Available online at https://www.sjomr.org

SCIENTIFIC JOURNAL
OF MEDICAL RESEARCH

Vol. 6, Issue 21, pp 26-33 , 2022

ISSN: 2520-5234

ORIGINAL ARTICLE

Correlation Between Serum Ferritin Level and Endocrine Disorder in Female Patients with Beta-Thalassemia Major In Kerbala, Iraq

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ARTICLE INFORMATIONS

Article history:
Received: 29 May 2021
Revised: 15 July 2021
Accepted: 28 July 2021
Published: 24 March 2022

Keywords:
Blood transfusion,
Beta-thalassemia major,
Ferritin, Hypogonadism,
Iron overload, Thalassemia.

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ABSTRACT

Background: Beta-thalassemia major (BTM) is an inherited disorder of hemoglobin production in which there is a complete or partial failure in synthesizing ß-globin chains. Although the mainly recommended treatment for ß-TM is blood transfusion, blood transfusion leads to many toxic complications like iron overload, subsequent tissues damage, and oxidative stress.

Objectives: This study aims to identify the serum ferritin levels in fifty female patients with beta-thalassemia that classified into different groups (primary, secondary amenorrhea, and normal menstruation) then examine the relationship between serum ferritin and biochemical parameters (LH, FSH, estradiol, T3, T4, and TSH). Finally, using transabdominal ultrasound to explore the size of ovaries and uterus and examine the secondary sexual characteristics in the subjects.

Material and methods: This study involved fifty females with BTM aged 14–24 years. The subjects were conducted at the thalassemia branch at the Children's teaching hospital, Karbala, Iraq. The study was carried out from July 2017 to November 2018. According to their amenorrhea status, the patients were classified into three groups: primary amenorrhea, secondary amenorrhea, and normal menstruation groups. Each female received a physical examination and a series of blood tests, and their hormone levels were studied (LH, FSH, estradiol, T3, T4, TSH, and ferritin level), along with an abdominopelvic ultrasound, and determined the changes in external secondary sexual characteristics, such as the development of the breasts, pubic and axillary hair. The relationships among these research variables were then analyzed using SAS 2012. Associations between the categorical variables were tested using the Chi-square, and Duncan's Multiple Range, where significance was accepted at $p < 0.05$.

Results: The results of beta-thalassemia major patients indicate that the females significantly do not have development of breast and axillary hairs compared with those who

CITATION: Al-khafaji KM, AL-Mosawy WF, Naser IH, Almusawi IMS. "Correlation Between Serum Ferritin Level and Endocrine Disorder in Female Patients with Beta-Thalassemia Major In Kerbala, Iraq". Sci. J. Med. Res. 2022;6(21):26-33. DOI: 10.37623/sjomr.v06i21.6

have ($p \leq 0.05$). We found the small size of ovaries and uterus (28 and 36%, respectively) of primary amenorrhea, (2 and 4%, respectively) of secondary amenorrhea and (20 and 4%, respectively) of normal menstruation with remarkable significant values ($p \leq 0.01$) between groups for both parameters. The results of LH hormone for the PA group (2.40 ± 0.36) and NM group (4.73 ± 0.75) were significantly lower ($p \leq .0001$) than those in the SA group (7.75 ± 1.90). While the levels of estradiol hormone for PA and SA groups (22.02 ± 2.83 and 16.8 ± 3.20 respectively) were significantly decreased ($p \leq .001$) when compared with NM groups (141.95 ± 49.75). The correlation analysis to evaluate the relationship between the LH in female beta_thalassemia major patients' group with normal menstruation revealed a significant positive correlation with FSH and serum T3. FSH showed a significant association with T3, and T3 showed a significant positive correlation with T4 and TSH. In the female beta_thalassemia major patient group with primary amenorrhea, the data reveals a significant positive association between FSH with LH, and a negative association with the TSH, while LH showed a significant negative association with TSH. However, serum T4 indicates a significant positive correlation with serum TSH.

INTRODUCTION

Thalassemia is a congenital hemolytic disease. It was classified as an inherited disease according to Mendel's laws. In this disorder, abnormal, defective hemoglobin (Hb) is produced. In erythroid precursors, the two chains of the Hb molecule don't pair together, leading to damage of one of the chains.[1] The classification of thalassemia depends on the type of the affected chain. It is called alpha-thalassemia when alpha-chain is damaged and beta-thalassemia when the damage affects the beta hemoglobin chain characterized by the most serious blood disorder since hemoglobin will not synthesize normally and ultimately abnormal erythrocyte producing severe anemia.[2] Males and females have similar rates of disease (Nang). Thalassemia patients usually suffer from severe anemia necessitating regular blood transfusions.

Alpha and *Beta-thalassemia* are the major two types of thalassemia. Alpha-thalassemia resulted from a defect in the synthesis and production of the alpha hemoglobin chain. This type is usually observed in patients who has a mutation in α-globin genes on a single chromosome.[3] There are two types of alpha-thalassemia, alpha-thalassemia major, which may be even during fetal life, leading to severe anemia that may ultimately affect the baby's life shortly after birth or before birth. The second type is hemoglobin H disease, which is milder than beta-thalassemia and does not need a blood transfusion.[4]

The second type of this disorder is beta-thalassemia, a type of genetic blood disease. The beta-chain of the hemoglobin is either absent or not fully synthesized. This situation will result in anemia caused by less hemoglobin production and erythrocytes. It could be classified to three types, major, intermediate, and minor beta-thalassemia.[5]

Beta-thalassemia Major

The signs and symptoms of beta-thalassemia usually appear between 6 months to two years of a baby life which include malnutrition, diarrhea, irritability, episodes of high body temperature, abdominal discomfort, hepatomegaly, and splenomegaly.[6] Patients with this type of thalassemia require a regular blood transfusion, which is not free of multiple complications due to iron overload. These complications include delayed growth in children, myocardiopathy, liver (fibrosis and cirrhosis), hypogonadism, parathyroid and thyroid insufficiency.[7] On the other hand, patients with infectious liver and iron overload are at risk of developing hepatocellular carcinoma, which may lead to death resulting from iron deposition in the cardiac tissues.[8]

Beta-thalassemia Intermedia

This type of thalassemia may not appear any symptoms until adulthood, while another patient may develop symptoms during childhood. Blood transfusion is the only useful therapy for some complications such as leg ulcers and gallstones. But if the patient's hemoglobin level is still sufficient (between 7 to 9 g/dL) may require only supporting therapy. As in beta-thalassemia major, iron overload could occur due to blood transfusion and can result in many serious complications.[9]

Beta-Thalassemia Minor

This type of thalassemia is known as the lowest dangerous as the other types. Most patients with beta-thalassemia minor usually have mild anemia with no symptoms.[10]

PATHOPHYSIOLOGY

Beta-thalassemia is a genetic disease, and babies can carry this disease if their parents are carriers.[11] This disorder results from a deficiency in either alpha or beta of one of the two hemoglobin chains, either alpha or beta. This is usually caused by multiple mutations in the responsible gene producing unhealthy hemoglobin molecules, which are unstable and undergo hemolysis or apoptosis of immature red blood cells. RBCs destruction can cause several serious problems due to the accumulation of hemoglobin contents in the body, such as cardiac and hepatic damage due to iron overload,[12] which are the main causes of death in patients with thalassemia.[13] In addition, iron overload can cause several other health problems such as endocrine disorders (hypogonadism) and hypothyroidism.

This study aims to identify the relation between serum ferritin levels and endocrine disorder (especially menstrual cycle) in female patients with beta-thalassemia major.

PATIENT AND METHODS

Fifty Iraqi females with B-thalassemia major (BTM). Patient's samples were collected from the thalassemia center/Children's Teaching Hospital, Kerbala/Iraq, during (July–December 2018). All patients are on blood transfusion (15 mL of RBCs/kg, at 2 to 3 weeks interval) to keep a hemoglobin concentration above 8 g/L before the transfusion process; all patients were on iron chelation therapy. The diagnosis of this disease was based on the hematological findings, i.e., peripheral blood evaluation and hemoglobin electrophoresis.

The age of the patient's groups was ranged from 14–24 years; patient history included demographic data, initiation, duration, and frequency of blood transfusion as well as chelation therapy, age, weight, height, gender, spleen status, blood group, parent's relationship, and the clinical signs and symptoms were collected and documented. All of the fifty female patients studied were on iron chelation therapy (Deferasirox) was given as an oral tablet.

The body mass index was also calculated and classified according to WHO classification as in Table 1.

Patients are classified according to their Amenorrhea status into three groups; primary amenorrhea (PA), secondary amenorrhea (SA), normal menstruation (NM) group as explained in Table 2.

Breast development and pubic and axillary hair were physically examined for each patient included in this study. In addition, Ovaries and uterus size were also observed and calculated by using abdominopelvic ultrasound with a full urinary bladder.

In the same time, hemoglobin concentration, ferritin levels were measured by using venous blood for each patient in addition to estradiol, T3, and T4 were examined by competitive immunoassay, while FSH, LH, and TSH were tested by two sites immuno enzymometric assay.

Table 1: Classification of BMI according to WHO.

BMI (kg/m2)	*Categories*
< 18.5	Underweight
18.5-24.9	Normal weight
25.0-29.9	Overweight
>30	Obese

Table 2: Classification of patients according to menstrual status

	Female Patient groups		
	PA	*SA*	*NM*
Number	28	10	12
Percentage	56%	20%	24%
Age (years) Range	14-24	14-24	14-24

PA: primary amenorrhea, SA: secondary amenorrhea, NM: normal menstruation

Statistical analysis

Data were analyzed by using SAS 2012. The study results were expressed as mean± standard error percentage (%) for the following variables (age, weight, height, hematological tests, serum ferritin levels, and biochemical parameters tests) by Chi-squared. The statistical significance of differences between patient groups were carried out by using (Duncan's Multiple Range) tests. The associations between variables were assessed by using Pearson's correlation coefficient (r). The p-values of difference ≤ 0.001.[14]

RESULTS

The total data and characteristics of 50 Beta-thalassemia major female patients are represented in Table 3. Patients ages with a range of 14–24 years. Thirty-five (70%) of female thalassemia patients found that their parents have Consanguinity, and thirty-four female patients (68%) have a family history. About forty (80%) is regular treatment with chelation therapy. While the frequency of blood groups, (20%) of group A, (22%) of group B, (6%) of group AB and (52%) of group O. The range of Hb is 6 → 11.6 and the mean is 8.750 ± 0.246 (Table 3).

The results of the distribution of BTM female patients indicated in Table 4, the percentage of BMI (36%) underweight, (58%) normal, and (2%) overweight. The prevalence of complications includes splenomegaly were (7.3%), splenectomy (70.7%), and hepatomegaly (22%), while the prevalence of hepatitis C virus was (14.6%), diabetes mellitus in the study (2.4%) and heart failure (4%).

Regarding to the secondary sex characteristics development (breast, pubic and axillary hairs) of BTM female patients (34 and 28%, respectively), which indicate that the females significantly ($p \leq 0.05$) does not have breast development, pubic and axillary hairs comparing with development of the breast, pubic and axillary hair (32 and 72%, respectively), the result visualizes in Table 5.

Table 3: General characterized of Beta-thalassemia major (BTM) female patients.

Characteristics		*N (%)*
Age (years)	Range	14 → 24
Consanguinity	Consanguineous	35/50 (70%)
	Non-consanguineous	15/50 (30%)
Family History	Present	34/50 (68%)
	Absent	16/50 (32%)
Chelation therapy regularity	Regular	40/50 (80%)
	Irregular	10/50 (20%)
Blood groups N (%)	A	10/50 (20%)
	B	11/50 (22%)
	AB	3/50 (6%)
	O	26/50 (52%)
Hb (g/dL)	Range	6 → 11.6
	M ± SD	8.750 ± 0.246

M: mean, SD: standard deviation, N: number

By using ultrasonic imaging, the uterus of ovarian size was examined for each patient and shown in Table 6.

Interestingly, the small sizes of ovaries and uterus were (28 and 36%, respectively) of primary amenorrhea, (2 and 4%, respectively) of secondary amenorrhea and (20 and 4%, respectively) of normal menstruation with remarkable significant values ($p \leq 0.01$) between groups for both parameters (Table 6).

The mean ± SE for FSH, LH, ESR, T3, T4, TSH hormones and ferritin levels are shown in Table 7. The results of LH hormone for the PA group (2.40 ± 0.36) and NM group (4.73 ± 0.75) were significantly lower ($p \leq .0001$) than those in the SA group (7.75 ± 1.90). At the same time, the levels of estradiol hormone for PA and SA groups (22.02 ± 2.83 and 16.8 ± 3.20 respectively) were significantly decreased ($p \leq .001$) when compared with NM groups (141.95 ± 49.75).

By using Pearson's correlation test to evaluate the relationship between the tested parameters in female BTM patients' group with normal menstruation (Table 8). The serum revealed positive significant correlation with serum FSH ($r = 0.84$, $p = \leq 0.01$), and with the serum T3 ($r = 0.84$, $p = \leq 0.01$). Serum FSH showed a significant positive association with serum T3 ($r = 0.72$, $P = \leq 0.01$).

While serum T3 showed a positive significant correlated with serumT4 and serum TSH respectively ($r = 0.76$, $p = \leq 0.05$ and $r = 0.38$, $P = \leq 0.01$ respectively).

Table 4: Distribution of BTM female Patients according to the status of BMI, spleen status, liver status, hepatitis C virus (HCV) infection, heart failure, and Diabetes Miletus.

BMI Status (Kg/m^2)	Underweight (n%)	18/50 (36%)
	Normal (n%)	29/50 (58%)
	Overweight (n, %)	1/50 (2%)
	Normal (n%)	39/50 (10%)
Spleen Status	Splenomegaly (n%)	4/50 (7.3%)
	Splenectomy (n%)	7/50 (70.7%)
Liver Status	Normal (n, %)	48/50 (78%)
	Hepatomegaly (n, %)	2/50 (22%)
Hepatitis HCV infection	Negative (n, %)	37/50 (85.4%)
	Positive (n, %)	13/50 (14.6%)
Diabetes mellitus	Normal (n, %)	45/50 (97.6%)
	Diabetes mellitus	5/50 (2.4%)
Heart failure	Normal (n, %)	48/50 (96%)
	Heart failure	2/50 (4%)

BMI: body mass index, n: number.

Table 5: Secondary sex characteristics development distribution for all BTM female patients.

Secondary characteristics development	*Groups*	*N (%)*	*Chi-square p-value*
Breast	developed	16/50 (32%)	p = 3.75 *
	not developed	34/50 (68%)	
Pubic and axillary hair	developed	36/50 (72%)	p = 3.77 *
	not developed	14/50 (28%)	

*: Significantly different ($p \leq 0.05$), N: number.

Table 6: Frequency distribution of BTM female patients according to the size of ovary and uterus.

	Female patient groups			*Chi-square*
Size	*PA N (%)*	*SA N (%)*	*NM N (%)*	*p-value*
Small ovary	14(50%)	1 (10%)	10(83%)	p = 10.18**
Normal ovary	13(46%)	9 (90%)	3(25%)	
Small uterus	18(64%)	2 (20%)	2(16%)	p = 13.98**
Normal uterus	8(28%)	9 (90%)	11(91%)	

PA=Primary amenorrhea, SA= Secondary amenorrhea, NM= Normal menstruation.
**: highly significantly different ($p \leq 0.01$).

Table 7: The hormonal assay and ferritin level in female BTM Patient groups with menstrual cycle as (mean ± SE).

Groups / *Hormonal tests*	*NM N=12*	*SA N=10*	*PA N=28*	*Chi-square p-value*
FSH (mIU/mL) M ± SE	7.37± 1.41 A	6.48± 0.48 A	5.11 ± 0.59 A	NS
LH (mIU/mL) M ± SE	2.40 ± 0.36 B	7.75 ± 1.90 A	2.40 ± 0.36 B	P = 0.0003
Estradiol (g/dL) M ± SE	141.95 ± 49.75 A	16.8 ± 3.20 B	22.02 ± 2.83 B	P = 0.001
T3 (ng/mL) M ± SE	1.28 ± 0.07 A	1.20 ± 0.07 A	1.23 ± 0.05 A	NS
T4 (ng/mL) M ± SE	70.15 ± 1.57 A	70.06 ± 4.41 A	72.03 ± 3.21 A	NS
TSH (ng/mL) M ± SE	4.25 ± 0.49 A	3.73± 0.06 A	5.85 ± 1.20 A	NS
Ferritin (ng/mL) M ± SE	4011 ± 672.06 A	2416 ± 647.75 A	3202 ± 438.56 A	NS

Results expressed as mean± standard deviation (SE).
**: highly significantly different ($P \leq 0.01$).
NS: Not Significant.
Data with the same letter are not significantly different (NS) level.
Means with the different capital letters are significant difference level.

Table 8: Correlation of biochemical parameters in female BTM Patient groups with normal menstruation.

Parameters	*FSH (mIU/mL)*	*LH (mIU/mL)*	*Estradiol (g/dl)*	*T3 (ng/mL)*	*T4 (ng/mL)*	*TSH (ng/mL)*	*Ferritin (ng/mL)*
FSH (mIU/mL)	r=1.00	r=0.84 P= ≤0.01	r=0.01	r=0.72 P= ≤0.05	r=-0.1894	r=-0.50	r=0.01
LH (mIU/mL)	r=0.84 P= ≤0.01	r=1.00	r=0.57	r=0.84 P= ≤0.01	r=-0.57	r=-0.46	r=-0.06
Estradiol (g/dL)	r=0.01	r=-0.57	r=1.00	r=0.45	r=-0.71	r=-0.40	r=-0.55
T3 (ng/mL)	r=0.72 p= ≤0.05	r=0.84 P= ≤0.01	r=0.45	r=1.00	r=0.76	r=0.84 P= ≤0.01	r=0.37
T4 (ng/mL)	r=-0.57	r=-0.55	r=-0.71	r=0.76 P= ≤0.05	r=1.00	r=0.61	r=-0.12
TSH (ng/mL)	r=0.50	r=-0.46	r=-0.40	r=0.38 P= ≤0.01	r=0.61	r=1.00	r=-0.09
Ferritin (ng/mL)	r=0.01	r=-0.06	r=-0.55	r=0.37	r=-0.12	r=-0.09	r=1.00

*: significantly different (P≤ 0.05).
**: highly significantly different (P ≤ 0.01).

Table 9: Correlation of biochemical parameters in female BTM Patient groups with secondary amenorrhea.

Parameters	*FSH (mIU/mL)*	*LH (mIU/mL)*	*Estradiol (g/dl)*	*T3 (ng/mL)*	*T4 (ng/mL)*	*TSH (ng/mL)*	*Ferritin (ng/mL)*
FSH (mIU/mL)	r=1.00	r=0.28	r=0.44	r=0.56	r=-0.08	r=-0.03	r=0.34
LH (mIU/mL)	r=0.28	r=1.00	r=0.41	r=0.48	r=0.66	r=0.13	r=-0.03
Estradiol (g/dL)	r=0.44	r=0.41	r=1.00	r=0.84 P= ≤0.05	r=0.30	r=-0.30	r=-0.45
T3 (ng/mL)	r=0.56	r=0.48	r=0.25	r=1.00	r=0.15	r=0.68	r=-0.04
T4 (ng/mL)	r=0.08	r=-0.66	r=0.30	r=0.15	r=1.00	r=-0.05	r=-0.76
TSH (ng/mL)	r=0.03	r=-0.13	r=0.30	r=0.68	r=-0.05	r=1.00	r=0.23
Ferritin (ng/mL)	r=-0.34	r=-0.03	r=-0.42	r=-0.04	r=-0.76	r=0.22	r=1.00

*: Significantly different (P ≤ 0.05).

Table 10: Correlation of biochemical parameters in female BTM Patient groups with primary amenorrhea.

Parameters	*FSH (mIU/mL)*	*LH (mIU/mL)*	*Estradiol (g/dl)*	*T3 (mg/dl)*	*T4 (mg/dl)*	*TSH (mg/dl)*	*Ferritin ng/mL*
FSH (mIU/mL)	r=1.00	r=0.86 P= ≤0.01	r=-0.19	r=-0.13	r=0.46	r=-76 P= ≤0.01	r=0.05
LH (mIU/mL)	r=0.86 P= ≤0.01	r=1.00	r=-0.05	r=0.36	r=-34	r=-0.48 P= ≤0.05	r=-0.09
Estradiol (g/dL)	r=0.01	r=-0.19	r=1.00	r=0.30	r=0.02	r=-0.03	r=-0.29
T3 (mg/dL)	r=0.13	r=0.36	r=0.30	r=1.00	r=0.31	r=0.20	r=0.17
T4 (mg/dL)	r=0.46	r=-0.34	r=0.20	r=0.31	r=1.00	r=-0.53 P= ≤0.05	r=-0.10
TSH (mg/dL)	r=0.76 P= ≤0.01	r=-0.48 P= ≤0.05	r=-0.03	r=0.20	r=0.53 P= ≤0.05	r=1.00	r=0.15
Ferritin ng/mL	r=-0.05	r=0.05	r=-0.29	r=0.17	r=-0.10	r=0.15	r=1.00

*: significantly different (p ≤ 0.05).
**: highly significantly different (p ≤ 0.01).

All of the tested parameters for female BTM patient group with secondary amenorrhea illustrated in Table 9 showed a non-significant correlation between them.

In addition, the correlation between biochemical parameters in the female BTM patient group with primary amenorrhea is illustrated by Table 10. However, the data reveals a significant positive association between serum FSH with serum LH (r = 0.86, p = ≤0.01), and negative association with serum TSH (r = 0.76, p = ≤0.01), while serum LH showed significant negative association with serum TSH (r = -0.48, p = ≤0.05). However, serum T4 indicates significant positive correlation with serum TSH (r = 0.53, p = ≤0.05).

DISCUSSION

Thalassemia is considered the most common hereditary disorder globally. The complications of Beta-thalassemia, such as cardiovascular disease and other serious complications resulting from such as congestive heart failure and chronic anemia, usually lead to death for the patients.[15] Blood transfusion and iron-chelation therapy have prolonged and improved the quality of life in patients with this disease, the improvement being mainly due to the decline in mortality by heart failures.[16] Iron deposition on the pituitary gonadotrophic cells followed by disruption of gonadotropin production is the important reason for hypo- gonadotrophic hypogonadism.

Table 3 in the present study showed in the female gender of thalassemia patients' blood group O is the highest percentage in thalassemia patients, followed by B blood group in females. Abid Al-Kader Abbas did a previous study during 2013 in Kirkuk found nearly similar results, his study revealed blood group O+" was the most common group (48.4%) in his patient's sample[17] "O+" was the most common In a previous study done in thalassemia unit in Mumbai, India; patients with blood group type O+ve were more affected than people with other blood groups especially if they have a family history of this disease.

Table 4 shows the complications of the disease or treatment (iron chelation therapy), which could be developed with time.[18-20] Findings of this study show that obesity is not a serious complication, especially for patients older than 10 years of age since they usually recommend underweight properties, which may be because of endocrinopathies like hypogonadism was reported in previous similar studies. In this study, the percentage of patients with heart failure was 4%. Most patients die from systolic dysfunction while other patients develop diastolic disorders. However, using appropriate cardiac medications with regular chelation therapy improve systolic function to a greater extent.[21] In those patients, the index was significantly higher than healthy individuals and increased with systolic left ventricular function worsening.[22] Treatment of iron overload by iron chelators such as deferoxamine can delay the process of cardiomyopathy in BTM patients. Some reports indicate that combined iron chelation therapy can reverse the process of heart failure and improve cardiovascular function in BTM patients.[23] Hepatitis C is transfusion-related infections. Transfusion-acquired HCV remains one of the most important problems among patients with thalassemia.[24] It is known that the hepatitis C infection is most probably related to the thalassemia patient's treatment.[25] Thalassemia patients may acquire hepatitis C from the administered infected blood with HCV. Prevalence of diabetes has been reported (2.4%) in the present study, only one patient has diabetes shown in table 4. the pathophysiology is still unclear, but it may attribute to the accumulation of iron in the pancreatic cells, caused less insulin secretion. Insulin resistance may be resulted by iron deposition in both liver (where iron deposits may interfere with insulin ability to suppress hepatic glucose production) and muscle (where iron deposits may decrease glucose uptake because of muscle damage).[26] Prevalence of splenomegaly has been reported (7.3%). B-thalassemia patients usually develop splenomegaly to reduce chronic anemia and its complications, but this will have more than positive effects on hemoglobin levels since it shows that this process will reduce hemoglobin concentration. Therefore, removing the spleen shows the better result in improving hemoglobin levels and total blood volume.[27]

Prevalence of developed breast has been reported (32%) as shown in Table 5 and the prevalence of developed pubic and axillary hair (72%). Papadim *et al*., who showed that hypogonadism is clinically diagnosed in females by the presence of primary or secondary amenorrhea without development or with development of secondary sexual characteristics. Absence of breast development is suggested to be due to hypogonadism.[28] The absence of pubic and axillary hair finding of this study is agreed with other studies such as Elsedfy *et al*., 2011[29] who showed that adrenal androgen declines with advancing puberty in thalassemia patients might explain the absence of pubic and axillary hair observed in this condition. Additionally, menarche is frequently delayed, breast development is often poor, and patients are frequently oligomenorrheic or amenorrheic, even if menarche occurs.[30] In addition, the reported case study by SH Wong and his colleagues[31] who study the patient case with the same findings. Ultrasonography image was used to investigate and assess the genital tract, including ovaries and uterus of our study's female subjects. Interestingly, findings show that the small size of ovaries and uterus (28 and36%, respectively) of primary amenorrhea, (2 and 4%, respectively) of secondary amenorrhea and (20 and 4%, respectively) of normal menstruation with highly significantly different values between groups for both parameters as shown in Table 6. In addition to iron-overload-induced endocrinopathies, the affected sexual maturation at puberty was found to be attributed to insufficient body fat and defective growth, altered pubertal development, and poor bone health.[32] Compared with control groups, beta-thalassemia patients develop ovarian dysfunction as a result of low estrogen levels or because of hypogonadotropic hypogonadism due to deposition of iron in the ovaries itself, which could lead to pubertal failure.[33,34] Iron overload still the main cause of these complications but the exact mechanisms still not very clear, there are a considerable number of evidences indicate that free radical formation and lipid peroxidation can lead to the damages of mitochondrial, lysosomes, and sarcoplasmic membranes. The presence of iron deposits and oxidative damage by free radicals affects the pituitary and ovarian follicles.[35]

Regarding our results shown in Table 7, the mean ±SE for LH hormone for PA group (2.40 ± 0.36) and NM group (4.73 ± 0.75) were significantly lower ($p \leq .0001$) than those in SA group (7.75 ± 1.90). While the levels of estradiol hormone for PA and SA groups (22.02 ± 2.83 and 16.8 ± 3.20 respectively) were significantly decreased ($p \leq .001$) when compared with NM groups (141.95 ± 49.75). One of the studies on thalassemia patients showed a high prevalence of hypogonadism (69%). They found a low serum level of gonadotropins (FSH and LH) in over 14-year-old patients with impaired puberty, which indicated that hypogonadotropic hypogonadism is responsible for this complication.[36] A study conducted by Soliman et al. in the Netherlands.[37] They concluded that starting the chelation therapy with deferoxamine before the age of 10 can significantly prevent gonadal dysfunction compared with the initiation of chelation therapy after the age of 10 (90% and 10%, respectively)),[38] carried out a study on thalassemia male and female patients of 4–18 years of age, where there was a significant increase in the mean serum levels of iron and Ferritin in thalassemia patients as compared to control groups Similarly,[39] Work also revealed that iron indices were

markedly increased in thalassemic patients, and the mean serum level of Ferritin were also raised as compared to control group.[40] Similarly, in our study, high serum ferritin levels were observed in thalassemic groups as compared to the control groups, which was similar to the results reported by ([41]), suggesting that increased serum ferritin levels are related to short stature and endocrinopathies. Regarding the mean values of LH, FSH, and Estradiol hormones, in this study, there was a highly significant decrease in patients groups (primary and secondary amenorrhea) compared with both control and normal menstruation groups. While, regarding the mean values of GnRH hormone, this study found no significant difference between each group of beta-thalassemia major compared with the control group as well as no significant difference among patients' groups table (8). These results agreed with MR Safarinejad 2010,[42] who reported the lower in basal 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.

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