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Evaluation of some Biochemical and Endocrine Profiles in transfusion-dependent Iraqi major β - thalassemia patients

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Abstract

Endocrine Abnormalities in β-thalassemia major are common disturbing complications, that need prompt management. Importance of this work was to evaluate the some biochemical parameters and endocrine hormones related to the pubertal maturity and fertility status in cases with thalassemia. A sixty patients [38 males and 22 females] with β - thalassemia major against 30 healthy subjects [17 males and 13 females] were enrolle . The Blood levels of, Leptin, Vitamin D, thyroid function test, parathyroid hormone, ghrelin, and sex hormones were determined in the blood. Body Mass Index (BMI) was also evaluate. Results showed that besides lower BMI, all hormones assessed were significantly lower in thalassemia β-major cases compared to healthy ($p<0.001$). Furthermore, the ghrelin/ Leptin ratio in female cases was lower than the values obtained in the controls ($p<0.001$) .Finally, significant negative correlations, ($p<0.05$) were detected between circulating levels of ghrelin and Follicle stimulating hormone (FSH), Luteinizing (LH) in male and female . The lower values of ghrelin and Leptin in thalassemia β-major possibly constitute another hormonal imbalance which may cntribute to the obstruction of growth and sexual maturation encountered in patients group. Hypothyroidism occurs in proportion of β-major thalassemia patients in the absence of clinical signs of hypothyroidism. Regular follow-up, for early detection and timely treatment of such complications could improve, the type of life of those patients.

Keywords: Thalassemia, Thyroid function, Vitamin D, Leptin, ghrelin.

تقييم بعض المعاملات الهرمونية والكيميائية في مرضى الثلاسيميا المعتمد على نقل الدم في العراق

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الخلاصة

الشذوذ الهرموني في بيتا ثلاسيميا الرئيسي هي تعقيدات شائعة تحتاج الى عناية .الهدف من هذه الدراسة هو تقييم بعض االمؤشرات الكيميائية والهرمونية المتعلقة في البلوغ والخصوبة في مرضى الثالاسيميا .سجلت هذه الدراسة، على مجموعة مرضى بيتا ثلاسيميا مكونة من 38 ذكور و اناث 22ومجموعة سيطرة مكونة من30 شخص بصحة جيدة 17 ذكور و13 اناث .حدد فيتامين د، لبتين، كيهرلين، اختبار وظيفة الغدة الدرقية، هرمونات الباراثايرويد والهرمونات الجنسية ،واظهرت النتائج انخفاض معنوي في مستويات الهرمونات لمجموعة الثلاسيميا ($p<0.001$). .وجد مستوى الكهرلين في المرضى الاناث اقل من القيمه المتحصلة من الاصحاء .القيمة المنخفضة من اللبتين ،LH, FSH و الكهرلين اظهرت انخفاض معنوي

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($p<0.05$) في مرضى الثلاسيميا .عدم التوازن الهرموني هذا يؤدي الى اعاقة النمو والبلوغ الجنسي في هؤلاء المرضى.قصور الغدة الدرقية يحدث في نسبة معنوية من مرضى الثلاسيميا. المتابعة المنظمة للتحري المبكر وعلاج بعض التعقيدات يمكن ان تحسن نوعية حياة هؤلاء المرضى.

Introduction

Beta-thalassemia represents a group of recessively inherited hemoglobin disorders detected by reduced synthesis of β-globin chain and it considered the most common genetic disorder worldwide[1] .The major type results in severe anemia , which needs regular blood transfusion . The combination of transfusion and chelation therapy has extended the life of thalassemia cases for fifth decades of life[2, 3]. Frequent transfusion of blood in turn can lead to iron overload which may result in hypogonadism , hypothyroidism, hypoparpthyroidism and other endocrine abnormalities.

The high morbidity and mortality in patients of thalassemia is the consequence of iron overload [4].The blood transfusion and chelation therapy has dramatically prolonged the life expectancy of these cases [5]. thus transforming thalassemia ß-major from a fatal disease of childhood rapidly to a chronic disease compatible with a prolonged life, On the other hand, iron overload, frequent blood transfusions, poor compliance to chronicity and therapy of the disease have in turn participitate to a whole spectrum of complications including hypogonadism, cardiac problems, hypothyroidism and diabetes mellitus, hypoparpthyroidism metabolic problems and other endocrine [6]. Primary hypothyroidism that may affect thalassemia patients is major lead to gland infiltration by iron overload. Central hypothyroidism caused by low secretion of Thyrotropin Stimulating Hormone (TSH) from the anterior pituitary gland, or by decreased secretion of Thyrotropin-Releasing Hormone (TRH) from the hypothalamus is less common[7]**.**

Pubertal fertility and development are assessed by a multi hormonal effect.,a functional damage in any of the components of this hormone directly affects reproduction in either gender. Previous research ,added two new hormone, ghrelin and Leptin [8-11], hormones secreted by gastrointestinal tract and adipose tissue. Besides their effect on fat metabolism and carbohydrate and appetite, these hormones acting on the hypothalamic pituitarygonadal axis, different effects on reproductive function, embryo, implantation, development, and clinically relevant conditions [12].

Leptin is produced by adipocyte cells and there is relationship between it and the feeding state in different animals. Stimulation of reproductive neuro-endocrine output is also correlated with increasing circulating levels of leptin[13].This hormone mainly acts on the hypothalamus [14], and its lack in the mice results in persistent immaturity as a result pituitary of hypothalamus . Malfunction many research have been published on leptin concentrations in different age of thalassemia patients and in all of them decreased level of leptin concentrations were observed [15-17], with different conclusions being reached depending on the study design and the cases cohort, ghrelin is the endogenous ligand of the growth hormone GH and has been implicated in the regulation of a wide range of endocrine and non-endocrine functions, including the control of GH secretion, energy balance ,food intake, and control of adipocity [18, 19]. Despite the link between fertility and,energy homeostasis . ghrelin has central role in the control of gonadal function in thalassemia patients has not been evaluated.. The risk of vitamin D deficiency in thalassemia increase with older and age [20]. patients with thalassemia have significantly worse vitamin D status, compared with age-matched healthy control. One-third of healthy adults consuming vitamin D fortified milk and multivitamin supplementation remain vitamin D-deficient [21]. Similarly, despite primarly awareness and routine prescription of daily vitamin D, deficiency of vitamin D in thalassemia remains difficult[22]. The alternative to daily supplementation is intermittent supervised therapy with high-dose vitamin D. Oral therapy is desirable to maintain long term acceptance of the therapy. In this content the aim of this study is to investigate the interconnections between ghrelin and Leptin and reproductive hormones at the various developmental stages in thalassemia patients.

Materials and methods

Sixty Iraqi patients(22 females and 38 males) with β-thalassemia major were involved in this study,with age range between (13-19) years old. These patients were registered as β-thalassemic major patients in "Thalassemia Unit" at (AL-Ibn Al-Ballade) Teaching Hospital" in Baghdad city, (Iraq). The diagnosis was distinguash by clinical symptoms, hematological test. All patients were on blood transfusion as a part of their treatment and DFO used chelating agent. A structural questioner

was made and include name, sex, age, region, religion, age at the time of diagnosis of thalassemia ß-major, total units of blood transfusions, whether the cases on iron chelators, if yes , duration of therapy. Hormonal therapy with Patients or those terminally ill, were excluded. Patient formed the study group were enrolled with 15-20 sex matched and age patients of non hematological and non-endocrinal were taken as control. Two cases and controls were assessed for serum thyroid hormones ,triiodothyronine (T3) ,thyroxine (T4), thyroid stimulating hormone(TSH),ferritin, iron, calcium, inorganic phosphate, intact Parathyroid hormone, vitamin D3, Leptin, gherlin and reproductive LH, FSH,Prolactin, Estradiol and testosterone, patients depend on thyroid profile thalassemia ß-major cases were divided to compensated hypothyroid, euthyroid, uncompensated hypothyroid and Blood samples were collected from individuals at the morning in test tubes and the serum separated by centrifugation after clotting. Some hormone and biochemical parameters were estimated as in the table.

Test	Company	Origin
Gonadotropins, LH,FSH	Monobind industry	USA
Estradiol, testosterone, prolactin	IBL	germany
Ieptin	DBC	canada
Ghrelin	Biovendor labo	germany
Vitamin D3	ichroma	korea

For all hormones, the standard protocols achieved by manufactures were followed

Statistical analysis: The data were analyzed using SPSS program (Version 17.0). T-test independent sample was used for compare between patients and control group. For various comparisons of circulating hormones of the various groups, one-way ANOVA was used Determination of correlations was applied using Pearson's two-tailed bivariate model-values less than 0.05 were considered as significantly differences.

Results and Discussion

The mean age (M±SD) of patients group was 15.92 ± 2.13 years and they received packed cell(PC) transfusions at an average of 13.42 ± 2.9 days as showed in Table- 1. Out of 60 cases, 26 were on chelation therapy and 14 were on no chelation. Five cases were taking desferrioxamine infusion, 8 were on deferiprone and 7 patient were on combined chelation therapy.

Table 1-Anthropometric and Physical examination in patients group

Parameter	Mean± SD	Min	Max	95%CI
Weight (kg)	55.52± 7.20	36.2	66	48.48-52.53
Height (cm)	152±8.13	139.2	174	152.56- 163.21
BMI (kg/m^2)	22.19±2.54	16.97	23.45	20.44-22.35
Transfusion interval (day)	13.42±2.9	10.45	14.25	13.59-18.90
Duration of transfusion (year)	13.12±4.71	10.4	16.3	16.34-18.62

Among patients group the mean serum T3 (1.18.32 ± 2.43 ng/ml) while the normal level was (1.36 ± 2.57 ng/ml). The mean of serum T4 in patients group (7.39 ± 1.56 µg/dl) and significantly lower ($p<0.001$) than that of control group (9.26 ± 2.28 µg/d).The mean serum thyroid stimulating hormone level was significantly higher ($p<0.01$) in patients group (4.59 ±1.44 mIU/L) compared with control group (2.42 ± 1.34 mIU/L).Among thalassemia patients 75% were euthyroid (n=45), 15% were compensated hypothyroid (n=9),10% were uncompensated, hypothyroid (n=6) and none was overt hyperthyroid or hypothyroid. While comparition the biochemical profile of euthyroid thalassemia (n=45) to hypothyroid thalassemic (n=15), it was found that the level of serum ferritin of hypothyroid cases (3115.46 ± 1121.21 ng/L) was comparable to that of euthyroid patient (3195.68 ± 1172.14 ng/L). It was also noticed there was non- significant correlation between serum thyroid

stimulating hormone level and iron overload, transfusion frequency among euthyroid and hypothyroid thalassemic patients(P>0.05) .

Table 2- Some biochemical and endocrine parameters for male and female β- major thalassemia and healthy subjects . (Data presented as Mean±SD)

Parameter	Male patients	Male control	Female patients	Female control
BMI (Kg/m^2)	22.19±2.14*	25.12±2.54	22.34±2.31*	24.19±2.33
Iron (ug/dl)	183± 23*	87± 11	119± 19*	55± 17
Ferritin (ng/ml)	3196±1541***	122.19±25.4	3212.19±1881***	121±11.54
Ca^{++}(mg/dl)	8.0 ± 1.95*	10.23± 1.73	7.9± 1.75*	9.8± 1.83
Phosphate(mg/dl)	4.6± 0.95*	3.5± 0.78	4.4± 0.91*	3.8± 0.65
Leptin (ng/ml)	2.40 ± 2.28*	4.30 ±1.99	4.39 ± 1.95*	7.30 ± 1.60
Ghrelin (pg/ml)	61.78 ±22.37*	87.91 ±42.44	80.73 ±29.10**	142.68 ±43.25
PTH (ng/ml)	44.39 ± 1.95*	54.39 ± 1.95	41.77 ± 1.87*	55.39 ± 2.78
LH (IU/L))	2 .29±2.54*	4.15 ±2.54	4.27±2.73*	8.94±1.87
FSH (IU/L)	2.40± 2.11*	7.43±2.96	3.51 ± 2.62*	8.47±2.91
Vitamin D3(ng/ml)	18.1±3.1	33±4.2	18.1±3.12*	26.1±3.55
Prolactin (ng/ml)	4.39±1.47	4.30±1.82	4.22±1.55*	4.19±1.67
Estradiol (nmol/L)	68.30±10.12	107.79±13.72 *	62.93 ±17.14*	101±17.21
Testosterone(µg/ml)	2.61±1.12	4.11±1.19	0.41±0.12*	0.61.1±1.11

P>0.05 : Not significance ,P<0.05 significant* ,P<0.01 significant**,p <0.001 high significant***

It was reported in many studies [23, 24] that thyroid damage occur. In this study clinical hypothyroidism was observed in 40% of thalassemia patients in this study. Although it was no correlation found between level of thyroid dysfunction and age and amount of blood transfusion . Similar results have been documented by(Jain et al), who observed that the level of thyroid dysfunction was not related to sex, age and hemoglobin levels, but observed high transfused iron load (units/kg/year) in patients with hypothyroid function. the difference was not statistically significant[25]. This show study that the level of thyroid dysfunction have no correlation with serum ferritin level [26].Similar observations have been found by others [27-29]. It was noticed a significant

hypothyroidism among thalassemia patients. In this study, it is important to monitor function of thyroid in these cases and institute prompt therapy when indicated.

The Mean of circulating follicle stimulating hormone (FSH) levels in healthy male control group where higher than patients group, while circulating levels of LH in male patients and control groups were comparable. Both FSH levels of male and female patients were significant lower ($p<0.001$) than the corresponding values in the healthy. Mean values of T4 and T3 in male and female patients were also significant lower ($P<0.001$) than the corresponding values obtained in the male and female controls as showed in Table- 2.

The values of Estradiol , steroid hormones and testosterone in patients group were also significant lower than the corresponding values in the control group ($p<0.01$). The values of ghrelin and Leptin were significant lower than the values obtained in the corresponding control group ($p<0.01$).To determine correlations between ghrelin or Leptin and each of hormones, Pearson's two-tailed bivariate correlation analysis was achieved for each group. The results showed distinct and significant ($p<0.05$) negative correlations between ghrelin and (LH) ($r= -0.42$),(FSH) ($r= -0.37$),Estradiol ($r= -0.31$) testosterone ($r= - 0.27$) for the male patients, while these correlations were observed in the other three categories . The ratio of Leptin/ ghrelin values were lower ($p<0.01$) in the female patients compared with the control females in control group(Table 2). Results also demonstrated no relationship between all parameters of reproductive hormone versus iron and ferritin levels in thalassemia patients except a positive correlation in ratio of LH/FSH with iron and ferritin levels in thalassemia patients, in addition a positive relation between iron and ferritin levels in thalassemia patients was detected.

Deficiency of Vitamin D was observed in 60 patients with β-major thalassemia while Vitamin D insufficiency was observed in 15 patient, data in this study demonstrate that the problem of inadequate vitamin D status in beta major thalassemia has persisted in this study. There was significant association between deficiency of vitamin D with hypothyroidism, observed deficiency of vitamin D may start early in thalassemia major even before hypoparpthyroidism is detected. deficiency of vitamin D lead to low bone mass in thalassemia. Thalassemia patients progressively develop iron overload [30]. it is possible in liver that a deficiency hydroxylation of vitamin D or in vitamin D absorption appears in older thalassemia patients .

With multiple transfusions, increasing of plasma iron can cause development tissue damage in the, endocrine glands, liver and other organs by generating free radicals, and causes oxidative stress state [31]. The body of human a weaked capacity to control iron overload, transfusion therapy is frequently associated with Iron overload in cases with thalassemia β- major .Iron accumulation in various tissues may leads to severe complications, including, hypogonadism, hypothyroidism, and hypoparpthyroidism [32]. In other studies showed a high prevalence of endocrine abberation in β-thalassemia cases[27]. The just method of iron overload control in transfusion dependent of Iron therapy of chelation in cases. Despite the use of therapy with iron chelation , peripheral endocrine tissues ,the pituitary gland, and gonad axis are susceptible to damage and iron deposition. Growth lately and puberty with decreases of final height happens frequently in this population. Sexual complexes such as delayed or arrested puberty and hypogonadism are the most endocrine complications. Stop progress puberty is defined as decline in sexual activity and azzoospermia in males and secondary amenorrhea in females . Iron sedimentation on the pituitary gonadotrophic cells which is followed by demolitionof gonadotrophin production is the large causes of hypogonadotrophic hypogonadism. Secondary hypogonadism becomes event later in life [33].

It has been recommended that decreasing circulating levels of FSH and LH among thalassemic cases is the result of reduced GnRH secretion result from inadequate pituitary stimulation [2, 34].This could be the cause for the detection of decreased levels of gonadotropins resulting in low circulating levels of gonadal steroids in these cases .Lately the results published from animal studies showed that both ghrelin and leptin have a role in gonadotropin releasing hormone(GnRH) production at different social reproductive stages [11], leptin and ghrelin showed disagree effects on pulsatile GnRH secretion[35]. Furthermore , plump children have early maturity that could be the effect of high levels of leptin. decreased levels of leptin could be one of the causes for delayed puberty among cases with β- major thalassemia, particularly among the male cases who have lower leptin levels than female cases. Expression of ghrelin has been demonstrated in mature Leydig cells of human and rat testis, as well as in steroidogenically active luteal and interstitialhilus cells

of the ovary [36]. Gonadal expression of ghrelin receptors was also observed in sertoli and Leydig cells of the testis and in follicular, interstitial hilus cells and luteal surface epithelial of the ovary., while Lebrethon (2003), reported that ghrelin decreased GnRH in the pre-pubertal period in male rats and in mature rats has no effect [11]. Fernandez -Fernandez et al ,(2005) explain the role of ghrelin on sexual puberty , by inhibition of LH secretion by ghrelin in vivo in the pre-pubertal males as well as gonadectomized male and female rats [36].whereas FSH remained unaffected .Tena-Sempere, (2000) showed that ghrelin could decreased circulating steroid hormones in pre-pubertal male rats [37].

Results of this work indicating that circulating ghrelin in Iraqi thalassemia patients both sexes are significantly lower than control group . According to results obtained from studies in rodent and higher animals, the levels of gonadotropins would be expected to be high [21], but in this study it was noticed that levels of circulating ghrelin .The interpretation is not apparent.However, we found negative and significant parallel correlations between ghrelin and FSH, LH, testosterone and Estradiol in male patients[36,37], data resembling those obtained in the rodent studies.

We also evaluated the ratio of Leptin to ghrelin compare with control group. The increasing of ghrelin in respect to Leptin could be the cause of the negative correlation observed between ghrelin and gonadotropins or steroid hormones in male cases. In assumption the balance of reproductive hormones in thalassemia beta-major patients is impaired . Low circulating levels of FSH and LH as well as of steroid hormones in both genderes are detected.

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