Serum ghrelin levels in patients with Behcet's disease

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Adv Dermatol Allergol 2016; XXXIII (6): 450–456
DOI:10.5114/ada.2016.63884

Abstract

Introduction: Behcet's disease (BD) is a chronic, relapsing, systemic vasculitis of unknown etiology.
Aim: To measure serum ghrelin levels in BD patients and healthy controls and to investigate its association with metabolic syndrome (MetS).
Material and methods: Thirty BD patients and 30 healthy individuals were enrolled in the study. Ghrelin levels were measured in blood samples using ELISA.
Results: The mean serum ghrelin level in BD patients (28.57 ±14.04) was significantly lower compared to healthy controls (40.72 ±23.21) (p = 0.01). The mean serum ghrelin level in BD patients who had MetS (24.18 ±12.73) was lower compared to BD patients who did not have MetS (30.77 ±14.45), but this difference was not significant (p > 0.05).
Conclusions: Ghrelin levels were lower in BD patients compared to healthy controls. There was no association between reduced ghrelin levels and MetS; however, there was a negative correlation between ghrelin levels and disease activity.

Key words: Behcet's disease, ghrelin, metabolic syndrome, insulin resistance.

Introduction

Behcet's disease (BD) is a multisystemic, chronic, inflammatory disease characterized with recurrent oral and genital ulcerations and uveitis attacks [1, 2]. While the disease etiopathogenesis is not fully known, emphasis is given to genetic factors, microbial agents, and endothelial cell dysfunction [1, 3, 4]. Recent studies suggest that the prevalence of metabolic syndrome in patients with BD is higher compared to the normal population [5].

Ghrelin was discovered by Japanese scientists in 1999 as an endogenous ligand for growth-hormone secretagogue (GHS-R), which is capable of inducing growth hormone secretion from the pituitary gland [6]. Ghrelin is a 28-amino acid, lipopeptide hormone secreted primarily from the stomach fundus. In addition to the stomach, ghrelin is also synthesized in the hypothalamus, pituitary gland, thyroid gland, small intestine, kidneys, heart, α-, β-, and ε-cells of the pancreas, the central nervous system, lung, placenta, gonads, immune system, breasts, and teeth [6–10]. Ghrelin's effect on appetite has been experimentally documented [7]. Immunohistochemical analyses have shown that ghrelin is endogenously expressed in α-, β-, and ε-cells of pancreatic islets, while GSH-R1a is primarily expressed in pancreatic α- and β-cells [11]. These findings indicate that the regulation of ghrelin and insulin levels are highly related. In addition, plasma ghrelin levels are low in obesity, and have been regarded as an important finding of metabolic syndrome, just like hyperinsulinemia and insulin resistance [12, 13].

Aim

This study aims to investigate ghrelin levels and the association between ghrelin and MetS in patients with BD in light of all available information.

Material and methods

Patient selection and patient characteristics

This study was carried out on 30 BD patients who were followed up at the Dermatology Department of the

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Received: 23.10.2015, **accepted:** 12.11.2015.

Faculty of Medicine at Firat University and at the Dermatology Department of Elazig Teaching and Research Hospital, and 30 healthy individuals were included as the control group. One-to-one interviews were conducted with 30 patients (15 males and 15 females) diagnosed based on International Behcet's Disease Study Group Criteria [14], and the patients' ages, genders, disease duration, medications, and history of smoking were recorded. BD-associated symptoms and/or findings (oral ulcer, genital ulcer, eye involvement, arthralgia, arthritis, acneiform skin lesions, erythema nodosum-like lesions, thrombophlebitis, gastrointestinal involvement, neurological involvement, and pathergy test results) were recorded. Thirty healthy individuals (15 males and 15 females), who were admitted to the hospital for annual check-ups with no systemic, dermatologic, rheumatologic, or neurological diseases (including family history) and did not use alcohol, recreational substances, or any medication, were included in the control group.

Exclusion criteria: 1) age < 18 years, 2) pregnancy, 3) acute or chronic infection, 4) acute or chronic neurological disorders (excluding Neuro-Behcet's disease), 5) polycystic ovary syndrome or amenorrhea, 6) hyperthyroidism or hypothyroidism.

Study plan

All enrolled patients were analyzed for age, gender, age of onset, duration of disease, history of smoking and alcohol use, height, weight, body mass index (BMI), and waist circumference. The BD-associated symptoms and/or findings (oral ulcer, genital ulcer, eye involvement, arthralgia, arthritis, acneiform skin lesions, erythema nodosum-like lesions, thrombophlebitis, gastrointestinal involvement, neurological involvement, and pathergy test results) were recorded. Patients' symptoms and/or findings were recorded as follows: active, inactive (previous), or not observed. Ghrelin levels may be affected by many metabolic factors, and therefore, patients and controls had similar BMI to decrease the different metabolic factors in BD patients.

Body mass index was calculated as weight/height (kg/m^2) and metabolic syndrome was diagnosed in the presence of central obesity in addition to two or more criteria of the International Diabetes Foundation [15]. In addition, insulin resistance was calculated based on the homeostasis model assessment of insulin resistance (HOMA-IR) formula. Cases with a HOMA-IR index of > 3.2 were diagnosed to have insulin resistance [16].

Collection and storage of biological samples

As ghrelin is a peptide hormone and can be broken down by proteases, aprotinin (500 Kallikrein units per ml) was added to plain biochemistry tubes before the collection of blood samples from the participants to prevent proteolysis [17]. Blood samples were collected between 9:00 am and 10:00 am after an overnight fast to avoid any effects associated with circadian rhythm. Serum ghrelin levels were analyzed using a human ghrelin kit (Cat. No. A05106; SPI-Bio, Montigny-le-Bretonneux, France) by the enzyme-linked immunosorbent assay (ELISA) method according to the manufacturer's instructions. According to the kit's supplier, the intra- and inter-assay coefficients of variation (CV) for this kit are < 7% and < 8.1%, respectively.

Statistical analysis

SPSS version 12.0 (SPSS, Chicago, IL) was used for statistical analyses. Data were presented as mean ± SD. Independent samples *t*-test and Mann-Whitney *U*-test were used to compare groups. In all analyses, *p*-values < 0.05 were considered statistically significant.

Results

Thirty BD patients and 30 healthy controls who were admitted to the Department of Dermatology of the Faculty of Medicine at Firat University and the Dermatology Department of Elazig Teaching and Research Hospital were included in the study. The age range in BD patients (25–54) and the control group (21–64) was similar. The disease duration ranged between 1 and 17 years in BD patients, while the mean disease period was 5.96 ±4.4 years. Female/male (F/M) ratio was 1 : 1 in both groups. There was no significant difference in age, gender, and BMI distribution between the groups ($p > 0.05$). The demographic and clinical features of patient and control groups are presented in Table 1.

Family history was positive in three patients with BD (10%), while none of the healthy controls had a history of BD in their families. The symptoms and/or findings of patients with BD were recorded as follows: active, inactive (previous), or not observed. The BD patients with at least two active systemic findings/symptoms were considered active patients. Based on this classification, 14 (46.7%) patients were active, and 16 (53.3%) were inactive. The

Table 1. Clinical findings of patient and control groups

Parameter	Behcet's disease	Control	*P*-value
N	30	30	
Gender (M/F)	15/15	15/15	> 0.05
Age* [year]	36.66 ±7.9	37.20 ±11.3	> 0.05
BMI* [kg/m^2]	24.08 ±1.57	24.47 ±1.63	> 0.05
BMI score*	2.23 ±0.43	2.33 ±0.47	> 0.05
Waist circumference [cm]	85.53 ±8.37	87.33 ±9.58	> 0.05

**Mean ± standard deviation.*

Table 2. Clinical properties of Behcet's disease patients

No.	Age	Gender	OU	GU	Eye	Joint	ENLL	Acne	DVT	CS	GIS	NI	Pathergy
1	37	M	2	2	0	0	0	0	0	0	0	2	2
2	30	F	1	1	0	2	1	0	0	0	0	0	0
3	40	F	2	2	1	0	0	2	0	0	0	0	0
4	39	M	1	1	1	0	0	0	0	0	0	0	1
5	39	M	1	1	2	2	0	0	0	0	0	0	0
6	32	F	1	1	1	0	0	0	0	0	0	0	1
7	45	M	1	1	0	0	2	0	0	0	0	0	1
8	45	F	2	1	1	0	0	0	0	0	0	0	0
9	34	M	2	0	2	2	0	1	0	0	0	0	2
10	29	F	2	1	0	2	1	0	0	1	0	0	0
11	38	F	1	1	0	0	2	0	2	0	0	0	0
12	28	M	1	1	1	0	1	0	0	0	0	0	0
13	54	F	2	2	2	2	0	2	0	0	0	1	2
14	39	F	2	2	2	2	0	0	0	0	0	0	0
15	36	M	1	1	1	1	0	0	1	0	0	0	0
16	52	M	1	1	1	0	0	0	0	0	0	0	0
17	31	F	2	2	1	0	0	0	1	0	0	0	0
18	43	M	1	1	1	1	0	0	0	0	0	0	0
19	28	M	2	1	0	0	1	1	0	0	0	0	0
20	54	F	2	2	1	0	0	0	0	0	0	0	2
21	31	M	1	1	0	1	0	0	0	0	0	0	1
22	27	M	2	1	1	2	1	0	0	2	0	0	0
23	31	F	1	1	0	0	0	0	0	0	0	0	1
24	25	M	2	2	1	0	0	0	0	0	0	0	2
25	34	F	1	1	0	0	0	0	0	0	0	0	1
26	31	F	1	2	1	0	0	0	2	0	0	0	0
27	42	F	1	1	1	1	0	1	0	0	0	0	0
28	40	M	2	1	1	0	1	0	0	0	0	0	0
29	39	F	1	2	1	2	1	0	0	1	0	0	2
30	54	M	1	1	1	1	0	0	0	0	1	0	0

CS – cardiovascular system, DVT – deeep vena thrombosis, ENLL – eritema nodosum like lesion, F – female, GIS – gastrointestinal system, GU – genital ulcer, M – male, NI – neurologic involvement, OU – oral ulcer, 0 – never happened, 1 – inactive, 2 – active.

clinical features of patients with BD are presented in Table 2. Nine (30%) BD patients used colchicine, and 9 (30%) BD patients used systemic corticosteroids, and immunosuppressive agents including azathioprine and cyclosporine in addition to colchicine. Twelve (40%) patients were not on any treatment.

The mean serum ghrelin level in BD patients (28.57 ±14.04) were significantly lower compared to healthy controls (40.72 ±23.21) ($p = 0.01$). Similarly, the mean serum glucose level in BD patients (80.10 ±1.84) was significantly lower compared to healthy controls (88.06 ±10.45, $p = 0.008$) (Table 3 and Figure 1).

An evaluation of the patients and the controls for metabolic syndrome (MetS) was observed in 12 (40%) BD patients and 12 (40%) controls. The mean serum ghrelin level in BD patients with MetS (24.18 ±12.73) was lower compared to BD patients without MetS (30.77 ±14.45), but this difference was not significant ($p > 0.05$). A grouping of BD patients for IR and/or MetS presence found no significant differences in serum ghrelin levels between the groups. However, an evaluation of all patients and controls demonstrated that the mean serum ghrelin level in BD patients with IR and/or MetS (28.64 ±17.90) was significantly lower compared to BD patients without IR and/or MetS (38.93 ±20.52) ($p = 0.03$) (Figure 2).

An examination of the association between disease activity and serum ghrelin, glucose, insulin, and C-peptide levels showed that the mean serum ghrelin level in active BD patients (26.97 ±15.52) was lower compared to inactive BD patients (29.98 ±12.95), but the difference between the groups was insignificant. Similarly, there was no significant difference in serum C-peptide and insulin levels between active and inactive BD patients ($p < 0.05$). However, the mean serum glucose level in active BD patients (72.14 ±7.57) was significantly lower compared to inactive BD patients (85.12 ±12.51, $p = 0.001$) (Figure 3).

Table 3. Laboratory findings of patient and control groups

Parameter	Behcet's disease	Control	*P*-value
Glucose* [mg/dl]	79.06 ±12.25	86.86 ±10.84	0.01
Triglycerides* [mg/dl]	133.76 ±53.38	131.70 ±83.31	> 0.05
LDL-cholesterol* [mg/dl]	114.43 ±26.94	108.67 ±25.49	> 0.05
HDL-cholesterol* [mg/dl]	45.44 ±11.54	46.28 ±12.17	> 0.05
Total cholesterol* [mg/dl]	170.76 ±39.81	181.00 ±21.66	> 0.05
Insülin* [µIU/ml]	8.39 ±6.83	9.01 ±3.85	> 0.05
C-peptid* [ng/ml]	2.57 ±1.27	2.11 ±0.74	> 0.05
HOMA-IR values*	1.66 ±1.39	1.99 ±0.93	> 0.05
Ghrelin* [pg/ml]	28.57 ±14.04	40.72 ±23.21	0.01

**Mean ± standard deviation.*

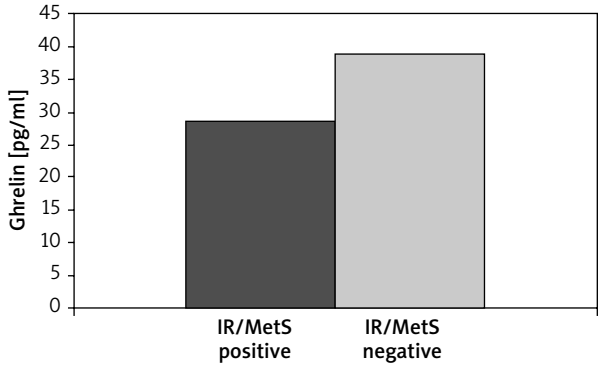

Figure 2. Mean serum ghrelin levels in groups with IR/MetS positive and IR/MetS negative

Behcet's disease may progress with multisystemic involvements, and different system involvements can be observed in each patient. Given that the oral mucosa is an important site regarding involvement in BD, the study evaluated the serum ghrelin levels in BD patients with and without active oral involvement. The mean serum ghrelin level was 30.50 ±16.73 in BD patients with oral involvement and 27.10 ±11.91 in BD patients without oral involvement; however, there was no significant difference between the groups ($p > 0.05$).

There was a positive correlation between ghrelin level and BMI, and ghrelin level and glucose level in BD patients ($r = 0.48$, $p < 0.05$; $r = 0.55$, $p < 0.001$, respectively). On the other hand, there was a negative correlation between ghrelin level and disease activity ($r = –0.45$, $p < 0.05$).

Discussion

Various metabolic functions of ghrelin have been defined since its discovery as a molecule that induces growth hormone secretion from the pituitary gland.

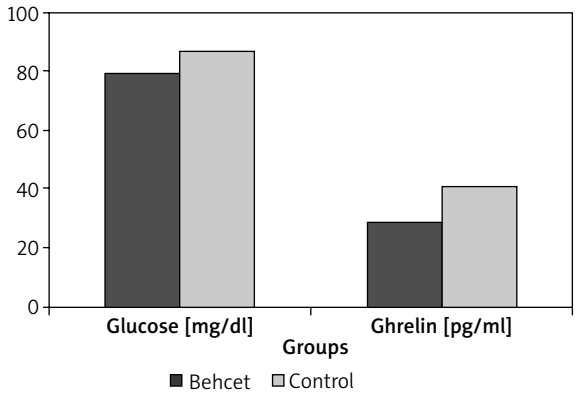

Figure 1. Mean serum ghrelin and glucose levels in groups

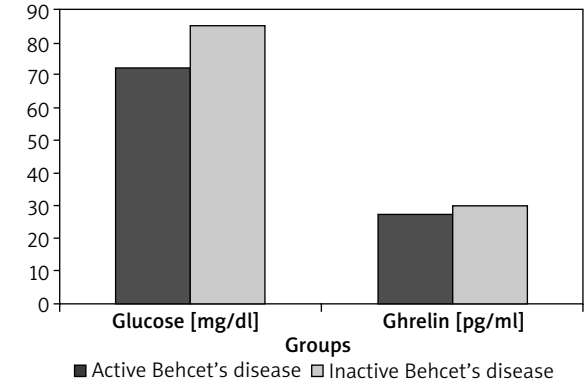

Figure 3. Mean serum ghrelin and glucose levels in active and inactive groups

Throughout the day, ghrelin levels increase in case of fasting, and decrease during fullness. Circulating ghrelin levels have been shown to have a regulatory effect on body weight in the long term [18]. A negative correlation between ghrelin levels and body weight has been shown in patients who are on low-calorie diets, have cancer-associated anorexia, and anorexia nervosa. It has also been reported that increased weight in patients with anorexia nervosa reduces ghrelin levels [19].

Serum ghrelin levels are low in patients with type 2 diabetes or insulin resistance [20]. Moreover, studies have shown that saliva ghrelin levels in type 2 diabetes patients are low, and this could serve as an important parameter to monitor the course of diabetes [10]. In fact, some authors suggest that a low ghrelin level is an indicator of MetS [21]. According to a study on premenopausal and non-diabetic women, there was a strong association between fasting ghrelin levels and subcutaneous lipid mass, while insulin resistance showed a negative correlation with ghrelin levels [20]. On the other hand, ghrelin levels were normal in children with type I diabetes [22]. Studies have shown that ghrelin levels are lower in obese individuals compared to those who are underweight. Weight loss through diet caused an increase in ghrelin levels [23].

In the present study, serum ghrelin levels were significantly lower in BD patients compared to healthy individuals. In addition, there was a positive correlation between serum ghrelin level and BMI in BD patients. This finding was consistent with various studies that have shown that serum ghrelin levels decrease in obese patients. Interestingly, while serum glucose levels were within the normal range, BD patients had significantly lower serum glucose levels compared to healthy controls. In addition, there was a positive correlation between serum ghrelin levels and serum glucose levels. These findings were interesting, as ghrelin levels are expected to increase during fasting, and decrease during fullness; however, both serum glucose and ghrelin levels were lower in the present study, which could be explained as follows: different studies have shown that ghrelin, glucose and insulin might display different behaviors in metabolic events, especially in case of external stimuli. Acute ghrelin administration induces hyperglycemia, and reduces insulin levels, both in humans and rodents [24]. In this case, ghrelin and glucose levels are elevated at the same time, while later on, the decrease in circulating ghrelin levels implies that glucose levels will also decrease in parallel. In addition, studies have shown that insulin decreases ghrelin levels. Following the gradual induction of hyperinsulinemia (1.2 and 4 μU/kg/s), ghrelin levels during normoglycemia decreased by 17%, 27%, and 33%, respectively. The finding that the induction of hyperglycemia during normoglycemia decreases ghrelin levels indicates that insulin might play an important role in regulating ghrelin levels [25]. On the other hand, when hyperglycemia was induced with 50 g of glucose administration, plasma ghrelin levels were reduced to the minimum level 30 min after the plasma glucose level returned to the normal level [26].

There is only a single study in the literature on serum ghrelin levels in BD patients. Koca *et al.* [27] carried out a study on 29 BD patients, and did not determine any significant difference in serum ghrelin levels between BD patients and healthy controls.

Polycystic ovary syndrome (PCOS) is accompanied by insulin resistance, and studies on PCOS patients have contradictory findings. Waśko *et al.* [28] found that PCOS patients had high ghrelin levels compared to the control group, reporting a negative correlation between ghrelin level and BMI, and insulin level. Another study reported that ghrelin levels in PCOS patients were not significantly different compared to the control group [29], while Pagotto *et al.* [30] reported that PCOS patients had lower ghrelin levels compared to obese patients. Schöfl *et al.* [31] found that ghrelin levels were lower in PCOS patients compared to healthy, obese, or underweight individuals. This study revealed that BD patients with MetS had lower ghrelin levels compared to controls, although this difference was not significant. Due to the insufficient number of patients with insulin resistance, no comparison could be made between BD patients with and without insulin resistance. However, when patients and controls are considered together, the study found that serum ghrelin levels were significantly lower in individuals who had IR and/or MetS compared to individuals who did not have IR and/or MetS.

Studies have shown variable serum ghrelin levels in chronic inflammatory diseases [32–34]. An increase in ghrelin levels has been shown in the case of active chronic inflammatory diseases such as ankylosing spondylitis, ANCA-dependent vasculitis, celiac disease, and inflammatory bowel disease [32, 34]. According to another study, serum ghrelin levels were higher in patients with psoriasis compared to healthy controls; however, this difference was not significant [35]. On the other hand, Otero *et al.* [33] reported that ghrelin levels were decreased in rheumatoid arthritis (RA) patients. Koca *et al.* found that serum ghrelin levels were lower in RA patients compared to BD patients. The authors argued that the decrease in ghrelin levels could be due to the high-dose corticosteroid treatment [26].

Neutrophil-mediated cytokine levels are elevated in BD patients [1, 3]. Ghrelin is well known to suppress the secretion of proinflammatory cytokines and chemokines, and the growth of inflammatory cells [36]. On the other hand, studies have shown that cytokines directly suppress ghrelin synthesis [37]. In this study, significantly lower ghrelin levels in BD patients, and also in active BD patients could be explained by elevated cytokine levels in BD patients. Few studies reported a negative correlation between ghrelin levels and the severity of psoriasis [35, 38]. Similarly, Koca *et al.* [27] stated that inflamma-

tion and cytokines could be a reason for lower ghrelin levels in RA. The same study also emphasized the fact that lower ghrelin levels may worsen the prognosis of RA [27]. This study found lower ghrelin levels in patients with active BD; however, this reduction was not statistically significant. On the other hand, the study detected a negative correlation between the ghrelin level and disease activity, and the ghrelin level and glucose level.

Studies on mice have shown that plasma ghrelin levels were higher in females compared to males. Similarly, studies on humans also showed that plasma ghrelin levels were higher in females [39]. In this study, ghrelin levels were also higher in women, and this finding was consistent with other studies in the literature.

Conclusions

Ghrelin is a recently-discovered anabolic hormone with side effects including ghrelin growth, appetite, fat accumulation, and increased gluconeogenesis and functions such as energy consumption and storage in the brain and in peripheral tissues. This study found a significant decrease in ghrelin levels in BD patients compared to healthy controls. The decrease in ghrelin levels could not be correlated with MetS; however, the study found a negative correlation between ghrelin levels and disease activity. Therefore, the researchers consider that ghrelin levels are reduced in BD due to a mechanism different than the development of MetS and inflammatory processes might be effective in this regard. Larger studies focusing on the relation between the immune system, severity of BD and ghrelin levels may be useful to clarify the etiopathogenesis of the disease and to improve activation markers for the patients with BD.

Conflict of interest

The authors declare no conflict of interest.

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