J Periodontol • August 2016

Chemerin as a Novel Crevicular Fluid Marker of Patients With Periodontitis and Type 2 Diabetes Mellitus

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Background: The objectives of the present study are to: 1) determine whether gingival crevicular fluid (GCF) chemerin is a novel predictive marker for patients with chronic periodontitis (CP) with and without type 2 diabetes mellitus (t2DM); 2) analyze the relationship between chemerin and interleukin (IL)-6 in periodontally healthy individuals and in patients with CP and with and without t2DM; and 3) evaluate the effect of non-surgical periodontal therapy on GCF chemerin levels.

Methods: Eighty individuals were split into four groups: 20 who were systemically and periodontally healthy (CTRL), 20 with t2DM and periodontally healthy (DM-CTRL), 20 systemically healthy with CP (CP), and 20 with CP and t2DM (DM-CP). Individuals with periodontitis were treated with non-surgical periodontal therapy. GCF sampling procedures and clinical periodontal measures were performed before and 6 weeks after treatment. Enzyme-linked immunosorbent assay was used to measure chemerin and IL-6 levels.

Results: Greater values for GCF chemerin and IL-6 levels were found in CP groups than in periodontally healthy groups, in DM-CP than in CP, and in DM-CTRL than in CTRL ($P<0.008$). GCF chemerin and IL-6 levels decreased following therapy in CP groups ($P<0.02$). A comprehensive overview of all groups showed a statistically significant positive correlation of chemerin with IL-6, glycated hemoglobin, sampled-site clinical attachment level, and gingival index ($P<0.05$).

Conclusions: In this study, periodontitis and t2DM induced aberrant secretion of chemerin, and non-surgical periodontal therapy influenced the decrease of GCF chemerin levels in patients with CP with and without t2DM. Furthermore, it suggests GCF chemerin levels may be considered a potential proinflammatory marker for diabetes, periodontal disease, and treatment outcomes. *J Periodontol 2016;87:923-933.*

KEY WORDS

Chemerin protein, human; diabetes mellitus; gingival crevicular fluid; interleukin-6; periodontitis; periodontal disease.

The term diabetes mellitus (DM) refers to a collection of metabolic diseases defined by the inefficient management of glucose metabolism.[1] This persistent hyperglycemic illness is linked to underlying ailments such as myopathies, neuropathy, macrovascular disease, delayed wound healing, and periodontitis.[1,2] With periodontitis, a persistent inflammatory disease, cytokines, chemokines, and inflammatory mediators are produced as tissue defense mechanisms attempt to fight off microbiologic invaders.[3] A number of investigations have shown evidence of a bidirectional link between DM and periodontitis.[4] Changes within the microflora and neutrophil management, alongside delayed wound repair, mean that having DM makes the chance of periodontal disease more likely.[5] It is thought that periodontitis makes it harder for the body to manage DM, but that periodontal treatments can result in healthier glycemic control. Research on the impact of non-surgical periodontal treatments for those who suffer with DM indicates a healthier periodontal condition and improvements to glycemic regulation.[6-8]

Periodontitis and DM seem to share a number of pathogenic features. For instance, they both present increased immuno-inflammatory responses with similar biologic mediators.[9] The progression of DM and periodontitis comes only after mild inflammation and an increase in plasma and gingival crevicular fluid (GCF) levels

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doi: 10.1902/jop.2016.150657

of proinflammatory markers such as C-reactive protein, tumor necrosis factor (TNF)-α, cytokines (interleukin [IL]-1β), IL-6, and prostanoids.[10] Within this environment, proteins (adipokines) produced by the adipose tissue and the tissue defense cells are likely to play a role in any kind of inflammatory reaction. In addition, the adipocytes generate inflammatory cytokines such as TNF-α and IL-6. Traditionally, the inflammatory cytokines were thought to have been generated by macrophages. They are, therefore, used to explain the connection between inflammation and resistance to insulin.[11]

Adipose tissue is an active endocrine organ. It produces a number of inflammatory cytokines, with the most important being adipokines. These cytokines influence insulin sensitivity, alter glucose and lipid metabolism, and impact inflammatory responses.[12,13] A number of these adipokines (leptin) play a part in anti-inflammatory processes relating to tissue defense. Others (resistin and visfatin) have a proinflammatory effect, and some (progranulin) play a proinflammatory and anti-inflammatory role at the same time.[14,15] The relationship between proinflammatory and anti-inflammatory adipokines can lead to a low-grade inflammatory condition, as occurs with periodontitis and DM.[16,17] If plasma levels of proinflammatory adipokines are raised, sufferers may be vulnerable to periodontal disease.[16] However, proinflammatory adipokine volumes can be lowered in patients with periodontitis and DM with the use of non-surgical periodontal treatment.[15,18]

Chemerin, an adipose tissue–specific adipokine was discovered recently. It plays an important part in adipocyte differentiation and development and also influences glucose, lipid metabolism, and inflammation levels.[12,13] It is produced by adipose tissue, the liver, epithelial cells, endothelium, fibroblasts, and keratinocytes.[19] Chemerin controls adipocyte differentiation and adipogenesis via the use of a receptor called chemokine-like receptor 1 (CMKLR1).[13] The process is thought to be involved in both proinflammatory and anti-inflammatory responses.[20] One of the earliest discoveries relating to the role of chemerin was associated with its chemoattractant properties for leukocytes, toward areas of inflammation. Plus, chemerin supports the connection of macrophages to extracellular matrix proteins and adhesion molecules. This aids the union of macrophages to tissue endothelium.[12] There is evidence for the proinflammatory influence of chemerin in that fluctuating chemerin volumes have been seen to correlate positively with recognized markers of inflammation such as TNF-α, IL-6, and C-reactive protein.[12] Thus, it can be identified within inflamed tissues and inflammatory fluids. Alongside these proinflammatory characteristics, experimental study[21] has shown evidence of the anti-inflammatory features of CMKLR1. In fact, an anti-inflammatory influence for chemerin/CMKLR1 was outlined in a mouse model for lipopolysaccharide-induced lung inflammation. In the research, the introduction of recombinant chemerin lowered lung tissue inflammation and alveolar infiltration by neutrophils, particularly in comparison to vehicle-tested mice.[21] To summarize, experimental research has found evidence to support the proinflammatory and anti-inflammatory influence of chemerin within immune cells.[20] This means that there is no clear consensus on whether chemerin participates more in the development of inflammation or its management.

The links among chemerin, DM, and obesity have been thoroughly investigated.[13,22,23] Fluctuating chemerin levels were found to be higher in patients with obesity and type 2 DM (t2DM). It was also suggested that insulin resistance may be a forecaster of chemerin levels, but not necessarily in accordance with body mass index (BMI) or fasting insulin.[22,24,25] Perhaps more significantly, chemerin has been associated with persistent micro- and macrovascular complications.[23] Although there is evidence to support the impact of chemerin on glucose homeostasis, the exact influence and participation is unknown. For the moment, researchers are unable to produce consistent findings. For instance, experiments with cultured 3T1-1–derived adipocytes have offered support for both the interruptive[26] and stimulatory[27] influence of chemerin on glucose uptake.

Two studies[19,28] investigated human chemerin levels during periodontal inflammation. The first[19] examined salivary levels of chemerin during periodontal inflammation. The second[28] looked at volumes of human chemerin within the GCF and tear fluid of patients with chronic periodontitis (CP) with and without t2DM. The studies[19,28] indicated that human chemerin should be treated as a potential GCF and tear-fluid marker of inflammatory activity in CP and DM.

The biologic links between DM and periodontal disease have been widely discussed.[1,2,4] This study focuses on the influence of chemerin within the pathogenesis, potentially connecting DM and periodontal disease. The authors theorize that chemerin may be valuable as an inflammatory facilitator for patients with CP with and without t2DM, and that non-surgical periodontal treatment might have a positive impact on chemerin levels. To the best of the authors' knowledge, chemerin levels of GCF in patients with t2DM and CP after non-surgical periodontal treatment have not been studied to date. Thus, the objective of this research is to: 1) identify the impact of chemerin on the pathogenesis of periodontal disease and DM by assessing GCF levels of IL-6, which exert a recognized proinflammatory influence on periodontal disease and

DM; and 2) analyze the impact of non-surgical periodontal treatment on GCF chemerin levels in patients with CP with and without t2DM.

MATERIALS AND METHODS

Study Population and Study Design

The study volunteers were chosen from a group of participants sourced by the Department of Periodontology, Faculty of Dentistry, Bülent Ecevit University (Zonguldak, Turkey). All of the volunteers were directed to the institution for either dental treatment or a dental examination from July 2014 to May 2015. Ethics approval for this study was granted by the Ethics Committee of the Faculty of Medicine, Bülent Ecevit University, Zonguldak, Turkey, in line with the Helsinki Declaration of 1975, as revised in 2013. The aim of the research was discussed with 80 volunteers, and consent was obtained from each participant whose age and sex demographics, per group, are shown in Table 1. Participants were split into four groups: 1) CTRL (20 healthy controls); 2) DM-CTRL (20 periodontally healthy individuals with t2DM); 3) CP (20 individuals without DM and with CP); and 4) DM-CP (20 individuals with both t2DM and CP).

Study inclusion requirements were: 1) aged ≥35 years and 2) a minimum of 20 natural teeth, not including third molars. Periodontal disease status was defined in line with clinical and radiographic guidelines from the 1999 classification of periodontal disease.[29] CP was identified as a minimum of six teeth exhibiting clinical attachment loss (AL) and a probing depth (PD) of ≥5 mm, positive for bleeding on probing (BOP) within multiple regions, and bone loss affecting >30% of the existing teeth on clinical and radiographic examination. The gingival index (GI) score for indications of inflammation (red color and swelling of the gingival margin) was ≤2 for the CP groups.[30] The periodontally healthy groups had a score of ≤3 mm and measured GI of 0 (absence of clinical inflammation). They presented no evidence of AL and no radiographic evidence of alveolar bone loss (for example, distance between the cemento-enamel junction and the bone crest was <3 mm in >95% of proximal tooth sites).

Patients with DM were defined as those with t2DM diagnosis of ≥1 year. They did not suffer with any additional systemic diseases, only t2DM. The glycemic condition of individuals diagnosed with t2DM was verified using their glycated hemoglobin A1c (HbA1c) and fasting plasma glucose (FPG) measures. To take part in the research, patients with DM had to exhibit HbA1c <8% and ≥6.5% (well controlled)[31] and FPG ≥126 mg/dL[32] at the start of the investigation. All patients with DM were given consistent volumes of oral antidiabetic agents and had not altered their treatments within the 3 months preceding the study.

Table 1.

Demographic Values in the Study Population and GCF Volumes Before and After Treatment Among the Groups [mean ± SD (median)]

Group	Sex (males:females)*	Age (years)*	BMI (kg/m^2)*	HbA1c (%)	FPG (mg/dL)	Duration (years)	GCF Volume (µL)	
							Baseline	After Treatment*
CTRL	11:9	48.80 ± 5.34 (50.50)	21.65 ± 1.52 (21.64)	4.97 ± 0.45 (5.00)	85.80 ± 9.40 (87.5)		0.27 ± 0.03 (0.27)	
CP	10:10	49.05 ± 4.71 (49.50)	22.64 ± 1.67 (22.65)	5.02 ± 0.59 (5.10)	87.75 ± 7.79 (87.00)		0.49 ± 0.03‡ (0.49)	0.32 ± 0.02 (0.31)
DM-CTRL	11:9	48.75 ± 5.71 (49.00)	22.53 ± 1.48 (22.57)	7.31 ± 0.37† (7.30)	159.81 ± 23.75† (165.00)	3.85 ± 0.75* (4.00)	0.28 ± 0.04 (0.28)	
DM-CP	11:9	47.35 ± 4.97 (48.50)	22.51 ± 1.42 (22.52)	7.28 ± 0.46† (7.25)	161.70 ± 23.44† (158.50)	4.05 ± 0.76* (4.00)	0.50 ± 0.03‡§ (0.50)	0.33 ± 0.03 (0.32)

HbA1c = hemoglobin A_{1c}; FPG = fasting plasma glucose.
Kruskal–Wallis/Bonferroni-adjusted Mann–Whitney; Bonferroni correction α = 0.05/6 = 0.008.
* No significant difference among groups (P >0.008).
† Significant difference from CTRL and CP groups (P <0.008).
‡ Statistically significant difference from CTRL and DM-CTRL groups (P <0.008).
§ No statistically significant difference from CP group (P >0.008).

HbA1c and FPG levels were examined for the non-diabetic individuals to verify their non-diabetic status (HbA1c $<6\%$ and FPG <10 mg/dL).[32] The anthropometric measurements taken included weight (kg) and height (m). These were measured as a way to determine BMI (weight divided by the square of height [kg/m^2]).[33] Obesity was described as BMI ≥ 30, overweight as BMI 25 to 29.9, and healthy weight as BMI 20 to 24.9 kg/m^2.[33] The DM conditions and BMI readings for all participants were assessed by an endocrinologist physician, Dr. F. Kuzu, Department of Endocrinology and Metabolic Diseases, Bülent Ecevit University, Zonguldak, Turkey.

Study exclusion parameters included: 1) pregnancy; 2) lactation; 3) smoking (current or former); 4) antibiotic treatment in the 6 months preceding the study; and 5) a systemic condition besides DM (cardiovascular disease, rheumatoid arthritis, osteoporosis, or immunologic disorders) that might influence development of periodontal disease. Also barring involvement was: 1) prescription of non-steroidal anti-inflammatory medications within the 6 months preceding the study; 2) need for antibiotic prophylaxis for dental processes; 3) non-surgical periodontal treatment during the 6 months preceding the study; 4) surgical periodontal treatment during the 12 months preceding the study; or 5) BMI >24.9 kg/m^2.

Clinical Measurements and Periodontal Treatment

Intra-examiner calibration was conducted before the study was launched. Before actual readings were taken, 10 individuals were randomly chosen and used to standardize the investigator (ŞBD). The investigator analyzed all clinical measurements on two specific dates, set 48 hours apart. The standardization of the clinician was judged to be suitable when two sets of readings were $<90\%$ identical at the millimeter level.[34]

The periodontal condition of participants was judged by examining plaque index (PI),[35] GI,[36] PD, clinical attachment level (CAL), and BOP[37] (considered positive if it showed signs within 15 seconds of contact). The degree of periodontal bone loss was calculated using full-mouth periapical radiographs. The clinical readings were carried out on six areas of every tooth (mesio-buccal, disto-buccal, mid-buccal, mesio-lingual, disto-lingual, and mid-lingual), via the use of a periodontal probe.[‡] The readings were taken in millimeters by the same investigator (ŞBD), who was masked to the overall study structure.

Participants were offered information and instructions relating to everyday plaque control. Patients with periodontitis underwent scaling and root planing (SRP), via use of manual scalers and curets,[§] while under local anesthetic. This treatment was carried out independent of adjunctive therapy and finished within 14 days of the start of investigation. Appointments were conducted twice weekly and lasted 45 to 60 minutes. Six weeks after SRP, clinical measurements and GCF sampling were taken again from the CP groups. Periodontal therapy was carried out by the same investigator (ŞBD).

Site Selection and GCF Collection

Clinical and radiologic examinations and sampling site selections were conducted by a single examiner (ŞBD). Samples were gathered the day after patients had been given clinical assessment. This was done to avoid mixing GCF and blood associated with the probing of inflamed areas. Two sites per individual were chosen from each group. GCF samples were taken from mesio-buccal or disto-buccal sites outside of single-rooted teeth. Samples were taken from these areas at baseline and 6 weeks after SRP from all groups. For CP groups, GCF sample procurement was taken from the areas with the most prominent clinical indications of inflammation (the highest GI score with BOP) and the highest PD with radiographic readings of bone loss. For healthy groups, GCF samples were acquired from areas that showed no clinical inflammation (GI = 0, without BOP). Before GCF sampling, all supragingival plaque was eliminated from the sample area with a sterile cotton tool. The site was then washed with water, sectioned off with cotton balls, and gradually air-dried to prevent contamination with saliva. Paper strips[‖] were positioned inside the crevice until a small amount of resistance was encountered and then allowed to remain in place for 30 seconds.[38] The process was conducted delicately to prevent manual damage to gingival tissues. 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, and liquid was weighed again, immediately after collection, to take evaporation into account.[39] Samples consisting of blood and saliva were disregarded. Two strips from each study participant were put into a single (coded) Eppendorf container, combined to form one sample, and quickly stored at –80°C until they could be evaluated.

Biochemical Analyses

For evaluation, 300 µL phosphate-buffered saline (pH 7.4) was incorporated into every GCF container. Containers were then vortexed and homogenized for 1 minute and centrifuged at 3.000 × g for 15 minutes at 4°C, and supernatants were gathered. Overall volumes of chemerin[¶] and IL-6[#] within the samples

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[‡] Williams probe, Hu-Friedy, Chicago, IL.
[§] Hu-Friedy.
[‖] PerioPaper; Oraflow, Smithtown, NY.
[¶] Cat. no. CK-E11406, Hangzhou, China.
[#] Cat. no. EK0410, Boster Biological Technology, Pleasanton, CA.

were evaluated using sandwich enzyme-linked immunosorbent assay carried out with commercially supplied equipment. All samples and standards were evaluated in duplicate, as recommended by the manufacturer.

Overall readings were calculated in picograms for chemerin and IL-6. The usual detection parameters for the chemerin and IL-6 evaluations, as advised by the manufacturer, spanned from a minimum of 10 pg/mL and 4.69 pg/mL, respectively, to a maximum of 3,000 pg/mL and 300 pg/mL. The lowest detection parameters (level of sensitivity) for the evaluation were 4.99 pg/mL for chemerin and <0.3 pg/mL for IL-6. Intra-assay and interassay coefficients of variation were <10% and <12% for chemerin and <4.9% and <5.5% for IL-6. Concentration of color was calculated at 450 nm, and the findings were determined using the standard curves carried by every evaluation kit. GCF chemerin and IL-6 concentrations were determined by dividing the total amount of chemerin (picograms) and IL-6 (picograms) by amount of the GCF (microliter). The readings for concentrations are presented as picograms per microliter.

Statistical Analyses

The primary outcome variable (GCF chemerin levels) was used as a way to determine the sample size calculation and judge the value of the investigation. Nevertheless, no sample size determination could be carried out, as no specific data were found in existing literature about GCF chemerin levels before the study was launched. Thus, estimates had to be taken from the authors' preliminary study (unpublished) that involved only 12 patients per group. It was judged that a sample size of 16 individuals, in line with the chemerin levels for each group, would enable a type II error level of $\beta = 0.20$ (80% power) and a type I error level of $\alpha = 0.05$ (5% probability). To eliminate the influence of prospective withdrawals (individuals leaving the study), a total of 20 individuals was included in every group.

The Shapiro–Wilk test was conducted to judge whether results were typically spread. Comparisons of biochemical and clinical parameters were evaluated via use of a Kruskal–Wallis non-parametric test. This was done by post-study group comparisons, in accordance with the Bonferroni-adjusted Mann–Whitney *U* test, after the typicality of results had failed. For the Bonferroni correction, $\alpha = 0.05/6 = 0.008$ was judged to be statistically valuable. The Wilcoxon signed-rank test with Bonferroni correction (paired observations) was used as a way to contrast the baseline readings with those taken after therapy. For comparison of paired data sets, $\alpha = 0.05/2 = 0.025$ was judged to be statistically valuable. χ^2 analysis was used to contrast the BOP percentage and the distribution of sex within the groups. The Spearman rank correlation test was used to identify the link between overall volumes of chemerin and IL-6 and HbA1c levels with the CAL and GI from sample areas. All comparisons were carried out via the use of statistical software.** $P < 0.05$ was determined to be statistically valuable.

RESULTS

Clinical Findings

No notable disparity of age, sex, or BMI across groups, or for duration of DM across groups with DM was observed. HbA1c and FPG levels were significantly greater in patients with t2DM than in patients without DM ($P < 0.05$) (Table 1). Full-mouth and sample-site PD, AL, BOP, PI, and GI were statistically greater among the CP groups (CP and DM-CP) than among the periodontally healthy groups (CTRL and DM-CTRL) ($P < 0.05$). Mean PD, CAL, BOP, PI, and GI levels were significantly lower among the CP groups after non-surgical periodontal therapy ($P < 0.05$) (shown in Table 2). GCF volumes were significantly greater among the CP groups than the periodontally healthy groups ($P < 0.008$). There was no real variation or discrepancy between the CP groups for GCF volume ($P > 0.008$). GCF volumes significantly decreased after non-surgical periodontal therapy for both CP groups ($P < 0.05$) (Table 1).

Biochemical Findings

Total amounts and concentration levels of chemerin are presented in Figure 1. The total amount and concentration of GCF chemerin was statistically significantly greater among CP groups than periodontally healthy groups ($P < 0.008$). The total amount and concentration of chemerin was statistically significantly greater among the DM-CP group than the CP group, and in the DM-CTRL group than the CTRL group, ($P < 0.008$). The total amount of chemerin dropped among the CP groups after SRP ($P < 0.03$).

Total amounts and concentration levels of IL-6 are presented in Figure 2. The total amount and concentration of IL-6 was significantly greater among CP groups than the periodontally healthy groups ($P < 0.008$). The total amount of IL-6 was significantly greater among the DM-CP group than the CP group, and in the DM-CTRL group than the CTRL group ($P < 0.008$). The total amount of IL-6 among CP groups dropped after SRP ($P < 0.03$).

Correlations

Correlation coefficients are presented in Table 3. A positive correlation was discovered between total amount of chemerin and IL-6 for all groups. A statistically valuable positive correlation was discovered among the total amount of chemerin with

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** SPSS, v.19.0, IBM, Chicago, IL.

Table 2.

Clinical Parameters Before and After Treatment (full-mouth and sampled-site periodontal examination) in Study Groups [mean ± SD (median)]

Group	PD (mm)	CAL (mm)	GI	PI	BOP (%)
CTRL					
Full-mouth	2.28 ± 0.29 (2.35)	2.36 ± 0.30 (2.36)	0.13 ± 0.07 (0.14)	0.15 ± 0.08 (0.15)	8.70 ± 2.51 (8.49)
Sampled sites	2.10 ± 0.38 (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)
CP					
Full-mouth					
Before treatment	4.09 ± 0.37 (4.09)	4.25 ± 0.35 (4.26)	2.34 ± 0.34 (2.39)	2.38 ± 0.32 (2.43)	75.83 ± 10.07 (75.25)
After treatment[‡]	2.61 ± 0.30 (2.57)	2.88 ± 0.38 (2.88)	0.81 ± 0.30 (0.81)	0.54 ± 0.30 (0.54)	8.91 ± 1.68 (8.93)
Sampled sites					
Before treatment	5.80 ± 0.59 (6.00)	6.20 ± 0.70 (6.00)	2.50 ± 0.51 (2.50)	2.60 ± 0.50 (3.00)	100.00 ± 0.00 (100.00)
After treatment[‡]	2.43 ± 0.37 (2.50)	2.85 ± 0.61 (3.00)	0.35 ± 0.49 (0.00)	0.30 ± 0.47 (0.00)	0.35 ± 0.49 (0.00)
DM-CTRL					
Full-mouth	2.23 ± 0.34* (2.21)	2.32 ± 0.36* (2.38)	0.15 ± 0.09* (0.17)	0.16 ± 0.10* (0.15)	9.13 ± 2.52* (8.33)
Sampled sites	2.10 ± 0.50* (2.00)	2.20 ± 0.57* (2.00)	0.00 ± 0.00* (0.00)	0.00 ± 0.00* (0.00)	0.00 ± 0.00* (0.00)
DM-CP					
Full-mouth					
Before treatment	4.11 ± 0.34[†] (4.08)	4.31 ± 0.36[†] (4.26)	2.29 ± 0.40[†] (2.42)	2.55 ± 0.33[†] (2.61)	70.29 ± 8.35[†] (67.59)
After treatment[‡]	2.67 ± 0.26 (2.63)	3.09 ± 0.43 (3.00)	0.76 ± 0.34 (0.81)	0.54 ± 0.31 (0.58)	9.24 ± 1.89 (9.45)
Sampled sites					
Before treatment	5.78 ± 0.55[†] (6.00)	6.15 ± 0.65[†] (6.00)	2.55 ± 0.51[†] (3.00)	2.50 ± 0.51[†] (2.50)	100.00 ± 0.00[†] (100.00)
After treatment[‡]	2.45 ± 0.46 (2.50)	2.98 ± 0.68 (3.00)	0.40 ± 0.50 (0.00)	0.35 ± 0.49 (0.00)	0.30 ± 0.47 (0.00)

Kruskal–Wallis/Bonferroni-adjusted Mann–Whitney; Bonferroni correction $\alpha = 0.05/6 = 0.008$.
* No statistically significant difference from CTRL group ($P > 0.008$).
† No statistically significant difference from CP group ($P > 0.008$).
‡ No statistically significant difference among groups ($P > 0.008$).

IL-6, HbA1c, sampled-site CAL, and GI for all groups, when every clinical group was evaluated at the same time ($P < 0.05$).

DISCUSSION

t2DM is a condition associated with a multitude of dangers; dyslipidemia, visceral obesity, and hyperglycemia are the key factors. Dyslipidemia is one of the most common causes of DM.[13,40] It is fair to argue that periodontitis facilitates its development within diabetic circumstances.[41] It has been suggested that some active molecules increased by periodontitis could disrupt the systematic lipid metabolism, but the exact process is a mystery. Unhealthy adipose tissue metabolism in t2DM sufferers might impact other organs, as a result of the creation of adipokines, TNF-α, IL-6, and other proinflammatory cytokines.[41,42]

Adipose tissue, once considered a basic fat storage area, is now known to be an active endocrine organ that generates many adipokines responsible for controlling lipid metabolism and inflammation.[41] Chemerin is a new adipokine that participates in both metabolic and immune dysfunction.[43] Emerging research[28] indicates that inflammatory cytokines might play a part in chemerin generation within the adipose tissue. A number of investigations[26,44] have demonstrated IL-β– and TNF-α–induced chemerin mRNA expression and production from adipocytes, and can be traced back to the increase of serum chemerin volumes in DM and periodontitis sufferers.[26,44] Abnormal production of chemerin might provoke or intensify the progression of t2DM and periodontitis. Plus, periodontitis could participate in the unhealthy expression of chemerin. This is likely to accelerate unhealthy lipid metabolism in patients with DM. Thus, the objectives of the research were: 1) to determine whether GCF chemerin is a new forecasting indicator for patients with CP with and without t2DM and 2) to determine whether non-surgical periodontal therapy has an impact on GCF levels of chemerin in patients with CP with and without t2DM.

A number of studies[13,22,44] have linked chemerin to various inflammatory markers in obesity and t2DM. An earlier investigation[45] found that plasma chemerin levels were greater among patients who are obese and overweight. Therefore, chemerin is recognized as a potential connection between obesity-related diseases and inflammation.[45] Within the present research, BMI was calculated, and obese individuals with

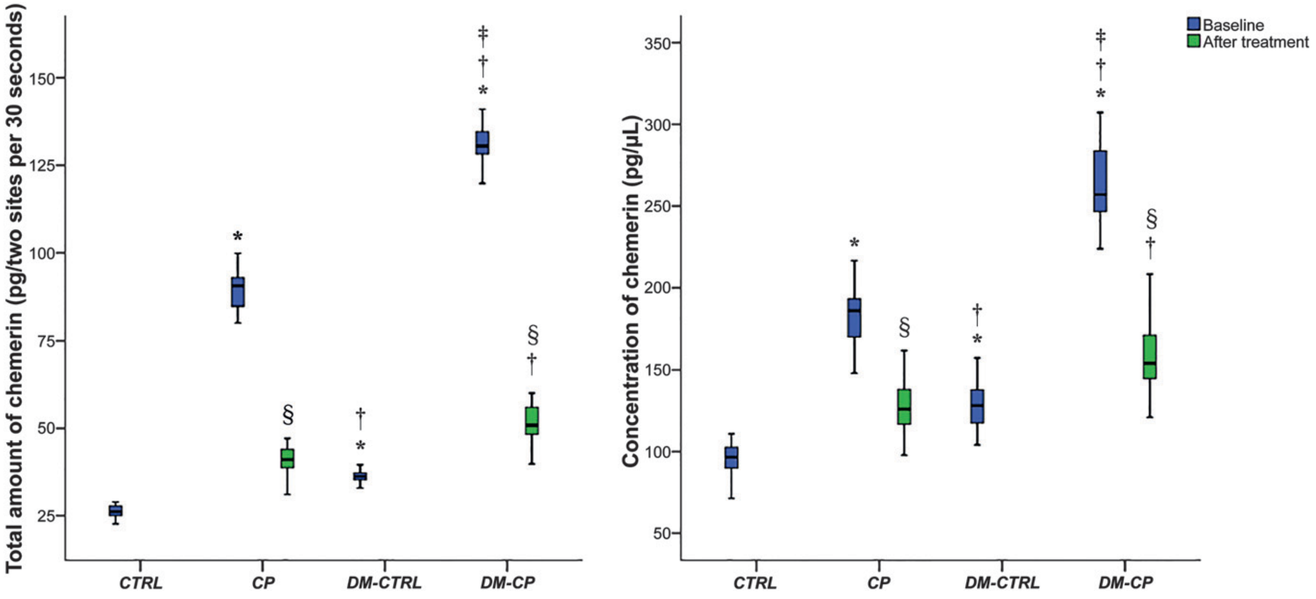

Figure 1.
Total amount and concentration levels of chemerin in GCF among groups. Data are presented as box and whisker plots. Median value is indicated by the line within the box plot. The box extends from the 25th to the 75th percentiles. Whiskers extend to show the highest and lowest values. **Statistically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

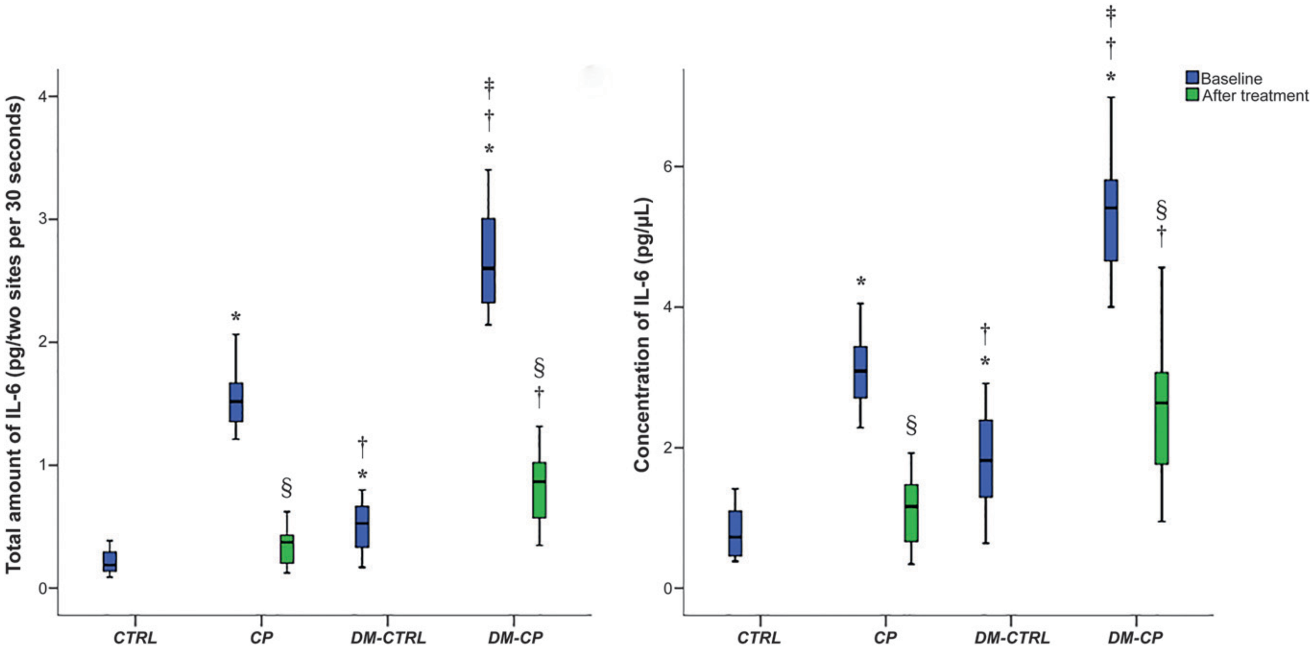

Figure 2.
Total amount and concentration levels of IL-6 in GCF among groups. Data are presented as box and whisker plots. The median value is indicated by the line within the box plot. The box extends from the 25th to the 75th percentiles. Whiskers extend to show the highest and lowest values. **Statistically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

Table 3.

Spearman's Rank Correlation (*r*) Among Groups With Respect to Chemerin, IL-6, HbA1c, and Sampled-Site CAL and GI

Group	Chemerin and IL-6	Chemerin and HbA1c	IL-6 and HbA1c	Chemerin and CAL	IL-6 and CAL	Chemerin and GI	IL-6 and GI
CTRL							
r	0.805*	0.144	0.315	0.373	0.361	NA	NA
P	<0.001*	0.55	0.18	0.11	0.12	NA	NA
CP							
r	0.860*	0.355	0.375	0.486*	0.514*	0.503*	0.521*
P	<0.001*	0.12	0.10	0.03*	0.02*	0.02*	0.02*
DM-CTRL							
r	0.588*	0.583*	0.546*	0.449*	0.328	NA	NA
P	0.006*	0.007*	0.01*	0.047*	0.16	NA	NA
DM-CP							
r	0.769*	0.589*	0.497*	0.622*	0.554*	0.584*	0.532*
P	<0.001*	0.006*	0.03*	0.003*	0.01*	0.007*	0.02*
All							
r	0.978*	0.425*	0.407*	0.813*	0.818*	0.854*	0.853*
P	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

NA = not applicable.
* Statistically significant ($P < 0.05$).

BMI >24.9 were eliminated from the study. Another investigation[46] discovered that chemerin levels are notably greater in men than in women. However, serum chemerin levels were lower in male individuals with t2DM. The research[46] proposed that chemerin levels are closely linked with glycemic status, after accounting for age, sex, and BMI. Thus, for the present research, the impact of age and sex on chemerin levels was kept minimal by incorporating a balanced amount of males and females and only choosing participants aged 35 to 60 years.

In recent years, researchers[47,48] have examined the link between serum chemerin levels in patients with t2DM. Chemerin serum levels were discovered to be higher within the serum of patients with t2DM than in those with normal glucose tolerance.[47] Some researchers[48,49] have demonstrated a connection between higher strengths of circulating chemerin and insulin resistance for patients with both type 1 and type 2 DM. In a similar outcome, chemerin levels in GCF were discovered to be greater in DM-CTRL groups than in CTRL groups within this study. The research identified chemerin levels in GCF, because GCF (which offers more data than indicators in saliva) is positioned close to the periodontal tissues in which periodontal disease emerges.[28] Özcan et al.[19] and Patnaik et al.[28] assessed chemerin levels during periodontal inflammation. Özcan et al.[19] found that salivary chemerin levels were notably greater among the periodontitis group than the healthy and gingivitis groups. Patnaik et al.[28] examined the volumes of GCF and tear-fluid chemerin in CP and t2DM. They discovered that chemerin levels in both fluids were greater among patients with t2DM and CP than periodontally healthy individuals. In line with these findings, GCF chemerin levels in this study were discovered to be greater among CP groups than periodontally healthy groups and greater among the DM-CP group than the CP group. However, Patnaik et al. could not identify variations in chemerin levels of patients with t2DM and normal glucose tolerance.[45] Bobbert et al.[43] discovered that chemerin can be a forecaster for t2DM. Nevertheless, inconsistent findings exist for the link between chemerin and DM. The present findings indicate that chemerin may also be generated locally within the periodontium and might represent chronic inflammation in patients with CP with and without t2DM. This would suggest that it plays a part in the pathogenesis of periodontitis and DM. Chemerin production could also be affected by the link between t2DM and periodontitis.

Periodontal disease and DM are very common chronic conditions, and inflammation might play a vital part in their connection. IL-6 plays an essential part in a number of chronic inflammatory conditions.[50] It participates in leukocyte recruitment, T-cell activation, and apoptosis. Increased volumes of IL-6 at the periodontal sites in patients with DM might be linked to the t2DM modulation of periodontal disease.[51] Researchers[9,52,53] have discovered that local amounts of

IL-6 are greater among patients with t2DM than nondiabetic individuals with periodontitis. Also, it has been demonstrated that serum and GCF IL-6 levels drop following early periodontal therapy for patients with periodontitis and t2DM.[50,54] More significantly, fluctuating chemerin volumes have been found to positively correlate with IL-6 and indicators of inflammation.[43] Thus, this research examined the volumes of IL-6 in GCF, as it has a recognized proinflammatory influence on patients with CP and t2DM. This is why the volumes of GCF chemerin levels were correlated with the GCF IL-6. Within this research, GCF IL-6 volumes were notably greater among CP and t2DM groups than the other control groups. GCF levels of IL-6 dropped after non-surgical periodontal therapy for CP groups. The findings demonstrate that periodontal therapy can notably lower chemerin and IL-6 levels in GCF, indicating a significant difference in all clinical periodontal parameters among both CP groups after treatment. The CP groups exhibited a notable drop in PD, AL, BOP, PI, and GI levels after periodontal therapy. The results of the research propose that non-surgical periodontal therapy could significantly alleviate periodontal inflammation. Plus, a statistically valuable positive correlation was discovered among the total amount of chemerin with IL-6, HbA1c, FPG, CAL, and GI for all groups, when all clinical groups were evaluated at the same time. Hence, it can be argued that chemerin exerts a proinflammatory influence on CP and t2DM, just like IL-6. Finally, the production of chemerin could be provoked by raising IL-6 volumes in individuals suffering from periodontitis and t2DM symptoms. The findings explain why chemerin should be considered therapeutically valuable with regard to treatment of periodontal disease. Further research involving a greater number of participants is vital if increasing GCF chemerin levels is to be treated as a risk-related variable for periodontal disease and t2DM.

CONCLUSIONS

This study examines the influence of periodontitis and t2DM on GCF levels of chemerin and the impact of non-surgical periodontal therapy on GCF chemerin volumes within various groups. Periodontitis and t2DM induced abnormal production of chemerin, and periodontal therapy is advantageous to the reduction of chemerin volumes. The findings indicate that GCF chemerin volumes could be valuable as diagnostic and prognostic variables for the efficiency of periodontal disease and DM therapies. However, additional longitudinal research incorporating greater sample sizes need to be conducted to verify these results and offer more insight into the influence of chemerin on the pathogenesis of periodontitis and t2DM.

ACKNOWLEDGMENTS

The researchers have no financial links to any of the equipment used in this investigation. This study was funded by the researchers. The authors express their gratitude to Dr. Fatih Kuzu, at the Department of Endocrinology and Metabolic Diseases, Faculty of Medicine, Bulent Ecevit University, for his support during the assessment of diabetic status and the calculation of BMI data. Dr. Kuzu reports no conflicts of interest associated with this research. The authors report no conflicts of interest related to this study.

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Submitted November 6, 2015; accepted for publication February 8, 2016.