

Original article

Serum ghrelin levels but not GH, IGF-1 and IGFBP-3 levels are altered in patients with fibromyalgia syndrome

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Abstract

Introduction: Both hypothalamo-pituitary-insulin-like growth factor-1 (IGF-1) axis and ghrelin levels may be altered in fibromyalgia syndrome (FMS) due to increased somatostatin tone. The aim of this study is to compare hypothalamo-pituitary-IGF-1 axis, ghrelin concentrations and their relations in premenopausal women with FMS and premenopausal healthy controls.

Methods: Seventy-five women (47 FMS and 28 healthy women) were enrolled in the study. Fasting plasma glucose, serum growth hormone (GH), insulin, C-peptide, IGF-1, insulin-like growth factor binding protein-3 (IGFBP-3) and ghrelin levels were measured. Depressive symptoms were assessed using Beck Depression Inventory. Pain intensity and sleep disturbance were recorded on a visual analog scale. The activity of daily living was assessed by fibromyalgia impact questionnaire.

Results: There were no significant differences in GH, IGF-1, IGFBP-3, glucose, insulin, and C-peptide levels between patients and controls ($p > 0.05$), whereas ghrelin levels were significantly lower in patients than controls ($p < 0.05$). Ghrelin levels were not correlated with GH, IGF-1, IGFBP-3, glucose, insulin, and C-peptide levels while they were positively correlated with tender point score and sleep disturbance score and negatively correlated with pain intensity score.

Conclusion: Our results suggest that low levels of ghrelin in FMS are not related to the changes in hypothalamo-pituitary-IGF-1 axis but may be related to some symptoms of FMS. Our results need to be clarified by further studies.

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1. Introduction

Fibromyalgia syndrome (FMS) is a common chronic and poorly understood clinical syndrome characterized by widespread musculoskeletal pain, with a prevalence of 2% in general population especially affecting middle-aged women [1].

Involvement of many systems and wide range of symptoms complicate the understanding of pathophysiological mechanisms underlying FMS. Some authors suggest an immunological basis for this syndrome [2], while others propose

a dysregulation between neuroendocrine, pain and stress pathways [3]. Evidence for hormonal perturbations in FMS came from the studies assigning a role for hypothalamo-pituitary-adrenal (HPA) axis as the final pathway linking the somatic and psychological symptoms seen in FMS [4,5]. HPA axis is closely related to growth hormone (GH) secretion since corticotropin releasing factor (CRF) and somatostatin have opposing actions on GH secretion.

Initial hypothesis that defects of GH and insulin-like growth factor-1 (IGF-1) secretion might play a role in the pathogenesis of FMS came from the idea that FMS patients had abnormalities in stages 3 and 4 of non-rapid eye movement (non-REM) sleep [6] and GH secretion predominantly occurred during these stages of sleep [7]. In fact, FMS and

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adult GH deficiency syndrome share common symptoms: poor general health, reduced exercise capacity, muscle weakness, impaired cognition and reduced lean body mass. While some studies reported low GH and IGF-1 production in FMS [8–11], other studies reported no changes in GH secretion [12–15] and one study reported increased GH but not IGF-1 levels [16].

Ghrelin is a 28-amino-acid peptide expressed and secreted mostly from stomach, but also from pituitary gland, hypothalamus and kidney [17]. Two main functions of ghrelin are stimulation of GH secretion and enhancing food intake. It has also been shown to stimulate corticotroph and lactotroph secretion, influence gastroenteropancreatic functions, reproduction, cardiovascular functions and regulate sleep and energy homeostasis [17]. Like other hormonal feedback mechanisms, it has been proposed that GH might also regulate ghrelin levels by negative feedback, however, several studies have failed to show this effect [18,19].

To date, only one study has shown that both basal GH and ghrelin levels are comparable among FMS patients and controls [20]. Given the fact that perturbations in GH and IGF-1 secretion in FMS are still awaiting to be solved and ghrelin may also contribute to these perturbations, we aimed to investigate whether hypothalamo-pituitary-IGF-1 axis and ghrelin levels in FMS patients are different from that of healthy controls. We compared GH, IGF-1, insulin-like growth factor binding protein-3 (IGFBP-3) and ghrelin levels as well as glucose, C-peptide and insulin levels of premenopausal women with FMS and healthy premenopausal women since glucose, C-peptide and insulin levels are also known to affect hypothalamo-pituitary-IGF-1 axis.

2. Methods

2.1. Subjects

Forty-seven ($n = 47$) premenopausal women who met the 1990 American College of Rheumatology criteria for the diagnosis of FMS were enrolled in the study. Twenty-eight ($n = 28$) age-matched demographically similar healthy premenopausal women were also selected as controls.

Exclusion criteria in subjects were as follows: (a) recent or past history of psychiatric disorders, (b) pregnancy, (c) subjects with inflammatory, endocrine, gastrointestinal or other chronic diseases, (d) use of glucocorticoids in the past year, and (e) a body mass index (BMI) greater than 40 kg/m^2 .

A structured interview was performed in all subjects before the introduction to the study to evaluate any psychiatric, endocrinologic or other chronic problems. All prescription and non-prescription medications as well as vitamins and herbal medicine were stopped at least two weeks prior to the study.

The approval from the Ethical Committee of Human Studies Research was obtained and written informed consent was also obtained from all patients as well as the healthy controls.

The demographic, functional and clinical characteristics including age, marital status, employment status, education level, and duration of symptoms were recorded. Pain intensity

was recorded on a visual analog scale (VAS) of 100 mm in length, the ends being assigned with “0 = no pain” and “100 = severe pain”. Another VAS of 100 mm in length was used to record nighttime sleep disturbance (0 = no problem, 100 = severe problem). Tender point examination was carried out by the exertion of a uniform amount of manual finger pressure, until fingernail bed blanches.

2.2. Health surveys

The depression rate was assessed by the beck depression inventory (BDI) in all patients and controls. The test consists of 21 questions scaled in a Likert format. The higher score shows increased depression of the subjects (0–63). It has been shown to be valid and reliable in the Turkish version [21]. The fibromyalgia impact questionnaire (FIQ) is widely used in fibromyalgia patients to evaluate both the clinical severity of the disease and the efficacy of different treatments and has been found to be valid and reliable in Turkish fibromyalgia patients [22,23].

2.3. Laboratory tests

After a thorough physical examination, full blood count, erythrocyte sedimentation rate, C-reactive protein and biochemical markers were evaluated for both groups. All blood samples were collected during early follicular phase of menstrual cycle early in the morning (08:30–10:30 am) after an overnight fast and serum was separated immediately by centrifugation; the serum samples obtained were stored at -20°C until required for assaying. Insulin (normal values $3\text{--}29.1 \mu\text{U/mL}$), GH (normal values $0.06\text{--}5.0 \text{ ng/mL}$), and C-peptide (normal values $1.1\text{--}5.0 \text{ ng/mL}$) (Diagnostic Products Corporation, Los Angeles, CA) were measured by chemiluminescence immunoassay (CLIA) with the Immulite 2000 analyzer (Diagnostic Products Corporation). Concentrations of fasting plasma glucose (normal values $70\text{--}110 \text{ mg/dl}$) (Roche Diagnostic GmbH, Mannheim, Germany) were determined with the Hitachi modular system autoanalyzer (Tokyo, Japan) and were measured by an enzymatic rate method. IGF-1 and IGFBP-3 were measured by ELISA (Biosource, Cat. No: KAPB2010 and KAPB2014, respectively) and ghrelin (octanoylated and deoctanoylated) was measured by radioimmunoassay (RIA) (Phoenix Pharmaceuticals, Cat. No: RK-031-30).

2.4. Statistical analyses

Values are expressed as the mean \pm standard deviation, median (minimum–maximum) and in numeric values. All statistical tests were two-tailed; $p < 0.05$ was taken as the level of statistical significance. Normality was tested using the Shapiro–Wilk test. Normally distributed data were analysed using the t -test for two independent samples, while the non-normally distributed data were analysed using the Mann–Whitney U test. Spearman’s correlation test was used for correlation analysis. We used the χ^2 test to assess frequency differences between the groups.

3. Results

Clinical characteristics of the patients and healthy controls are presented in Table 1. There were no significant differences in age, height, weight, BMI and duration of education between women with FMS and control women ($p > 0.05$). On the other hand, the mean pain intensity, sleep disturbance, BDI, and tender point scores were significantly higher in the FMS compared with the control group ($p < 0.05$).

There were no significant differences in GH, IGF-1, IGFBP-3, insulin, and C-peptide levels between patients and controls ($p > 0.05$) whereas ghrelin levels were significantly lower in patients than in controls ($p < 0.05$) (Table 2).

Neither GH nor IGF-1 was correlated with age, BMI, glucose, insulin levels, tender point score, FIQ score, sleep disturbance and pain intensity scores in both the patient and the control groups (data not shown). A positive correlation between the tender point score ($r = 0.32$, $p < 0.05$), sleep disturbance score ($r = 0.31$, $p < 0.05$) and ghrelin levels, negative

Table 1
Baseline characteristics of patients and healthy control subjects

Variable	Fibromyalgia ($n = 47$), mean \pm SD	Controls ($n = 28$), mean \pm SD	p -Value
Age (years)*	38.21 \pm 9.80	37.78 \pm 8.29	0.93
Height (cm)*	160.02 \pm 5.41	162.03 \pm 5.84	0.16
Weight (kg)*	63.65 \pm 11.14	61.07 \pm 10.67	0.52
Body mass index (kg/m ²)*	24.97 \pm 4.65	23.31 \pm 3.82	0.24
Disease duration (years)	3.03 \pm 2.55	–	
FIQ score	74.70 \pm 1.88	–	
Pain intensity (VAS)**	6.82 \pm 1.53	2.64 \pm 1.90	0.001
Sleep disturbance (VAS)**	7.57 \pm 1.89	1.71 \pm 0.89	0.001
Tender point score (1–18)**	15.25 \pm 0.34	2.49 \pm 2.18	0.001
BDI score**	14.85 \pm 8.31	7.78 \pm 5.01	0.001
Marital status	[No (%)]		0.44
Married	38 (81)	22 (79)	
Single	8 (17)	6 (21)	
Divorced	1 (2)		
Highest educational level achieved	[No (%)]		0.35
Elementary school	14 (30)	10 (36)	
Secondary school	5 (10)	6 (21)	
High school	14 (30)	5 (18)	
University	14 (30)	7 (25)	
Employment status	[No (%)]		0.73
Homemaker	26 (56)	15 (54)	
Retired	5 (11)	1 (4)	
Employed	12 (24)	12 (42)	
Student	4 (9)		
Duration of education (years)	10.39 \pm 3.95	9.21 \pm 4.02	0.41

* $p > 0.05$; ** $p < 0.05$.

Values are means \pm standard deviation, except where indicated as number and percentage of the group. VAS: visual analog scale; FIQ: fibromyalgia impact questionnaire; and BDI: beck depression inventory.

Table 2
Serum hormones and scores in patients and healthy control subjects

	Fibromyalgia ($n = 47$), median (min–max)	Controls ($n = 28$), median (min–max)	p -Value
Fasting plasma glucose (mg/dL)	90.00 (69–117)	88 (68–110)	0.23
Growth hormone (ng/mL)	0.49 (0.05–9.17)	0.56 (0.04–7.23)	0.82
Insulin (μ U/mL)	8.94 (3.05–27.40)	9.61 (3.96–28.40)	0.46
C-peptide (ng/mL)	1.98 (0.69–5.18)	2.37 (0.60–7.00)	0.27
IGF-1 (ng/mL)	389.90 (187.34–1044.49)	325.60 (154.95–691.74)	0.10
IGFBP-3 (ng/mL)	2777.05 (1968.99–3451.46)	2649.59 (1913.21–3275.36)	0.39
Ghrelin (ng/mL)	150 ^a (90–320)	200 ^a (110–620)	0.001

Values are median (min–max). IGF-1: insulin-like growth factor-1; IGFBP-3: insulin-like growth factor binding protein-3.

^a Significantly different from control value ($p < 0.05$).

correlation between pain intensity score ($r = -0.30$, $p < 0.01$) and ghrelin levels were found in both the patient and the control groups (Table 3).

4. Discussion

This study evaluates the hypothalamo-pituitary-IGF-1 axis and its relation with ghrelin levels in patients with FMS. GH, IGF-1 and IGFBP-3 levels, age, height, weight, BMI, marital and education status were comparable between FMS patients and healthy controls. We found that FMS patients had significantly higher tender point, pain intensity, sleep disturbance and BDI scores compared to the control subjects.

In our study, the patients with FMS had significantly higher sleep disturbance score than the control subjects. Because GH is secreted during stages 3 and 4 of non-REM sleep, it has been hypothesized that patients with FMS might have GH deficiency. Bennett et al. [8] were the first to demonstrate a coexistence of FMS and adult GH deficiency. In a population of

Table 3
Spearman's correlation of ghrelin with other hormones and clinical severity and distress variables (r -values)

	r -Value
IGF-1	-0.015
IGFBP-3	0.062
Insulin	-0.096
GH	0.096
Glucose	-0.093
Age	-0.103
Body mass index	-0.130
Tender point score*	0.322
FIQ	0.071
BDI	-0.067
Sleep disturbance*	0.314
Pain intensity**	-0.305

* $p < 0.05$; ** $p < 0.01$.

IGF-1: insulin-like growth factor-1; IGFBP-3: insulin-like growth factor binding protein-3; FIQ: fibromyalgia impact questionnaire; and BDI: beck depression inventory.

500 female FMS patients and 152 age-matched controls, they were able to show that IGF-1 levels in FMS were significantly lower. Later on, in a randomized, double-blind, placebo-controlled trial in 50 women with FMS and low IGF-1 levels, they were able to show that, daily GH therapy resulted in an increase in IGF-1 levels, improvement in FIQ score and tender point score [24]. However, several other studies have found no evidence for abnormal regulation of hypothalamo-pituitary-IGF-1 axis [12–15]. Buchwald et al. [13] could not demonstrate any significant difference in IGF-1 and IGFBP-3 levels between patients with FMS and healthy controls. McCall-Hosenfeld et al. [14] also could not find a difference in GH and IGF-1 axis in premenopausal women with FMS and controls. Our results are in accordance with Buchwald et al. and McCall-Hosenfeld et al. that we found no significant differences in basal GH, IGF-1 and IGFBP-3 between patients with FMS and healthy controls.

The major mediator of GH action is IGF-1, which is released from liver in response to GH. IGF-1, together with its major binding protein, IGFBP-3, reflects the overall daily secretion of GH. Although our results are based on single measurements of GH, IGF-1 and IGFBP-3, most previous studies conducted so far were done in a similar manner [16,20,25,26]. In fact, IGF-1 levels are used widely in clinical practice. By measuring serum IGFBP-3, levels of which correlate well with GH action and IGF-1 levels, we showed that all GH, IGF-1 and IGFBP-3 were comparable between the patient and the control groups. Age, BMI, C-peptide levels, insulin levels and hyperglycemia also affect GH and IGF-1 secretion. In our study, all parameters were also comparable between the patient and control groups. So, we do not think that our results are confounded with these parameters. Yet, both GH and IGF-1 were not correlated to any of these parameters in both the patient and the control groups.

The identification of ghrelin has added complexity to the understanding of regulation of GH secretion. The regulation of ghrelin secretion is also poorly understood. Plasma ghrelin levels increase in food deprivation, malnutrition, anorexia nervosa, hypoglycemia and leptin administration and decrease with food intake and obesity [17]. Although, ghrelin is a potent stimulator of GH secretion, it is not known whether there is a feedback regulation between GH and ghrelin and whether GH regulates ghrelin secretion. In a recent study, Janssen et al. [18] did not observe elevated ghrelin levels in GH deficient patients and GH replacement did not modify ghrelin levels after one year. The existence of somatostatin receptors in stomach raises the possibility that somatostatin might regulate ghrelin secretion as well as it regulates GH secretion at hypothalamo-pituitary level. Norrelund et al. showed a 70–80% reduction in ghrelin levels during intravenous somatostatin infusion in healthy subjects [27]. Only moderate doses of somatostatin were able to decrease ghrelin levels profoundly. Broglio et al. [28] showed that somatostatin exerted strong inhibitory effects on ghrelin secretion during 120 min of somatostatin infusion in healthy subjects. The effects of somatostatin infusion on ghrelin secretion were similar to its effects on insulin and GH secretion. However, at the end of

infusion, insulin and GH secretion recovered despite persistently suppressed ghrelin levels. Later on, these findings were confirmed by Barkan et al. [19]. They suggested that somatostatin is a strong inhibitor of ghrelin and through suppression of ghrelin levels it might suppress GH secretion.

In our study, ghrelin levels in FMS patients were significantly lower than the control subjects. We also did not find a relation between GH and ghrelin nor did we find a relation between ghrelin, IGF-1 and IGFBP-3 in both patients and controls. Therefore, we suggest that low levels found in FMS patients were not influenced by hypothalamo-pituitary-IGF-1 axis. Our results may be reconciled by stating that the lower levels of ghrelin in patients with FMS might be due to increased somatostatin tone observed in FMS discussed above. The finding that GH levels were comparable between two groups does not rule out this possibility since GH secretion is pulsatile and may not reflect true differences between the groups. However, we cannot explain why IGF-1 and IGFBP-3 were also comparable between the groups. The evidence from the literature suggests that regulation of ghrelin secretion might be different from that of GH and IGF-1 secretion and does not necessarily include a feedback mechanism. Our patients had mean disease duration of three years. Given the fact that somatostatin inhibits ghrelin levels more potently than GH levels, changes in ghrelin levels might have started long before the changes in GH, IGF-1 and IGFBP-3 levels.

To date, only one study comparing ghrelin levels in patients with FMS and healthy subjects has been reported [20]. Otero et al. compared plasma GH and ghrelin levels in 19 FMS patients and 14 age, gender and BMI matched healthy controls. Neither GH nor ghrelin levels were different from controls. However, as they stated GH levels were measured at individual time points. In this study, we compared IGF-1 and IGFBP-3 besides GH and ghrelin to make a better understanding of GH secretion perturbations. We also correlated ghrelin to BMI, age, insulin, pain intensity, sleep disturbance, tender point, BDI and FIQ scores which are thought to be the confounders that might affect ghrelin levels. Ghrelin was positively correlated with sleep disturbance and tender point scores and negatively correlated with pain intensity score in both groups. We cannot explain by now whether these correlations are significant clinically and whether ghrelin levels are related to some of the symptoms of FMS since same correlations were also observed in control group.

Our results suggest that regulation of the hypothalamo-pituitary-IGF-1 axis is similar in premenopausal women with FMS and healthy premenopausal women. However, significantly reduced ghrelin levels observed in FMS patients and the relation between ghrelin levels and symptomatology of FMS need to be clarified with further studies.

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