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Original Article

A Multicenter ICET-A Study on Age at Menarche and Menstrual Cycles in Patients with Transfusion-Dependent Thalassemia (TDT) who Started Early Chelation Therapy with Different Chelating Agents

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Abstract. ***Introduction*: To evaluate the effect of early chelation therapy (≤ 3 years) with a variety of chelating agents on age at menarche and menstrual characteristics in patients with transfusion-dependent thalassemia (TDT).**
***Design:* A retrospective multicenter study promoted by the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine (ICET-A).**
***Setting*: Eight of 13 International Thalassemia Centers (61.5%) in the ICET-A Network participated.**
***Patients:* Fifty-seven female TDT patients, aged 11 to 26 years, and with early iron chelation therapy, were eligible for the present study. They were enrolled from one center from Iran (33 patients), 3 centers from Bulgaria (9), 1 from Greece (8), one from Oman (4), 1 from Cyprus (2), and 1 from Italy (1). Seven patients were excluded, four still prepubertal (age 12-14 years) and 3 with primary amenorrhea. Therefore 50 patients were finally enrolled.**
***Results:* All fifty TDT patients developed spontaneous menarche at a mean age of 14.2 ± 2.24 years**

(range 9 – 20). A significant positive correlation was observed between age at menarche and serum ferritin levels (r: 0. 41, p=0.005). Regular menstrual cycles were reported from 32 (64%) patients, of whom 28 (83.3%) get menarche at age ≤ 14 years. Complications were more frequent in patients older than 14 years at menarche and in those with secondary amenorrhea.
Conclusions: **Age at menarche greater than 14 years was a forerunner of menstrual irregularities and associated complications in 36% of patients despite precocious chelation therapy. The poor adherence to treatment, to be demonstrated in future studies, could explain the finding.**

Keywords: Transfusion-dependent thalassemia, Menarche, Menstrual cycles, Iron chelation therapy (ICT), Iron overload, treatment adherence.

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Introduction. Medical advancements with regular blood transfusion therapy, iron-chelating therapies (ICTs) and imaging methods have improved the life expectancy of children and adolescents suffering from transfusion-dependent thalassemia (TDT); however, as patients approach the age of puberty, they often develop growth retardation and disorders of pubertal development [delayed puberty, arrested puberty, late menarche and acquired hypogonadotropic hypogonadism (AHH)], particularly if the disease is poorly controlled with regular ICT.[1]

Up to now, the effects of ICT on the age of menarche and subsequent menstrual cycles have been evaluated mainly in patients who started ICT between 5 and 10 years of age[2,3] and only in a small number of cases under the age of 5 years.[4]

Menarche is a significant event that marks the onset of sexual and reproductive maturation in girls and is considered a surrogate marker of general good health in subjects affected by chronic disorders. Menarche typically occurs within 2–3 years after thelarche (breast budding) between the ages of 10 and 15 years, corresponding to - 2 standard deviation (SD) and + 2 SD, respectively. The 95th percentile for menarche is 14.5 years, although many textbooks define primary amenorrhea as an absence of menses at 16 years.[5] In practice, at or before the age of 15, menarche is experienced by 98% of girls.[6]

Menstrual regularity patterns include three main dimensions: bleeding frequency, duration, and intensity. By the third year after menarche, 60–80% of menstrual cycles are 21–34 days long.[7]

The main aim of this retrospective study was to ascertain, in TDT patients over 11 years who started iron chelation therapy early at ≤ 3 years, the percentage of subjects with spontaneous pubertal development, the patient's age at menarche, and the characteristics of subsequent menstrual cycles.

Method used for the preparation and distribution of the questionnaire. A multicenter international study using an ad hoc questionnaire in accordance with the Declaration of Helsinki was proposed at the beginning of September 2021 by the Coordinator (VDS) of the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine (ICET-A) (first step).

After the first draft preparation, the questionnaire was discussed by e-mail, and the final version was validated at the beginning of October 2021 by 4 endocrinologists (De Sanctis V, Soliman AT, Tzoulis P, and Di Maio S) and 2 hematologists (Daar S and Kattamis C) (second step).

Thirteen centers active in the ICET-A Network were invited to participate in the study. The questionnaire was distributed by mail to the Centers that accepted the invitation, and the deadline for sending the requested data was fixed to the end of December 2021 (third step).

The questionnaire included the following information: patients' demographics and anthropometrics data, age at first transfusion, age at the start of chelation therapy, serum ferritin level (SF) at the start of ICT and at menarche, associated endocrine and non-endocrine complications, date at menarche or absence (primary amenorrhea), menstrual pattern information collected on the basis of patients' self-reporting, namely menstrual history during the three months preceding the last observation [cycle regularity, cycle length (short and long), duration of menstrual bleeding, and amount of menstrual flow, and age at

secondary amenorrhea (SA)]. Socioeconomic status, based on parental education and occupation at the time of last observation, behavioral patient habits (smoking, alcohol consumption), patient's physical activity, mothers' and sisters' age at menarche, and menses patterns were not included in the questionnaire.

The following were excluded from the study: (a) bone marrow transplanted patients; (b) those who were HIV positive; (c) patients with a clinical history of isolated menarche; (d) patients who had died before the study; (e) those with mental illness (depression, anxiety disorders, eating disorders, and addictive behavior), and (f) patients with chronic kidney diseases.

Material and Methods.

Definitions of menarcheal age and menstrual disorders. A menarcheal age was considered precocious before age 10 and later above the age of 14. The age of 14 years, equal to approximately + 1.25 SD from the mean, was chosen as the threshold for defining a menarche as "late" because an age at menarche more than 1 SD from the mean and progressively closer to + 2 SD, represents a greater risk of pathology and because the maternal menarche ages of individual patients were not available.

Primary amenorrhea was defined as the absence of menses at 16 years.

The menstrual cycle period interval was defined as the number of days from the first day of one menstrual period to the first day of the next menstrual period [short cycle interval: ≤ 21 days; long cycle interval ≥ 35 days and < 90 days (oligomenorrhea)]. Frequent menstrual bleeding was defined as more than four episodes in 90 days, heavy and prolonged menstrual bleeding was the presence of excessive menstrual blood loss (approximated to the number of pads per day), short menses and light menstrual bleeding (hypomenorrhea) was defined when the menstrual cycle was 2 days or less; the absence of menstruation for more than 3 months, at any time after menarche, in the presence of documented AHH, was classified as SA.[4,6-9] Gynecologic age was defined as the age in years at last observation minus age at menarche.

Patients with primary or SA were evaluated for basal pituitary–gonadal axis (HPG) integrity and by the exclusion of other endocrine/non-endocrine complications. The diagnosis of AHH was characterized by low levels of estradiol (E2) in the presence of low or inappropriately normal gonadotropin (LH and FSH) serum levels.[10]

Anthropometry and assessment of associated endocrine complications. Height and weight were measured using a standard technique. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Height and weight were measured according to international recommendations.[11] BMI was evaluated based on the World Health Organization (WHO) recommendations: underweight (<18.5 kg/m^2); normal range (18.5–24.9 kg/m^2); overweight (25.0– 29.9 kg/m^2); obese (≥ 30 kg/m^2).[12]

Associated endocrine complications. Associated endocrine complications were assessed and defined according to the I-CET position statement published in 2013.[13]

According to the American Diabetes Association, prediabetes was defined as follows: impaired fasting glucose (IFG) when fasting plasma glucose (FPG) was between 5.6–6.9 mmol/L and impaired glucose tolerance (IGT) when the 2-h plasma glucose (2-h PG) value during a 75 g oral glucose tolerance test (OGTT) was between 7.8–11.0 mmol/L. Diabetes mellitus was confirmed by FPG ≥7.0 mmol/L and/or a 2-h PG value during a 75 g OGTT of ≥11.1 mmol/L.[14]

The assessment of iron overload (IOL). The assessment of iron overload (IOL) was evaluated by SF. The manufacturer's normal reference range values in females were 15–150 ng/mL. Iron overload was arbitrarily classified as mild (SF < 1,000 ng/mL), moderate (SF: ≥1,000 ng/mL and < 2,000 ng/mL) or severe (SF: ≥ 2000 ng/mL).[4] Duration of chelation was defined as the age at the last observation minus the age at the start of chelation.

Statistical analysis. Differences for continuous variables were analyzed with an independent-sample t-test for normally distributed variables and with the Mann-Whitney test in case of non-normality. Qualitative variables were compared by using the chi-squared test. The Statgraphics XVII software for Windows was used for all statistical analyses. A P value < 0.05 was considered as significant.

Ethics. The study was designed in accordance with the Helsinki Declaration; all participants were informed about the nature and purpose of the study. Each patient or their legal guardian agreed to participate in this study and gave consent after a brief session to explain the aims. Confidentiality, anonymity, and non-transmissibility of detailed personal patients' data were assured. The retrospective study was exempted from institutional Ethics Committee approval.[15] No identifiable private patient information was collected, and an anonymized dataset was analyzed.

Results.

Data collected and participating Centers. Eight of the 13 Thalassemia Centers of the ICET-A Network participated in the study, reporting data on fifty-seven female TDT patients aged 11 to 26 years. They were from Iran (33 patients; Shiraz), Bulgaria (Sofia n = 5, Plovdiv n = 2, Varna n = 2), Greece (8 patients; Athens),

Oman (4 patients; Muscat), Cyprus (2 patients; Nicosia), and Italy (1 patient; Catanzaro). Seven patients were excluded: 4 (2 from Cyprus, 1 from Greece and 1 from Iran) were still prepubertal (age 11-14 years), and 3 (2 from Iran, 1 from Bulgary) had primary amenorrhea. All started chelation therapy at or before the age of 3 years. Therefore, 50 patients were enrolled. They were born between 1995 and 2008 and aged 19.5 ± 4.2 years (range: 11 – 26). Their main clinical, therapeutic (ICT), and laboratory characteristics are summarized in **Table 1**.

Age at diagnosis and age at the start of transfusion were reported in all 50 patients. SF levels were available in 44/50 patients at the start of chelation therapy, 45/50 adolescents at menarche, and 7/8 patients at the age of SA. BMI was reported at the age of menarche and SA.

Table 1. Main clinical, laboratory, and therapeutic characteristics in 50 TDT patients with spontaneous menarche. Results are reported as mean ± standard deviation (DS) and range.

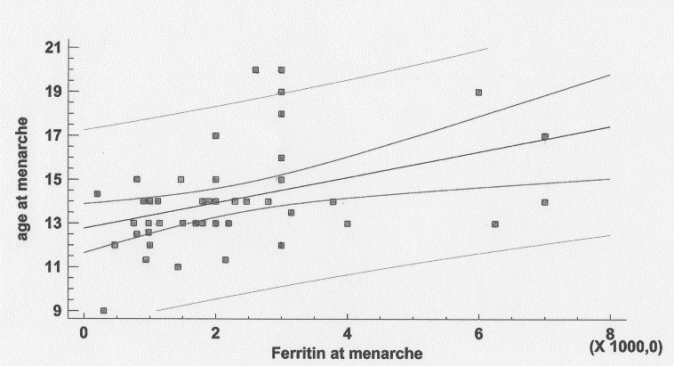
Age at diagnosis of TDT (years)	1.03 ± 0.72 (0.16 - 3)
Age at start of transfusions (years)	1.08 ± 0.74 (0.16 - 3)
Age at start of iron chelation therapy (years)	1.7 ± 0.9 (0.25 - 3)
Age at spontaneous menarche (years)	14.2 ± 2.24 (9 - 20)
Body mass index at menarche (kg/m^2) (data available in 45 patients)	19.7 ± 3.2 (15.4 - 28.2)
Serum ferritin at menarche (years) (data available in 45 patients)	2,281 ± 1,620 (467 - 7,000)
Number of patients on treatment with desferrioxamine (DFO)	22
Number of patients on treatment with deferiprone (DFP)	2
Number of patients on treatment with deferasirox (DFX)	26

Spontaneous menarche. Fifty patients developed spontaneous menarche at a mean age of 14.2± 2.24 years (range 9 – 20). At the start of chelation therapy, 22 patients had received desferrioxamine mesylate (DFO), 26 patients deferasirox (DFX), and 2 patients deferiprone (DFP).

Among the 26 patients receiving DFX, 22 had normal menstrual cycles and achieved menarche at 13.12 ± 0.73 years when their SF level was 1834 ± 1325 ng/ml; at the start of chelation therapy, their SF was 1365 ± 985 ng/ml, not significantly different from SF levels at menarche (p = 0.27).

No correlation was found between BMI and age at menarche (n = 45, r = -0.26; p= 0.065), while a significant inverse correlation was found between weight percentile and age at menarche (n = 48; r = -0.39, p = 0.0057). In 9 of 47 patients whose data were available, the standing height at menarche was ≤ 3° percentile: 7/22 (31.8%) patients treated with DFO; 2/23 (8.7%) patients treated with DFX (p= 0.07) and 0/2 treated with DFP. SF levels in these 9 patients were 2345 ± 1908 ng/dl (range 300 – 7000). Unfortunately, the height of the parents of these 9 as well as the other patients was not available.

Figure 1. Correlation between age at menarche and serum ferritin level in 45 patients (r = 0. 41, P= 0.005).



A significant correlation was observed between age at menarche and SF levels in 45 patients (r: 0. 41, p= 0.005) (**Figure 1**).

In particular, the mean age at menarche was 12.7 ± 1.7 (n = 10), 13.3 ± 1.1 (n = 11), and 15.0 ± 2.5 (n = 24) years in patients with mild SF (<1,000 ng/mL), moderate SF (between 1,000 and 1,999 ng/mL), and severe SF (from ≥ 2,000 to 7,000 ng/mL), respectively (p= 0.0067. In the ten patients with mild iron overload, SF levels at the start of chelation (970 ± 601 ng/dl) were not different from SF levels at menarche (p= 0.23).

Menstrual characteristics and chelation therapy. Thirty-two of 50 patients (64%) reported regular menstrual cycles; their SF levels at the start of chelation (1394 ± 906 ng/ml) were not different from SF levels at menarche (2073 ± 1522 ng/ml, P = 0.28). Their ages at menarche were 13.14 ± 0.97 years vs 15.78 ± 2.7 years in 18 patients with irregular cycles, p = 0.0003, of whom 7 (14%) with oligomenorrhea, 3 (6%) with short/light menses (hypomenorrhea), and 8 (16%) with SA. No patient reported heavy and prolonged menstrual bleeding (**Table 2**).

Regular menstrual cycles were more frequent in patients who experienced menarche at age 14 years or less (82.3%) than in those who experienced menarche at age greater than 14 years (25%; p < 0.001) (**Table 2**). The group of 16 patients with menarche > 14 years of age had a significantly higher frequency of oligomenorrhea (37.5% vs 2.9%; p= 0.0028) and hypomenorrhea (6% vs. 0%; p= 0.03) (**Table 2**).

The severity of IOL was not significantly associated with the characteristics of the menstrual cycles (**Table 3**).

Moreover, regular menstrual cycles were more frequent in patients treated with DFX (68.7%; P= 0.004). Their mean age at menarche was 13.12 ± 0,74 years, significantly lower than the ages at menarche of 20 patients not on DFX = 14.9 ± 2.8 years, p = 0.0173, while their mean SF at menarche, 1840 ± 1356 (range 600 –

Table 2. Characteristics of menstrual cycles according to age at menarche (≤ 14 years or >14 years) in 50 TDT patients.

Characteristics of menstrual cycles	34 patients who had menarche ≤ 14 years	16 patients who had menarche > 14 years	P-value
Regular cycles (N and %)	28 (82.3%)	4 (25.0 %)	<0.001
Frequent menstrual bleeding	0	0	-
Short-term cycles (N and %) (< 21 days)	0	0	-
Oligomenorrhea (N and %) (> 45 days)	1 (2.9%)	6 (37.5%)	0.0028
Short/light menses (N and %) (<2 days)	0	3 (6.0%)	0.03
Secondary amenorrhea (N and %)	5 (14.7%)	3 (18.7%)	0.69

Table 3. Characteristics of menstrual cycles in relation to the severity of iron overload at menarche in 45 TDT patients (in five patients the serum ferritin level was not available).

Characteristics of menstrual cycles	10 patients with serum ferritin: < 1,000 ng/mL	11 patients with serum ferritin: 1,000 – 1,999 ng/mL	24 patients with serum ferritin: 2,000 – 7,000 ng/mL	P-value
Regular cycles, n	8	8	15	0.1
Short cycles (< 21 days)	0	0	0	0.99
Oligomenorrhea (> 45 days)	0	1	4	0.12
Heavy/prolonged menstrual bleeding	0	0	0	0.99
Short/light menses (<2 days)	0	0	2	0.32
Secondary amenorrhea	2	2	3	0.99

6250) was not different from SF levels, 2611 ± 1690 (range 300 – 7000) ng/ml of patients not on DFX, p = 0.12.

Gynecological age was 5.4 ±.3.27 years (range 1-12 years; median 4.7 years). Only one of them developed SA.

Secondary amenorrhea was reported in 8 patients at 17.9 ± 3.5 years (range 14-23) and preceded by oligomenorrhea. Their menarcheal age was reported as 14.2 ± 2.3 years when the registered mean SF level was 1,828 ± 1,014 ng/mL. Seven of them had received DFO and one DFX from an early age. None had received two chelating drugs until the last observation (**Table 4**). At the time of SA, their gynecological age was 7.8 ± 3.2 years (range 2 – 14; median 8 years).

At the last observation, forty-three patients remained on monotherapy, while 7 patients were receiving two ICTs (5 patients: DFO + DFP, 1 patient: DFP + DFX, and 1 patient: DFO + DFX).

Associated complications. At last observation, the presence of associated complications was more prevalent in patients with SA [6/8 (75%)] and in those who achieved menarche after the age of 14 years [11/16 (68,7%)].

Glucose dysregulation was the commonest associated complication (12/50; 24%), followed by thyroid disorders (6/50; 12%, 2 patients with secondary hypothyroidism and 4 with primary hypothyroidism of whom 1 had goiter), hypoparathyroidism (1/50; 2%), and heart failure due to iron overload associated with IGT; her SF at menarche had been 7000 ng/ml (1/50; 2%).

Table 4. Menstrual history in relation to iron chelation therapy from the start of treatment to last observation.

	Patients with regular menstrual cycles (n)		At last observation (n)	
Chelating agent at the start of treatment	**Monotherapy at last observation**	**Second chelating drug added**	**Patients with irregular menstrual cycles**	**Patients with secondary amenorrhea**
(*) Desferrioxamine (DFO) - 22 Pts.	8 (^) (8/32 = 25%)	DFP in 4 patients	7	7
(**) Deferasirox (DFX)- 26 Pts.	22 (^) (22/32= 68.7%)	DFO in 1 patient and DFP in 1 patient	3	1
(***) Deferiprone (DFP)- 2 Pts.	2 (2/32 = 6.25 %)	DFO in 1 patient	0	0
Total (50 patients)	**32 (64.0%)**	**7 out of 32 (21.8%)**	**10 (20.0%)**	**8 (16.0%)**

Legend = (^): p= 0,0004; **(*):** 40 -50 mg/kg/day subcutaneously 6 h/day (4 - 6 days/week); **(**):** dispersible tablet (DT) formulation 20–30 mg/kg/once daily and film coated tablet (FCT):14 mg/kg/once daily. Dose equivalence:20 mg/kg/day of DT = to 14 mg/ kg/day FCT = to ~75 mg/kg/day of DFP, and = to ~ 40 mg/kg/day of DFO); **(***):** 70-80 mg/kg/ day, given orally 7 days/week.[15]

Discussion. In our retrospective study on 50 adolescents and young adults with TDT who started early ICT, the prevalence of hypogonadism due to AHH was much lower (7.5%) than previous reports in which the prevalence reached much higher values, even 38% (19). These data confirm the importance of early onset of chelation therapy to attain normal sexual maturation.[4] At the start of chelation therapy, 22 patients received DFO, 26 DFX, and 2 patients DFP. However, their mean age at menarche (14.2 ± 2.24 years; range 9 – 20) was higher compared to data reported in the general population in Iran (13.2 years),[16] Bulgaria (12.7 years),[17] Greece (12.2 years),[18] Oman (13.3 years),[19] and Italy (12.4 years).[20]

Although compliance to ICT was not assessed in the present study, the efficacy of early start of chelation, as assessed by serum ferritin levels at menarche, showed that 10/45 of patients preserved low SF levels <1,000 ng/mL. Their age at menarche 12.6 ± 2 years was significantly lower compared to 14.9 ± 2.4 years of the others 35 patients, p= 0.0425. Their SF at menarche (712 ± 272 ng/ml) was not different from SF at the start of chelation therapy (970 ± 601, p > 0.05). Eight had regular cycles, while 2 got secondary amenorrhoea at ages 15 and 17 years after menarche at 9 and 15, respectively.

Seven of the 10 were on treatment with DFX. Thus, late menarche was reported in patients with severe iron overload (SF: >2,000 ng/mL) compared to those with SF < 1,000 ng/mL (12.7 years, close to the mean age at menarche) (p= 0.0067). These data indicate that, despite the availability of oral ICT, treatment adherence is still a serious risk factor during pubertal age. Therefore, considerable attention should be given to factors contributing to non-adherence to ICT during adolescence and early adulthood, particularly those related to patients' education.

Notably, one patient had menarche at 9 years (BMI: 18.7 kg/m², SF: 300 ng/mL), and 9 patients had very late menarche [>16years of age, mean 18.5, range 17-20 years, BMI: 20.3 kg/m² (range 16.25 - 27), mean SF: 4,000 ng/mL (range: 3,000 – 7,000 ng/mL]. Although precocious menarche is an event not reported before in thalassemias, extremely late menarche has been reported by Psihogios et al. (at 25 years),[21] Safarinejad et al. (at 16.8 ± 2.1years),[22] and Abd et al. (> 18 years in 2 patients).[23] Unfortunately, no data were reported on their BMI and SF level at menarche.

In this study population, a significant correlation was found between weight percentile and age at menarche. However, no significant correlation was found between BMI and age at menarche, contrary to what we observed previously on puberty and menstrual cycles in thalassemia.[4] In the early 1970s, Frisch and Revelle[24] suggested a "critical weight" theory, pointing out the relationship between weight and pubertal timing.[25]

Most likely, the mechanism behind the association between weight status and time of sexual maturation, at least for menarche as an endpoint, may be more complex than a direct causal relation. As suggested by Bratke et al.,[25] subcutaneous fat tissue like triceps (TSF) and subscapular skinfold (SSF) could show a stronger relation with menarche than the BMI, which measures both fat mass and lean mass.

Late menarche in patients with TDT could be caused by other conditions, such as functional hypogonadotrophic hypogonadism (FHH).[26] FHH has a wide range of etiologies (stress, excessive exercise or restrictive eating habits, chronic disease, e.g., anorexia nervosa, inflammatory bowel disease, celiac disease, chronic renal disease, and cystic fibrosis) that can inhibit the gonadotrophic axis by various mechanisms. It is assumed that disorders of secretion of various neuropeptides: neuropeptide Y (NPY), corticotropin-releasing hormone (CRH), leptin, ghrelin, and β-endorphin may cause a disorder of pulse GnRH secretion that results in the impairment of gonadotropin pulse secretion.[27]

Fernandez-Fernandez et al.,[28] who have investigated the role of ghrelin on sexual maturation, showed that ghrelin inhibits LH secretion in vivo in prepubertal males as well as gonadectomized male and female rats, whereas FSH remained unaffected. Moreover, Moshtaghi-Kashanian and Razavi[29] have hypothesized that a decreased leptin/acylated-ghrelin ratio may constitute one additional mechanism involved in delayed puberty, irregular menses, and amenorrhea.

In our study, irregular periods (oligomenorrhea and hypomenorrhea; 20%) were associated with older age of menarche (> 14 years) and other endocrine complications. Furthermore, in 8 patients, SA was diagnosed at the age of 14 – 23 years (17.9 ± 3.5 years; range 14-23) and was preceded by oligomenorrhea in the absence of signs of hyperandrogenism: ascertaining the absence of clinical (hirsutism) or biochemical evidence of hyperandrogenism in late adolescents and young women with oligomenorrhea is essential to rule out the frequent polycystic ovary syndrome or other less common ovarian or adrenal disorders.

These findings support the concept that oligomenorrhea may represent a serious menstrual dysfunction that should be diagnosed early. However, at the time of SA, the mean SF level was not statistically significantly different from the mean SF registered at menarche (1,385 ± 313 vs. 1,828 ± 1,014; p= 0.29), although there was a trend for reduction. Therefore, independent of SF risk stratification into lower or higher-risk subgroups, certain tissues, and cell types might be more sensitive to NTBI and iron-mediated toxicity.

In a previous study, we have documented that the duration of the menstrual history of patients was strictly correlated to the age at the start of s.c. ICT with DFO. In particular, the duration was 12.5 ± 8.9 years (range: 1.4 -28, median: 15 years) in 24 patients who started

chelation therapy < 5 years (13 with preserved menses and 11 with SA), 7.2 ± 8.8 years (range: 0. 6 – 28.2, median: 3 years) in 54 patients who started treatment from 5 to 12.5 years, and 3.1 ± 2.3 years (range 2 - 8, median 3 years) in 8 patients who started treatment > 12.5 years (p=< 0.01) (4). In this study, only 1 of 8 TDT patients on treatment with DFX developed SA. However, it is important to note that the 22 patients with regular cycles reported in this study and treated with DFX had a relatively short gynecological age (mean: 5.7 years).

Unfortunately, only a few patients enrolled in our study were treated with DFP in mono or combined chelation therapy. Therefore, we are just at the beginning of a long journey that will give us new insights over the years. Additional studies are also needed to assess better the negative effects of IOL and the positive effects of efficient ICTs on the reproductive system.

Glucose dysregulation (GD) prevailed among the endocrine complications and was more frequent in patients with SA. Although the mechanisms by which estrogen deficiency may alter insulin action in humans are not completely understood, animal studies have shown that estrogens increase glucose transport and glucose utilization in muscle cells.[30-31] These findings provide a basis for further research to explore the effects of estrogen deficiency on GD and offer an indication for potential therapeutic interventions.

Our work has several limitations. The first is the lack of data on SF levels between menarche and the last observation in young women who were still menstruating; moreover, we have no data on parents height and regarding ICT adherence except the low SF levels (<1,000 ng/mL) on menarche. Other inconsistencies may be attributable to our retrospective study's relatively small sample size, which may affect the statistical power of our observations. Moreover, the study did not cover some information, such as the severity of genotype and hematological phenotype that varies among populations, girls' education, family history, and lifestyle habits.

From another perspective, this study has several strengths: (a) it is the first investigation focusing on a multicenter study on menarche and menstrual cycles in patients with TDT who started early chelation therapy with different ICT regimes; (b) it encompasses patients with TDT treated and followed at the reference centers for hemoglobinopathies in their own countries; (c) Third, the preliminary results offer several ideas and reflections for future studies.

Conclusions. Early chelation alone does not necessarily coincide with efficient chelation in childhood and puberty because a non-small proportion of patients, equal to 36%, still had menstrual irregularities despite an early start of chelation by the age of 3 years. A key finding of this study was the significant positive correlation between age at menarche and SF level (r: 0. 41, p= 0.005), with a mean 2-year delay in the onset of menarche in females with severe iron overload compared with those with SF < 2,000 ng/ml. Late menarche (>14 years), related to high SF levels, was still frequent in most Centers and was a forerunner of irregular menstrual cycles and associated complications.

A possible explanation is poor adherence to therapy, evidenced by high ferritin values; however, the study design did not include this aspect.

Finally, an interesting finding was that a significantly higher percentage of females on treatment with DFX (68.7%) had normal menstrual cycles compared to DFO-treated ones (25%; p= 0.0004).

This acquisition could raise the intriguing question of the superiority of DFX over other chelating drugs. However, the design of our study and the low number of subjects treated with the various chelating drugs require a robust, specific perspective study before it can be recommended.

Adherence to ICTs is a key prerequisite for positive treatment outcomes and is especially important for those patients who require treatment regimens throughout their lifetime. A specific study that also includes compliance indicators will be able to confirm this insight.

Families, teenagers, and young adults must understand the importance of regular and constant ICT and should be conscious that even short periods of interruption or irregular adherence to ICT can have late deleterious effects on the H-P-G axis and other organs. Neglecting its importance, despite the innovative and expensive therapies for which the National Health Services pay high costs annually through their public funds, may lead to complications, hospitalization, and decreased quality of life.

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