Role of Ghrelin Hormone in Physical and Sexual Maturation in Children with Beta Thalassemia

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Abstract: Background: Thalassemia is a common genetic blood disorder associated with multiple endocrine disorders due to iron overload secondary to chronic hemolysis and recurrent blood transfusion. Ghrelin hormone regulates a lot of functions including growth hormone secretion, food intake and energy balance. This study aimed to assess serum ghrelin and its role in growth and puberty in children with β thalassemia and its correlation with iron overload. **Patients and Methods:** This cross sectional study was carried out on **60** children with β-thalassemia major including 32 males and 28 females with their age range 11 - 18 years and mean age value of 13.608 ± 1.880 years and **60** healthy children as control group including 28 males and 32 females with their age range 11 - 17 years and mean age value of 13.933 ± 1.780 years. For all patients the following were done: complete clinical evaluation including anthropometric data and Tanner staging, complete blood count, assessment of serum iron status, thyroid function, insulin like growth factor 1 (IGF-1), follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (males), estrogen (females) and ghrelin hormone. **Results:** There were significantly lower weight, Z score of weight, height, Z score of height and body mass index in patients compared with controls. There was delayed puberty in patients compared with controls as evidenced by more Tanner stage I and II in patients versus more Tanner stage IV and V in controls. Serum ghrelin level was significantly higher in patients compared with controls (mean serum ghrelin was 2220.333 ± 1818.194 in patients versus. 1413.733 ± 1040.498 in controls with P value of 0.027). FSH, LH, testosterone, estrogen and IGF-1 levels were significantly lower in patients compared to controls (mean FSH level was 1.44 ± 1.15 mIU/ml in patients versus 5.727 ± 2.349 mIU/ml in controls with p value <0.001, mean LH level was 1.286 ± 1.319 mIU/ml in patients versus 6.47 ± 2.55 mIU /ml in controls with p value <0.001, mean testosterone level was 1.304 ± 0.973 ng/ml in male patients versus 4.664 ± 2.246 ng/ml in controls with p value <0.001, mean estrogen level was 27.361 ± 11.015 pg/ml in female patients versus 213.813 ±87.762 pg/ml in controls with p value <0.001, mean IGF-1 level was 96.378 ± 46.673 ng/ml in patients versus 411.900 ± 131.937 ng/ml in controls with p value <0.001). There were significantly higher serum ferritin and iron and significantly lower TIBC in patients than controls, (mean serum ferritin was 3825.933 ± 1959.174 ng/ml in patients versus 85.367 ± 16.994 ng/ml in controls with p value <0.001, mean serum iron was 490.133 ± 171.445 ug/dl in patients versus 107.333 ± 19.505 ug/dl in controls with p value <0.001, mean serum total iron binding capacity was 117.083 ± 51.541 ug/dl in patients versus 270.533± 16.827 ug/dl in controls with p value <0.001. There were significant negative correlations between serum ghrelin and BMI, FSH, LH, testosterone and estrogen (r = -0.288 and p value of 0.026 for BMI and serum ghrelin, r = -0.302 and p value of 0.019 for FSH and serum ghrelin, r = -0.275 and p value of 0.033 for LH and serum ghrelin, r = -0.380 and p value of 0.032 for testosterone and serum ghrelin and r = -0.478 and p value of p 0.010 for estrogen and serum ghrelin. There were non significant negative correlations between serum ghrelin level and weight, height and serum ferritin of studied patients. **Conclusions**: Significant negative correlations between serum ghrelin level and BMI and sex hormones of studied patients may arouse our attention to the role of ghrelin in physical and sexual maturation in thalassemic patients. **Recommendations**: Extensive multicenter studies on thalassemic children to find the link between delayed puberty, growth retardation and increased serum ghrelin level. Children with thalassemia must undergo regular assessment by a team work of multi-specialties including hematology, endocrinology, nutrition and cardiology.

[Eman M Elaskary, Shebl S Shebl, Adel A Hagag, Mona M Watany. **Role of Ghrelin Hormone in Physical and Sexual Maturation in Children with Beta Thalassemia.** *Nat Sci* 2019;17(9):129-140]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 15. doi:10.7537/marsnsj170919.15.

Keywords: β-Thalassemia, iron overload, endocrine dysfunction, ghrelin hormone

1. Introduction

Thalassemias are heterogeneous group of inherited disorders characterized by reduced or absent synthesis of one or more globin chains of the hemoglobin molecule. (1) The major types of thalassemia are alpha- and beta-thalassemia which are classified according to the nature of defective globin. (2)

Beta thalassemia major is a significant public health problem in Egypt where over 1–5 million newborns are expected to be affected with this disorder and it is considered the most common genetically determined chronic hemolytic anemia

(85.1%) in our locality. The mainstay of treatment is based on adequate, safe blood transfusions and prevention of iron overload. (3)

The human body lacks active mechanism to excrete excess iron, therefore repeated blood transfusions lead to iron overload in these patients.

Excess iron can deposit in body organs particularly in pancreas, liver, pituitary, and the heart. To avoid iron overload, thalassemia patients are treated with iron chelators concomitantly with the blood transfusions. (4)

Pubertal development and fertility are determined by a multi-hormonal effect. A functional defect in any of the components of this hormonal complex directly affects puberty and reproduction in either gender. Ghrelin is a hormone secreted by the gastrointestinal tract, besides its effect on carbohydrate and fat metabolism and appetite, this hormone acts on the hypothalamic-pituitary gonadal axis, exerts various effects on reproductive function, implantation and embryo development. (5)

Ghrelin is the endogenous ligand of the growth hormone (GH) secretagogue receptor and has been implicated in the regulation of a large array of endocrine and non-endocrine functions, including the control of GH secretion, food intake, energy balance and control of adipocity. (5)

Short stature among patients with thalassaemia is a problem in developing countries due to several factors, such as inadequate blood transfusion, iron overload, abnormal growth hormone (GH) secretion, hypothyroidism, zinc deficiency, deferoxamine toxicity, inadequate treatment, and noncompliance of patients. (6)

The exact role of Ghrelin in the growth process of children is unclear. Its effect in control of GH secretion has not yet been clarified. (7)

Aim of the work

The aim of this work was to assess serum ghrelin and its role in growth and puberty in children with β thalassemia and its correlation with iron overload.

2. Patients and Methods

This cross sectional study was carried out after approval from ethical committee of research center of Tanta University and obtaining written consents from the parents of all children included in this study and was conducted on **60** children with transfusion dependent β-thalassemia major including 32 males and 28 females with their age ranged from 11 to 18 years and mean age value of 13.608 ± 1.880 years who attended to Hematology Unit, Pediatric Department, Tanta University Hospital in the period from September 2017 to September 2018. This study included also **60** healthy children as a control group including 28 males and 32 females with their age ranged from 11 to 17 years and mean age value of 13.933 ± 1.780 years.

Inclusion criteria:

Children with transfusion dependent β-Thalassemia major aged 11- 18 years with iron overload.

Exclusion criteria:

Children with other types of chronic hemolytic anemias as sickle thalassemia and sickle cell anemia.

All patients in this study were subjected to the following:

1. Complete history taking with special account on age of diagnosis of thalassemia, frequency of blood transfusion and iron chelation therapy (types and regularity).

2. Thorough clinical examination with special account on pallor, jaundice, mongoloid facies, splenomegaly or splenectomy, hepatomegaly, anthropometric measurements including body weight, height, body mass index (BMI), Tanner staging for assessment of puberty and assessment of testicular volume in male patients with beta thalassemia by orchidometer that consists of a string of twelve numbered wooden or plastic beads of increasing size from about 1 to 25 milliliters. The beads are compared with the testicles of the patients, and the volume is read off the bead which matches most closely in size. Pre-pubertal sizes are 1–3 ml, pubertal sizes are considered 4 ml and up and adult sizes are 12–25 ml. Small testes can indicate either primary or secondary hypogonadism. (8)

3-Laboratory investigations

Specimen collection and preparation:

Ten ml of venous postprandial pre-transfusion blood were collected using sterile needles through gentle venipuncture after sterilization of puncture site by alcohol, 2 ml of the sample was collected on 20 uL EDTA for CBC which was done on Leishman stained blood smear with evaluation using ERMA PCE-210 N cell – counter' (9) and HPLC using Automatic Glycohemoglobin Analyzer ARKRAY ADAMSTM A1c HA-8180T, Japan. (10)

The remaining 8 ml was collected in a plain tube that was allowed for clotting in a water bath at 37ºc. After clotting, centrifugation was done at 1500 x for 10 minutes.

Separated serum was collected in three tubes. The 1st tube for assessment of serum iron, iron binding capacity (11) and serum ferritin using kits for measuring serum ferritin (Ferritin AccuBind™ ELISA test system). (12) The second tube for assessment of serum levels of FSH, LH, testosterone in males, estrogen in females and thyroid function using TOSOH immunoassay system, Japan. (13) The third tube for assessment of IGF-1 and serum ghrelin levels. IGF-1 was analyzed using Human insulin-like growth

factors 1(IGF-1) commercial ELISA Kit Planegg – Germany.

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). (14) Serum ghrelin was measured using Human Ghrelin GHRELIN commercial ELISA Kit Planegg – Germany. The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). (15)

4. Chelation Therapy in studied patients:

Patients were treated with deferasirox in a dose of 20-30 mg/kg/day once daily preferably before meals (16) or desferrioxamine by 20-40 mg/kg/day for 6 days /week but in case of persistently high serum ferritin levels above 3000 ng/ml, deferasirox is combined with desferrioxamine in a dose of 20-40 mg/kg/day for 10 days per month either by subcutaneous infusion using infusion pump in 8-12 hours/day or by continuous intravenous infusion for 8-10 hours/day (combined chelation therapy). (17)

Statistics

Statistical analysis of the present study was conducted, using mean, standard error, student t- test, Chi-square, Linear Correlation Coefficient tests by SPSS V17.

Results

Pallor and jaundice were the most common presenting symptoms in thalassemic patients, while hepatomegaly and splenectomy were the most common signs.

Weight, Z score of weight, height, Z score of height and body mass index were significantly lower in patients compared with controls **(table I)**.

Table (I): Demographic data, anthropometric measurements and Tanner staging in studied groups.

Parameters		Patients (n=60)	Controls (n=60)	t or X2	P-value
Age (Years)	Range	11 - 18	11 - 17	-0.787	0.434
	Mean ±SD	13.608 ± 1.88	13.933 ± 1.78		
Weight (kg)	Range	26 - 61	36 - 68.7	-6.825	<0.001*
	Mean ±SD	37.083 ± 8.884	50.233 ± 8.045		
Z score Weight	Range	-2.7 - -0.4	-1.2 - 0.2	-7.726	<0.001*
	Mean ±SD	-1.458 ± 0.596	-0.540 ± 0.368		
Height (cm)	Range	126 - 163	138 - 176	-7.156	<0.001*
	Mean ±SD	141.175 ± 9.018	155.667 ± 9.136		
Z score Height	Range	-5 - -0.3	-1.2 - 0.7	-8.303	<0.001*
	Mean ±SD	-2.283 ± 1.117	-0.510 ± 0.479		
BMI (kg/m2)	Range	14.3 - 25	16 - 24.9	-3.855	<0.001*
	Mean ±SD	18.418 ± 2.772	20.607 ± 1.979		
Tanner Stage				48.696	<0.001*
Stage I		28 (46.67 %)	0 (0%)		
Stage II		24 (40 %)	4 (13.33%)		
Stage III		3 (5%)	6 (20 %)		
Stage IV		3 (5%)	6 (20 %)		
Stage V		2 (3.33%)	14 (46.67%)		
Testicular Volume/ml		Male Patients (n = 32)	Male Controls (n =28)	-9.598	<0.001*
Range		1 - 12	8 - 25		
Mean ±SD		5.063 ± 3.005	17.071 ± 5.484		

* Significant difference (P < 0.05)

Significantly higher prevalence of delayed menstruation (primary amenorrhea) in female patients and significantly lower testicular volume in male patients compared with controls **(table I)**.

There were microcytic hypochromic anemia with reticulocytosis and significantly higher total leucocytic count and platelet count in patients compared with controls **(table III)**.

There were significantly higher serum ferritin and serum iron and significantly lower TIBC in patients than controls **(table IV)**.

There were non significant differences between patients and controls as regard TSH, Free T3 and Free T4 levels **(table V)**.

FSH, LH, testosterone, estradiol and IGF-1 levels were significantly lower in patients compared to controls **(table V)**.

Serum ghrelin level was significantly higher in patients compared with controls with significant negative correlations between serum ghrelin level and BMI, FSH, LH, testosterone and estradiol levels of studied patients **(tables V, VI) (figures 1,2,3)**.

There were non significant negative correlations between serum ghrelin and weight, height and serum ferritin and non significant positive correlation between serum ghrelin and IGF1 of studied patients **(table VI)**.

There were significant negative correlations between serum ferritin and Z score.

WT, HT, Z score Ht, FSH, estradiol and IGF1 but there were non significant negative correlations between serum ferritin and weight, LH and testosterone of studied patients **(figures 4,5,6)**.

Table (II): Clinical data, chelation therapy and frequency of blood transfusion in studied patients

Parameters	**Patients (n=60)**
Clinical data	
Pallor	60 (100%)
Jaundice	60 (100%)
Hepatomegaly	43 (71.67%)
Splenectomy	43 (71.67%)
Mongoloid Facies	40 (66.67%)
Splenomegaly	17(28.33%)
Types of iron chelators	
SC Desferroxamine (Desferal)	15 (25 %)
Oral Deferasirox (Exjade)	26 (43.33 %)
Combined chelation therapy	19 (31.67 %)
Regularity of iron chelators	
Regular	19 (31.67%)
Irregular	41 (68.33 %)
Frequency of blood transfusion	
Every 2 Weeks	11 (18.33 %)
Every 3 Weeks	19 (31.67 %)
Every 4 Weeks	16 (26.67 %)
Every >4 Weeks	14 (23.33 %)

Table (III): Comparison between patients and controls as regard pre-transfusion complete blood count

		Groups						T-Test	
		Patients (n=60)			Controls (n=60)			T	P-value
Hb (gm/dl)	**Range**	6.5	-	10.1	11	-	13.4	-21.354	<0.001*
	Mean ±SD	7.803	±	0.967	12.087	±	0.735		
RBCS (million /mm^3)	**Range**	2.2	-	3.9	3.6	-	4.8	-17.253	<0.001*
	Mean ±SD	2.707	±	0.399	4.157	±	0.323		
HCT %	**Range**	18	-	30	35	-	44	-21.254	<0.001*
	Mean ±SD	23.247	±	3.076	40.061	±	2.431		
MCV (fL)	**Range**	55	-	77	77	-	90.6	-13.992	<0.001*
	Mean ±SD	68.017	±	5.510	83.957	±	4.123		
MCH (pg)	**Range**	15	-	26	28.2	-	32	-17.397	<0.001*
	Mean ±SD	21.100	±	2.653	29.840	±	0.997		
MCHC%	**Range**	30	-	35	31	-	36	6.32	0.852
	Mean ±SD	30.1	±	4.3	31.3	±	3.1		
Retic%	**Range**	3	-	7	0.4	-	2	17.962	<0.001*
	Mean ±SD	4.892	±	1.208	0.820	±	0.389		
Platelets (thousand mm^3)	**Range**	150	-	620	164	-	420	3.323	0.001*
	Mean ±SD	349.967	±	132.425	263.467	±	73.767		
WBCs (thousand mm^3)	**Range**	4.9	-	19.2	4.3	-	11	11.541	<0.001*
	Mean ±SD	13.095	±	2.855	6.363	±	2.014		

HCT = Hematocrit, MCV= Mean corpuscular volume, MCH= Mean corpuscular hemoglobin, MCHC= Mean corpuscular hemoglobin concentration, WBCs= White blood cells.

Table (IV): Comparison of serum iron status between patients and controls.

		Groups						T-Test	
		Patients(n =60)			Controls (n =60)			T	P-value
Serum Iron (ug/dl)	Range	150	-	750	82	-	143	12.156	<0.001*
	Mean ±SD	490.133	±	171.445	107.333	±	19.505		
Serum Ferritin (ng/ml)	Range	545	-	7638	56	-	120	10.428	<0.001*
	Mean ±SD	3825.933	±	1959.174	85.367	±	16.994		
TIBC (ug/dl)	Range	35	-	225	243	-	301	-15.851	<0.001*
	Mean ±SD	117.083	±	51.541	270.533	±	16.827		

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		Groups						T-Test	
		Patients (n =60)			Controls (n =60)			T	P-value
TSH(µIU/mL)	Range	0.9	-	5.5	1.7	-	5.5	-2.218	0.029*
	Mean ±SD	3.003	±	1.226	3.590	±	1.088		
Free T3 (pg/ml)	Range	2.7	-	5.9	3.2	-	5.6	-1.646	0.103
	Mean ±SD	4.065	±	1.120	4.443	±	0.809		
Free T4 (ng/dl)	Range	0.9	-	1.4	0.9	-	1.4	1.496	0.138
	Mean ±SD	1.158	±	0.172	1.103	±	0.145		
FSH (mIU/ml)	Range	0.1	-	3.8	2.5	-	11.5	-11.657	<0.001*
	Mean ±SD	1.440	±	1.150	5.727	±	2.349		
LH (mIU/ml)	Range	0.1	-	5.2	2.5	-	12.5	-12.745	<0.001*
	Mean ±SD	1.286	±	1.319	6.470	±	2.550		
Testosterone (ng/ml)	Range	0.02	-	3.4	0.7	-	8.1	-7.139	<0.001*
	Mean ±SD	1.304	±	0.973	4.664	±	2.246		
Estradiol (pg/ml)	Range	11.5	-	54	88	-	380	-11.186	<0.001*
	Mean ±SD	27.361	±	11.015	213.813	±	87.762		
IGF-1(ng/ml)	Range	25.3	-	300	183	-	612	-16.633	<0.001*
	Mean ±SD	96.378	±	46.673	411.900	±	131.937		
Serum Ghrelin (pg/ml)	Range	578	-	9932	100	-	5075	2.249	0.027*
	Mean ±SD	2220.333	±	1818.194	1413.733	±	1040.498		

TSH = Thyroid stimulating hormone, T3 = Triiodothyronine, T4= Thyroxine, FSH= Follicle stimulating hormone, LH = Luteinizing hormone, IGF-1= Insulin like growth factor 1

Table (VI): Correlations between serum ghrelin and anthropometric measurements, serum ferritin, FSH, LH, testosterone, estradiol and IGF1 of studied patients.

Correlations		
	Serum Ghrelin (pg/ml)	
	r	P-value
Weight (kg)	**-0.135**	**0.303**
Height (cm)	**-0.058**	**0.662**
BMI (Kg/m2)	**-0.288**	**0.026***
Serum Ferritin (ng/ml)	**-0.050**	**0.703**
FSH (mIU/ml)	**-0.302**	**0.019***
LH (mIU/ml)	**-0.275**	**0.033***
Testosterone (ng/ml)	**-0.380**	**0.032***
Estradiol (pg/ml)	**-0.478**	**0.010***
IGF-1 (ng/ml)	**0.033**	**0.800**

BMI = body mass index, FSH= Follicle stimulating hormone, LH = Luteinizing hormone, IGF- 1= Insulin like growth factor 1.

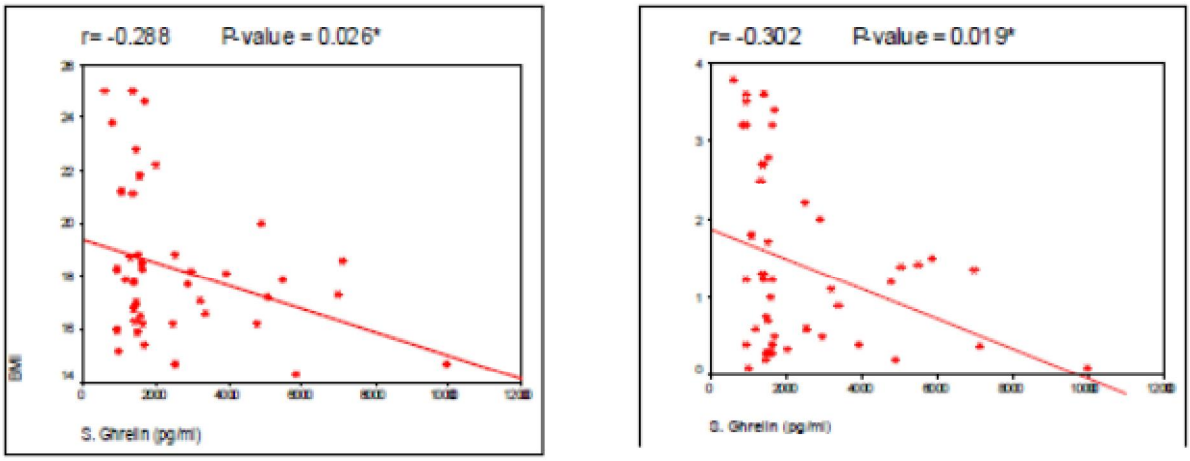

Figure (1): Correlation between serum ghrelin and BMI (left) and between serum ghrelin and FSH levels of studied patients (right).

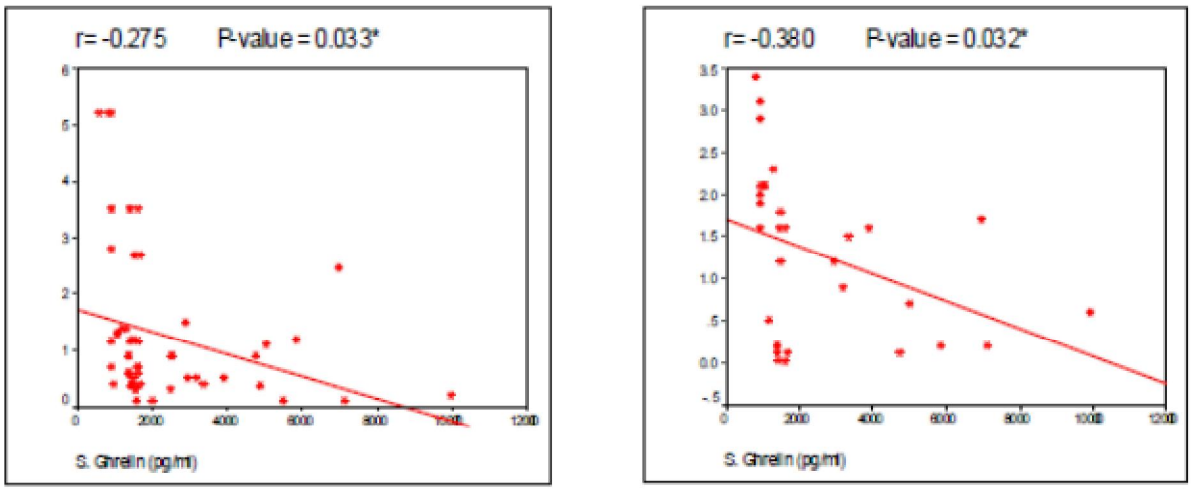

Figure (2): Correlation between serum ghrelin and LH levels (left) and between serum ghrelin and testosterone of studied patients (right)**.**

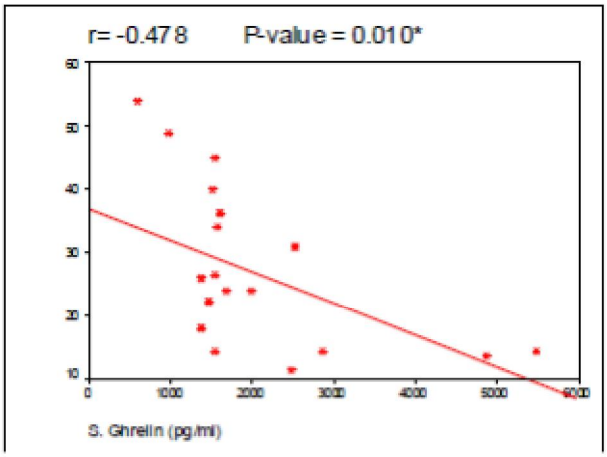

Figure (3): Correlation between serum ghrelin and estradiol of studied patients.

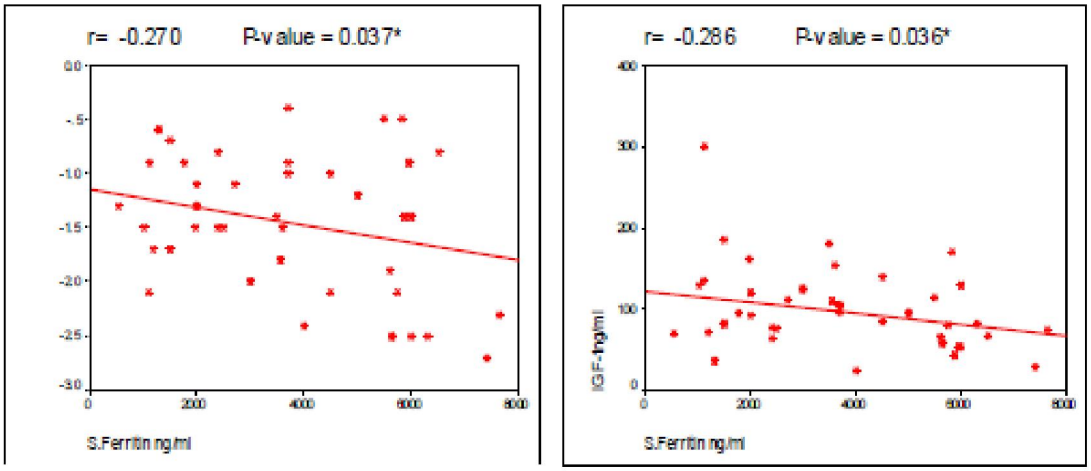

Figure (4): Correlation between serum ferritin and Z score of weight (left) and between serum ferritin and IGF1 of studied patients (right).

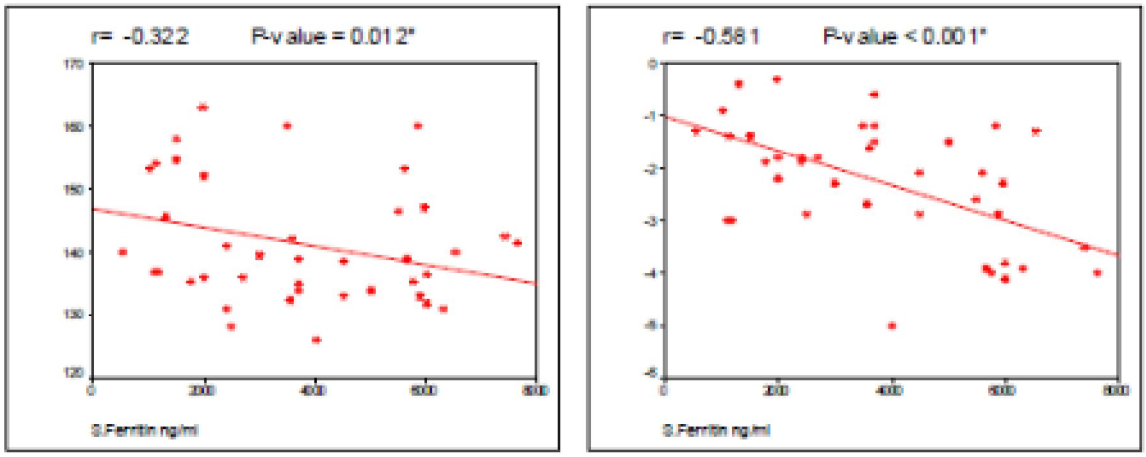

Figure (5): Correlation between serum ferritin and height (left) and between serum ferritin and Z score of height of studied patients (right).

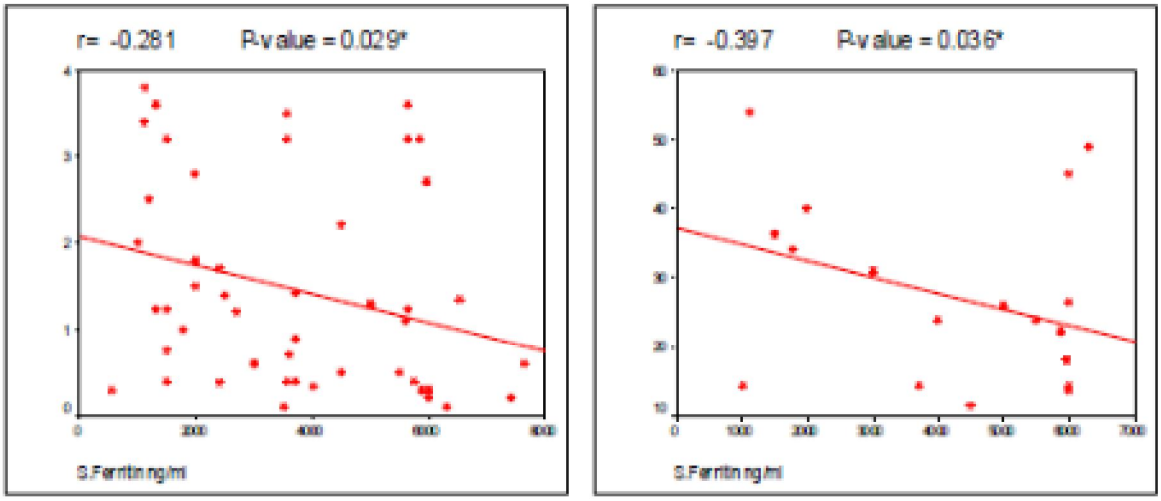

Figure (6): Correlation between serum ferritin and FSH (left) and between serum ferritin and estradiol of studied patients (right).

4. Discussion

In the current study, there were significantly lower anthropometric measurements including weight, Z score of weight, height, Z score of height and BMI in patients compared with control group. These results come in agreement with **Pemde et al 2011** (18) who reported that 13.37 % of their patients were underweight (weight-for-age *z*-scores < -2), one-third of patients had short stature (height-for-age *z*-scores < -2) and one-quarter of patients were undernourished, **Fahim et al 2013** (19) who found significantly lower mean body weight and height of patients than controls, short stature in 49% of patients and abnormal BMI in 43% of patients (BMI <18.5) and **Al-Naama et al 2016** (6) who found significantly lower BMI, BMI *Z*-score, height, and weight in patients than controls. Growth retardation in thalassemic children could be attributed to chronic anemia caused by inadequate transfusion, hypoxia, and other endocrine disorders which occur due to iron overload causing failure of puberty and consequent growth retardation **(Dumaidi et al., 2015)** (20), folate and zinc deficiencies **(Pemde et al., 2011)**. (18)

As regard to BMI, our results are in agreement with **Eissa and El-Gamal 2014** (21) who found significantly lower BMI in patients compared with controls which was explained by chronic nature of the disease. However, **Mohey-El-deen et al 2014** (22) disagree with our study as they found no significant difference between patients and controls as regard BMI. As physical growth is affected in transfusion-dependent thalassemic patients so minimizing the iron overload in these patients should be warranted for them to have normal growth and development.

In the current study, there was delayed puberty according to tanner staging in thalassemic patients compared with control group. This is in agreement with **El Beshlawy et al 2008** (23) who found failure of puberty in 71.4% of boys and 33.3% of girls and arrested puberty in 28.6% of boys and 66.7% of girls. All females included in their study had amenorrhea either primary in 88.9% or secondary in 11.1%, **Al-Naama et al 2016** (6) who found delayed puberty in 63% of male patients ≥ 14 years of age and 54.5% of female patients ≥ 13 years of age. In contrast, all individuals in the control group exhibited normal pubertal development and **Dhouib et al 2018** (24) who found delayed puberty in 42% of studied patients.

Hypogonadotropic hypogonadism is largely explained by the toxic effect of iron overload secondary to chronic blood transfusions because the human body lacks a mechanism to excrete excess iron **(Ouederni et al., 2017)**. (25)

In the current study there was significantly higher prevalence of amenorrhea (78.5%) in thalassemic female patients compared with controls. Our study was in agreement with **Merchant et al 2011** (26) who reported primary amenorrhea in 87.5% of βTM girls and **Sutay et al 2017** (27) who found no cases and 26.3% of controls had attained menarche in the age group of 8-12 years whereas 11.4% of cases and 93.3% of controls in the age group of >12 years had attained menarche.

In this study there was significantly lower testicular volume in thalassemic male patients compared with controls. This is in agreement with **Soliman et al 1999** (28) who found that only 27% of male patients had testicular development (volume > 3ml) also they found that males who had spontaneous testicular development had significantly smaller testicular volume than normal controls.

In our study, there were significantly higher serum ferritin and serum iron and significantly lower serum total iron binding capacity in studied patients compared with controls which could be explained by frequent packed RBCs transfusion and irregular iron chelators intake. This is in agreement with **Eissa and El-Gamal 2014** (21)**, Faruqi et al 2015** (29) and **Hagag et al 2016** (30) who found the same results.

Iron overload in beta thalassemia could be explained by two main mechanisms, increased iron absorption due to ineffective erythropoiesis and repeated blood transfusion **(Bhagat et al., 2012)**. (31)

In the present study there was significantly higher serum ghrelin level in patients compared with control group with significant negative correlations between serum ghrelin and BMI, FSH, LH, testosterone in studied male patients and estradiol levels in studied female patients**.**

Our results come in agreement with **Karamifar et al 2010** (32) who found significantly higher serum ghrelin in βTM than controls, in thalassaemia intermedia serum ghrelin level was not significantly different from βTM or control group. However, in contrast to our study, no significant correlation was found between ghrelin serum level and BMI.

Karamifar et al 2010 (32) considered the higher serum ghrelin level in the TM group as a compensatory response to growth retardation or partial resistance to ghrelin that leads to its increased level. The results of their study did not show any relation between ghrelin serum concentration and short stature, which is consistent with our results.

In contrast to our study, **Moshtaghi-Kashanian and Razavi 2009** (5) and **Majeed, 2017** (33) who found significantly lower ghrelin level in patients compared to healthy controls and they concluded that the lower value of ghrelin in patients with β-thalassemia may constitute another hormonal imbalance which may contribute to impaired growth and sexual maturation encountered in these patients.

In the current study there was significantly lower IGF-1 level in patients compared to control group.

Our results come in agreement with **Vogiatzi et al 2009** (34) who reported that 71% of all thalassemic patients had IGF-1 concentrations below normal and **Merchant et al 2011**(26) who reported that 51.43% of their thalassemic patients had low IGF-1 for age.

Many factors contribute to decreased level of IGF□1 including: GH deficiency, neurosecretory dysfunction of GH and partial resistance to GH (hepatic siderosis and bone), and/or IGF□1 resistance, delayed and/or failure of puberty due to hypogonadism with lack of stimulatory actions of sex steroids on pituitary release of GH and hepatic release of IGF□1 and attenuation of pubertal growth spurt, under□nutrition due to hyper□metabolism with a degree of caloric deficiency (macronutrient deficiency) or micronutrient deficiency (vitamin D, zinc), insufficient blood transfusion with significant periods of anemia and inadequate iron chelation with iron overload of the pituitary gland (GH, LH, FSH, TSH deficiencies), liver (systemic IGF□1 deficiency) and growth plate (local IGF□1 deficiency) and the co□occurrence of other endocrine disorders such as hypothyroidism and diabetes mellitus **(Soliman et al., 2015)**. (35) In the current study there were significantly lower levels of FSH, LH, testosterone in male patients and estradiol in female patients compared to controls.

Our results come in agreement with **Mula-Abed et al 2008** (36) who reported low levels of FSH, LH, estradiol (in females) and testosterone (in males) in 50 % of patients, **Merchant et al 2011** (26) who found low FSH in 14.29% of patients, low LH in 2.86% of patients, low estradiol in 43.75% of girls and low free testosterone levels in 89.47% of the boys, **Siripunthana et al 2015** (37) who reported significantly lower serum testosterone in patients compared to control group and **Sutay et al 2017** (27) who found significantly lower FSH, LH and estradiol in their female patients compared to controls.

Our results also come in agreement with **Perera et al 2010** (38) who found low level of FSH and LH in 24 % of patients and low level of testosterone and estradiol in 55% of their patients. They found that early transfusion with regular chelation therapy maintain normal gonadal functions and gonadal hormones secretion, while irregular chelation therapy leads to iron overload and impairment of gonadal hormones secretion due to iron deposition in secretory gonadotrophin cells of pituitary gland or secretory cells of gonads resulting in primary gonadal failure or hypogonadotropic hypogonadism.

It has been suggested that low circulating levels of LH and FSH among thalassemic patients is the result of impaired GnRH secretion resulting in inadequate pituitary stimulation **(Valenti et al., 1995)**. (39)

Hypogonadotrophic hypogonadism could be explained also by iron toxicity on adipose tissue, with resulting impaired synthesis of leptin and decreased its physiological role in sexual maturation than in normal children in whom leptin levels increase dramatically in early puberty and stimulate the hypothalamic-pituitary gonadal axis **(Skordis et al., 2006)**. (40) Other possible causes of hypogonadotrophic hypogonadism may include liver disorders, chronic hypoxia, diabetes mellitus and zinc deficiency **(Al-Rimawi et al., 2005)**. (41)

In contrast to our results **Siripunthana et al 2015** (37) found no significant difference in serum LH and FSH between pubertal patients and controls

suggesting intact hypothalamic– pituitary–gonadal axis. This suggests impaired Leydig cell function in β-thalassemia, although puberty is normal. They attributed their results to adequate treatment with blood transfusion at an appropriate time, effective iron chelation and relatively younger patients in their study compared with other studies, **De Sanctis et al 2008** (42) found no significant difference in FSH, LH and testosterone between thalassemia patients and control group and **Al-Hakeim et al 2011** (43) found no significant difference in FSH and testosterone between thalassemia patients and control group which could be explained by younger age of studied patients in their study compared with other studies and definite disturbances in serum hormone levels occur in thalassemia patients at the time of puberty due to changes in the iron indices.

In the current study no significant differences were found between patients and control group as regard TSH, free T3 and free T4. This is in agreement with **Al- Hakeim et al 2011** (43) who found no significant differences between healthy subjects and thalassemia patients as regard free T3, free T4, and TSH and concluded that the effect of iron overload is dependent upon the duration of the disease and the frequency of blood transfusion and that the disease progression is slow. The age of the patients in their study was relatively low and, therefore, the number of blood transfusions was also low. Many researches found that definite disturbances in serum hormone levels occur in thalassemia patients at the time of puberty due to changes in the iron indices (44) and **Mula-Abed et al 2008** (36) found primary hypothyroidism in only 3.3% of their patients and **Sharma et al 2017** (45) found hypothyroidism in only 4.7 % of patients with thalassemia.

The thyroid pituitary axis seems to be less sensitive to iron deposition than gonadal and GH axis. Hence, secondary hypothyroidism is rare in thalassaemic patients.

Our results come in contrast with **Ghosh et al 2008** (46) who reported subclinical hypothyroidism in 23.52% of patients, **Merchant et al 2011** (26) who found subclinical hypothyroidism in 20% of their patients, as indicated by high TSH and normal T3 and T4 levels and **Drema et al 2017** (47) who found subclinical hypothyroidism in 24% of patients and overt hypothyroidism in 2% of patients and explained the high prevalence of hypothyroidism in Indian patients with thalassemia by suboptimal chelation due to high cost of iron chelation therapy and poor compliance.

Iron deposition in the thyroids with subsequent thyroid dysfunction occurs with varying frequency depending on the region, quality of management and treatment protocols. (47)

Conclusions:

There were significant negative correlations between serum ghrelin level and BMI and sex hormones of studied patients which may arouse our attention to the role of ghrelin in physical and sexual maturation in thalassemic patients.

Recommendations:

Strict follow up of serum iron status for early detection of iron overload. Extensive multicenter studies on thalassemic children to find the link between delayed puberty, growth retardation and increased serum ghrelin level. Children with thalassemia must undergo regular assessment by a team work of multi-specialties including hematology, endocrinology, nutrition and cardiology.

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7/24/2019