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EFFECT OF BETA-THALASSEMIA MAJOR ON REPRODUCTIVE HORMONES LEVELS AND OVARIAN STATUS IN SAMPLE OF IRAQI FEMALE

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ABSTRACT
Blood transfusion the main cause that lead to tissue injury in patients with β- Thalassemia major by secondary iron overload. This study aimed to investigate the changes in some reproductive hormones levels and ovarian status in sample of Iraqi adult female with beta thalassemia major recruited from the Abin Al-Balady Hospital in Baghdad. Forty adult female patients (aged 17-24 years) and 20 ages matched healthy controls female were enrolled in this study. The levels of serum follicle stimulating hormone (FSH), prolactin (PRL), luteinizing hormone (LH), Estardiol (E2), Anti-mullerian hormone (AMH) and ferritin were measured by using Ichroma™ kits. The results of ferritin level revealed a significant ($p \leq 0.05$) increase when compared with control, also, a significant ($p \leq 0.05$) decrease, in the levels of (FSH), (LH), (PRL), (E2) and (AMH) was observed when compared with control. In all patients, (AMH), (PRL) and estradiol (E2), showed significant ($p \leq 0.05$) negative correlation with ferritin, while (FSH) and (LH), showed non-significant ($p \geq 0.05$) negative correlation with ferritin. The data confirm high reduction in sex hormones in beta thalassemia major female, which could be attributed to iron overload in pituitary gland and related reproductive organs.

KEYWORDS: Beta thalassemia, Iron overload, (AMH), Female Reproductive Hormone.

INTRODUCTION

Female patients with β-thalassemia major usually suffer from hypogonadotropic hypogonadism associated with amenorrhea, anovulation, and infertility, attributed to the iron effect on the pituitary gland as well as on the female reproductive organs. Iron overload in β-thalassemia major, resulted from repeated blood transfusions[1]. In female with β-thalassemia major the main cause of infertility are the iron overloud in the hypothalamic-pituitary-ovarian axis and reproductive organs. Direct iron deposition in reproductive system and hypothalamic-pituitary axis induced oxidative stress and then lipid peroxidation in cell organelles and membranes[2]. The byproducts of lipid peroxidation, like malondialdehyde impair function of the cell and damage DNA[3]. The normal functioning of reproductive organs and infertility in females were affected by the role of reactive oxygen species [4]. Reactive oxygen species play a regulatory role in various pathways of signaling transduction in maturation of oocyte, endometrial cycle, implantation, embryogenesis, and pregnancy[5].Ovarian function depends on the maintenance and development of ovarian follicles and play a major role in maintain the fertility[6] accumulation of the non heme iron lead to oxidative stress in ovarian stromal tissue and aging of the ovary [7]. Transfusional iron overload are the main cause of pituitary iron overload in the beginning of life and were independently predictive of hypogonadism [8].The (FSH) and estradiol levels in the follicular phase in women suffer from hypogonadotrophic hypogonadism are not reliable as markers to evaluate the function of gonads. Different studies have measured the response to gonadotrophin-releasing hormone and gonadotrophin stimulation tests by measuring estradiol to evaluate gonadal function [9, 10, 11], although ovarian function has been reported to be unaffected in patient with β-thalassemia major and hypogonadotrophic hypogonadism [9] many studies have reported impaired gonadal function in womwen with β-thalassemia major[10,11]. Anti-Müllerian hormone primarily secreted by the granulosa cells of growing follicles and is a predictor of ovarian aging. Anti-Müllerian hormone is a sensitive marker of ovarian reserve and function of granulosa cell[12]. Growing follicles number may indirectly reflect the size of the primordial follicle pool, which constitutes the ovarian reserve[13]. AMH levels are constant during the menstrual cycle and can be reliably detected in the serum[14]. Levels of serum AMH significantly decline with age and it is levels also have an excellent correlation with the count of antral follicle, as determined by vaginal sonography, therefore, serum Anti-Müllerian hormone levels can be used as a marker of ovarian reserve[13].

MATERIALS & METHODS

Subjects and collection of blood

Blood samples were collected from forty adult female, (age 17–24 years) with β-thalassemia major. They were recruited from thalassemia centre in Abin Al-Balady Hospital in Baghdad. The study was conducted from December 2015 to April 2016. Blood samples obtained from 20 adult healthy female (ages 17–24) were used as controls. Five (ml) of blood were drawn for each biochemical and hormonal study. Samples of blood were left at room temperature for about 30 minute, after that centrifugation was done at 3000 (r.p.m.) for 10-15 minute

to separate the serum. The separated serum was stored at – 20̊ C

Assay methods

Determination of ferritin, follicle stimulating hormone (FSH), prolactin (PRL) luteinizing hormone (LH), estradiol (E2) and Anti-Müllerian hormone (AMH): According to procedures recommended by: ichroma™ ferritin kit, ichroma™ FSH kit, ichroma™ LH kit and ichroma™ PRL kit from Boditech Inc. Korea and ELISA kit for estradiol (E2) hormone by using Enzyme -Linked Immunosorbent Assay (ELISA).

Statistical Analysis

The results were analized by using the (SPSS). Analysis of variance by ANOVA table as well as excretion mean and standard deviation depending on basic statistic method.

RESULTS

The results represented by table (1) and figure (1), showed a significant ($p \leq 0.05$) increase in the ferritin serum in β-thalassemia major (3431.08 ±547.10 ng/ml) when compared to control (484.4 ±155.58 ng/ml). a significant ($p \leq 0.05$) decrease was found in the level of serum Prolactin (11.67 ± 4.20 Ng/ml) , Estradiol (136.83 ±65.69 pmol /L), FSH(3.52 ± 2.17 mIU/ml), LH (2.08 ±1.79 mIU/ml) and AMH (22.45 pmol/L) in patients when compared to control(485.4 ± 156.68 pmol/L),(18.12 ±3.93 Ng/ml),(5.42 ±1.73 mIU/ml), (9.61 ±3.32 mIU/ml) and (33.25 pmol/L) respectively table (1) and figures (2,3,4 and 5). The results in table (2) showed a significant ($p \leq 0.05$) negative correlation in PRL (r= -0.3171), AMH (r= -0.2981) and Esterdiol (r= -0.0721), with ferritin, while there was non-significant ($p \geq 0.05$) negative correlation in LH (r= - 0.5243) and LH (r= -0.2916) with ferritin.

TABLE 1: Levels of Pituitary and Gonadal Hormones in Female Patients with Beta-Thalassemia Major and Control

	Groups	
Parameters	Healthy control	Beta-Thalassemia Major Patients
Estradiol E2 (pmol /L)	484.4 ± 155.58	136.83 ± 65.69*
Prolactin PRL (Ng/ml)	18.12 ± 3.93	11.67 ± 4.20*
LH (mIU/ml)	5.42 ± 1.73	2.08 ± 1.79*
FSH (mIU/ml)	9.61 ± 3.32	3.52 ± 2.17*
AMH (pmol /L)	33.250±2.341	22.45±2.012*
Ferritin (Ng/ml)	158.25 ± 32.72	3431.08 ± 547.10*

Values are expressed as mean ± S.D

* Values are statistically significant (P <0.05) as compared with control.

TABLE 2: Correlation between ferritin and pituitary and gonadal hormones in female with β-Thalassemia major

Correlation		r- value	Significant
Estradiol E2	ferritin	- 0.0721	Sig.
Prolactin	ferritin	- 0.3171	Sig.
AMH	ferritin	- 0.2981	Sig.
LH	ferritin	- 0.5243	NS
FSH	ferritin	- 0.2916	NS

Sig.: Significant $p \leq 0.05$, NS: Non significant $p \geq 0.05$

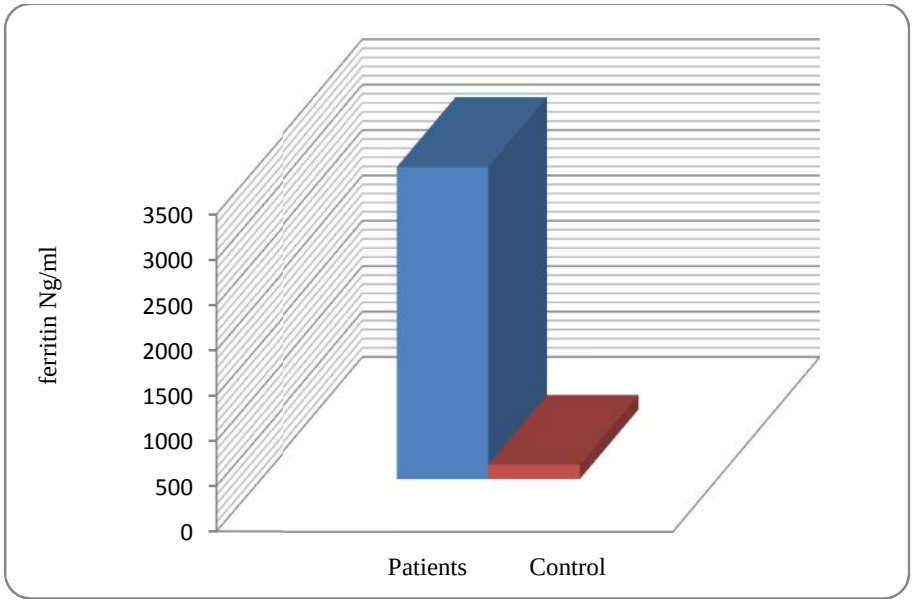

FIGURE 1: Ferritin concentration in β-Thalassemia major female

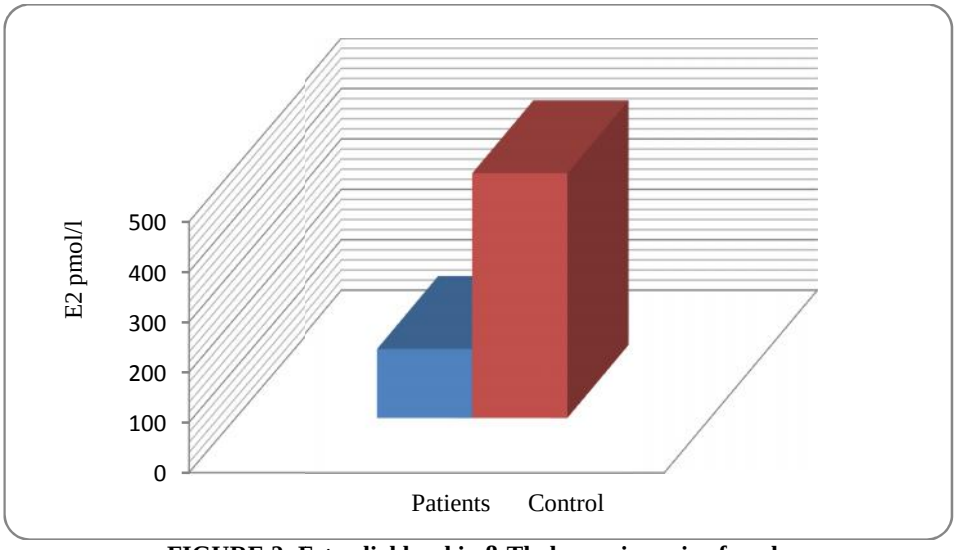

FIGURE 2: Estradiol level in β-Thalassemia major female

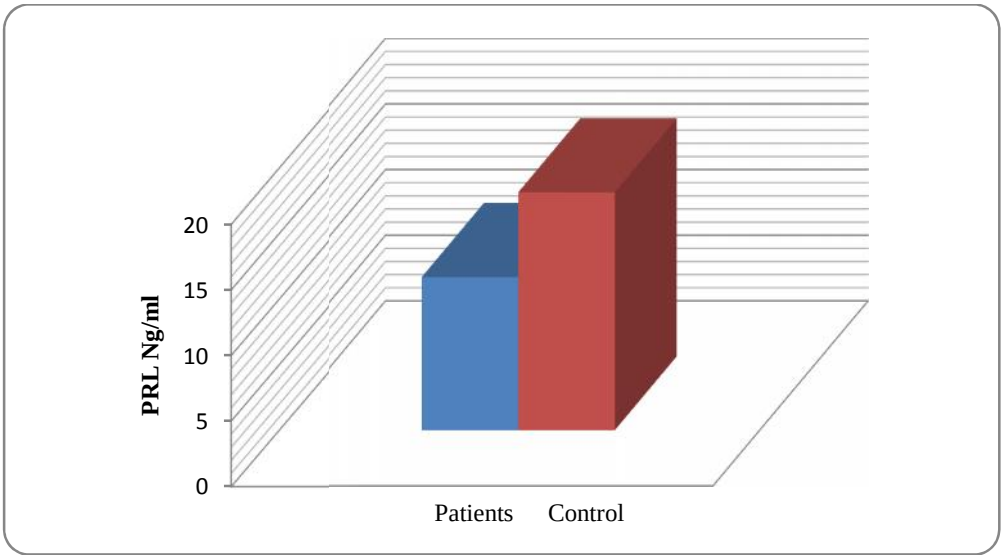

FIGURE 3: Prolactin level in β-Thalassemia major female

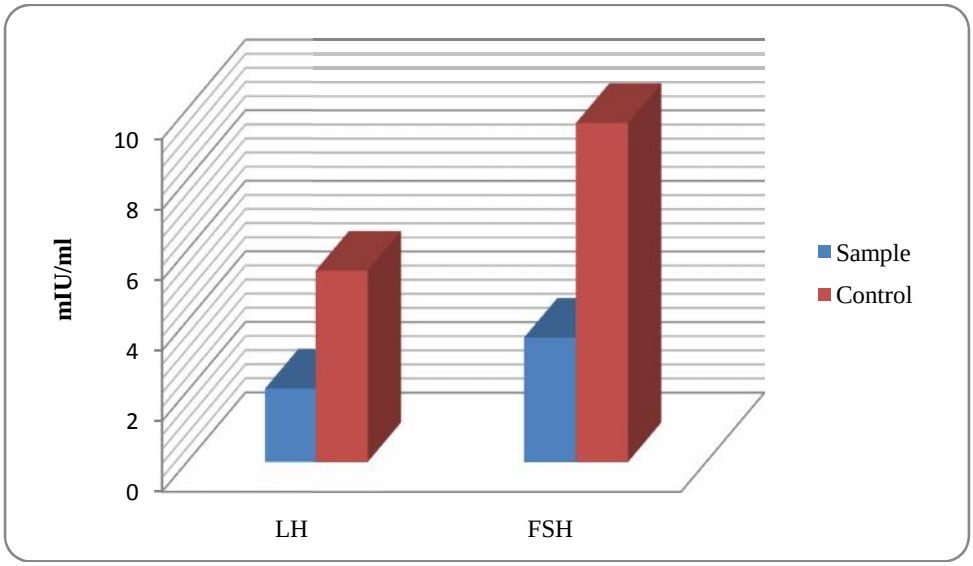

FIGURE 4: FSH and LH level in β-Thalassemia major female

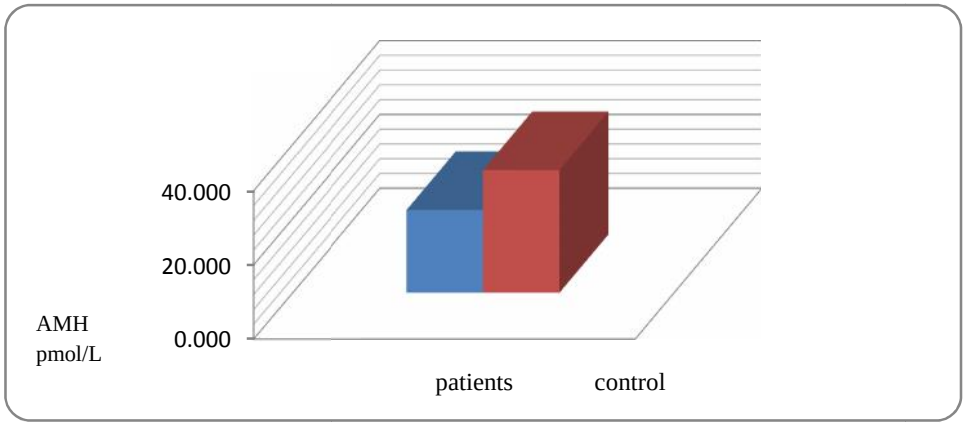

FIGURE 5: AMH level in β-Thalassemia major female

DISCUSSION

Because of advances in the current medical care systems, females with β-thalassemia major who receive life-long blood transfusions now live longer. Therefore the reproductive endocrinology and fertility requirements for such women are becoming more important. The results of this study showed a significant decrease in the level of pituitary and ovarian hormones, and this may provide additional strong evidence to suggest that iron overload are at high risk of being associated with impaired function of gonad. No direct evidence correlating the Infertility in females that suffers from β-thalassemia major with oxidative stress[15]. The catabolic iron catalyzes the production of free radicals, resulting in oxidative stress in lipid membranes, lysosomes, mitochondria, DNA, proteins. This is maybe induced oxidative stress and direct effect in many organs, like, pituitary gland, hypothalamus, and female reproductive organs, another organs indirectly effected such as, pancreas and liver, and this will contributing to the impaired the processes of metabolism of serum antioxidants and hormones [16]. Reactive oxygen species -induced apoptosis is involved in the regression of corpus luteum at the end of the non-fertile cycle [17].Conditions such as polycystic ovary syndrome, hydatidiform mole, preeclampsia, spontaneous abortions, endometriosis, embryopathies, preterm labor, and unexplained fertility also affected by oxidative stress [18]. Oxidative stress affects the production of steroid hormones; especially estradiol which is an important predictor of ovarian response, the abnormal production of estradiol could be impaired ovarian functions in β-thalassemia major females[19]. Oxidative stress modulates the age-related decline the infertility in patients suffer from β-thalassemia [20]. In a study on thalassemia patients group, after GnRH test, the levels of FSH, estradiol and LH were significantly lower than control [21]. Therefore, the hypothesis that long-term blood transfusion was in these women may lead to deposition of iron in the ovaries and reduce the ovarian reserve. In the current study, the results revealed that the level of serum Anti-Müllerian hormone as a marker of ovarian reserve, were lower in female with β-thalassemia major when compared with healthy female. AMH is exclusively secreted from the granulosa cells of the ovary after menarche and has high reproducibility and consistency over the cycle. It has been suggested that Anti-Müllerian hormone is a good marker for predicting ovarian reserve, and clinically it is used as a predictor of ovarian response before starting gonadotropin treatment for female who undergo assisted reproductive treatments as well as being considered the most applicable predictor when compared with other tests that are commonly used for determined the ovarian reserve [13, 22, 23, 24, 25] AMH has been reported to be a sensitive marker of diminished ovarian reserve related to aging[26, 27].The AMH level and ovarian volume could be considered as surrogate markers to represent ovarian reserve. In conclusion, the results of current study have demonstrated that female with β-thalassemia major were found to have lower serum AMH levels than control of a similar age. The serum Anti-Müllerian hormone levels were inversely related to the serum ferritin levels in women with β-thalassemia.

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