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ORIGINAL ARTICLE

Patients with multiple sclerosis have higher levels of serum ghrelin

M. Said Berilgen[1], Serpil Bulut [1], Bilal Ustundag [2], Aslan Tekatas[1] & Ahmet Ayar[3]

[1] Department of Neurology, Firat University Faculty of Medicine, Elazig, Turkey.
[2] Department of Biochemistry, Firat University Faculty of Medicine, Elazig, Turkey.
[3] Department of Physiology, Firat University Faculty of Medicine, Elazig, Turkey.

Correspondence to: Dr. M. Said Berilgen
University of Firat, Faculty of Medicine (TIP FAKULTESI),
Department of Neurology,
TR-23119, Elazig, TURKEY
FAX: +90 424 237 91 38
TEL: +90 424 237 00 00 Ext: 2609
EMAIL: msberilgen@yahoo.com

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Abstract

In addition to metabolic and neuroendocrine actions, the recently discovered hormone ghrelin has been found to have inhibitory effects on inflammatory processes. This novel finding suggests possible involvement of the peptide in the pathogenesis of inflammatory disorders including the inflammatory demyelinating disease of the central nervous system, multiple sclerosis (MS). The aim of the present study was to evaluate serum ghrelin levels in patients with MS.
Serum ghrelin levels were measured in 40 MS patients and 20 controls. Control subjects were selected from healthy individuals, matched for age, sex and BMI. Fasting plasma levels of ghrelin were determined by radioimmunoassay.
Serum ghrelin level was significantly higher in MS group (226.16 ± 35.84 pg/ml, n=40) than that in the control group (113.04 ± 11.28 pg/ml, n=20, $P<0.001$). Both, relapsing remitting and secondary progressive MS patients had ghrelin levels significantly higher than controls, while there was no significant difference between the ghrelin levels of patients with these two categories of MS.
This study for the first time shows that patients with MS have higher levels of ghrelin and this increase in circulating ghrelin level may function against the proinflammatory process in these patients.

Introduction

Multiple sclerosis (MS) is a chronic, progressive, inflammatory demyelinating disease of the central nervous system (CNS), namely the brain and spinal cord. It is one of the leading causes of non-traumatic disability among the young adults, affecting more than 2.5 million people worldwide [11]. Although MS commonly regarded as an autoimmune disorder, occurring in genetically predisposed individuals, the exact pathogenesis of the disease remains unclear as the factor(s) triggering the pathological changes in MS remain poorly understood [10,11].

MS is characterized by multifocal areas of demyelination with relative preservation of axons, decreased oligodendrocyte numbers, and multiple patches of gliotic scarring [11]. Initially in the

disease course, MS involves recurrent bouts of CNS inflammation that causes destruction of myelin sheath covering axons, and the axons themselves. The process results with multiple clusters of hard sclerotic tissue, which interrupt and sometimes stop signal traveling through the nerve cells, resulting in various neurologic symptoms occurring at different locations throughout the body and preventing body functions from occurring [10,11].

There are four basic categories of MS: relapsing remitting (RR-MS), secondary progressive (SP-MS), primary progressive and progressive relapsing [10,11]. The RR-MS is the most common but the least severe form, is characterized by acute attacks, which are often followed by full recovery and the individual usually remains well until the next episode.

Although the exact cause of inflammation remains unclear, the lines of evidence suggests that there is no one single cause for the disease. According to the two currently most widely held hypothesis on the cause of MS, it is either an autoimmune disorder or infectious disease, or a combination of both [8,10]. Recent studies suggest that T-cell-mediated or T-cell-plus-antibody-mediated autoimmune responses may account for the etiologies of inflammation; while others suggest a primary disorder involving virus or toxin-induced demyelination rather than autoimmunity in a subset of patients [10,11]. There is also evidence that MS appears to be mediated by CD4+ T cells, specific T cell receptor bearing lymphocytes, adhesion molecule expression, and cytokine secretion [10,11].

Recently, the polypeptide hormone ghrelin is discovered as the peptide hormone that potently stimulates release of growth hormone from the anterior pituitary, and has been isolated from stomach, hypothalamus and other tissues [4]. Although the predominant source of ghrelin is epithelial cells lining the fundus of the stomach, it is widely expressed in other tissues, and in this way it may have both endocrine and paracrine effects [4]. It is now known that this novel gastrointestinal hormone not only have a role in the control of GH secretion, it may have other important physiological functions such as food intake, gastric acid secretion, and gastric motor activity. Furthermore, in a recent study it was discovered that ghrelin exerts anti-inflammatory effects by inhibiting the secretion of both acute and chronic cytokines including IL-1, IL-6, TNF-alpha, IFN-gamma, and chemokines in human endothelial cells *in vitro* and *in vivo* in mouse model of sepsis and inflammation [1,7]. Ghrelin and the GHSR signaling system were detected in human T cells, B cells and neutrophils, regardless of maturity of cell types [4]. Additionally, a role for ghrelin in neuroimmunological control is implicated in the adaptive response directed at balancing the pro-inflammatory and anti-inflammatory pathways [3]. These novel anti-inflammatory actions of ghrelin suggest that this peptide could play a modulatory role in inflammatory disorders including multiple sclerosis.

This study, for the first time, examined the serum ghrelin levels of patients with MS and compared with healthy body mass index (BMI)-matched controls.

Materials and methods

The protocol of this study was approved by the local Ethics Committee. Patients were informed about the study protocol and signed consent was obtained before participating. A total of 40 prospective patients admitted for routine visits who were previously diagnosed, based on McDonald diagnostic criteria for MS, and being followed at the Department of Neurology at Firat University Medical Center, were enrolled to the study [9].

They were a total of 40 patients, 31 women and 9 men. Of the patients 29 had RR-MS. Of these 21 women and 8 men with a mean age of 33.9 ± 8 years (range 23–52y). The mean expanded disability status scale (EDSS) was 1.8 ± 1, and the mean duration of the disease was 5.3 ± 3.2 years. Ten of these patients were treated with Rebif 44 mcg (Interferon (IFN) Beta-Ia delivered subcutaneously), 5 of these patients were treated with Betaseron (IFNbeta-1b) and the other 5 patients were treated with Copaxone (glatiramer acetate injection) while the remaining 9 patients were not receiving any treatment. The remaining 11 patients had SP-MS, 10 women and 1 men with a mean age of 36.9 ± 9 years (range 31–51). The mean expanded disability status scale (EDSS) was 6.1 ± 2.2, and the mean duration of the disease was 10.7 ± 6.2 years. Of these patients 5 were treated with Betaseron (IFNbeta-1b) while others not receiving any treatment..

Exclusion criteria were as follows: presence of any concurrent psychiatric disease; regular drug use including oral contraceptives except for interferons or glatiramer acetate, presence of other diseases (neurological, endocrinological, rheumatological, hematological, acute or chronic infectious and/or inflammatory), liver or kidney dysfunction, substance or alcohol abuse, or a body mass index (BMI) over 25.

The control group consisted of 20 healthy volunteers matched for age (34.6 ± 7, range 25–53 years), gender (12 female, 11 male) and BMI.

Each patient underwent detailed history and clinical examination prior to being enrolled in the study. Magnetic resonance imaging (MRI) of the brain was performed in all patients. Baseline laboratory investigations included white blood cell count, red blood cell count, creatinine, electrolytes, GOT, GPT, AP, γGT, partial thromboplastin time, sedimentation rate, C-reactive protein, serum iron, transferrin, and glucose. Thyroid function tests were performed, and serum levels of cholesterol and triglycerides were measured. Serum lipid levels were specifically measured in this study due to their effect on the serum ghrelin levels. Pelvic ultrasonography was performed to exclude polycystic ovary disease in the female participants. Height and weight were measured in all patients and healthy control subjects.

Table 1: Measured parameters (mean ± SD) in the patients with multiple sclerosis and controls.

	Total MS population (n=40)	RR-MS group (n=29)	SP-MS group (n=11)	Control (n=20)	*p*
Ghrelin (pg/ml)	226.16 ± 35.84[a]	228.34 ± 37.05[a]	220.42 ± 33.40[a]	113.04 ± 11.28	<0.001
BMI (kg/m^2)	24.83 ± 4.23	24.94 ± 3.84	24.72 ± 4.62	23.95 ± 0.72	NS
Triglyceride (mg/dL)	152.12 ± 55.32	151.13 ± 47.09	154.72 ± 75.60	157.40 ± 28.93	NS
Cholesterol (mg/dL)	197.57 ± 37.63	198.06 ± 46.51	185.16 ± 36.58	186.35 ± 37.23	NS

[a]: *P*<0.001 compared to the control group (One-Way ANOVA), NS: nonsignificant
BMI: Body mass index, RR-MS: relapsing remitting, SP-MS: secondary progressive

Serum ghrelin levels were measured in blood samples collected from each participants antecubital veins at 8 a.m. after a 12-h of fasting. The blood samples were immediately centrifuged, stored at –80 °C until analyzed. Serum ghrelin levels were measured by a commercially available radioimmunoassay (RIA) kit (Phoenix Pharmaceuticals Inc, Phoenix, AZ, USA) that employs I-125 labeled bioactive ghrelin as a tracer and a rabbit polyclonal antibody against full-length octanoylated human ghrelin. The assay was able to detect both ghrelin and des-octanoyl-ghrelin. The sensitivity of the assay was 30 pg/ml, and the intra- and interassay coefficients of variation were < 5% and < 14%, respectively.

Data were statistically evaluated with SPSS. Comparisons between-group were performed using One-Way ANOVA, and the Tukey B and Scheffé tests were used as post hoc tests. Data are expressed as mean ± standard deviation. $P<0.05$ was considered as significant.

Results

In the group of 40 MS patients, serum ghrelin levels were significantly higher than in the healthy control group ($p<0.001$, One-Way ANOVA). When the group of 29 patients with RR-MS and the group of 11 patients with SP-MS were compared to the control group separately, their serum ghrelin levels were also significantly higher than the control group ($p<0.001$ in both groups, One-Way ANOVA). There was no significant difference between the serum ghrelin levels of RR-MS and SP-MS patients ($p>0.05$, One-Way ANOVA).

In terms of serum cholesterol, triglyceride levels and BMI values, no significant differences were found between the overall group of 40 MS patients and the control group ($p>0.05$, One-Way ANOVA). When the RR-MS group and the SP-MS group were compared separately to the control group in terms of serum cholesterol, triglyceride levels and BMI values, no significant differences were found ($P>0.05$, One-Way ANOVA). These findings in the MS patients and healthy controls are summarized in Table 1.

Discussion

To our knowledge, this is the first study to examine serum ghrelin levels in MS patients. In the overall group of 40 MS patients included in the study, serum ghrelin levels were found to be significantly higher than in the control group. This was also valid when serum ghrelin levels of patients with RR-MS and SP-MS were compared to the control group.

Te pathogenesis of MS remains unknown [10,11]. However, it is widely accepted that MS is an autoimmune disease, which is at least in part caused by T cell mediated mechanisms, can be triggered by environmental factors, and is related to the immunogenetic background of an individual and associated with multiple genes [15]. The basis for the autoimmune hypothesis comes from several lines of evidence, including the animal model experimental allergic encephalomyelitis (EAE), the nature of the pathological changes, and the presence of a dysimmune state in patients with MS [10]. The immune pathogenesis of MS centers on the T lymphocyte. The CD4+ T cell subset can be further subdivided into T helper (Th) 1 and 2. Th 1 cells secrete the proinflammatory cytokines, interleukin (IL) 2, interferon gamma (IFNγ) and tumor necrosis factor alfa (TNFα) [10]. Elevated level of these proinflammatory cytokines including TNFα and IFNγ has been reported in MS, being increased before episodes [12]. Additionally, *in vitro* studies has shown that TNFα induces demyelinization [14].

Ghrelin is a newly discovered hormone, with a potent orexigenic action, controlling energy expenditure, adiposity, and growth hormone secretion [1,7]. Additionally, nonfunctional receptor mRNA variant identified as GHSR type 1b has recently been identified within a wide variety of tissue including lymphoid organs [6]. Expression of ghrelin and the GHSR signaling system in human T, B lymphocytes and neutrophils has been documented [2]. Another study has been demonstrated that ghrelin exerts both specific and selective inhibitory effects on the expression and production of the inflammatory cytokines including IL-1β, IL-6, and TNFα by human peripheral blood mononuclear cells and T cells [1,7]. It has recently been reported that an increase in the level of circulating leptin within a murine MS model regulates inflammatory anorexia and disease susceptibility [4]. Moreover, it has been reported that in EAE fasting-induced suppression of leptin levels dramatically attenuates the onset of EAE [5,13].

The higher levels of plasma ghrelin in patients with MS were independent of BMI, serum cholesterol and triglycerides as they were not significantly different from those values of the control group. Since some

of the MS patients were not receiving any treatment but still had higher plasma ghrelin levels, and lack of information for the possible interaction of agents used in MS treatment (interferons and glatiramer acetate) with ghrelin levels, it is unlikely that the higher serum ghrelin levels were due to MS therapy.

In conclusion, results from this study indicated that patients with MS have higher plasma levels of ghrelin. This may have risen to support the anti-inflammatory capacity of the patients. There is a need for further studies involving larger groups of patients for a more conclusive statement on possible role of ghrelin on the pathogenesis and serving as a target for treatment strategies of MS.

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