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ORIGINAL ARTICLE

Chemerin and interleukin-6 levels in obese individuals following periodontal treatment

U Balli[1], F Ongoz Dede[2], S Bozkurt Dogan[3], Z Gulsoy[1], E Sertoglu[4]

[1]*Department of Periodontology, Faculty of Dentistry, Bulent Ecevit University, Zonguldak;* [2]*Department of Periodontology, Faculty of Dentistry, Ordu University, Ordu;* [3]*Department of Periodontology, Faculty of Dentistry, Yıldırım Beyazıt University, Ankara;* [4]*Department of Medical Biochemistry, Gulhane Military Medical Academy, Istanbul, Turkey*

OBJECTIVE: To investigate changes in the levels of gingival crevicular fluid (GCF) chemerin and interleukin-6 (IL-6) in both obese/non-obese individuals with periodontitis following non-surgical periodontal therapy.

METHODS: Individuals (n = 80) were split into four groupings according to periodontal/anthropometric parameters: (i) periodontal healthy without obesity; (ii) chronic periodontitis (CP) without obesity; (iii) periodontal healthy with obesity; and (iv) CP with obesity. Individuals with periodontitis were treated with non-surgical periodontal therapy. Both GCF sampling procedures and clinical periodontal measures were performed prior to treatment and 6 weeks thereafter. Enzyme-linked immunosorbent assay was utilized to measure both chemerin and IL-6 levels.

RESULTS: Greater values for chemerin and IL-6 were observed in obese individuals compared to their non-obese controls and in individuals with CP compared to their periodontal healthy controls ($P < 0.008$), which decreased following therapy ($P < 0.05$). A positive correlation was found between chemerin and IL-6 in obese groups ($P < 0.05$). A comprehensive overview of all groups showed a statistically significant positive correlation among chemerin, along with IL-6, waist-to-hip ratio, body mass index, and clinical attachment levels ($P < 0.05$).

CONCLUSION: It could be concluded that chemerin levels may act as both diagnostic and prognostic indicators. Chemerin may also play an integral part in the pathologic mechanisms that relate adipokines to both periodontal disease and obesity.

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Keywords: chemerin protein; human; interleukin-6; periodontal disease; obesity; gingival crevicular fluid

Correspondence: Umut Balli, Department of Periodontology, Faculty of Dentistry, Bulent Ecevit University, 67600 Kozlu, Zonguldak, Turkey. Tel: +90 530 3255749, Fax: +90 372 2613403, E-mail: umutballi@gmail.com
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Introduction

Obesity can be classed as a disease whereby superfluous fat accumulates within the human body to excessive levels in which health can be hindered (Formiguera and Canton, 2004). It is thought to be a low-grade systemic inflammation and has been linked with inflammatory disorder, identified through increased pro-inflammatory proteins (Cancello and Clement, 2006). Despite the contradictory findings, the association between obesity and periodontitis has been shown in many studies, with evidence that greater body mass index (BMI) and waist-to-hip ratio (WHR) are relative to elevated gingival inflammation, clinical attachment loss, and local inflammatory response, that is, increased acute-phase proteins and pro-inflammatory cytokines (Al-Zahrani *et al*, 2003; Linden *et al*, 2007; Ylostalo *et al*, 2008; Khader *et al*, 2009; Kushiyama *et al*, 2009; Gorman *et al*, 2012; Goncalves *et al*, 2015b). The association found in obesity and periodontal disease is hypothesized to be due to the amassing of white adipose tissue (WAT) and increased secretion levels of adipokines from WAT.

White adipose tissue is not only an energy storage organ but also a major key endocrine and secretory organ with a number of metabolic activities (Trayhurn and Beattie, 2001). It secretes numerous immune-modulatory adipokine molecules: adiponectin, leptin, visfatin, resistin, chemerin, omentin, visceral adipose tissue-derived serpin, tumor necrosis factor-α, and interleukin-1β (IL-1β), IL-6 (Vazquez-Vela *et al*, 2008; Wozniak *et al*, 2009). These adipokines, including a variety of pro- or anti-inflammatory cytokines, peptide hormones, and enzymes, appear to be involved in a wide range of biological processes (Wozniak *et al*, 2009; Kloting and Bluher, 2014; Fasshauer and Bluher, 2015). As a whole, adipokines demonstrate much potential for application in therapy relative to obesity and its associated diseases (Fasshauer and Bluher, 2015).

Chemerin was first isolated from human inflammatory fluids and has since been positively associated with a number of diseases involving chronic inflammation (Bozaoglu *et al*, 2007; Jin *et al*, 2015; Patnaik *et al*, 2015). It aids in the management of pro-inflammatory cytokine production, interleukin (IL)-1β, tumor necrosis factor-alpha (TNF-α), IL-6, IL-8, and matrix metalloproteinase-13

(Iannone and Lapadula, 2011). In obese and/or diabetic humans, chemerin has been found in high levels, especially when abdominal adiposity is also a factor (El-Mesallamy *et al*, 2011; Chakaroun *et al*, 2012). Additionally, recent data indicate chemerin-derived peptides have anti-inflammatory properties (Yamawaki *et al*, 2012), leading us to conclude that chemerin could be a factor in the onset as well as the recovery of inflammatory diseases. It is hypothesized here that chemerin may be the functional connection between such diseases as periodontitis and obesity.

The function of IL-6 in periodontal disease has been thoroughly investigated; yet, to our knowledge, no research has hitherto been undertaken to assess chemerin levels in gingival crevicular fluid (GCF) of those with chronic periodontitis (CP) (obese and non-obese individuals) or has investigated the relationship between the IL-6 and chemerin in periodontal disease. The hypothesis is that if chemerin has a role in the pathogenesis of inflammatory events, individuals who have periodontitis with or without obese would present the increased levels of chemerin and IL-6, and also, elevated levels of the adipokines would decrease after treatment. Our primary objective in this clinical study therefore was to investigate how the obesity/periodontitis and non-surgical periodontal treatment affect GCF chemerin levels in periodontitis and to understand how chemerin may be utilized as a potential diagnostic/prognostic biological indicator for periodontal disease. Secondly, we also aimed to discern the relation between chemerin and IL-6, a highly functional, pro-inflammatory adipocytokine.

Materials and methods

Study population

Eighty individuals who had been admitted to the Periodontology Department of Bulent Ecevit University, Faculty of Dentistry (from July 2014 until May 2015), took part in this study. Based on the periodontal and anthropometric measurements, individuals were categorized into four groups: (i) normal-weight individuals featuring a healthy periodontium (Group CTRL); (ii) normal-weight individuals with generalized CP (Group CP); (iii) obese individuals featuring a healthy periodontium (Group O-CTRL); and (iv) obese individuals with generalized CP (Group O-CP). Each provided written consent following briefing of the accepted guidelines approved by the Ethics Committee of the University (Protocol ID: 2014-115-17/06; ClinicalTrials.gov ID: NCT02660814). We used CONSORT (Consolidated Standards of Reporting Trials) 2010 statement and its check list for quality assessment.

Inclusion and exclusion criteria

For all individuals, the following inclusion criteria were adhered to: (i) individuals were between 30 and 49 years of age; (ii) with the exclusion of third molars, had > 20 natural teeth; (iii) glycosylated hemoglobin (HbA1c) levels of < 6.5%; and (iv) fasting plasma glucose (FPG) levels of < 100 mg dl^{-1}.

The exclusion criteria were as follows: aggressive periodontitis, periapical pathologies, exposure to mechanical force as a result of occlusion/orthodontics, and systemic diseases such as cancer, human immune-deficiency virus, diabetes mellitus, or additional diseases which may interfere with adipokines levels and the periodontal conditions, exposure to high-grade steroid therapies, radiation/immuno-suppressive therapies, pregnancy, lactation, smoking over the past 5 years, allergic reaction to any kind of drug, etc. Participants featured no history of either periodontal or drug therapies within the preceding 6 months, namely anti-inflammatory treatments, antibiotic courses, or other pharmacological treatments.

Primary and secondary outcome variables

Primary variables were changes in both chemerin and IL-6 levels following periodontal treatment. Secondary variables include clinical attachment levels (CAL), probing pocket depth (PPD), bleeding on probing (BOP), gingival index (GI), and plaque index (PI).

Assessment of obesity

Anthropometric measurements were ascertained by specialist, with BMI and WHR taken as parameters for obesity according to the World Health Organization criteria (World Health Organization, 2008). For BMI, the height (m) to weight (kg) ratio (kg m^{-2}) was used (Zimmermann *et al*, 2013). WHR was obtained through waist circumference (cm) to hip circumference (cm) ratios (Zimmermann *et al*, 2013).

Abdominal obesity is identified when WHR ≥ 0.85 for females and ≥ 0.90 for males. Inclusion criteria for groups with typical weight were $20 \leq$ BMI < 25 kg m^{-2}, with WHR under that which is determined for obesity (World Health Organization, 2008). For obese groups, inclusion criteria were $30 \leq$ BMI < 40 kg m^{-2}, with a respective WHR ≥ 0.85 for females and ≥ 0.90 for males (Zimmermann *et al*, 2013).

Periodontal examinations

Diagnoses for periodontal conditions were based on the International World Workshop for a Classification of Periodontal Disease and Conditions (Armitage, 1999). All individuals were subjected to radiography as well as a full examination inclusive of the PPD, CAL, GI (Löe and Silness, 1963), BOP (Ainamo and Bay, 1975), and PI (Silness and Löe, 1964). Those with CP were subject to a full examination, again following with scaling and root planing (SRP). The inclusion criteria for healthy individuals were a GI = 0, PPD ≤ 3 mm, and no indication of attachment/bone loss ascertained through clinical/radiographic examination. Inclusion criteria for those with CP were inflammation (red color with a swollen gingival margin), GI ≥ 2, PPD, and CAL ≥ 5 mm, with bone loss affecting > 30% of existing teeth on clinical/radiographic examination.

Intra-examiner reproducibility

PPD and CAL were measured, and PI, GI, and BOP scores were recorded using a Williams periodontal probe (Hu-Friedy, Chicago, IL, USA). Each examination, group determination, site selection, and GCF sampling were all carried out by a single investigator (F.O.D.), blind to the research aims and study population. Preceding measurement, ten individuals were chosen at random for calibration. Individuals were assessed twice with a 48-h interval. Should baseline and +48-h measurements fall within a < 10% mm range, then researcher measurements were considered to be acceptable (Schwarz *et al*, 2006).

Periodontal treatment

Once baseline GCF samples were taken, individuals with CP were subjected to non-surgical periodontal treatment, SRP by means of manual scaling and curets with local anesthetic (Hu-Friedy). Periodontal treatment was completed in 2–3 visits for 2 weeks based on each patient's requisites, and each visit was 45–60 min long. No pharmacological interventions were prescribed as part of the course of treatment. Patients were given oral hygiene instructions, using the modified Bass approach, toothpaste, and interdental tools. The same researcher administered treatment (F.O.D).

Collection of samples

For each group, two sites within each individual were chosen for sampling, with GCF taken from the mesiobuccal or distobuccal sites of single-rooted teeth. Baseline and +6 weeks samples were taken from the same sites following SRP within individuals who had CP. To avoid contamination of GCF with blood as a result of probing an inflamed area, each examination and site selection was made 2 days prior to the collection of GCF samples. In the healthy group, baseline samples were obtained from sites with no clinical inflammation. For those with CP, samples from the most inflamed sites (those with the greatest GI index with BOP and those with high PPD values) were collected, in addition to those with radiographic evidence of alveolar bone loss. Before the sampling, the selected sites were initially isolated with cotton rolls, saliva

was removed, and any supragingival plaque eliminated by means of a sterile curette. GCF samples were obtained via intracrevicular methods (Griffiths, 2003), using filter paper (Periopaper; ProFlow, Inc. Amityville, NY, USA). Strips were positioned within the crevice until resistance was perceived, at which point they were left for 30 s. The degree of GCF on the strips was calculated by weighing the collected liquid. The strips were put into closed and numbered plastic microcentrifuge cases. The liquid was weighed again, immediately after collection, to take evaporation into account (Tuter *et al*, 2010; Dogan *et al*, 2016). Those with visible saliva or blood were subsequently discarded, with two from each individual were placed in a singular Eppendorf tube and made into a unified sample, which was immediately stored at −80°C until analysis.

Quantification of chemerin and IL-6

For the assay procedure, 300 μl of neutral (pH 7.4) phosphate-buffered saline was administered to each sample tube. They were then vortexed and homogenized for 1 min, then centrifuged at 3000***g*** for 15 min at 4°C. Supernatants were then obtained, and chemerin levels (Hangzhou Eastbiopharm Co., Ltd., Hangzhou, China) and IL-6 (Boster Biological Technology Co., Ltd., Pleasanton, CA, USA) were analyzed via a sandwich enzyme-linked immunosorbent assay via commercially accessible kits. All samples/standards were assayed in duplicate following industry recommendations.

Levels were ascertained in pg for both chemerin and IL-6. Detection limits as purported by the manufacturer ranged from 10 and 4.69 pg ml^{-1}, respectively, to a maximum of 3000 ng l^{-1}/300 pg ml^{-1}. Minimum sensitivity levels reported for the assay were < 4.99 ng l^{-1} and < 0.3 pg ml^{-1} for chemerin and IL-6, respectively. Intra- and interassay coefficients of variation were 10%and 12%, and 4.9% and 5.5% for chemerin and IL-6, respectively. Color intensity was measured at 450 nm, with results ascertained through the standard curves provided by each kit.

Statistical analysis

Sample size and study power were both determined on the basis of the primary outcome variable, namely the levels of GCF chemerin and IL-6. However, due to the lack of accurate data on GCF chemerin levels prior to research commencement, sample size could not be calculated. Instead, the data on IL-6 levels reported by Zimmermann *et al* (2013) (minimum difference of 0.10 with 2.63 pg standard deviation between obese and non-obese individuals with periodontitis) were relied upon to determine the sample size necessary for appropriate study power. Hence, for IL-6 levels, a sample consisting of 19 participants in every group was established to accommodate a type II error level of β of 0.20 (80% power) and a two-tailed type I error level of α of 0.05 (5% probability). Twenty participants were recruited for each group to make up for any potential withdrawals. A power of 86% was obtained through *a posteriori* power calculation.

The normal distribution of data was assessed with the Shapiro–Wilk test. Meanwhile, the Kruskal–Wallis nonparametric test was conducted to analyze comparisons between biochemical and clinical parameters. The failure of normality of data prompted the application of the Bonferroni-adjusted Mann–Whitney *U*-test for *post hoc* group comparisons. Regarding the Bonferroni correction, $\alpha = 0.05/6 = 0.008$ was established to have statistical significance. The values at baseline and the post-therapy values were compared through the Wilcoxon signed-rank test (paired observations). The BOP percentage and gender ratio among groups were compared through chi-squared analysis. Furthermore, the correlations between the total quantity of chemerin, IL-6, BMI, and WHR with clinical periodontal parameters were evaluated with Spearman's rank correlation test. Statistical software (version 19.0; SPPS Inc Chicago, IL, USA) was employed to carry out all tests. Statistical significance was established for $P < 0.05$.

Results

Clinical findings

Table 1 gives the demographic values of the study population. BMI and WHR figures were greater in patients with obesity than those of normal weight ($P < 0.05$). Factors of age, gender, HbA1c, and FPG for all categories revealed no statistical significance ($P > 0.05$). Table 2 indicates the clinical findings. CAL, PPD, BOP, GI, and PI taken from both sample sites and the full mouth were greater in the periodontitis groups than the healthy groups ($P < 0.05$). As predicted, significant improvements in clinical parameters were observed in the periodontitis groups following SRP at both the full and site-specific levels ($P < 0.05$). There were no significant differences in periodontal clinical parameters following therapy between CP groups in the full mouth and at the sample sites ($P > 0.05$).

Biochemical findings

Total chemerin levels are represented in Figure 1, which indicates a higher value in those with CP than those without ($P < 0.008$). Total chemerin levels were also higher in obese subjects compared to their normal-weight counterparts ($P < 0.008$). Analyses following periodontal therapy showed a significant reduction in chemerin levels for periodontitis patients ($P < 0.025$). Further, chemerin levels were significantly greater in obese patients with periodontitis, in comparison with individuals of normal-weight periodontitis group following SRP ($P < 0.05$).

Figure 2 denotes the GCF levels of IL-6 in study groups. Total amount of IL-6 was found to be significantly greater in patients with CP than healthy individuals ($P < 0.008$) and was found to decrease following SRP ($P < 0.025$). Moreover, IL-6 levels between CP groups following SRP were also statistically significant ($P < 0.008$). IL-6 levels were also much greater in obese subjects in comparison with their control groups ($P < 0.008$).

Table 1 Demographic values in the study population

	Sex[a] *(male:female)*	*Age*[a] *(years)*	*BMI (kg* m^{-2}*)*	*WHR*	*HbA1c*[a] *(%)*	*FPG*[a] *(mg* dl^{-1}*)*
CTRL	11:9	37.06 ± 3.80 (37.00)	22.75 ± 1.29 (22.94)	0.81 ± 0.03 (0.81)	5.09 ± 0.42 (5.20)	91.39 ± 8.26 (90.00)
CP	10:10	39.67 ± 3.87 (38.00)	22.61 ± 1.35 (22.69)	0.83 ± 0.04 (0.83)	5.15 ± 0.49 (5.20)	92.67 ± 5.76 (91.50)
O-CTRL	11:9	38.17 ± 3.82 (38.00)	33.99 ± 2.28[b] (33.85)	0.94 ± 0.05[b] (0.93)	5.17 ± 0.44 (5.25)	94.78 ± 6.20 (95.50)
O-CP	9:11	40.56 ± 4.11 (39.00)	33.80 ± 2.11[b] (33.21)	0.95 ± 0.04[b] (0.96)	5.20 ± 0.49 (5.30)	96.33 ± 7.45 (96.50)

BMI, body mass index; WHR, waist-to-hip ratio; HbA1c, glycosylated hemoglobin; FPG, fasting plasma glucose; CTRL: normal-weight individuals with a healthy periodontium; CP: normal-weight individuals with generalized chronic periodontitis; O-CTRL: obese individuals with a healthy periodontium; O-CP: obese individuals with generalized chronic periodontitis. Data are expressed as the mean ± standard deviation (median).
[a]No significant difference among groups ($P > 0.008$).
[b]Significant difference from CTRL and CP groups ($P < 0.008$). Kruskal–Wallis/Bonferroni-adjusted Mann–Whitney *U*-test. Bonferroni correction $\alpha = 0.05/6 = 0.008$.

Table 2 Clinical parameters before and after treatment (full mouth and sampled sites periodontal examination) in the study groups

	Before treatment						*After treatment*					
	PPD (mm)	*CAL (mm)*	*GI*	*PI*	*BOP (%)*	*GCF (μl)*	*PPD*^{cd}*(mm)*	*CAL*^{cd}*(mm)*	*GI*^{cd}	*PI*^{cd}	*BOP*^{cd} *(%)*	*GCF*^{cd} *(μl)*
Full-mouth CTRL	2.10 ± 0.27 (2.14)	2.14 ± 0.51 (2.00)	0.13 ± 0.07 (0.13)	0.08 ± 0.04 (0.08)	7.67 ± 1.28 (7.67)							
Sampled sites CTRL	1.92 ± 0.53 (2.00)	2.20 ± 0.47 (2.00)	0.00 ± 0.00 (0.00)	0.00 ± 0.00 (0.00)	0.00 ± 0.00 (0.00)	0.27 ± 0.02 (0.28)						
Full-mouth CP	4.10 ± 0.42 (4.12)	6.14 ± 0.74 (6.00)	2.30 ± 0.40 (2.34)	2.33 ± 0.36 (2.42)	71.66 ± 8.10 (70.94)		2.71 ± 0.43 (2.60)	3.15 ± 0.55 (2.96)	0.77 ± 0.30 (0.82)	0.60 ± 0.36 (0.51)	9.12 ± 1.77 (8.90)	
Sampled sites CP	5.83 ± 0.69 (6.00)	6.20 ± 0.70 (6.00)	2.22 ± 0.43 (2.00)	2.22 ± 0.43 (2.00)	100.00 ± 0.00 (100.00)	0.48 ± 0.07 (0.47)	2.58 ± 0.43 (2.50)	2.94 ± 0.66 (3.00)	0.33 ± 0.49 (0.00)	0.22 ± 0.43 (0.00)	0.22 ± 0.43 (0.00)	0.32 ± 0.09 (0.30)
Full-mouth O-CTRL	2.14 ± 0.28[a] (2.15)	2.56 ± 0.25[a] (2.25)	0.14 ± 0.05[a] (0.15)	0.11 ± 0.05[a] (0.11)	8.08 ± 1.53[a] (7.81)							
Sampled sites O-CTRL	2.08 ± 0.67[a] (2.00)	2.11 ± 0.63[a] (2.00)	0.00 ± 0.00[a] (0.00)	0.00 ± 0.00[a] (0.00)	0.00 ± 0.00[a] (0.00)	0.28 ± 0.04[a] (0.28)						
Full-mouth O-CP	4.05 ± 0.26[b] (4.06)	4.37 ± 0.32[b] (4.41)	2.18 ± 0.35[b] (2.27)	2.25 ± 0.41[b] (2.34)	68.18 ± 10.86[b] (65.73)		2.76 ± 0.32 (2.91)	3.23 ± 0.51 (3.21)	0.76 ± 0.37 (0.82)	0.59 ± 0.32 (0.49)	9.10 ± 2.13 (8.65)	
Sampled sites O-CP	5.94 ± 0.62[b] (6.00)	6.17 ± 0.66[b] (6.00)	2.22 ± 0.43[b] (2.00)	2.17 ± 0.38[b] (2.00)	100.00 ± 0.00[b] (100.00)	0.49 ± 0.06[b] (0.51)	2.67 ± 0.42 (3.00)	3.06 ± 0.57 (3.00)	0.33 ± 0.49 (0.00)	0.28 ± 0.46 (0.00)	0.28 ± 0.46 (0.00)	0.34 ± 0.03 (0.34)

CTRL: normal-weight individuals with a healthy periodontium; CP: normal-weight individuals with generalized chronic periodontitis; O-CTRL: obese individuals with a healthy periodontium; O-CP, obese individuals with generalized chronic periodontitis; PPD, probing pocket depth; CAL, clinical attachment levels; BOP, bleeding on probing; GI, gingival index; PI, plaque index; GCF, gingival crevicular fluid.

[a]No statistically significant difference from CTRL group ($P > 0.008$).

[b]No statistically significant difference from CP group ($P > 0.008$).

[c]No statistically significant difference between groups ($P > 0.008$).

[d]Statistically significant differences from before treatment ($P < 0.025$). Kruskal–Wallis/Bonferroni-adjusted Mann–Whitney *U*-test (Bonferroni correction $\alpha = 0.05/6 = 0.008$) Wilcoxon signed-rank test ($P < 0.05$). Data are expressed as the mean ± standard deviation (median).

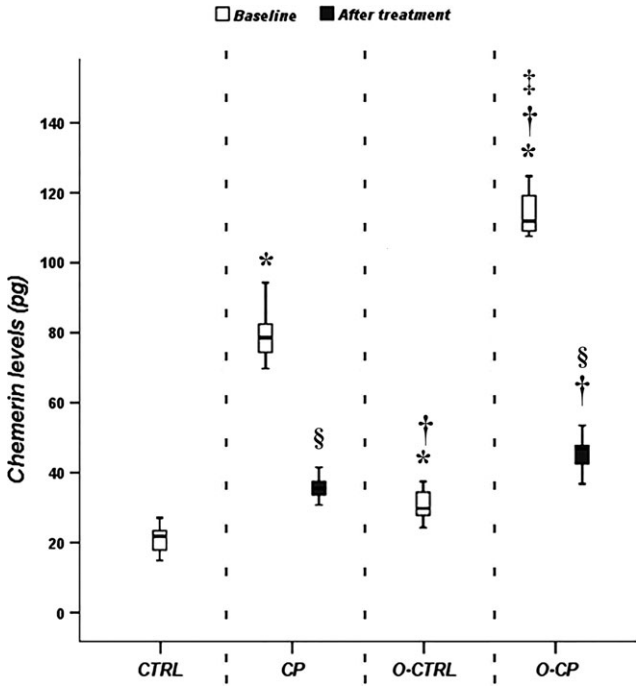

Figure 1 Total amount of Chemerin in GCF. *Statistically significant difference from CTRL (Bonferroni-adjusted Mann–Whitney *U*-test). †Statistically significant difference from CP (Bonferroni-adjusted Mann–Whitney *U*-test). ‡Statistically significant difference from O-CTRL (Bonferroni-adjusted Mann–Whitney *U*-test). §Statistically significant difference from baseline (Wilcoxon signed-rank test). Data are presented as box and whisker plots. The median value is indicated by the line within the box plot. The box extends from the 25th to the 75th percentiles. Whiskers extend to show the highest and lowest values

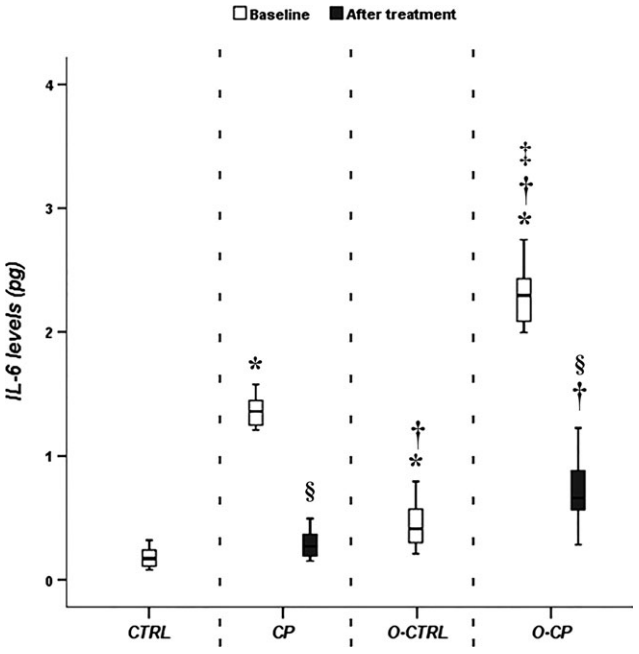

Figure 2 Total amount of IL-6 in GCF. *Statistically significant difference from CTRL (Bonferroni-adjusted Mann–Whitney *U*-test). †Statistically significant difference from CP (Bonferroni-adjusted Mann–Whitney *U*-test). ‡Statistically significant difference from O-CTRL (Bonferroni-adjusted Mann–Whitney *U*-test). §Statistically significant difference from baseline (Wilcoxon signed-rank test). Data are presented as box and whisker plots. The median value is indicated by the line within the box plot. The box extends from the 25th to the 75th percentiles. Whiskers extend to show the highest and lowest values

Correlations

Correlation coefficients are displayed in Tables 3 and 4. A moderate positive correlation between total chemerin and IL-6 levels was found in O-CTRL and O-CP groups ($P < 0.05$). When all clinical groups were examined together, positive correlations of statistical significance from moderate to strong emerged for the presence of chemerin with IL-6, BMI, WHR, and CAL ($P < 0.05$). Chemerin moderately positively correlated with PPD $\geq$ 6 mm sites, but for the O-CP group only, whereas WHR moderately correlated with 4 $\leq$ PPD $\leq$ 5 mm and PPD $\geq$ 6 mm sites ($P < 0.05$). This moderate relationship was also observed for IL-6 and BMI with the presence of 4 $\leq$ PPD $\leq$ 5 mm and PPD $\geq$ 6 mm sites in CP groups ($P < 0.05$).

Discussion

Our results showed that total amount of GCF chemerin and IL-6 was higher in obese individuals compared to their non-obese controls and in individuals with CP compared to their periodontal healthy controls. In addition, these adipocytokines were decreased following therapy in both CP groups. Moreover, GCF chemerin was correlated with clinical parameters in CP individuals with obesity.

In this study, age as a variable was largely minimized through a narrowed age-range criteria, gender by equal distribution, and the researchers were blind to other confounding variables. HbA1c and FPG were evaluated to prevent the effects of glucose metabolism in adipokines.

By increasing GCF volume in diseased areas, GCF elements would be diluted when locally secreted in the GCF (Griffiths, 2003; Lin *et al*, 2005). The GCF volume whereby concentration is affected directly is a dependent variance, as such, data working in concentration values shift to dependent variables in quantifying a GCF element (Lin *et al*, 2005). As a result, quantifying biological indicators by sampling time within the GCF could be a more better, valid, and reliable diagnostic and prognostic. Therefore, the current research is founded primarily on total data amount.

It has been established that obesity is linked to chronic low-grade systemic inflammation (Ouchi *et al*, 2011). Also, it has been suggested that natural killer T, mast, and B cells, as well as T lymphocytes may enter adipose tissue and interfere with adipocytes and macrophages (Anderson *et al*, 2010). Adipocytes enlarge in obese individuals, with adipose tissue experiencing molecular/cellular changes which affects both metabolism and inflammation (Ernst and Sinal, 2010). Serum levels of inflammatory indicators TNF-α and C-reactive protein are heightened for obese individuals (Park *et al*, 2005). IL-6 also has proinflammatory biological functions in both obesity/periodontitis (Zimmermann *et al*, 2013). About one-third of total IL-6 is produced by adipose tissues, and it is plausible that

Table 3 The Spearman's rank correlation (*r*) among groups with respect to chemerin, IL-6, BMI, WHR, and sampled site CAL and GI

	Chemerin to IL-6	*Chemerin to BMI*	*IL-6 to BMI*	*Chemerinto WHR*	*IL-6 to WHR*	*Chemerinto CAL*	*IL-6 to CAL*	*Chemerinto GI*	*IL-6 to GI*
CTRL	0.384	0.350	0.369	0.174	0.382	0.163	0.353	NA	NA
CP	0.413	0.330	0.416	0.368	0.405	0.430	0.545[a]	0.438	0.580[a]
O-CTRL	0.509[a]	0.445	0.328	0.348	0.434	0.206	0.379	NA	NA
O-CP	0.572[a]	0.598[a]	0.506[a]	0.718[b]	0.593[a]	0.493[a]	0.523[a]	0.489[a]	0.516[a]
All groups	0.956[b]	0.409[a]	0.410[a]	0.498[a]	0.509[a]	0.792[b]	0.814[b]	NA	NA

CTRL: normal-weight individuals with a healthy periodontium; CP: normal-weight individuals with generalized chronic periodontitis; O-CTRL: obese individuals with a healthy periodontium; O-CP: obese individuals with generalized chronic periodontitis; IL-6, interleukin-6; BMI, body mass index; WHR, waist-to-hip ratio; CAL, clinical attachment levels; GI, gingival index; NA, not applicable.
[a]Moderate correlation with statistically significant ($P < 0.05$).
[b]Strong correlation with statistically significant ($P < 0.05$).

Table 4 The Spearman's rank correlation (*r*) among groups with respect to chemerin, IL-6, and number of deep sites in CP group

	Chemerin to $4 \leq PPD \leq 5$	*Chemerin to* $PPD \geq 6$	*IL-6 to* $4 \leq PPD \leq 5$	*IL-6 to* $PPD \geq 6$	*BMI to* $4 \leq PPD \leq 5$	*BMI to* $PPD \geq 6$	*WHR to* $4 \leq PPD \leq 5$	*WHR to* $PPD \geq 6$
CP	0.248	0.444	0.470*	0.522*	0.491*	0.506*	0.392	0.407
O-CP	0.424	0.491*	0.482*	0.548*	0.514*	0.547*	0.496*	0.511*

CP: normal-weight individuals with generalized chronic periodontitis; O-CP: obese individuals with generalized chronic periodontitis; PPD, probing pocket depth; IL-6, interleukin-6; BMI, body mass index; WHR, waist-to-hip ratio.
*Moderate correlation with statistically significant ($P < 0.05$).

elevated secretion of IL-6 in obese individuals leads to metabolic problems (Ouchi *et al*, 2011). Here, as hypothesized, IL-6 was considerably increased in CP groups and obese patients. Following periodontal therapy, IL-6 levels were reduced in CP groups.

Chemerin is a chemo-attractant adipokine which has pro- and anti-inflammatory behavior, dependent on the enzymatic cleavage function via differing protease classes in biological events (Yoshimura and Oppenheim, 2008; Lehrke *et al*, 2009; Luangsay *et al*, 2009; Kaneko *et al*, 2011; Yamawaki *et al*, 2012). It also regulates production of pro-inflammatory cytokines, namely IL-1β, TNF-α, IL-8, and MMP-13, associated with periodontitis (Iannone and Lapadula, 2011). Chemerin levels were found to increase in overweight individuals and to relate positively to BMI and WHR (Bozaoglu *et al*, 2007; Weigert *et al*, 2010). This is consistent with our finding that chemerin and IL-6 were increased with obesity and positively correlated with BMI and WHR. In addition, BMI and WHR positively correlated with the number of PPD with 4–5 mm and with 6 mm or more in the present study. Recker *et al*, 2015 reported that waist circumference, but not BMI and fat%, was positively correlated with the severity of periodontitis, whereas Saxlin *et al*, 2011 found that all three parameters were positively correlated with the number of teeth with deepened periodontal pockets. These findings could be interpreted to indicate that increased body weight has a detrimental effect on periodontal status.

Four clinical studies have explored the involvement of chemerin in periodontitis (Ozcan *et al*, 2015, 2016; Patnaik *et al*, 2015; Dogan *et al*, 2016). (Duzagac *et al*, 2015). Patnaik *et al* (2015) reported that human chemerin levels increased from periodontally healthy to CP to individuals with type-2 diabetes/CP. A clinical study found chemerin levels in the saliva of those with periodontitis were correlated with the degree of tissue destruction, and that for these individuals, chemerin levels were higher (Ozcan *et al*, 2015). Between healthy groups and those with gingivitis, no difference was found, which corroborates our results which denoted that with periodontitis, GCF chemerin levels are higher (Ozcan *et al*, 2015). It was also examined here whether non-surgical periodontal therapy affected GCF chemerin levels. Our results are not directly comparable with any other, for no other research has as yet considered non-surgical periodontal therapy effects on chemerin in obese individuals. However, two recent clinical studies have examined non-surgical periodontal therapy effects on chemerin levels in saliva and GCF (Dogan *et al*, 2016; Ozcan *et al*, 2016). Another clinical study by Ozcan *et al* (2016) showed that chemerin levels in saliva were higher in periodontitis individuals than those of healthy individuals and decreased at 6 months after periodontal therapy (Ozcan *et al*, 2016). In our recent study on GCF chemerin levels in individuals with diabetes mellitus, we have demonstrated that chemerin levels in GCF increased with both periodontitis and diabetes mellitus and dropped after SRP (Dogan *et al*, 2016). In the present study, chemerin levels decreased following treatment, which is consist with other studies. Taken as a whole, it may be speculated that chemerin could play a pro-inflammatory part in periodontitis pathogenesis as IL-6. In contrast to biochemical findings, we did not find any difference in the response to periodontal treatment in terms of clinical parameters between obese and non-obese individuals; this finding is similar to studies by Duzagac *et al* and Ongoz Dede *et al* (2015), (2016). Two clinical studies conducted by Goncalves *et al*, 2015a,b indicated obese patients with CP demonstrated lower reduction in

probing depth than non-obese individuals with CP 6 months after SRP; a difference was not observed for 3 months.

Reduction in the levels of chemerin and IL-6 is directly associated with improved clinical outcomes. In addition, mostly moderate correlation of these adipokines with clinical parameters may put forward the idea that decreasing their levels or inhibiting their increment may result in preventing the progression of periodontal disease. Also, management of adipocytokines levels can help improve preventive, diagnostic, and therapeutic strategies against the periodontal disease. Furthermore, these adipocytokines may be a potential part of the relationship between obesity and periodontitis. Certainly, the mechanisms of this relationship and clinical implications are needed to be addressed further studies and intervention.

In this study, we compared four groups and we found higher GCF chemerin levels in obese versus non-obese individuals. Also, chemerin and IL-6 levels are the highest at obese individuals with chronic periodontitis. This study also found GCF chemerin levels were positively correlated with BMI, WHR, CAL, and GI, which were mostly moderate. When quantitative data and correlation findings were evaluated together, the results suggest that chemerin as an adipocytokine is involved in the periodontitis pathogenesis. Periodontal treatment was found to be beneficial on the levels of adipocytokines in GCF. Considering this results in light of similar studies, chemerin may prove new and promising options for understanding and management of the relation between obesity and periodontal disease. However, further longitudinal studies need to be undertaken, with a larger study population, in order to understand the role of chemerin and confirm these results that chemerin may play an active part in the pathogenesis of periodontitis.

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Authors contributions

Umut Balli helped in directing of all study phases, ensuring the implementation of the study calendar, follow-up phases of the study and evaluating the results of the study. Figen Ongoz Dede helped in implementation of the clinical research and providing of samples follow-up. Seyma Bozkurt Dogan helped on selecting of enrolled patients to the study and evaluating the results of the study. Zehra Gulsoy helped in supporting to clinical phases. Erdim Sertoglu helped in preparation the samples for biochemical analyses and evaluating the ELISA tests.

References

Ainamo J, Bay I (1975). Problems and proposals for recording gingivitis and plaque. *Int Dent J* **25**: 229–235.

Al-Zahrani MS, Bissada NF, Borawskit EA (2003). Obesity and periodontal disease in young, middle-aged, and older adults. *J Periodontol* **74**: 610–615.

Anderson EK, Gutierrez DA, Hasty AH (2010). Adipose tissue recruitment of leukocytes. *Curr Opin Lipidol* **21**: 172–177.

Armitage GC (1999). Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* **4**: 1–6.

Bozaoglu K, Bolton K, McMillan J *et al* (2007). Chemerin is a novel adipokine associated with obesity and metabolic syndrome. *Endocrinology* **148**: 4687–4694.

Cancello R, Clement K (2006). Is obesity an inflammatory illness? Role of low-grade inflammation and macrophage infiltration in human white adipose tissue. *BJOG* **113**: 1141–1147.

Chakaroun R, Raschpichler M, Kloting N *et al* (2012). Effects of weight loss and exercise on chemerin serum concentrations and adipose tissue expression in human obesity. *Metabolism* **61**: 706–714.

Dogan SB, Balli U, Dede FO, Sertoglu E, Tazegul K (2016). Chemerin as a novel crevicular fluid marker of periodontitis patients with type 2 diabetes mellitus. *J Periodontol* doi: 10.1902/jop.2016.150657.

Duzagac E, Cifcibasi E, Erdem MG *et al* (2015). Is obesity associated with healing after non-surgical periodontal therapy? A local vs. systemic evaluation. *J Periodontal Res* doi: 10.1111/jre.12340.

El-Mesallamy HO, El-Derany MO, Hamdy NM (2011). Serum omentin-1 and chemerin levels are interrelated in patients with type 2 diabetes mellitus with or without ischaemic heart disease. *Diabet Med* **28**: 1194–1200.

Ernst MC, Sinal CJ (2010). Chemerin: at the crossroads of inflammation and obesity. *Trends Endocrinol Metab* **21**: 660–667.

Fasshauer M, Bluher M (2015). Adipokines in health and disease. *Trends Pharmacol Sci* **36**: 461–470.

Formiguera X, Canton A (2004). Obesity: epidemiology and clinical aspects. *Best Pract Res Clin Gastroenterol* **18**: 1125–1146.

Goncalves TE, Feres M, Zimmermann GS *et al* (2015a). Effects of scaling and root planing on clinical response and serum levels of adipocytokines in patients with obesity and chronic periodontitis. *J Periodontol* **86**: 53–61.

Goncalves TE, Zimmermann GS, Figueiredo LC *et al* (2015b). Local and serum levels of adipokines in patients with obesity after periodontal therapy: one-year follow-up. *J Clin Periodontol* **42**: 431–439.

Gorman A, Kaye EK, Apovian C, Fung TT, Nunn M, Garcia RI (2012). Overweight and obesity predict time to periodontal disease progression in men. *J Clin Periodontol* **39**: 107–114.

Griffiths GS (2003). Formation, collection and significance of gingival crevice fluid. *Periodontol 2000* **31**: 32–42.

Iannone F, Lapadula G (2011). Chemerin/ChemR23 pathway: a system beyond chemokines. *Arthritis Res Ther* **13**: 104.

Jin CH, Yi KW, Ha YR *et al* (2015). Chemerin expression in the peritoneal fluid, serum, and ovarian endometrioma of women with endometriosis. *Am J Reprod Immunol* **74**: 379–386.

Kaneko K, Miyabe Y, Takayasu A *et al* (2011). Chemerin activates fibroblast-like synoviocytes in patients with rheumatoid arthritis. *Arthritis Res Ther* **13**: R158.

Khader YS, Bawadi HA, Haroun TF, Alomari M, Tayyem RF (2009). The association between periodontal disease and obesity among adults in Jordan. *J Clin Periodontol* **36**: 18–24.

Kloting N, Bluher M (2014). Adipocyte dysfunction, inflammation and metabolic syndrome. *Rev Endocr Metab Disord* **15**: 277–287.

Kushiyama M, Shimazaki Y, Yamashita Y (2009). Relationship between metabolic syndrome and periodontal disease in Japanese adults. *J Periodontol* **80**: 1610–1615.

Lehrke M, Becker A, Greif M *et al* (2009). Chemerin is associated with markers of inflammation and components of

the metabolic syndrome but does not predict coronary atherosclerosis. *Eur J Endocrinol* **161**: 339–344.

Lin SJ, Chen YL, Kuo MY, Li CL, Lu HK (2005). Measurement of gp130 cytokines oncostatin M and IL-6 in gingival crevicular fluid of patients with chronic periodontitis. *Cytokine* **30**: 160–167.

Linden G, Patterson C, Evans A, Kee F (2007). Obesity and periodontitis in 60–70-year-old men. *J Clin Periodontol* **34**: 461–466.

Löe H, Silness J (1963). Periodontal disease in pregnancy. I. prevalence and severity. *Acta Odontol Scand* **21**: 533–551.

Luangsay S, Wittamer V, Bondue B *et al* (2009). Mouse ChemR23 is expressed in dendritic cell subsets and macrophages, and mediates an anti-inflammatory activity of chemerin in a lung disease model. *J Immunol* **183**: 6489–6499.

Ongoz Dede F, Bozkurt Dogan S, Balli U, Avci B, Durmuslar MC, Baratzade T (2016). Glutathione levels in plasma, saliva and gingival crevicular fluid after periodontal therapy in obese and normal weight individuals. *J Periodontal Res* doi: 10.1111/jre.12349.

Ouchi N, Parker JL, Lugus JJ, Walsh K (2011). Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* **11**: 85–97.

Ozcan E, Saygun NI, Serdar MA, Kurt N (2015). Evaluation of the salivary levels of visfatin, chemerin, and progranulin in periodontal inflammation. *Clin Oral Investig* **19**: 921–928.

Ozcan E, Saygun NI, Serdar MA, Bengi VU, Kantarci A (2016). Non-surgical periodontal therapy reduces saliva adipokines and matrix metalloproteinases levels in periodontitis. *J Periodontol* doi: 10.1902/jop.2016.160046.

Park HS, Park JY, Yu R (2005). Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. *Diabetes Res Clin Pract* **69**: 29–35.

Patnaik K, Pradeep AR, Nagpal K, Karvekar S, Singh P, Raju A (2015). Human chemerin correlation in gingival crevicular fluid and tear fluid as markers of inflammation in chronic periodontitis and type-2 diabetes mellitus. *J Investig Clin Dent* doi: 10.1111/jicd.12181.

Recker EN, Brogden KA, Avila-Ortiz G *et al* (2015). Novel biomarkers of periodontitis and/or obesity in saliva–an exploratory analysis. *Arch Oral Biol* **60**: 1503–1509.

Saxlin T, Ylostalo P, Suominen-Taipale L, Mannisto S, Knuuttila M (2011). Association between periodontal infection and obesity: results of the health 2000 survey. *J Clin Periodontol* **38**: 236–242.

Schwarz F, Bieling K, Latz T, Nuesry E, Becker J (2006). Healing of intrabony peri-implantitis defects following application of a nanocrystalline hydroxyapatite (Ostim) or a bovine-derived xenograft (Bio-Oss) in combination with a collagen membrane (Bio-Gide). A case series. *J Clin Periodontol* **33**: 491–499.

Silness J, Löe H (1964). Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* **22**: 121–135.

Trayhurn P, Beattie JH (2001). Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. *Proc Nutr Soc* **60**: 329–339.

Tuter G, Serdar M, Kurtis B *et al* (2010). Effects of scaling and root planing and subantimicrobial dose doxycycline on gingival crevicular fluid levels of matrix metalloproteinase-8, -13 and serum levels of HsCRP in patients with chronic periodontitis. *J Periodontol* **81**: 1132–1139.

Vazquez-Vela ME, Torres N, Tovar AR (2008). White adipose tissue as endocrine organ and its role in obesity. *Arch Med Res* **39**: 715–728.

Weigert J, Neumeier M, Wanninger J *et al* (2010). Systemic chemerin is related to inflammation rather than obesity in type 2 diabetes. *Clin Endocrinol (Oxf)* **72**: 342–348.

World Health Organization (2008). *Waist circumference and waist–hip ratio: report of a WHO expert consultation*. World Health Organization: Geneva : 1–47.

Wozniak SE, Gee LL, Wachtel MS, Frezza EE (2009). Adipose tissue: the new endocrine organ? A review article. *Dig Dis Sci* **54**: 1847–1856.

Yamawaki H, Kameshima S, Usui T, Okada M, Hara Y (2012). A novel adipocytokine, chemerin exerts anti-inflammatory roles in human vascular endothelial cells. *Biochem Biophys Res Commun* **423**: 152–157.

Ylostalo P, Suominen-Taipale L, Reunanen A, Knuuttila M (2008). Association between body weight and periodontal infection. *J Clin Periodontol* **35**: 297–304.

Yoshimura T, Oppenheim JJ (2008). Chemerin reveals its chimeric nature. *J Exp Med* **205**: 2187–2190.

Zimmermann GS, Bastos MF, Dias Goncalves TE, Chambrone L, Duarte PM (2013). Local and circulating levels of adipocytokines in obese and normal weight individuals with chronic periodontitis. *J Periodontol* **84**: 624–633.