indicating the likely effect of suppression of ovarian activity secondary to HH in addition to the primary damage to the ovary. As most women had assessment of their ovarian reserve when they had been well-chelated and ferritin levels had stabilised – we failed to demonstrate any correlation between the levels of ferritin, liver iron concentration, cardiac T2* and ovarian reserve markers at the time point when the assessment occurred.

Table 3
Correlations of AMH and AFC levels with other clinical and biochemical parameters (FSH – follicle stimulating hormone; AMH – anti-Mullerian hormone; AFC - antral follicle count. [a] denotes significant difference).

	AMH		AFC	
	r	p	r	p
Age	−0.44	0.07	−0.52	0.0319[a]
FSH	0.18	0.48	−0.01	0.96
Ferritin	−0.42	0.09	−0.19	0.46
Liver iron concentration	−0.10	0.69	0.10	0.68
Cardiac T2*	0.38	0.12	0.10	0.67
AFC	0.75	0.0004[a]	–	–
AMH	–	–	0.75	0.0004[a]

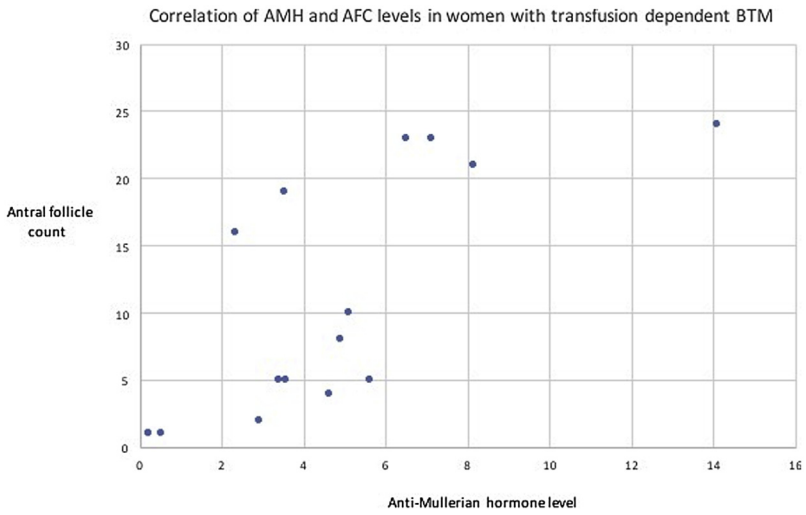

Fig. 1. Correlation of AMH and AFC levels in women with transfusion dependent BTM (X values – AMH levels and Y values – AFC; n = 17; r = 0.7552 and p = 0.0004).

We found that the levels of serum AMH were significantly lower in women with BTM as compared to age and BMI matched women with HH due to other causes, suggesting that the iron overload is an independent cause of ovarian damage apart from its indirect effect of suppression of ovarian activity due to HH.

It is important to recognise limitations of serum AMH as a marker of reproductive capacity. Serum levels of this hormone do not accurately predict natural conception and livebirth, and the rate of decline in ovarian reserve (and AMH) can vary considerably between individual women [24]. Other limitations of our study include small sample size and a mixed study design, which included retrospective data analysis. We could not stratify patients according to the type of chelators due to small sample size and HRT they were receiving. Also, we had to choose our age-matched controls from healthy women who were due to start fertility treatment for male factor subfertility as this was a non-intervention retrospective data comparison study within our unit. But these women did not have any known haematological or medical conditions and the cause of subfertility was male factor related.

It appears that subfertility in women with BTM could therefore be attributed to multiple factors such as iron deposition and iron-induced oxidative stress in hypothalamus, pituitary and ovaries as well as the iron overload in other organs such as heart, liver and pancreas contributing to the impaired metabolism of hormones, serum antioxidants and impaired general health. It is possible that the ovarian damage had started with accumulation of small amounts of iron right from an early stage (pre-puberty or puberty) as ovarian follicles are highly sensitive to endocrine disrupters such as free radicles or chemotherapy [4,8,25].

Further studies are therefore required to elucidate the mechanisms by which iron overload can adversely affect the ovarian reserve, and the best treatment strategies to prevent such damage. One useful approach which might shed light on the pathophysiology and natural history of damage to the hypothalamo-pitutary-ovarian axis, might be to sequentially perform ovarian reserve as well as endocrine testing every few years in women with BTM starting from adolescence, and correlating this information to their disease activity and/or iron overload. This knowledge could be vital for understanding whether early intensified chelation treatment could potentially prevent or reverse the ovarian damage similar to that noted in the cardiac tissue [6,26].

Conclusion

The long-held belief that the gonad might be spared from effects of transfusional iron overload in women with BTM is questionable. The two most discriminatory markers of ovarian reserve – serum AMH level and AFC were significantly lower in women with transfusion dependent BTM as compared to age-matched healthy controls suggesting a direct impact of the disease activity or iron overload on the ovary. Low ovarian reserve could therefore be an important contributor towards subfertility in many women with BTM. Supressed levels of AMH were also noted in women without HH having regular menstrual cycling suggesting a likely deleterious effect of the background diagnosis and/or treatment (transfusional iron overload) on ovarian reserve.

Authorship & contributorship

VST, RB, AJE and RC participated in the study design. VST, RB, AJE, EM and RC reviewed and contributed to the study protocol. VST, RB, AJE, EM and RC participated in the data collection and preparation of the draft manuscript. All authors have approved the final version of the manuscript.

Conflict of interest

None of the authors have any conflicts of interest to declare.

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