Odontology (2014) 102:59–67
DOI 10.1007/s10266-012-0100-3

ORIGINAL ARTICLE

Ghrelin levels in chronic periodontitis patients

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Received: 28 March 2012 / Accepted: 5 December 2012 / Published online: 5 January 2013

Abstract Ghrelin is a peptide hormone that has modulatory effects on the immune system. This study was designed to evaluate plasma ghrelin levels in patients with chronic periodontitis and to investigate if a relationship exists between ghrelin and periodontal parameters, serum cytokines, and bone turnover markers. Thirty-five chronic periodontitis patients (CP) and periodontal healthy individuals (C) were included in this study. Periodontal parameters were recorded. Blood samples were obtained to determine the levels of total and acylated ghrelin, interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), the soluble receptor activator nuclear factor kappaB ligand (sRANKL), alkaline phosphatase (ALP), and osteocalcin (OSC). Plasma levels of total and acylated ghrelin were significantly elevated in the CP group compared with the C group ($p < 0.05$). The difference was significant only between males in the two groups (groups were compared with respect to gender) ($p < 0.05$). There was no difference between the groups regarding the levels of serum sRANKL, TNF-α, and ALP. A relative increase in the serum levels of IL-1β and a decrease in the serum levels of OSC of the CP group were observed ($p < 0.05$). In addition, positive correlations between total ghrelin/ALP and total ghrelin/acylated ghrelin were discovered. We found no direct correlation between ghrelin levels and periodontal parameters. Our results indicate an increase of total and acylated ghrelin levels in patients with chronic periodontitis. Further, studies in larger populations (which could include ghrelin levels in gingival tissue, gingival crevicular fluid, and saliva) are needed in order to confirm the role of ghrelin in periodontal disease.

Keywords Ghrelin · Chronic periodontitis · Alkaline phosphatase · Osteocalcin · Pro-inflammatory cytokines

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Introduction

Ghrelin is a recently described peptide hormone that is secreted predominantly by the stomach. Substantially lower amounts have also been detected in other organs, cells, and tissues such as the pituitary gland, salivary glands, teeth, heart, cells of the immune system, and osteoblasts [1–3].

Two major forms of ghrelin are present in tissue and blood: Des-acylated ghrelin and acylated ghrelin. Both forms of ghrelin have important physiological roles in growth hormone secretion, food intake, and energy metabolism [4]. Acylation is necessary for the binding of ghrelin to the growth hormone secretagogue receptor (GHS-R1a) and stimulation of growth hormone expression [1]. Recent studies have demonstrated that the anti-inflammatory activity of ghrelin is related to the level of acylation [5, 6].

Major research studies on ghrelin have primarily focused on its effects on endocrine function. Recent findings suggest evidence of ghrelin having modulatory effects on the immune system and bone metabolism [7–9]. Ghrelin down-regulates the lipopolysaccharide (LPS) induced pro-inflammatory cytokine production (including interleukin

IL-1β and tumor necrosis factor [TNF)-α) and exhibits strong anti-inflammatory activity [10].

In addition to anti-inflammatory activity, ghrelin also stimulates the differentiation and proliferation of osteoblastic cells and enhances bone formation [7–9]. Deng et al. [11] showed that ghrelin significantly increases the expression of alkaline phosphatase (ALP), osteocalcin (OSC), and collagen type-1 (all markers of osteoblast differentiation). In addition, ghrelin levels were positively correlated with osteoprotegerin (OPG) levels and negatively correlated with the soluble receptor activator nuclear factor kappa-B (sRANKL) levels [12].

Higher ghrelin levels have been reported in chronic inflammatory diseases such as ankylosing spondylitis, Crohn's disease, and inflammatory bowel disease [13]. However, ghrelin levels were decreased in type 2 diabetes mellitus, obesity, and metabolic syndrome [14]. Such studies suggest that circulating ghrelin levels are influenced by the presence of systemic inflammation, although the understanding of the exact mechanisms of the regulation of ghrelin secretion is only beginning to emerge.

Periodontitis is a chronic inflammatory disease characterized by the loss of connective tissue attachment and bone surrounding the teeth in response to bacterial accumulation [15]. Although microorganisms are the primary etiologic agents, chemical mediators of inflammation are responsible for the destruction of periodontal tissues. Studies have documented significantly elevated serum and tissue levels of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α in subjects with periodontitis [16, 17]. Increased levels of pro-inflammatory cytokines also contribute to the alveolar bone loss by inducing RANKL expression and suppressing OPG expression [18]. Furthermore, recent studies suggest that periodontitis affects not only the tooth supporting apparatus, but also the overall systemic health status of a patient [19, 20]. Due to the systemic effects of periodontitis, we considered that periodontitis may affect circulating ghrelin levels (similarly to other chronic inflammatory diseases). To date, there is no study evaluating the circulating ghrelin levels in patients with periodontitis.

The aims of the present study were to (I) evaluate the circulating levels of ghrelin in patients with chronic periodontitis and (II) determine whether ghrelin levels were related to other clinical periodontal parameters (serum cytokine levels and serum bone-turnover markers).

Materials and methods

Study population

This study was carried out in 70 subjects (age, 29–42 years): 35 chronic periodontitis patients (CP) [11 women (F) and 24 men (M)] and 35 individuals without periodontitis (C) (12 F and 23 M). The subjects were selected from individuals who attended Süleyman Demirel University, Faculty of Dentistry, Department of Periodontology for dental treatment.

Exclusion criteria were as follows: Current or ex-smoking; pregnancy; currently lactating; oral contraceptive drug usage; current hormone replacement therapy; menopause; periodontal therapy in the past 6 months; antibiotic use; immune-modulation or anti-inflammatory drug usage in past 3 months; existence of a major systemic disease and/or immune system abnormality; or aggressive periodontitis and oral pathology.

All participants were subjected to a detailed, systemic examination to determine the individual's medical status. The initial examination included measuring serum lipid profiles and blood glucose levels. Serum lipid profiles and fasting blood glucose (FBG) levels were analyzed in Süleyman Demirel University, Faculty of Medicine, and Department of Biochemistry. Data relating to age, gender, and body mass index (BMI) (kg/m^2) were also recorded.

Subjects with a body mass index (BMI) of >18.5 and <25; FBG <110 mg/dL; high-density lipoprotein cholesterol (HDL) >35 mg/dL; low-density lipoprotein cholesterol (LDL) <130 mg/dL; total cholesterol (TC) <200 mg/dL; and triglycerides (TRG) <200 mg/dL were included in the study.

The inclusive enrollment dates of the study were from July 2009 to July 2010. The study protocol was approved by the local ethics committee (date: 10.02.2009, number: 02). All eligible subjects were informed in detail concerning the study and written consent was obtained.

Periodontal parameters

The following clinical parameters were recorded during periodontal examination: Plaque index (PI) [21], gingival index (GI) [22], percentage of bleeding on probing sites (BOP %), probing depth (PD), and clinical attachment levels (CAL) of each tooth by the same clinician (G.Y.). All assessments were performed using a periodontal probe (Willams periodontal probe, Hu-Friedy, Chicago, IL, USA).

Following periodontal recordings, subjects were divided into two groups as periodontal healthy (C) and chronic periodontitis (CP). The diagnosis was based on the clinical and radiographic criteria stated and described on the 1999 Consensus Classification of Periodontal Diseases [23]. Subjects with CP should have at least 14 teeth, and more than 30 % of the sites should have a PPD ≥4 mm and clinical attachment loss ≥4 mm. Periodontal healthy individuals should have a mean GI < 1, mean percentage of BOP ≤ 25 %, and no sites of attachment loss.

Laboratory analysis

Venous blood was collected early in the morning before breakfast and after overnight bed rest (between 9.00 and 10.00 am). Assays were performed for plasma levels of total ghrelin, acylated ghrelin, serum sRANKL, IL-1β, TNF-α, ALP, and OSC.

In order to measure the total and acylated ghrelin levels, 2 mL of venous blood was collected in a chilled EDTA tube and 40-μL aprotinin (Sigma-Aldrich, USA) was added for every 1 mL of blood. Samples were immediately centrifuged at 3500 rpm for 10 min at +4 °C. Supernatants were then transferred into separate tubes, 100 μL of 1 N HCl per mL of collected plasma was added, and immediately centrifuged at 3500 rpm for 5 min at +4 °C. Supernatants were then transferred in Eppendorf tubes and stored at −80 °C until analysis. Aprotinin and hydrochloric acid were added to prevent the degradation of acylated ghrelin by proteases. Plasma total and acylated ghrelin levels were determined using commercially available ELISA kits (Millipore Human Ghrelin Active/Total ELISA kit, USA).

In order to evaluate the serum levels of sRANKL, IL-1β, TNF-α, ALP, and OSC, 8 mL venous blood was collected in biochemical tubes. For sRANKL, IL-1β, and TNF-α analyses, samples were centrifuged at 4000 rpm for 4 min at room temperature, and then supernatants were transferred into separate Eppendorf tubes and stored at −80 °C until analysis. Serum TNF-α, IL-1β and sRANKL levels were measured utilizing commercial ELISA kits (Diasource TNF-α/IL-1β Human EASIA kit, Belgium; Biovendor sRANKL Human ELISA kit, Czech Republic).

For ALP and OSC analyses, samples were centrifuged at 10000 rpm for 4 min at room temperature. The serum ALP level was determined by a spectrophotometer method (Olympus AU 2700, Japan) and the OSC level was assessed using an electrochemiluminescence immunoassay method (ELECSYS 2010, Japan) immediately after sample collection.

Statistical analysis

Power analysis was performed using statistical software (NCSS/PASS 2000 Dawson Edition, NCSS, Kaysville, UT). The significance level was 0.05, the standardized difference was 0.50, and the statistical power of the study was >70 % for this study.

All statistical analyses were performed using the statistical software (SPSS 15.0, Chicago, IL, USA). Kolmogorov–Simirnov test was used to calculate the normality distribution of the data and Levene's variance homogeneity test was performed to determine the equality variances of groups. The results indicated that the data were normally distributed and equality of variance was maintained across the groups. Therefore, the differences between the groups, including all parameters, were tested using an independent sampling *t* test. The combined effects of gender and periodontal status on the levels of total and acylated ghrelin were analyzed using a 2-level factorial model of analysis of variance. A Chi-square and risk estimation test was used for determining the associations between qualitative variables (periodontal condition and total/acylated ghrelin). Correlations between serum, systemic, and clinical periodontal parameters were determined using Pearson's correlation analysis. Quantitative data were recorded as a mean ± standard deviation. Qualitative data were presented as frequency and percentage. *P*-values of less than 0.05 were considered statistically significant.

Results

A total of 70 subjects, 35 patients with chronic periodontitis (11 F, 24 M) and 35 controls without periodontitis (12 F, 23 M), were included in this study. All of the subjects included in this study completed our evaluations. The mean ages for the CP and C groups were 36.91 ± 2.78 and 35.54 ± 3.72 years, respectively. Table 1 shows the subject characteristics of the study groups. There were no

Table 1 Subject characteristics of study groups (mean ± SD)

	C ($n = 35$) (12F/23 M)	CP ($n = 35$) (11F/24 M)	*p* value
Age	35.54 ± 3.72	36.91 ± 2.78	0.085
BMI (kg/m^2)	21.95 ± 1.21	22.30 ± 1.20	0.230
FBG (mg/dL)	90.49 ± 6.73	93.74 ± 7.36	0.057
TC (mg/dL)	164.60 ± 26.44	168.17 ± 23.32	0.410
TRG (mg/dL)	93.91 ± 39.40	95.20 ± 35.29	0.886
HDL (mg/dL)	50.91 ± 9.34	50.37 ± 11.34	0.828
LDL (mg/dL)	97.57 ± 30.54	100.19 ± 24.88	0.693

C systemically and periodontally healthy control group, *CP* chronic periodontitis group, *BMI* body mass index, *FBG* fasting blood glucose, *TC* total cholesterol, *TRG* triglyceride, *HDL* high density lipoprotein cholesterol, *LDL* low density lipoprotein cholesterol

Table 2 Clinical periodontal parameters of study groups (mean ± SD)

Periodontal parameters	C ($n = 35$) (12F/23 M)	CP ($n = 35$) (11F/24 M)	p value
GI	0.16 ± 0.08	1.33 ± 0.34	**0.000**
PI	0.23 ± 0.09	2.10 ± 0.65	**0.000**
BOP %	6.91 ± 5.77	93.75 ± 11.04	**0.000**
PD	1.66 ± 0.15	4.11 ± 0.44	**0.000**
CAL	1.68 ± 0.17	4.90 ± 0.87	**0.000**

C systemically and periodontally healthy control group, *CP* chronic periodontitis group, *PI* plaque index, *GI* gingival index; *BOP%* bleeding on probing, *PD* probing depth, *CAL* clinical attachment level

Statistically significant difference was shown in bold ($P < 0.05$)

significant differences in age, gender, BMI, TC, HDL, LDL, TRG, and FBG between the groups ($p > 0.05$).

Periodontal parameters of the study groups are given in Table 2. All clinical parameters were significantly lower in the C group than in the CP group ($p < 0.05$). In the C group, there were no differences with regard to gender ($p > 0.05$); but in the CP group, PD and CAL levels were significantly higher in men than in women ($p < 0.05$).

Table 3 illustrates serum parameters of the study groups and subgroups according to gender. Serum OSC level was lower and IL-1β level was higher in the CP groups than in the C groups ($p < 0.05$). These differences were significant only between the male subgroups ($p < 0.05$). There were no significant differences in terms of ALP, sRANKL, and TNF-α levels between the groups or the subgroups ($p > 0.05$).

As shown in Table 4, the CP group had higher total and acylated ghrelin levels than the C group ($p < 0.05$), and these differences were significant only between the male subgroups ($p < 0.05$). Although there was no statistically significant difference, the women in the CP group had higher total ghrelin and lower acylated ghrelin levels than did those in the C group ($p > 0.05$).

Furthermore, in the C group, both total and acylated ghrelin levels were higher in female subjects (however, these differences were not statistically significant [$p > 0.05$]). On the contrary, in the CP group, the male subjects had higher total and acylated ghrelin levels.

Owing to the fact that total and acylated ghrelin levels were affected by a combination of periodontal status and gender, the effects of gender on the ghrelin levels were tested with a 2-level factorial model of analysis of variance. The interaction between periodontal condition and gender can be explained by the statistically significant differences for the levels of acylated ghrelin ($p < 0.05$), but not for total ghrelin ($p > 0.05$) (Table 5).

For further analysis, participants were divided into two groups on the basis of a median of high or low total/acylated ghrelin levels. Chi-square and risk estimation tests were performed considering plasma total or acylated ghrelin levels as dependent variables and periodontal disease as an independent variable. According to these analyses, the presence of periodontitis was associated with increased levels of total and acylated ghrelin (OR, 1.78; 95 % CI, 0.70–4.49; OR, 2.25; 95 % CI, 0.87–5.86, respectively) (Table 6).

Table 3 Serum parameters of study groups and subgroups by gender (mean ± SD)

Serum parameters	Group	n (C/CP)	C	CP	p value
ALP	Total	35	59.14 ± 16.63	60.80 ± 13.71	0.651
	Female	12/11	51.25 ± 12.02	56.00 ± 15.34	0.416
	Male	23/24	63.26 ± 17.41	63.00 ± 12.62	0.953
OSC	Total	35	18.00 ± 5.49	14.68 ± 3.49	**0.004**
	Female	12/11	16.74 ± 6.26	13.92 ± 1.36	0.158
	Male	23/24	18.66 ± 5.06	15.03 ± 4.10	**0.010**
sRANKL	Total	35	193.49 ± 112.01	233.17 ± 129.81	0.175
	Female	12/11	215.67 ± 101.97	205.36 ± 76.66	0.788
	Male	23/24	181.91 ± 117.39	245.92 ± 147.70	0.108
IL-1β	Total	35	77.49 ± 87.09	163.13 ± 190.02	**0.016**
	Female	12/11	81.37 ± 65.25	110.00 ± 101.36	0.426
	Male	23/24	75.46 ± 85.34	187.48 ± 216.66	**0.025**
TNF-α	Total	35	1.11 ± 0.77	1.55 ± 1.50	0.125
	Female	12/11	1.18 ± 0.66	0.98 ± 0.89	0.549
	Male	23/24	1.07 ± 0.83	1.81 ± 1.67	0.062

C systemically and periodontally healthy control group, *CP* chronic periodontitis group, *ALP* alkaline phosphatase, *OSC* osteocalcin, *sRANKL* soluble receptor activator of nuclear factor-kappaB, *IL-1β* interleukine-1β, *TNF-α* tumor necrosis factor-α

Statistically significant difference was shown in bold ($P < 0.05$)

Table 4 Plasma total and acylated ghrelin levels of study groups and subgroups by gender (mean ± SD)

Serum parameters	Group	*n* (C/CP)	C	CP	*p* value
Total ghrelin	Total	35	391.29 ± 212.30	514.61 ± 297.96	**0.050**
	Female	12/11	482.07 ± 204.54	502.46 ± 341.48	0.862
	Male	23/24	343.93 ± 204.69	520.18 ± 283.62	**0.019**
Acylated ghrelin	Total	35	158.12 ± 74.36	197.54 ± 71.25	**0.027**
	Female	12/11	187.69 ± 66.14	165.60 ± 55.21	0.397
	Male	23/24	142.69 ± 75.05	212.19 ± 73.95	**0.003**

C systemically and periodontally healthy control group, *CP* chronic periodontitis group

Statistically significant difference was shown in bold ($P < 0.05$)

Table 5 Subject characteristics of study groups from two-level factorial analysis of variance model (mean ± SD)

Dependent variable	Gender	Group	*n*	Mean	Standard deviation	%95 Confidence Interval		*p* value
						Lower bound	Upper bound	
Total ghrelin	Female	–	23	492.27	258.40	384.70	599.84	0.363
	Male	–	47	432.05	258.18	356.86	507.25	
	–	C	35	413.00	271.90	321.23	504.27	0.139
	–	CP	35	511.32	278.06	417.49	605.15	
	Female	C	12	482.07	258.14	333.29	630.86	0.240
		CP	11	502.46	258.13	347.06	657.86	
	Male	C	23	343.92	258.16	236.46	451.39	0.240
		CP	24	520.18	258.13	414.98	625.39	
Acylated ghrelin	Female	–	23	176.64	70.59	147.25	206.03	0.965
	Male	–	47	177.44	70.54	156.89	197.98	
	–	C	35	165.19	74.31	140.12	190.26	0.191
	–	CP	35	188.89	75.96	163.26	214.53	
	Female	C	12	187.69	70.53	147.04	228.34	**0.013**
		CP	11	165.60	70.51	123.14	208.06	
	Male	C	23	142.69	70.55	113.33	172.05	**0.013**
		CP	24	212.19	70.55	183.44	240.93	

C systemically and periodontally healthy control group, *CP* chronic periodontitis group

Statistically significant difference was shown in bold ($P < 0.05$)

In the CP group, there was a positive correlation between total ghrelin and ALP ($r = 0.352$, $p = 0.038$). Total ghrelin also correlated positively with acylated ghrelin ($r = 0.347$, $p = 0.041$). The levels of total and acylated ghrelin did not correlate with serum inflammatory markers or clinical periodontal parameters ($p > 0.05$).

Discussion

In this study, we evaluated the plasma levels of total and acylated ghrelin in chronic periodontitis patients. Furthermore, we examined the relationship between plasma total and acylated ghrelin levels, clinical periodontal parameters, and serum markers of bone turnover and inflammation.

Following the discovery of the various effects of ghrelin outside of the known effects on appetite regulation, most studies have focused on the role of ghrelin in inflammation. In most research studies, ghrelin has been found to have inhibitory effects on inflammatory cell proliferation and pro-inflammatory cytokine production [10, 24–26]. Kodama et al. [27] observed that administration of ghrelin suppresses neutrophil accumulation and production of myeloperoxidase, IL-8, and TNF-α. It that Administration of exogenous ghrelin has also been reported to suppress the production of IL-1β and TNF-α and stimulate the production of IL-10. Additionally, the protein levels of IL-4 and IL-13 were observed to increase after stimulation of T-cells with ghrelin [28]. Shimizu et al. [29] showed that ghrelin administration in rat endothelial cells improves

Table 6 Chi-square and risk estimation results considering total and acylated ghrelin

Independent variable	Total ghrelin		*p* value*	Odds ratio (OR) (%95 CI)	Acylated ghrelin		*p* value*	Odds ratio (OR) (%95 CI)
	<367.4	≥367.4			<166.9	≥166.9		
Periodontal condition								
C	20 (57.1)	15 (42.9)	0.232	1	21 (60.0)	14 (40.0)	0.094	1
CP	15 (42.9)	20 (57.1)		**1.78** (0.70–4.58)	14 (40.0)	21 (60.0)		**2.25** (0.87–5.86)

* Chi-square test

C systemically and periodontally healthy control group, *CP* chronic periodontitis group

Statistically significant difference was shown in bold ($P < 0.05$)

endothelial dysfunction. Similarly, Chow et al. [26] stated that ghrelin shows anti-inflammatory activity against LPS in human vascular smooth muscle and endothelial cells.

In a recent study, oral epithelium and fibroblasts were shown to produce ghrelin, and higher concentrations of ghrelin to be present in gingival crevicular fluid (GCF) compared with salivary concentrations in subjects without periodontitis. Additionally, ghrelin was found to suppress IL-8 production by TNF-α- or LPS-stimulated oral epithelial cells [30].

Apart from the determination of ghrelin concentrations in GCF, salivary, and periodontal tissues in patients without periodontitis [30, 31], to our knowledge, this is the first study designed to evaluate the circulating levels of total and acylated ghrelin in patients with chronic periodontitis. According to our results, the CP group had higher total and acylated ghrelin levels in comparison with the C group. Hataya et al. [32] reported that administration of LPS decreased the levels of ghrelin in the early phase, but repeated LPS administration caused an increase in ghrelin levels. In addition, increased ghrelin levels were reported in inflammatory diseases such as ankylosing spondylitis, inflammatory bowel disease, and celiac disease [13, 33, 34]. Poykko et al. [35] reported that plasma ghrelin levels correlated positively with the early phase of atherosclerosis formation. It was suggested that expression of pro-inflammatory cytokines during inflammation induces ghrelin expression [36]. Mafra et al. [37] showed that the serum total ghrelin concentration correlated positively with TNF-α and IL-6 levels. Similarly, Sung et al. [38] reported that systemic alteration of TNF-α affected circulating ghrelin levels. However, some diseases such as type 2 diabetes mellitus, obesity, and metabolic syndrome caused a decrease in the plasma ghrelin levels [14, 39, 40].

All of these studies reported that circulating ghrelin levels could be affected by inflammation and cytokine production; however, it is still unknown how the severity of inflammation and/or phase of the disease progression could affect ghrelin levels. Moreover, in the majority of studies, only total ghrelin levels were evaluated and no attempts were made to assess the levels of acylated or des-acylated ghrelin. Acylation of ghrelin is crucial for anti-inflammatory activity [41]. Therefore, in addition to total ghrelin, acylated ghrelin levels were also considered in our study.

When assessing the influence of chronic periodontitis as an independent variable on total and acylated ghrelin levels, the odds ratio values were 1.78 and 2.25 for total and acylated ghrelin, respectively. Higher total and acylated ghrelin levels were found in the CP group compared with those in the C group. However, total and acylated ghrelin levels did not correlate with any clinical periodontal parameters. Therefore, we could not comment on the relationship between increased plasma ghrelin levels and chronic periodontitis. Furthermore, based on the findings of previous studies reporting a variety of activity of ghrelin in different tissues or organs, it could be concluded that the effects of ghrelin are cell specific [26, 29]. In order to clarify the relationship between ghrelin levels and chronic periodontitis, a study that compares the plasma ghrelin levels, gingival tissue ghrelin levels, and/or GCF would be valuable.

Ghrelin is also associated with bone metabolism. Determination of ghrelin and GHS-R1 in rat osteoblast shows that osteoblastic cells are responsive to ghrelin signaling. Recent studies have reported that ghrelin induces differentiation and proliferation of osteoblasts [7]. In addition, ghrelin was observed to induce gene expressions of ALP and OSC.Local ghrelin administration in rat calvarial defects was stated to increase the production of ALP and OSC [11]. On the basis of these findings, it could be concluded that ghrelin plays a role in enhancing bone formation [7–9].

On the other hand, other studies have reported that there was no direct association between ghrelin and parameters of bone metabolism (such as OSC or pro-collagen type-1 amino-terminal pro-peptides) [42, 43]. However, a recent study has shown that ghrelin is positively correlated with OPG and negatively correlated with sRANKL in patients with chronic renal disease [12]. In contrast, Di Carlo et al. [42] have reported that there was no correlation between ghrelin and RANKL or OPG in postmenopausal women.

In our study, there was a significant positive correlation between total ghrelin and ALP in patients with chronic

periodontitis, but plasma total and acylated ghrelin levels were not associated with OSC and sRANKL. Bone destruction in periodontitis is episodic, and bone resorption is followed by bone apposition [44]. Our patients had moderate periodontitis. The positive correlation between ALP and ghrelin may be related to bone metabolic activity in periodontal disease. It may be necessary to evaluate patients on the basis of periodontitis severity and/or aggressive periodontitis to assess this relationship. Owing to the fact that levels of OPG were not assessed in this study, we could not draw any conclusions as to the effects of ghrelin on the ratio of sRANKL/OPG in patients with chronic periodontitis.

Previous studies have reported that plasma ghrelin levels are affected by several factors such as BMI, age, and gender [39, 40]. In the literature, conflicting findings have been reported concerning the effects of gender on the levels of ghrelin. Some of these studies have shown that gender did not have any influence on the ghrelin levels [45, 46], while others suggested that ghrelin levels were higher in women [40, 47].

In the present study, it was found that both total and acylated ghrelin levels were higher in women in the C group, but this finding was not statistically significant. Moreover, in women, acylated ghrelin levels were lower in the CP group than in the C group; however, this finding was also not statistically significant. In this regard, the small numbers of female subjects in the groups poses a limitation on the study. When gender was considered separately, men in the CP group had significantly higher total and acylated ghrelin levels than those in the C group.

When the effects of gender and periodontal status on ghrelin levels were analyzed together, we found that acylated ghrelin was significantly associated with gender in the presence of periodontitis. This finding suggests that ghrelin levels may be affected by sex hormones and inflammation and that its expression is regulated by different mechanisms in men and women. Similar to our suggestion, Cicero et al. [48] remarked that ghrelin had specific interactions with sex hormones and that the ratio of testosterone/estrogen was one of the main determinants of ghrelin levels in women. Greenman et al. [49] also stated that ghrelin correlated positively with testosterone in both men and postmenopausal women.

Plasma ghrelin levels are influenced by age as well as gender. Besides finding a negative correlation between ghrelin and age [40], no association between ghrelin and age has been reported [47]. Contrary to this, Purnell et al. [45] stated that ghrelin was positively correlated with age. In our study, all participants were between 29 and 42 years of age, and we found no correlation between plasma ghrelin levels and age.

Plasma ghrelin levels have also been reported to be influenced by BMI, blood lipid profiles, and FBG (with a negative correlation reported on all 3 parameters) [39]. For this reason, in our study (and regardless of personal declarations), plasma lipid profiles, FBG levels, BMI, and individual medical status were recorded. Furthermore, patients with systemic diseases such as diabetes mellitus, obesity, and cardiovascular disease (all known to affect the plasma levels of ghrelin) were excluded.

In conclusion, both the total and acylated ghrelin levels were higher in chronic periodontitis group. When groups were compared according to sex, these differences were significant only between the male groups, and acylated ghrelin levels tended to decrease in females with chronic periodontitis. These findings suggest that ghrelin and its isotypes may be affected by gender and may display different activity during inflammation. Further studies on larger populations, including exclusively women or men are necessary to clarify whether ghrelin has any role in the pathogenesis of periodontitis. Additionally, comparing plasma ghrelin levels with GCF levels, salivary levels, and gingival tissue levels in patients with different types and severity of periodontitis (and investigation of the effects of periodontal treatment on the levels of ghrelin and its isotypes) may be useful for assessing the activity of ghrelin.

Acknowledgments The authors are grateful to The Unit of Scientific Research Projects of Süleyman Demirel University, Isparta, Turkey, for supporting the study. This study was financially supported by The Unit of Scientific Research Projects of Süleyman Demirel University, Isparta, Turkey, with the project number 1774-D-09.

Conflict of interest The authors declare that they have no conflict of interests.

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