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The Role of Reproductive Hormones on Fertility with Beta-Thalassemia

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Abstract: ***Beta thalassemia major is a common genetic blood disorder which is the most severe form of thalassemia, and an inherited disease resulting from homozygosity or compound heterozygosity for beta thalassemia with severe reduction or total absence of beta globin chains. Blood transfusions are mandatory for survival of beta thalassemia major patients which leads to iron overload, subsequent tissue damage and oxidative stress.Failure of pubertal growth, delay or absence of sexual development, amenorrhea, sexual dysfunction and infertility due to hypogonadism are well-recognized disturbances of the hypothalamic " pituitary " gonadal axis in β-thal patients.***

Keywords: Beta thalassemia major, serum iron, reproductive hormones

1. Introduction

Thalassemia syndrome is a heterogeneous group of inherited disorders which result from a genetic abnormality of globin production, there is decreased or absence of synthesis of either α or β-globin chain of adult hemoglobin, HbA (α2β2)]1[. Thalassemias are classified according to which chain of the hemoglobin molecule is affected into α thalassemias and β thalassemia]2[. Beta thalassemia is a hereditary anemia resulting from defects in the production of β-globin chains. Depending on clinical severity, three forms are distinguished, namely, thalassemia major, thalassemia intermedia and thalassemia minor]3[. Beta thalassemia major is a common genetic blood disorder which is the most severe form of thalassemia with significantly large number of children dying each year throughout the world]4[. It is an inherited disease resulting from homozygosity or compound heterozygosity for beta thalassemia with severe reduction or total absence of beta globin chains. Blood transfusions are mandatory for survival of beta thalassemia major patients which leads to iron overload, subsequent tissue damage and oxidative stress]5[.

Failure of pubertal growth, delay or absence of sexual development, amenorrhea, sexual dysfunction and infertility due to hypogonadism are well-recognized disturbances of the hypothalamic " pituitary " gonadal axis in β-thal patients]6[. The history and clinical findings are substantiated by the laboratory investigations, results confirmed it's diagnosis when the circulating gonadotropins are inappropriately low and the levels of estradiol are extremely below the normal values]6[. Aberrant gonadotrophin response to gonadotropins releasing hormone (GnRH) administered acutely and in a pulsatile fashion strongly indicates failure of gonadotroph cells, which seem to be extremely vulnerable to iron damage]7[.

2. Aim of the Study

It in investigate of iron overload effect on hypothalamic – pituitary – gonadal axis function in Patients with beta-thalassemia major and its impact on uterine size by examination for presence of secondary sexual characteristics and hormonal indicators (LH, FSH , Estradiol and GnRH) and using of transabdominal ultrasound to determine uterine size.

3. Patients and Healthy Control

After approval of the study by ethical committee of college of medicine and consent of patients an explained to them. The study was conducted during the period from October 2014 to July 2015 at Babylon Maternity and pediatric teaching Hospital of Hilla city. The samples were taken from Hereditary Bleeding Center of Maternity and pediatric teaching Hospital.

Table 1: Number and age of the 55 patients and 20 controls in the study

	Patient groups			control group
	Primary amenorrhea	Secondary amenorrhea	Normal menstruation	
Number	20	19	16	20
Age (years)	18-28 years	18-30 years	18-29 years	18-30 years

4. Blood Collection

The collection of blood was performed for β-thalassemia major patients and healthy control in Hereditary Bleeding disorders Center in Hilla city, ten milliter (ml) of blood were drawn for each biochemical and hormonal study. Blood samples were left at room temperature for about 30 minute, after that centrifugation was done at 3000 round per minute (r.p.m.) for 10-15 minute to separate the serum and stored at –20 ^{0}C until analysis. The blood sample of all patient groups and control collected at early follicular phase (3-5 days).

5. Assay methods

Determination of serum iron according to proceure recommended by the iron kit from Spectrum , Egypt by using spectrophotometer and read at 623 nm. Determination of ferritin, glutathione (GSH), follicle stimulating hormone (FSH), luteinizing Hormone (LH), estradiol (E2) and human gonadotropin releasing hormone (GnRH): According to procedures recommended by the FSH, LH and E2 hormone kit from Monobind lnc, USA, ,while, GSH and GnRH kit from company Elabscience , USA. by using Enzyme Linked Immunosorbent Assay (ELISA). Ultrasound examination: Transabdomonal ultrasound was used in this study. Transabdominal ultrasound performed with 3.5 megahertz (MHz) transducer with a full urinary bladder.

6. Results

Table (6.1): Secondary sex characteristics development distribution for all thalassemic patients

Secondary characteristics development		Patient groups		
		PA	SA	NM
Breast	Not developed	20 (100%)	____	____
	Developed	____	19 (100%)	16(100%)
Pubic and axillary hair	Not developed	20 (100%)	----------	---------
	Developed	____	19 (100%)	16 (100%)

PA= Primary amenorrhea, SA= Secondary amenorrhea, NM= Normal menstruation

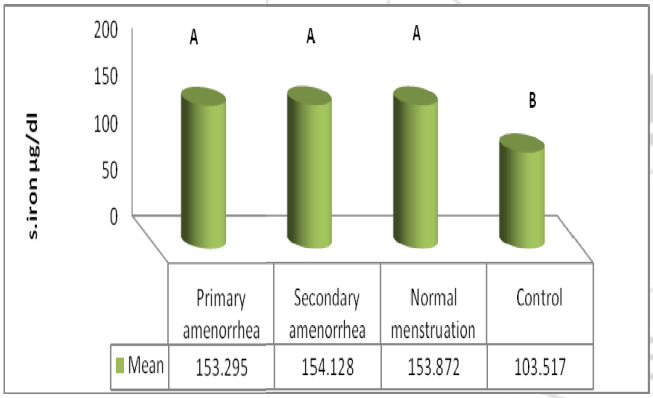

Figure (6.1): The mean values of serum iron in patient groups and control group.

-Values with different capital letters indicate significant difference at p<0.001 level

.-Values with same letter indicate no significant difference p>0.05.

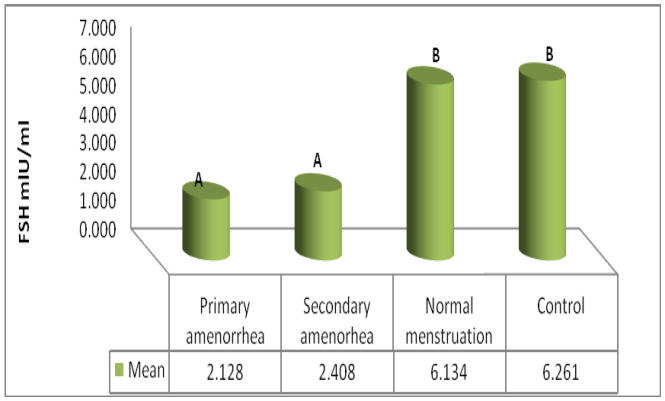

Figure (6.4): The mean values of Follicle Stimulating

Hormone in patient groups and control group.

-Values with different capital letters indicate significant difference at p<0.001 level.

-Values with same letter indicate no significant difference p>0.05.

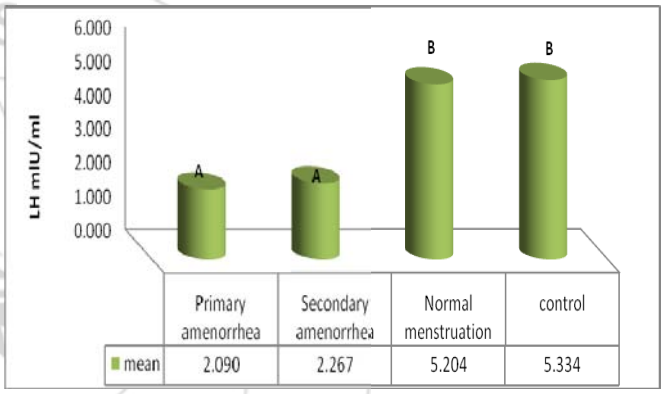

Figure (6.5): The mean values of Luteinizing Hormone in patient groups and control group.

-Values with different capital letters indicate significant difference at p<0.001 level.

-Values with same letter indicate no significant difference p>0.05.

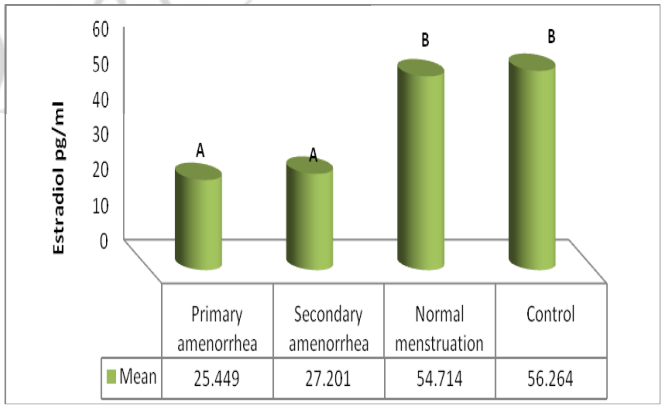

Figure (6.6): The mean values of Estradiol in patient groups and control group.

-Values with different capital letters indicate significant difference at p<0.001 level.

-Values with same letter indicate no significant difference p>0.05.

Table (6.2): The values of GnRH for all studying groups

	Patient groups			Control group
	PA	SA	NM	
GnRH pg/dl	738.2 ±129.4	740.6 ±130.2	742.4 ±126.7	739.2 ±127.4

-No significant difference among all studying groups ($p>0.05$).
PA= Primary amenorrhea, SA= Secondary amenorrhea, NM= Normal menstruation

Table (6.3): Frequency distribution (number, n and percentage, %) of beta thalassemic patients and control groups according to size of uterus

Uterine size (length and width)	Patient groups			Control
	PA	SA	NM	
Uterine length (equal or more than 5 cm)	A 0 (0%)	A 0 (0%)	B 16 (100%)	B 20 (100%)
Uterine length (less than 5 cm)	A 20 (100%)	A 19 (100%)	B 0 (0%)	B 0 (0%)
Uterine width (more than 2)	A 0 (0%)	A 0 (0%)	B 16 (100%)	B 20 (100%)
Uterine width (less than 2)	B 20 (100%)	B 19 (100%)	B 0 (0%)	B 0 (0%)

PA= Primary amenorrhea, SA= Secondary amenorrhea, NM= Normal menstruation

-Values with different capital letters indicate highly significant difference at $p\leq0.001$ level.
-Values with same letters indicate no significant difference $p>0.05$.

7. Discussion

Regarding the secondary sex characteristics development (breast, pubic and axillary hair) , the results in the present study have shown that 100% of primary amenorrhea had not development of the breast, pubic and axillary hair with significant difference than both groups of secondary amenorrhea and normal menstruation 100% whose had development of secondary sex characteristics (breast, pubic and axillary hair) as shown in table (6.1). These results agreed with papadim *et al.*, (2002)[8] who showed that hypogonadism is clinically diagnosed in a female by the presence of primary or secondary amenorrhea without development or with development of secondary sexual characteristics. Absence of breast development suggestive to be due to hypogonadism]9[. While, absence of pubic and axillary hair agreed with Elsedfy *et al.*, 2011]10[who showed that adrenal androgen production declines with advancing puberty in thalassemic patients and might explain the absent development of pubic and axillary hair observed in this condition. Menarche is frequently delayed, breast development is often poor, and patients are frequently oligomenorrheic or amenorrheic, even if menarche occurs]11[.

Iron is essential trace element present in almost all cells of the body. Human body requires iron for the synthesis of oxygen carrying protein called haemoglobin found in red blood cells, and myoglobin which is also a protein found in muscles. It also takes part in the production of other important proteins in the body such as for DNA synthesis and cell division]12[. When iron is present in excess amounts in the body it will lead to hemochromatosis, which may a be primary or secondary. Secondary hemochromatosis occurs in diseases like thalassemia due to iron overload especially in thalassemia major where repeated blood transfusions are required]13[.

The serum iron concentration was highly significant increased in patients with β-thalassemia compared to control group figure(6.1). This significant increase level of serum iron probably due to repeated blood transfusions and increased iron absorption from gastrointestinal tract]14[. Our results are in agreement with the study done by (Al-Muhammadi, 2011)]15[. The patients with beta thalassemia major have sever anemia due to ineffective erythropoiesis which is primary reason for iron overload and blood transfusion is secondary to it. Thus, increased iron may increase the potential of oxidative injury to erythrocyte and cell organelles . Patients with beta thalassemia major undergo from massive distraction of abnormal red blood cells in reticuloendothelial system, and those patients become undergo from sever anemia]16[.

FSH and LH, secreted by the gonadotropin cells of anterior pituitary gland, glycoprotein hormones each with a molecular weight of 30 kilodaltons, are required in homeostasis of fertility regulation via the hypothalamic-pituitary- gonadal axis which has been well established in both women and men. In women, FSH exerts its effect directly on ovarian granulosa cells, essential for growth and maturation of ovarian follicles while LH is required for rupturing of Graafian follicle and ovulation]17[. Estradiol is the most potent natural estrogen produced by the Graafian follicle of the ovary and the placenta and in smaller amounts by the adrenals, and the male testes. Target organs for estradiol include the follicles, uterus, breast, vagina, urethra, hypothalamus, pituitary and skin. Exerts its effect on the development of adult primary and secondary sexual characteristics]17[. Gonadotropin-releasing hormone (GnRH), also known as Luteinizing-hormone releasing hormone(LHRH) and luliberin, is a tropic peptide hormone responsible for the release of FSH and LH from the anterior pituitary. GnRH is synthesized and released from neurons within the hypothalamus]17[.

Regarding the mean values of LH, FSH and E2 hormones, in this study, there was highly significant decrease in patients groups (primary and secondary amenorrhea) compared with both control and normal menstruation groups figure (6.2),(6.3) and (6.4). While, regarding the mean values of GnRH hormone, this study found no significant difference between each group of beta thalassemia major compared with control group as well as no significant difference among patients groups table (6.2). These results agreed with (safainejad, 2010)]18[, who reported the decrease in basel LH and FSH in thalassemic groups (primary and secondary amenorrhea) compared to normal menstruation and the same pattern emerged after administration of GnRH. As well as the serum estradiol concentration of thalassemic groups (primary and secondary amenorrhea) was lower than normal menstruation. Explanation of these results may be due to

damage to the hypothalamic –pituitary- gonadal axis is most likely localized at a central level., the classic knowledge is that in transfusion-dependent β-thalassemia patients, increased iron deposition in the pituitary gland has a cytotoxic effect, leading mainly to hypogonadotropic-hypogonadism due to pituitary hyporesponsiveness to GnRH]19[. Papadimas *et al*., (2002)]8[have reported a higher incidence of hypogonadism in Greek subjects, manifested a menstrual irregularities and hypoestrogenemia was demonstrated in 95 of cases. However, only 74 of patients developed primary or secondary amenorrhea. Pignatti *et al*., (2005)]20[had reported hypogonadism in 55% Italian patient with thalassemia major. Argyropoulou *et al*., (2000)]21[reported that patients with β- thalassemia had subnormal serum LH and FSH responses to gonadotropin hypogonadism, also Chatterjee *et al*., (1998)]22[showed that patients had very low FSH and LH levels and failed to respond to GnRH challenge test indicating severe gonadotropin insufficiency. Thalassemia major, a severe hemolytic anaemia due to a genetic defect in the synthesis of haemoglobin chain, can produce hypopituitarism. This hypopituitarism leads to hypogonadotropic hypogonadism, an endocrinopathy occurring secondary to iron overload]23[. The iron overload is a consequence of frequent blood transfusion, which is the most important treatment modality for thalassemia major. Other possible causes of hypogonadism in beta thalassemia major include liver disorders, chrouic hypoxia, diabetes mellitus and zinc deficiency]24[. Female patients with βTM usually suffer from hypogonadotropic hypogonadism (HH) associated with amenorrhea, anovulation, and infertility, attributed to the iron effect on the pituitary gland as well as on the female reproductive system]25[.

The most common endocrine abnormalities with iron overload includes: hypogonadism , growth hormone deficiency and diabetis mellitus .Variable incidence of hypothyroidism and hypoparathyroidism , have been less commonly reported]26[.

Ultrasonography is the most frequently used imaging investigation in the assessment of the female genital tract. Most often the uterus and ovaries are evaluated with the help two dimensional transabdominal or endovaginal transonography]27[.

Regarding the size of uterus(length and width) , transabdomonal ultrasound in the present study have shown that 100% of primary and secondary had small uterus size (length and width) less than 5 cm and less than 2cm respectively with highly significant decrease than control group and normal menstruation 0% table (6.3).

These results agreed with (Karabulut et al., 2010 and Naredi et al., 2011)]28[-]9[, who reported small size uterus in primary and secondary amenorrhea as compared with normal menstruation and control group.

8. Conclusion

Beta thalassemia major patients could present with primary amenorrhea with hypogonadotrophic hypogonadism(HH) of early onset which characterized by subnormal LH, FSH and estradiol hormone levels without development of secondary sex characteristics and small uterine size, secondary amenorrhea with hypogonadotrophic hypogonadism (HH) of late onset which characterized by subnormal LH, FSH and estradiol hormone levels with development of secondary sex characteristics and small uterine size and normal menstruation with healthily functioning gonads which characterized by normal LH, FSH and estradiol hormone with full development of secondary sex characteristics and normal uterine size, this disorders may be due to multiple blood transfusion, iron overload and irregular chelation therapy.

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