Original Research

Myocardial iron is strongly associated with reproductive function in beta-thalassemic women under chelation therapy

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Summary

Objective: To evaluate gynaecological features of a group of transfusion dependent beta-thalassemic women and to analyze their reproductive function with the morpho-functional features of genital female tract. Materials and Methods: Cross-sectional study in a University Hospital and Tertiary Care Center. Fifty-nine transfusion-dependent beta-thalassemic women in reproductive age, were divided into two groups: to group A were assigned women with spontaneous menarche (n=44), while to group B were allocated patients with inducted menarche. Data on demographic characteristics, iron overload in liver and heart (evaluated with T2* RMN method), chelation therapy, and reproductive function were collected. Difference in demographic characteristics, chelation therapy, iron status, and reproductive function between groups were assessed, together with correlation analysis of iron overload. Results: Patients in group B had a worse reproductive function and a higher myocardial iron overload, than patients in group A. Moreover multivariate logistic regression showed a significant correlation between uterine biometry, FSH, E2, parity and hearth iron T2*HSIV, but not with liver iron. Conclusion: The present data shows that myocardial iron loading could be putative of prolonged and severe iron overload in the female genital tract, impairing fertility and reproductive function in patients affected with beta-thalassemia major under chelation therapy.

Key words: Beta-thalassemia major; Myocardial iron overload; Liver iron overload; Reproductive function; Chelation therapy.

Introduction

Beta thalassemia major (BTM) is the most common hemoglobin disorder in the world, with a great prevalence in people of Mediterranean origin, Arabs, and Asians [1, 2]. It has been estimated that about 1.5% of the global population (80 to 90 million people) are carriers of beta thalassemia, with about 60,000 symptomatic individuals born annually, the great majority being in the developing world. The total annual incidence of symptomatic individuals is estimated at one in 100,000 throughout the world and one in 10,000 people in the European Union [1].

Advances in the primary care of BTM by optimal blood transfusion and chelation therapy have improved survival and quality of life of patients into adulthood [1, 2] Nevertheless, iron may accumulate at multiple sites, such as heart, liver, pituitary gland, thyroid, and so on. Therefore, the majority of these patients may develop multiple endocrine disorders.

The commonest abnormality is hypogonadotropic hypogonadism (HH), which is expressed either with sexual infantilism and primary amenorrhoea or with delayed puberty and secondary amenorrhoea [3]. These conditions are the expression of hypothalamic-pituitary gonadal (HPG) axis dysfunction [3, 4]. This is an observational study aiming at evaluating gynaecological features of a group of transfusion dependant BTM women and to analyze their reproductive function with the morpho-functional features of genital female tract.

Materials and Methods

This observational study was conducted at the Department of the Women, the Child and of General and Specialized Surgery of the Second University of Naples and at the Department of Haematology of Cardarelli Hospital in Naples, between February 2011 and February 2013. The study protocol received institutional review board (IRB) approval before the beginning of the study, in accordance with the Code of Ethics of the Declaration of

Table 1. — *Characteristics of the 59 beta-thalassemic patients enrolled in the study. 44 women had spontaneous menarche (group A) and 15 women had inducted menarche (group B).*

Patients' characteristics	Group A (n=44)	Group B (n=15)	*p* value
Age (years)	37.8 ± 8.7	38.5 ± 6.9	0.786
Weight (kg)	57.3 ± 9.2	55.9 ± 8.9	0.641
Height (cm)	153.8 ± 8.5	157.2 ±7.9	0.653
BMI (kg/m^2)	23.4 ± 3.4	24.1 ±5.1	0.237
Ferritin level (<1500 µg/L) Onset of transfusional	1136 ± 352	1279 ± 478	0.871
Therapy (months)	12 ± 6	13 ± 5	0.785
Age of menarche	13.2 ± 1.4	17,3 ± 3,4	<0.0001
Ongoing hormonal therapy	56.8%	93.3%	<0.0001

Data are shown as means and standard deviations, and percentages. Statistical significance was set at *p* < 0.05.

Table 2. — *Differences between group A (n= 44 patients with spontaneous menarche) and group B (n= 15 patients with inducted vaginal bleeding).*

PARAMETER	Group A (n= 44)	Group B (n=15)	Statistics	95%CI	*p* value
Patients who underwent Ovarian Stimulation Cycles	20.0% (n=3)	20.4% (n=9)	OR 1.060	0.243-4.621	0.937
Pregnancy Rate (%)	36.3% (n=16)	6.7% (n=1)	OR 8.000	7.100-10.300	0.028
Uterine Biometry (mm)	67.4 ± 13.7	55.9 ± 12.4	Student *t*-test 2.872	3,870-19,129	0.020
T2 *HSIV (msec)	31.9 ± 11,7	23.2 ± 13.6	Student *t*-test 2.386	0.831-16.569	0.043
T2 *L (msec)	8.6 ± 7.2	7.9 ± 5.8	Student *t*-test 0.340	-3.004-4.404	0.735

OR: Odds ratio. CI: Confidence interval. Significance was set at *p* < 0.05.

Helsinki. Fifty-nine transfusion dependant BTM women, aged between 18 and 50 years, under chelation therapy were enrolled. Patients were divided into two groups: in group A women (n=44) who had a spontaneous menarche and in group B women (n=15) who had inducted vaginal bleeding thanks to hormonal replacement therapy. Demographic characteristics, together with data on hematological conditions, frequency of blood transfusions, kind of chelation therapy, serum ferritin levels, iron over-load in liver and heart (evaluated with T2* RMN method), thromboembolic risk evaluation, and bone density by MOC-DEXA of the vertebral district and of the right and left femur, were collected.

Data on menarche (age and spontaneity of menarche or vaginal bleeding), menses, estro-progestins replacement therapy, IUD, pregnancies (spontaneous or obtained with ovarian stimulation), menopausal status, pelvic infections together with pelvic ultrasound were examined. The HPG function was studied analyzing basal levels of luteinizing hormone (LH), follicular stimulating hormone (FSH) estradiol (E2), prolactin (PRL), anti-Müllerian hormone (AMH), and thyroid profile (evaluation of blood levels of TSH, fT3, fT4). This was undertaken on day 2 of menses in women with a regular menstrual cycle, or on any random day in those with amenorrhea.

Absence of menarche above the age of 15 was considered as delayed puberty. Oligomenorrhea was defined as menses at intervals higher than 35 days. Secondary amenorrhea was defined as the absence of menstruation for more than six months at any time after menarche. Infertility was defined as absence of pregnancies after one year of unprotected sexual intercourses. Statistical calculations were performed using the Statistical Package for the Social Sciences (SPSS) (version 20.0). No patients had missing data. Appropriated sample size for this observational study

was calculated to achieve a precision of 0.05 for sensitivity, on the basis of the international literature assessing a reproductive failure occurring in the 30% of BTM women, the total needed sample size per group was of 14 women. Data distribution was assessed by the Shapiro-Wilks test and showed a parametrical distribution. Descriptive statistics were assessed by calculating frequencies, means and standard deviations. Student *t*-test and chi-square test were used to compare data between study groups. Multivariate logistic regression was performed in order to verify the presence of association between following independent variables: age, menarche (spontaneous or inducted), age at menarche, FSH, LH, E2, parity, and uterine biometry, and heart and liver iron overload (dependent variable). Significance was set at *p* < 0.05.

Results

Demographic characteristics of the patients depicted in Table 1 show that the two study groups were homogeneous with regards to age, weight, height, BMI, onset of transfusion therapy, and ferritin level. Otherwise they showed a significant difference between age at menarche and ongoing hormonal therapy. Evaluating fertility, no difference was noted among patients who underwent ovarian stimulation cycles in group A (20.0% of them, n=9), and in group B (20.4% of them, n=3) (*p* value: 0.937) (Table 2). Otherwise, in group A (n=44), 16 (36.3%) had at least one pregnancy, whereas 28 of them (63.7%) were infertile. In group B (n=15), 14 (93.3%) patients were infertile, whereas only

one of them (6.7%) had one pregnancy. The pregnancy rate difference between groups resulted significant with a *p* value of 0.028 (Table 2).

Uterine biometry data, collected at pelvic ultrasound scan, and T2*HSIV values for heart MRI resulted in a statistically significant difference (*p* value: 0.020 and 0.043), while no difference existed between values of T2* for liver MRI (*p* value: 0.735) (Table 2).

Multivariate logistic regression, performed in order to verify the presence of association between following independent variables: age, menarche (spontaneous or inducted), age at menarche, FSH, LH, E2, parity, and uterine biometry, and heart and liver iron overload (dependent variables) showed a significant correlation between uterine biometry, FSH, E2, parity, and heart iron T2*HSIV, but not with liver iron (Table 3).

Discussion

In patients with BTM, long-term transfusion therapy for the correction of anemia results in toxic iron overload, which is generally proportional to the number of transfused units and is cumulative [5, 6]. Uncontrolled iron overload has serious clinical consequences resulting in significant morbidity, including liver damage, cardiac complications, and endocrine dysfunction [5, 6].

Currently, the improvement in the management of BTM include the possibility to achieve a pregnancy and to have a regular function of the reproductive system, as infertility represents an important and actual issue in BTM patients [7]. With an early and proper onset of chelation therapy, regular ferritin levels, and acceptable myocardial and liver iron values, it is possible to achieve a pregnancy with almost favorable outcome [7]. The value of myocardial MRI-T2* in predicting cardiovascular risk is well-established [6], and a myocardial T2* of < 20 ms (a marker of significant myocardial iron loading) has been associated with increased risk of left ventricular dysfunction [5]. Nevertheless, its utility in determining the risk of extra-cardiac complications of iron overload is less clear. Myocardial iron loading (cardiac T2* < 20 ms) has been reported in a cross-sectional study by Au *et al.* [8] as being strongly associated with diabetes mellitus, hypogonadism, hypothyroidism, and hypoparathyroidism in BTM patients.

The present data show a strong correlation between reproductive function and heart iron overload. Indeed, patients with a lower cardiac iron showed a better reproductive outcome, with the same level of ferritin and the same percentage of patients who underwent ovarian stimulation cycles. Therefore, the apparent lack of correlation between reproductive function and liver iron load can be explained by the hepatic and extrahepatic distribution of iron in transfusional overload, together with the differential influence of chelation therapy on cardiac and hepatic iron stores [8]. Indeed, in the early stages of transfusional iron

Table 3. — *Multivariate logistic regression of age, menarche (spontaneous or inducted), age at menarche, FSH, LH, E2, parity, and uterine biometry, and heart and liver iron overload (dependent variables).*

Independent variable	*p* value[#]	
Age	0.347	0.465
Menarche	0.239	0.381
Age at menarche	0.876	0.501
FSH	0.041	0.103
LH	0.067	0.184
E2	0.023	0.230
Parity	0.016	0.286
Uterine Biometry	0.022	0.459
Dependent variable	T2* HSIV[a]	T2*L[b]

This table reports the results of the multivariate logistic analysis, showing no statistically significant association between age, menarche (spontaneous or inducted), age at menarche, FSH, LH, E2, parity, and both heart and liver iron. Only uterine biometry resulted strongly associated with heart iron.

[#] *p* value: statistic significance value was set at < 0.05
[a] T2* HSIV: Myocardiac T2*
[b] T2* L: Liver T2*
*Before performing multivariate analysis, a correlation matrix that showed no significant collinearity (0.45) between heart and liver iron was performed.

overload, iron accumulation occurs predominantly in reticuloendothelial (RE) cells. Internal redistribution of iron to hepatocytes occurs in more advanced stages of transfusional overload, as hepatocytes are in dynamic equilibrium with plasma transferrin-bound iron released from the RE cells, and are also capable of non-transferrin-bound iron (NTBI) uptake from plasma when the capacity of apotransferrin to bind iron is exceeded [9].

When the NTBI rises to a critical level, non-transferrin mediated and unregulated influx of iron into other extra-hepatic parenchymal cells also occurs, notably cardiac myocytes and endocrine cells, resulting in the iron toxic effects within these tissues [10]. Therefore, in unchelated patients, the concentration of iron in myocardium is directly related to the number of transfused red cell units and to liver iron [11]. However, once chelation therapy begins, this relationship may be lost, as hepatic iron stores are fastly emptied, in contrast to cardiac iron deposits, which may take many years of intensive chelation therapy to be depleted [12]. The relationship between myocardial and liver iron load may also be influenced by the type and duration of action of the iron chelator. Although over the past decade many attempts have been conducted to overcome the problem of this lag in iron loading and unloading of the extra-hepatic tissues with respect to the liver by looking at the liver T2* longitudinally, it could not be possible to establish the significance of liver iron loading in the development of diabetes mellitus and hypogonadism. This may be because many of the present patients have late initial assessment of liver iron load and prior hepatic siderosis which had resolved at the time of initial liver T2* might still be missed while the endocrinopathy remains. Thus, the present data are consistent with these reports. It therefore appears that

the presence of myocardial iron loading as demonstrated on MRI-T2* has a clinical significance that extends beyond its unquestioned prognostic value for sideriotic cardiomyopathy. Consistent with other studies [13, 14], the present one suggests that myocardial iron loading could be putative of prolonged and severe iron overload in the female genital tract, possibly due to similar iron loading mechanisms. When myocardial iron overload is present, iron deposition in the pituitary is likely to have been present for an even longer period [13, 14], and the potential for concurrent or subsequent development of iron-related endocrine complications together with reproductive issues is high [15-17]. Abnormal myocardial T2* therefore serves as a good surrogate measure of extrahepatic iron loading in pituitary gland. The iron overload in the pituitary gland leads to hypogonadism and subsequently to an abnormal pubertal development that reflects also on fertility and pregnancy outcome [18]. Therefore, myocardial iron is less susceptible to rapid decrements in response to chelation compared with liver iron; it is more robust than liver iron as a marker of historical severe iron overload in patients actively receiving chelation therapy. At a molecular level, the iron-induced oxidative stress, mainly responsible of hearth iron damage, may also act both on the HPG axis and the female genital tract. Indeed, some studies showed how antioxidant capacity linked to glutathione S-transferase (GST) activity is impaired in BTM patients [19-21]. Therefore, iron-induced oxidative stress is related to GSTM1/GSTT1 genotype, with patients expressing GSTM1 null genotype having a worse hearth performance, this being a predisposing factor for myocardial iron overload in BTM patients with low body iron, as assessed by lifelong serum ferritin levels [19-21]. Future perspectives could include genetic analysis of GSTM1/GST1 in relation to an eventual reproductive failure. Thus, the potential role of antioxidants substances, which activity has been reported to be dependent on GSTM1/GST1 genotype [22], as putative scavengers to preserve and/or restore fertility potential in BTM women, should be investigated.

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