

Research paper

Ghrelin and leptin levels in relation to puberty and reproductive function in patients with beta-thalassemia

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OBJECTIVE: Recently published animal studies indicate that leptin and ghrelin play a role in puberty initiation and progress as well as in reproduction. The aim of our study was to evaluate the relation of these two hormones to the pubertal maturity and fertility status in patients with β -thalassemia. **DESIGN:** Blood levels of leptin, acylated ghrelin and sex hormones were determined in 97 (59 males and 38 females) beta-thalassemic patients, aged 18-23 years and in 50 healthy subjects (27 males and 23 females) matched for age. Body Mass Index (BMI) was also assessed. **RESULTS:** Besides lower BMI, all the hormones evaluated were significantly lower in β -thalassemic patients compared to controls ($p < 0.001$). Furthermore, the leptin/ghrelin ratio in female patients was lower than the values obtained in the controls ($p < 0.001$). Finally, significant negative correlations ($p = 0.050$) were detected between circulating levels of acylated-ghrelin and LH, FSH and sex hormones in female and male patients. **CONCLUSIONS:** The lower values of leptin and ghrelin in patients with β -thalassemia possibly constitute another hormonal imbalance which may contribute to the phenotype of impaired growth and sexual maturation encountered in these patients. The findings on the ghrelin levels constitute a novel observation.

Key words: Acylated-ghrelin, Beta-thalassemia, Fertility, Leptin, Puberty, Reproductive hormones

INTRODUCTION

Following the application of desferrioxamine (desferal) as an effective iron chelator, survival of patients with β -thalassemia has significantly improved. However, due to its large molecular size, desferal

permeability into the cells is relatively slow and its efficacy as an intracellular iron chelator is limited.¹ This is why many transfusion dependent homozygote beta-thalassemic patients continue to develop progressive accumulation of iron overload despite the fact that they use desferal regularly. Gradual increase of iron deposition in different organs could be responsible for tissue damage and a number of complications including impaired growth and puberty delay in these patients.²⁻⁵

Pubertal development and fertility are determined

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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. Recent research added two new members to this hormonal complex, namely leptin and ghrelin,⁶⁻¹¹ hormones secreted by adipose tissue and the gastrointestinal tract, respectively. Besides their effect on carbohydrate and fat metabolism and appetite, these hormones acting on the hypothalamic-pituitary-gonadal axis, exert various effects on reproductive function, implantation, embryo development, and clinically relevant conditions.⁶

Leptin is produced by adipocytes and is closely related to the feeding state in different animals. Stimulation of reproductive neuroendocrine output is also associated with increased circulating levels of leptin.¹² This hormone primarily acts on the hypothalamus and its deficiency in the ob/ob mouse results in persistent immaturity as a result of hypothalamic-pituitary malfunction.¹³ To our knowledge, several studies have been published on leptin levels in different age groups of thalassemic patients¹⁴⁻¹⁶ and in all of them low leptin levels were observed, with variable conclusions being reached depending on the study design and the patient cohort.

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 adiposity.¹⁷ Despite the proven link between energy homeostasis and fertility, the potential role of ghrelin in the control of gonadal function in thalassemic patients has not been assessed. To achieve this goal, we searched for the interconnections between ghrelin or leptin and reproductive hormones at the various developmental stages. Blood levels of leptin, acylated-ghrelin (active form) follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone, testosterone and estradiol were determined in homozygote beta-thalassemic patients regularly attending the special thalassemic clinic in the city of Kerman (Iran), and in controls.

SUBJECTS AND METHODS:

The Ethics Committee of Kerman University ap-

proved the protocol of this study, which is in accordance with the internationally accepted principles as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and the US guidelines (NIH publication #85-23, revised in 1985). Ninety-eight homozygote beta-thalassemic patients, aged 18-23 years attending the special clinic participated in this study. In these patients the diagnosis of beta-thalassemia major had been made at the age of 4-12 months and since that time they have been receiving packed red blood cell transfusions regularly. The number of blood pack cells transfusion received ranged from 20-50 packs per year depending on the severity of the anemia and their age. The average amount of desferrioxamine used was 10-40 vials (500 mg) per week, depending on the blood volume they received to maintain a hemoglobin level of 12g/dl. Fifty-nine of these patients were males with impaired sexual development and 38 were females with irregular menses or amenorrhea. Fifty healthy college students, aged 18-23 years (27 males and 23 females) matched for age and gender were used as controls

All male participants were requested to come fasting for sample collection in order to have a uniform sampling of the levels of reproductive hormones. Females in the control group were asked to come fasting within the third week past their menses. Since female patients had irregular menses or were suffering from amenorrhea, samples were collected either before their routine blood transfusion or within the third week past their last menses.

Five ml of fasting blood were collected and divided into two tubes to prepare plasma for measurement of acylated-ghrelin using EDTA as anticoagulant¹⁸ and serum for evaluation of other hormones. Samples were centrifuged at 800 g for 5 minutes and plasma or serum was kept at -70° till the time of assay. Serum levels of gonadotropins (FSH and LH) and ferritin were determined in all participants using Elisa kits manufactured by Monobind Industry (Lake Forest, CA, USA). Serum levels of steroid hormones (progesterone, testosterone and estradiol) were also determined in all samples using Elisa kits purchased from IBL, Germany. For leptin determination, we used the Elisa kit manufactured by DBC Diagnostic Biochem (Canada). Plasma acylated-ghrelin was determined using the Elisa kit from BioVendor Labo-

ratory Medicine Industry (Czech Republic). For all hormones the standard protocols provided by the manufacturers were followed.

Data were analyzed using the SPSS (Version 14.0) program. The independent sample t-test was used for comparison of the two groups after checking for normal distribution of the data. For multiple comparisons of circulating acylated-ghrelin and leptin of the four groups, one-way ANOVA (post Hoc Tukey's model) was used. Determination of correlations was carried out using Pearson's two-tailed bivariate model. p-values less than 0.05 were considered as significant differences.

RESULTS

The patients and control subjects were divided into two groups according to their gender. Height, weight and BMI of patients were significantly ($p < 0.001$) lower than their respective values in the control group. Furthermore, as was expected, serum ferritin levels of all patients were significantly higher than those of the healthy participants ($p < 0.001$).

Mean circulating FSH levels in healthy male controls were higher than LH, while circulating levels

of LH and FSH in male patients were comparable. Both LH and FSH of male patients were significantly lower ($p < 0.001$) than the corresponding values in the controls. Mean values of LH and FSH in female patients were also significantly lower ($P < 0.001$) than the corresponding values obtained in the female controls.

The values of steroid hormones progesterone, testosterone and estradiol in patients were also significantly lower than the corresponding values in the controls ($p < 0.001$). The values of leptin and acylated-ghrelin were significantly lower than the values obtained in the corresponding healthy controls ($p < 0.001$).

To determine correlations between leptin or ghrelin and each of reproductive hormones, Pearson's two-tailed bivariate correlation analysis was carried out for each group. The results showed distinct and significant ($p < 0.050$) negative correlations between acylated-ghrelin and LH ($r = -0.40$), FSH ($r = -0.39$), estradiol ($r = -0.30$) or testosterone ($r = -0.28$) for the male patients, while these correlations were not observed in the other three groups (Figures 1 and 2). The ratio of leptin/acylated-ghrelin values were lower ($p < 0.001$) in the female patients compared with the healthy females (Table 1).

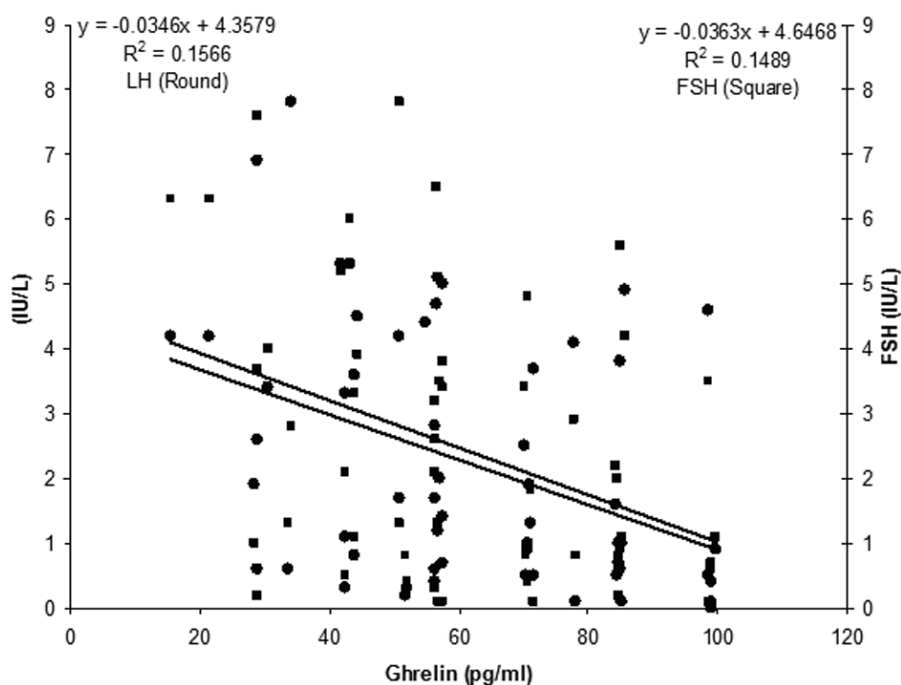


Figure 1. Negative correlation between ghrelin and LH or FSH in male homozygote beta-thalassemic patients ($p < 0.001$).

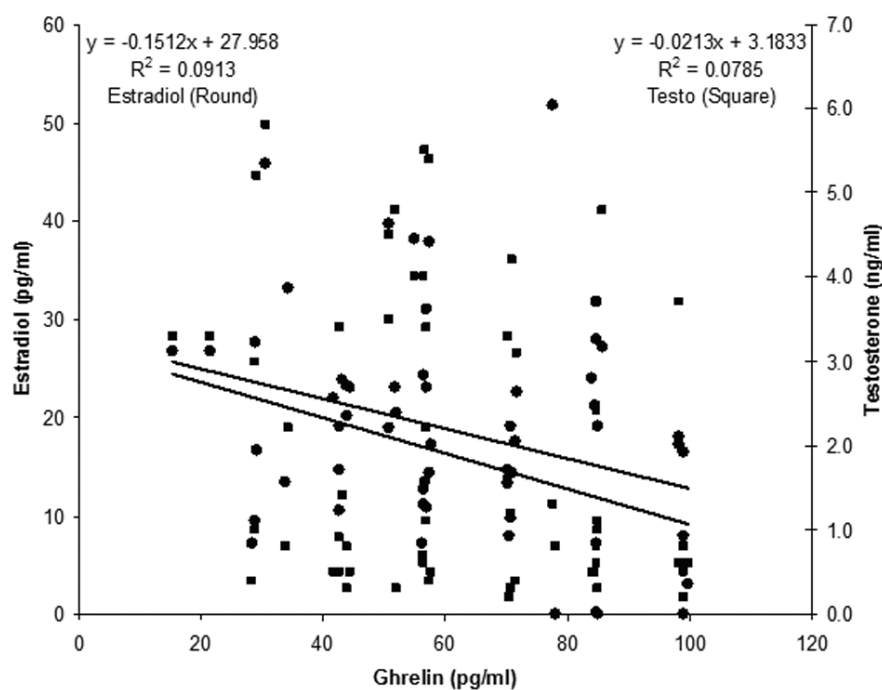


Figure 2. Negative correlation between ghrelin and estradiol or testosterone in male homozygote beta-thalassemic patients ($P < 0.001$). To convert to SI units multiply by 3,671 for estradiol and 3,467 for testosterone.

Table 1. Various parameters in the two groups (patients vs controls) by gender. Data presented as mean (SD)

Parameters	Male Patients (N=59)	Male Controls (N=27)	Female Patients (N=38)	Female Controls (N=23)
Age (years)	20.05 (1.93)	19.78 (1.72)	20.34 (2.02)	19.96 (1.61)
Weight (kg)	44.32 (9.38)	67.67 (9.28) *	46.86 (7.80)	55.61 (7.04) *
Height (cm)	149.9 (9.55)	171.74 (7.05) *	150.5 (7.81)	160.30 (6.65) *
BMI (kg/m ²)	19.54 (2.56)	22.96 (2.92) *	20.56 (2.28)	21.71 (3.05) *
Ferritin (ng/ml)	4196 (2402)	38.89 (22.96) *	4907 (1881)	23.16 (8.15) *
LH (IU/L)	2.22 (1.96)	4.15 (1.91) *	4.27 (2.53)	5.94 (1.87) *
FSH (IU/L)	2.40 (2.11)	7.43 (2.96) *	3.51 (2.60)	10.47 (3.91) *
Progesterone (nmol/L)	0.64 (0.45)	0.95 (0.57)*	3.85 (5.11)	19.30 (10.78)*
	0.20 (0.14)	0.30 (0.18)	1.21 (1.67)	6.07 (3.39)
Testosterone (nmol/L)	6.49 (5.90)	19.09 (5.17)*	1.46 (0.62)	2.53 (0.97)*
	1.87 (1.70)	5.50 (1.49)	0.42 (0.18)	0.73 (0.28)
Estradiol (nmol/L)	68.30 (41.10)	107.79 (37.21)*	120.93 (77.14) 32.95	270.11 (87.64)*
	18.61 (11.20)	29.37 (10.14)	(21.02)	73.60 (23.88)
Leptin (ng/ml)	2.40 (2.28)	4.30 (1.99) *	4.39 (1.95)	17.30 (5.60) *
α Ghrelin (pg/ml)	61.78 (22.37)	87.91 (42.44) *	80.73 (29.10)	142.68 (43.25) *
Ratio of Leptin/ α - Ghrelin	44.21 (45.23)	58.07 (33.11)	62.39 (36.84)	131.90 (60.97)*

* Significant differences ($p < 0.05$) between patients and gender matched controls. N: number of participants in each group, α ghrelin: acylated-ghrelin.

DISCUSSION

Puberty constitutes a distinct developmental stage characterized by physiological, anatomical and psychological alterations and comprises a preparatory step for reproduction. Age at puberty is determined by genetic and environmental factors. Delayed puberty in thalassaemic patients has previously been reported.¹⁹⁻²¹ The patients in the present study were pubertal (18-23 years old). All female patients had their menarche, but they were suffering from secondary amenorrhea or irregular menses at the time of sample collection, while male patients were suffering from impotence or did not have the secondary characteristics fully developed.

It has been suggested that low circulating levels of LH and FSH among thalassaemic patients is the result of impaired GnRH secretion resulting in inadequate pituitary stimulation.^{21,22} This could be the reason for the detection of low levels of gonadotropins resulting in low circulating levels of gonadal steroids in our patients.

Recently published results from animal studies showed that both leptin and ghrelin have a role in GnRH production at different reproductive stages.^{7,9,13,24-27} Additionally, leptin and ghrelin showed opposing effects on pulsatile GnRH secretion.⁹ Furthermore, obese children have early puberty that could be the effect of high circulating levels of leptin.²⁸⁻³¹ Low levels of circulating leptin could be one of the reasons for delayed puberty among patients with β -thalassaemia, particularly among the male patients who have lower leptin levels than female patients.

Expression of ghrelin has been demonstrated in mature Leydig cells of rat and human testis, as well as in steroidogenically active luteal and interstitial hilus cells of the ovary.²⁶ Gonadal expression of acylated-ghrelin receptors was also shown in Sertoli and Leydig cells of the testis and in follicular, luteal, surface epithelial and interstitial hilus cells of the ovary.²⁶ Vulliemoz et al²⁴ showed that ghrelin can inhibit GnRH pulse activity in ovariectomized adult monkey, while Lebrethon et al⁹ reported that ghrelin reduces GnRH in the pre-pubertal period in male rats and has no effect in mature rats. Fernandez-Fernandez et al,^{27,32} who have investigated the role of ghrelin on sexual maturation, showed that ghrelin inhibits LH

secretion *in vivo* in the pre-pubertal males as well as gonadectomized male and female rats, whereas FSH remained unaffected. Tena-Sempere^{17,33} also showed that ghrelin could reduce circulating steroid hormones in pre-pubertal male rats.

Our results indicating that circulating acylated-ghrelin in thalassaemic patients (both sexes) are significantly lower than in healthy individuals constitute a novel observation. According to data obtained from studies in rodent and higher animals, the levels of gonadotropins would be expected to be high in our patients who have low levels of circulating ghrelin, which was not the case. The interpretation is not apparent. However, we found negative and significant parallel correlations between ghrelin and LH, FSH, testosterone and estradiol in male patients, data resembling those obtained in the rodent studies.^{17,27,32,33} We also evaluated the ratio of leptin to ghrelin in the four groups of participants. This set of data showed that the leptin/ghrelin ratio of patients (both genders) was lower than in the respective control group. The excess of ghrelin in respect to leptin could be the cause of the negative correlation observed between ghrelin and gonadotropins or steroid hormones in male patients. Furthermore, these data also indicate that a balance between leptin and ghrelin is essential for appropriate puberty timing, a fact that was documented before the discovery of these hormones.³⁴

A positive correlation between leptin and BMI was detected only in the female control group, a finding in agreement with previous reports.³⁵ Since the BMI of the patients is lower than that of the gender matched control group, it could be concluded that adipose tissue is unable to maintain adequate leptin production when a higher leptin secretion is required, suggesting that the inappropriately low leptin secretion or decreased leptin/ghrelin ratio could contribute to the relevant pathology, namely the irregular menses or amenorrhea of the thalassaemic females. Obviously, more data are needed for a valid interpretation of our findings, which may have relevance to other forms of irregular menses or amenorrhea.

In conclusion, the balance of reproductive hormones in homozygous beta-thalassaemic patients is impaired. Low circulating levels of LH and FSH as well as of steroid hormones in both sexes are detected.

The reduced levels of reproductive hormones in β -thalassemic patients could be the result of intercellular iron overload and/or disturbance of the immune system.³⁵ Nevertheless, leptin and ghrelin values could also play a role in sexual maturity and fertility problems observed in these patients. We hypothesize that a decreased leptin/acylated-ghrelin ratio may also constitute one of the mechanisms involved in delayed puberty, irregular menses or amenorrhea not only in beta-thalassemic patients but also in non-thalassemic subjects.

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