EFFECT OF IRON OVERLOAD AND IRON CHELATORS ON ANTI-MÜLLERIAN HORMONE LEVEL IN EGYPTIAN FEMALE PATIENTS WITH TRANSFUSION-DEPENDENT B-THALASSEMIA

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ABSTRACT

Objective: This work aims to determine the effect of iron overload on serum Anti-Müllerian hormone (AMH) levels in females with transfusion-dependent β-thalassemia by measuring serum ferritin and to investigate the effects of iron chelation therapy including oral deferiprone (DFP) and subcutaneous deferoxamine (DFO) in the management of transfusion-related iron overload together with reproductive function.

Methods: A total of 90 female patients with thalassemia major (TM), thalassemia intermedia (TI) and thalassemia minor (T minor) were selected to investigate AMH by enzyme-linked immunosorbent assay and ferritin by immunoradiometric assay.

Results: Serum AMH level was lower in female patients with transfusion-dependent β-thalassemia than in T minor also, ferritin was 25 fold more in TM compared to T minor (3088.0±2497.6 ng/ml vs. 120.3±36.2 ng/ml). There was a significant negative correlation of AMH with ferritin in TM (r=−0.949, *p<0.001), in TI (r=−0.378, *p=0.039), and in T minor (r=−0.754, *p<0.001). The present finding implied a significantly higher ferritin and lower AMH in TM and TI that used DFO than DFP.

Conclusion: The results demonstrated that females with TM and TI were found to have lower serum AMH levels than T minor and inversely related to the serum ferritin levels in all thalassemic groups. Furthermore, it demonstrated that DFP was more efficient than DFO in removing excess iron and reducing the deleterious effect of excess iron to the reproductive system, which leads to fertility preservation of female patients with transfusion-dependent β-thalassemia.

Keywords: Anti-müllerian hormone, Ferritin, Iron overload, β-thalassemia, Deferoxamine, Deferiprone.

INTRODUCTION

β-thalassemias are a group of hereditary blood disorders characterized by anomalies in the synthesis of the beta chains of hemoglobin resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals [1,2]. Thalassemia was first recognized as a disorder by researchers in the United States and Italy, in 1925 [3]. The name of the disease is derived from the Greek words, "thalassa" meaning sea and "haima" meaning blood due to the mistaken notion that the disease was restricted to individuals of Mediterranean origin [3]. β-thalassemia is prevalent in Mediterranean countries, the Middle East, Central Asia, India, Southern China, and the far East as well as countries along the North coast of Africa and in South America. 300,000-500,000 children with a severe hemoglobin disorder are born each year, 30% of them have thalassemia. 50,000-100,000 children with beta-thalassemia major (TM) die each year in middle- and low-income countries [4]. In Egypt, β-thalassemia is the most common cause of chronic hemolytic anemia. The carrier rate varies between 5.5% and 9%. It was estimated that 1000/1.5 million/year live births have β-thalassemia [5].

β-thalassemia includes three main forms: TM, variably referred to as "cooley's anemia" and "Mediterranean anemia," thalassemia intermedia (TI) and thalassemia minor (T minor) also called "beta-thalassemia carrier", "beta-thalassemia trait" or "heterozygous beta-thalassemia." Individuals with TM usually present within the first 2 years of life with severe anemia, requiring regular red blood cell transfusions. Symptoms in untreated individuals with TM are growth retardation, pallor, jaundice, poor musculature, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoiesis, and skeletal changes that result from expansion of the bone marrow [6].

Treatment includes blood transfusions to correct anemia and iron chelating therapy to control iron overload [7-9]. Secondary iron overloading frequently results in target-organ toxicity such as heart failure, osteoporosis, or hypogonadism [10]. Two iron-chelating agents are approved to use: Subcutaneous deferoxamine (DFO) and synthetic oral deferiprone (DFP) [11]. The combination of the two iron chelators seems to maximize the efficacy producing additive and synergistic effects in iron excretion [12-14].

Advances in transfusions and iron chelation therapy have significantly improved the long-term survival and quality of life for women with transfusion-dependent β-thalassemia [15-18], evaluation of reproductive function in such women has become an emerging clinical issue [19,20]. Anti-müllerian hormone (AMH), also known as müllerian inhibiting substance, a member of the transforming growth factor-β superfamily, is primarily secreted by the granulosa cells of growing follicles [21]. Serum AMH levels have an excellent correlation with the number of antral follicles as determined by vaginal ultrasound [22,23].

Subjects

This study was conducted on 90 females that clinically diagnosed with β-thalassemia, 30 with TM, 30 with TI, and 30 with T minor. They were selected from hematology clinic-Cairo University (according to Scientific and Technological Cooperation Protocol between Faculty of Medicine, Cairo University, and Atomic Energy Authority, 2004). Written informed consent was obtained from all of the participants or their parents before blood sampling. The study was conducted in accordance with the guidelines set by the European Economic Community (EEC) regulations (Revised Directive 86/609/EEC) and approved by the Ethical

Committee at National Center for Radiation Research, Egyptian Atomic Energy Authority, Cairo, Egypt (NCRR-EAEA). Their age ranged from 10 to 25 years. The females with TM and TI received a blood transfusion every 3-4 weeks (transfusion characteristic and duration of transfusion were similar in all females). Females were prescribed DFO 40-50 mg/kg per infusion over 8-12 hrs, 5-6 days/week, or received DFP in regular oral dose (75 mg/kg/day when serum ferritin was <2500 ng/mL and 100 mg/kg/day when serum ferritin was >2500 ng/mL).

For regularly transfused patients (TM and TI), blood samples were collected at least 2 weeks from the previous blood transfusion, and they were instructed to leave off from taking their chelator therapy for 24 hrs before blood sampling. Iron chelation therapy whether oral DFP (kelfer and ferriprox) or subcutaneous DFO (desferal), weight, height, and body mass index (BMI) of patients were recorded z-scores for weight, height, and BMI for females between 10 and 19 years were calculated using the World Health Organization reference data [24].

METHODS

Serum AMH was measured by enzyme-linked immunosorbent assay according to the manufacturer's manual of a commercial kit purchased from EIAab Company, Catalog No: E0228h, China.

Serum ferritin was measured by immunoradiometric assay using a commercial kit purchased from Beckman Coulter, Immunotech, Czech Republic.

Statistical methods

The collected data were coded, tabulated, and statistically analyzed using IBM statistical package for social sciences statistics software version 22.0, IBM Corp, Chicago, USA, 2013. Mean, standard deviation, 95% confidence interval, and minimum and maximum values were calculated for various parameters. Inferential analyses were performed for quantitative variables using independent T-test in cases of two independent groups, ANOVA test for more than two independent groups with *post hoc* Tukey test; homogenous groups, while correlations were done using Pearson correlations. The level of significance was taken at $p<0.050$ is significant, otherwise is nonsignificant.

RESULTS

A total of 90 patients were included in this study. Clinical history of study groups is summarized in Table 1. 30 patients had TM with a mean age (14.2 ± 3.7), 30 had TI with a mean age (13.3 ± 3.3), and 30 had T minor with a mean age (15.6 ± 3.9). There were no significant (0.242) differences between subjects with TM, TI, and T minor with respect to age as shown in Table 1. BMI z-score was significantly (<0.001) different among all studied groups; lowest in TM, followed by TI and highest in T minor. Ferritin was 25 fold more in TM than T minor (3088.0±2497.6 ng/ml vs. 120.3±36.2 ng/ml). Ferritin was significantly different among all studied groups as shown in Table 1. AMH (in all cases, prepubertal and postpubertal) was significantly highest in T minor, followed by TI and least in TM with no significant difference between TM and TI as shown in Table 1. There was a significant negative correlation between AMH and ferritin in TM (r=−0.949, *p<0.001) as shown in Fig. 1a, in TI (r=−0.378, *p=0.039) as shown in Fig. 1b, and in T minor (r=−0.754, *p<0.001) as shown in Fig. 1c. Patients with TM that used DFO (n=14) had lower AMH (0.4±0.2 ng/mL) and higher ferritin (4235.7±3238.9 ng/mL) than DFP (n=16) that had higher AMH (0.6±0.1 ng/mL), and lower ferritin (2083.8±814.3 ng/mL). Also, patients with TI that used DFO (n=10) had lower AMH (0.8±0.1 ng/mL) and higher ferritin (1405.0±846.7 ng/mL) than DFP (n=20) that had higher AMH (1.0±0.4 ng/mL), and lower ferritin (727.5±288.4 ng/mL).

DISCUSSION

Iron overload is one of the most important complications of regular blood transfusion. Excess iron is extremely toxic to all cells of the body and can cause serious and irreversible organic damage [25]. In thalassemia, effective management of iron overload requires monitoring both of iron
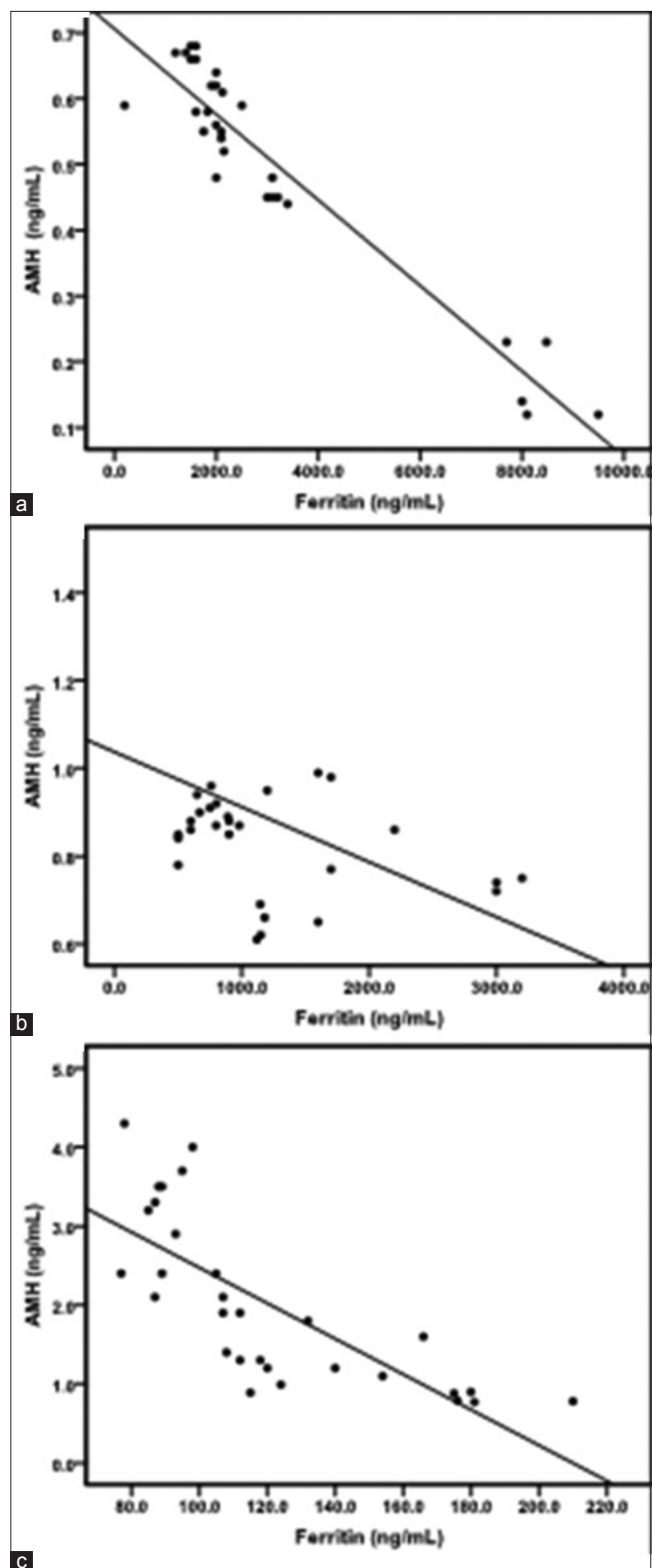
Fig. 1: (a-c) Correlations between ferritin and anti-müllerian hormone among thalassemia major, intermedia and minor, respectively

toxicity and the effects of excessive chelation [26]. Serum ferritin is the most commonly employed test to evaluate iron overload in β-TM.

The present result revealed that ferritin was 25 fold more in TM than T minor (3088.0±2497.6 ng/mL vs. 120.3±36.2 ng/mL) and the data were significantly different among all studied groups. Despite our patients

Table 1: Comparison between studied groups regarding history and clinical characteristics

Variables	TM (n=30)	TI (n=30)	Minor (n=30)	p
Family history	25 (83.3%)[a]	9 (30.0%)[b]	2 (6.7%)[c]	0.001*
Crisis	12 (40.0%)[a]	0 (0.0%)[b]	0 (0.0%)[b]	0.001*
Splenectomy	21 (70.0%)[a]	10 (33.3%)[b]	0 (0.0%)[c]	0.001*
Bony pain	30 (100.0%)[a]	25 (83.3%)[b]	0 (0.0%)[c]	0.001*
Fractures	6 (20.0%)[a]	0 (0.0%)[b]	0 (0.0%)[b]	0.002*
Dyspnea	30 (100.0%)[a]	3 (10.0%)[b]	0 (0.0%)[b]	0.001*
Palpitation	27 (90.0%)[a]	3 (10.0%)[b]	0 (0.0%)[b]	0.001*
Heart failure	2 (6.7%)[a]	0 (0.0%)[a]	0 (0.0%)[a]	0.129
Chelator				
DFO	14 (46.7%)[a]	10 (33.3%)[a]	-	0.292
DFP	16 (53.3%)	20 (66.7%)	-	
Transfusion age (months)				
Mean±SD	5.6±1.5[a]	11.1±1.8[b]	-	0.001*
Range	3.0-9.0	8.0-15.0	-	
95% CI	5.0-6.1	10.5-11.8	-	
Age (years)				
Mean±SD	14.2±3.7[a]	13.3±3.3[a]	14.8±3.8[a]	0.242
Range	10.0-25.0	10.0-25.0	12.0-25.0	
95% CI	12.8-15.6	12.1-14.5	13.4-16.3	
BMI z -score				
Mean±SD	−0.93±1.08[a]	−0.32±0.56[b]	0.85±0.87[c]	0.001*
Range	−3.49-0.89	−1.72-0.76	−1.49-1.91	
95% CI	−1.35-0.50	−0.53-0.10	0.50-1.19	
Ferritin (ng/mL)				
Mean±SD	3088.0±2497.6[a]	1179.2±775.2[b]	120.3±36.2[c]	0.001*
Range	200.0-9500.0	350.0-3200.0	77.0-210.0	
95% CI	2155.4-4020.6	889.7-1468.6	106.7-133.8	
AMH (All) ng/mL				
Mean±SD	0.5±0.2[a]	0.9±0.3[a]	2.0±1.1[b]	0.001*
Range	0.1-0.7	0.6-1.8	0.8-4.3	
95% CI	0.4-0.6	0.8-1.0	1.6-2.4	
AMH prepubertal ng/mL				
Mean±SD	0.6±0.1[a]	0.9±0.3[a]	2.0±1.1[b]	0.001*
Range	0.2-0.7	0.6-1.8	0.9-4.3	
95% CI	0.5-0.6	0.8-1.0	1.4-2.5	
AMH postpubertal ng/mL				
Mean±SD	0.4±0.2[a]	0.8±0.1[a]	2.1±1.1[b]	0.019*
Range	0.1-0.7	0.7-1.0	0.8-4.0	
95% CI	0.2-0.5	0.7-1.0	1.4-2.7	

*p<0.050, [a,b,c]homogenous groups had similar letters, TM: Thalassemia major, TI: Thalassemia intermedia, AMH: Anti-müllerian hormone, SD: Standard deviation, CI: Confidence interval, BMI: Body mass index, DFP: Deferiprone, DFO: Deferoxamine
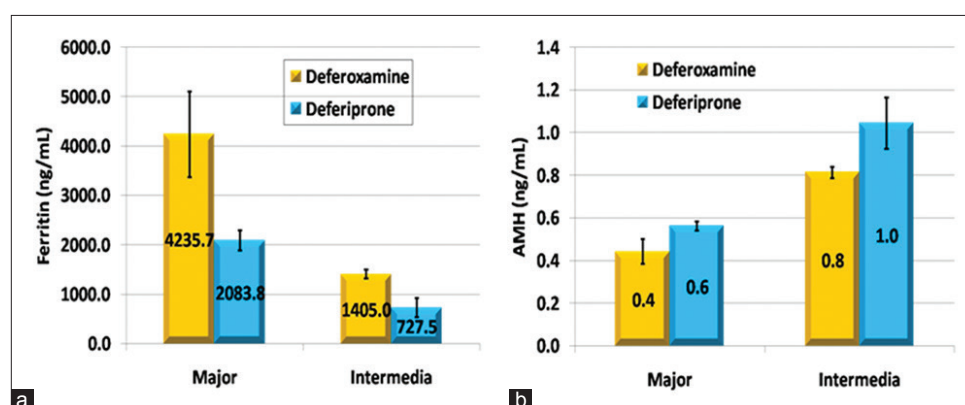

Fig. 2: (a and b) Comparison between studied chelators among thalassemia major and thalassemia regarding ferritin and anti-müllerian hormone (AMH) levels respectively

regularly using iron-chelating agents, the mean ferritin level in TM was 3088 ng/mL and in TI was 1179.2 ng/mL, which is above the desired level of 1000 ng/mL. Thus, the iron chelation could not be achieved as desired. These results agree with Pemde *et al.* [27] who stated that the mean ferritin level in patients with TM was 3138 ng/mL. Furthermore, Li *et al.* [28] stated that the mean ferritin level was 2729 ng/mL in 11-19-year-old transfusion-dependent TM patients.

In our study, BMI z-score was significantly (p<0.001) different among all studied groups; lowest in TM, followed by TI and highest in T minor. Our findings were in agreement with the previous studies, Salih *et al.* [29] demonstrated that BMI of thalassemic patients is significantly lower than those in control group. Fahim *et al.* [30] reported that BMI was low in 43% of patients with β-TM than in controls. Furthermore, Hashemi *et al.* [31] reported low BMI in 18.6% of their patients with β-TM.

However other reports; claimed that the mean BMI of their thalassemic patients were in the normal range and insignificantly different than controls [32,33].

In thalassemia, long-term red blood cell transfusions in females may lead to iron deposition in the ovaries and further reduce the ovarian reserve. AMH concentration, unlike other ovarian hormones, has been reported to be constant throughout the menstrual cycle [34] and significantly decline with age [35]. Because of this consistency and reliability, serum AMH levels can be used as a marker of ovarian reserve [23]. Lee and co-worker [36] reported that AMH and chronological age were more accurate than basal follicle-stimulating hormone, antral follicle counts (AFC) and BMI to evaluate causes of infertility in the prediction of live birth rate. It has been documented that women with lower AMH and AFC produce a significantly lower number of oocytes compared with women with higher levels. AMH shows significant promise to serve as such a marker in thalassemia women with iron overload and seems a better marker than AFC [37]. The current results demonstrated that females with transfusion-dependent β-thalassemia were found to have lower serum AMH levels than T minor. There was a significantly negative correlation between AMH and ferritin in all studied groups. These results agree with Chang *et al.* [38] who stated low AMH concentration in women with transfusion-dependent β-thalassemia when compared with healthy women of a similar age, also noted that serum ferritin was significantly and inversely related to the AMH concentrations. While Singer *et al.* [37] demonstrated that levels of AMH were mostly normal.

The present finding implied a significantly higher ferritin and lower AMH in TM and TI that used DFO than DFP. The disadvantages of DFO are that it is not orally bioavailable, fast rate of metabolism necessitating prolonged parenteral infusions, poor compliance, chelates zinc (sometimes causing clinical zinc deficiency), and high cost [39].

Data of El-Beshlawy [40] who reported the Egyptian experience with the use of different oral iron chelators on thalassemia patients, showed unacceptable toxicity with the exception of DFP and ICL670. El-Beshlawy *et al.* [41] found that the toxicity of DFP was mild to moderate and acceptable. Recent and ongoing studies have demonstrated that DFP, a small molecule that permeates all tissues, is more efficient in removing cardiac iron and improving cardiac function than DFO [42,43].

CONCLUSION

In conclusion, the results demonstrated that females with TM and TI were found to have lower serum AMH levels than T minor and inversely related to the serum ferritin levels in all thalassemic females. The results of this study are consistent with the hypothesis that the ovarian reserve might be impaired in females with transfusion-dependent β-thalassemia because of iron overload. Furthermore, it demonstrated that DFP was more efficient than DFO in removing excess iron and reducing the deleterious effect of excess iron to the reproductive system, which leads to fertility preservation of female patients with transfusion-dependent β-thalassemia.

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