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The role of interleukin-20 on inflammatory stress and periodontal tissue destruction in patients with metabolic syndrome and periodontitis

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Abstract

Background There is an increasing occurrence of periodontitis and metabolic syndrome (MetS), which is resulting in a decline in the overall quality of life. Both disorders can occur together since they are both linked to insulin resistance and systemic inflammation. However, evidence for a role of interleukin (IL)-20 in this comorbidity is very limited. This cross-sectional study aimed to comprehensively investigate, for the first time, the levels of RANKL/OPG, MMP-8 and OSI as well as the role of IL-20 in patients with MetS and periodontitis.

Methods The study included a total of 80 individuals, divided into four groups: 20 individuals who were healthy both systemically and periodontally, 20 individuals who were systemically healthy but had periodontitis, 20 individuals who had MetS but were periodontally healthy, and 20 individuals who had both MetS and periodontitis. Periodontal clinical parameters (plaque index, gingival index, bleeding on probing, clinical attachment loss, probing pocket depth) were evaluated. Gingival crevicular fluid (GGF) and serum samples were collected and used for biochemical assays. Enzyme-linked immunosorbent assay was used to determine the levels of IL-20, receptor activator of nuclear factor kappa B ligand (RANKL)/osteoprotegerin (OPG), matrix metalloproteinase-8 (MMP-8) and oxidative stress index (OSI).

Results IL-20 levels measured in serum and GCF were statistically significantly highest in patients with MetS and periodontitis ($p = 0.001$). Significant positive correlation was observed between serum and GCF IL-20 values and periodontal parameters ($p < 0.05$). There was a positive correlation between RANKL and RANKL/OPG levels and IL-20 and clinical parameters ($p < 0.05$). OSI values were found to be increased in the presence of both periodontitis and MetS ($p = 0.001$) and were positively correlated with serum and GCF IL-20 ($p < 0.05$).

Conclusions These data from the study suggest a correlation between IL-20 and both MetS and periodontitis. IL-20 may potentially worsen the condition of periodontal tissue by increasing both the oxidative stress levels, periodontal collagen degredation and the ratio of RANKL to OPG.

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Trial registration This study was registered on ClinicTrials.gov (NCT06092853), 2023-10-10, retrospectively registered.
Keywords MetS, Periodontitis, IL-20, RANKL, Oxidative stress

Background

Periodontitis is a chronic inflammatory disease that arises from a combination of many conditions. Periodontitis is characterized by the progressive degradation of the structures that provide support to the teeth and is associated with the accumulation of harmful plaque [1]. It is the primary cause of tooth loss in adults. Periodontitis arises from intricate interactions between pathogenic subgingival biofilms and the immune-inflammatory responses of the host [2]. Periodontitis has been found to be linked to several systemic illnesses, such as cardiovascular disease (CVD), diabetes mellitus (DM), and metabolic syndrome (MetS) [3].

Metabolic syndrome is a cluster of concurrent illnesses that increase the likelihood of CVD and also result in a twofold increase in the risk of developing type 2 diabetes [3]. The most common components of Mets are abdominal obesity, hypertension, and hyperglycemia. It is estimated to impact around 10–84% of the global population and approximately 25% of the population in affluent nations [4].

There has been a rise in the number of studies examining the correlation between periodontitis and MetS. This is due to both conditions being related with insulin resistance and systemic inflammation. Therefore, they may be comorbid [3]. Most studies on this issue reported that periodontitis might lead to or exacerbate MetS [5–7]. Nesbitt et al. [5] concluded that patients with moderate-to-severe alveolar bone loss as evident by radiography were significantly more likely to have MetS than those with minimal or no bone loss. A study by Morita et al. [6] found that deeper periodontal pockets were related with the positive transformation of one or more metabolic constituents over a 4-year period. Furthermore, Lopez et al. [7] claimed that reducing periodontal inflammation might reduce C-reactive protein (CRP) levels in patients with MetS. Iwasaki et al. [8] found that MetS increased the risk of the onset and progression of periodontitis by 2.6 times. Similarly, having more components of MetS was associated with more common and extensive periodontitis [3].

One of the commonalities between MetS and periodontal disease is their relation with oxidative stress, which leads to inflammation and individuals with both MetS and periodontal disease have increased levels of inflammatory markers in the bloodstream [9]. Increased cytokine concentrations and oxidative stress caused by periodontitis may lead to decreased insulin sensitivity. Decreased insulin sensitivity is considered an important condition that may lead to MetS [9]. Oxidative stress is assessed by measuring total antioxidant status (TAS) and total oxidant status (TOS) because it is not practically possible to measure different oxidant molecules separately [10].

Interleukin (IL)-20 is a cytokine classified under the IL-10 family. Prior studies have shown that tumor necrosis factor-alpha (TNF-α) and IL-1β can stimulate the release of IL-20, namely from cells like synoviocytes and macrophages. Multiple studies have demonstrated that IL-20 is involved in the pathogenesis of inflammatory conditions such as atherosclerosis, psoriasis, and rheumatoid arthritis (RA) [11]. IL-20 has recently been identified as a crucial element in controlling the processes of angiogenesis, chemotaxis, osteoblastogenesis, and osteoclastogenesis. A study conducted on patients with RA revealed that IL-20 increased the production of IL-6, IL-1β, IL-8, monocyte chemotactic protein-1 (MCP-1), and TNF-α [12]. IL-20 can also increase the level of the receptor activator of nuclear factor kappa B (RANK) in cells that are precursors to osteoclasts. Treatment with anti-IL-20 mAb 7E can prevent this. Research indicates that IL-20 has a role in controlling the process of osteoclast differentiation through many mechanisms. Osteoblasts release osteoprotegerin (OPG) to counter the heightened bone resorption. OPG interacts with RANKL and inhibits the formation of osteoclasts [12]. In our previous study, GCF and serum IL-20 levels were found to be statistically significantly higher in individuals with periodontitis than in periodontally healthy patients [13].

The study was planned with the hypothesis that IL-20 may be associated with MetS and periodontitis. In this context, the present study aimed to comprehensively investigate the changes in IL-20 and RANKL/OPG, MMP-8 and OSI levels, which are important biomarkers associated with periodontal tissue destruction, and the relationship between IL-20 and these parameters in patients with MetS and periodontitis. This was achieved by comparing the amounts of RANKL/OPG in GCF and serum, as well as the levels of TAS, TOS, and MMP-8 in serum, among people.

Methods

Ethics statement

The Non-Invasive Clinical Research Ethics Committee of Recep Tayyip Erdoğan University granted ethical permission (2022/27). The study was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2013. Each participant in the study received a comprehensive description of the study's goals and content, and they readily signed consent forms.

Study population

This study was conducted on a sample of 80 patients who were admitted to the Periodontology Clinic at Recep Tayyip Erdoğan University's Faculty of Dentistry between January 2022 and October 2022. It was a cross-sectional study design. The study's sample size was determined using the G*Power tool, with an 80% statistical power, an effect size of 0.4 (F-test effect size Large value was taken), and a margin of error of 0.05. The sample size was determined by considering four groups and MMP-8 levels were used as the primary outcome variable [14]. A total of 200 patients were screened, 50 patients for each group. Forty-two patients for the systemically and periodontally healthy group, 39 patients for the systemically healthy and periodontitis group, 37 patients for the metabolic syndrome and periodontally healthy group and 44 patients for the metabolic syndrome and periodontitis group met the inclusion criteria. A total of 80 patients, 20 for each group, were included in the study according to the order of applied to the clinic.

Inclusion criteria for the study were determined as being between 20 and 65 years of age and having at least 20 teeth, having teeth that had successful endodontic treatment (asymptomatic, without apical lesions), not using removable prosthesis and orthodontic appliances, and not having mucocutaneous diseases involving the oral region. The exclusion criteria of the study were; using anti-inflammatory, antibiotic and antioxidant drugs in the last 6 months, receiving periodontal treatment in the last 6 months, smoking, having an autoimmune disease or cancer, breastfeeding or being pregnant, using removable prosthesis and orthodontic appliances, and having mucocutaneous diseases involving the oral region.

All patients were divided into four study groups: the systemically and periodontally healthy control group (CG) ($n=20$); systemically healthy individuals with periodontitis (PG) ($n=20$); periodontally healthy individuals with MetS (MG) ($n=20$), and individuals with both MetS and periodontitis (MPG) ($n=20$).

Study groups were established according to the criteria outlined in the 2017 Classification of Periodontal Diseases [15] and the 2005 Metabolic Syndrome Diagnostic Criteria of the Endocrinology and Metabolic Society of Turkey [16]. Accordingly, inclusion criteria for Mets were determined as having at least one condition among impaired glucose tolerance, diabetes mellitus, and insulin resistance and at least two of hypertension (diastolic blood pressure of >85 mm Hg, systolic blood pressure of >130 mm Hg, or use of antihypertensives), dyslipidemia (triglyceride level of >150 mg/dL or high-density lipoprotein [HDL] level of <50 mg/dL for women and <40 mg/dL for men), and abdominal obesity (abdominal circumference of >80 cm for women and >96 cm for men or body mass index (BMI) of >30 kg/m^2). The criteria for systemically healthy individuals were defined as participants who did not report metabolic, psychiatric, autoimmune, etc. diseases in their self-reports and felt themselves to be healthy, excluding exclusion criteria.

The periodontally healthy control group included patients with probing pocket depth (PPD) of 3 mm or less, no clinical attachment loss (CAL), no radiographic evidence of bone loss, and minimal bleeding on probing of 10% or less. The periodontitis group (stage III-IV periodontitis, grade C) included patients with a PPD of 6 mm or greater, interdental CAL of 5 mm or greater, moderate ridge defect, a history of losing 4 or fewer teeth due to periodontal problems, and radiographic evidence of bone loss extending to the middle or apical thirds of the root. Furthermore, the percentage of bone loss per age was computed for each periodontitis grade, and all instances were categorized as grade C, exhibiting a bone loss per age exceeding 1.0%.

Examiner calibration

Measurements of clinical periodontal parameters were performed by a single experienced periodontist (R.S.). Intraexaminer calibration exercises were performed on forty patients before the study in two different periods, 1 week apart. The intraclass correlation coefficient (ICC) was used to examine the intraobserver agreement between the first and second measurements performed by the same examiner (R.S.). ICC values were 0.983 for PPD and 0.997 for CAL. The ICC values for all measurements were above 0.90, and indicate excellent reliability.

Clinical measurements

To assess the clinical periodontal status, the PPD, CAL, bleeding on probing index (BOP) (Ainamo & Bay, 1975) [17], gingival index (GI) (Löe & Silness, 1963) [18], and plaque index (PI) (Silness & Löe, 1964) [19] of all teeth of the patients were measured. PPD and CAL were assessed on six dental surfaces: mesiobuccal, distobuccal, midbuccal, mesiopalatal/mesiolingual, distopalatal/distolingual, and midpalatal/midlingual. PPD is determined by measuring the distance between the gingival margin and the deepest point of the sulcus. The measurement of CAL involved determining the distance from the cemento-enamel junction to the base of the periodontal pocket. Data on BOP, PI, and GI scores were obtained from four distinct regions (mesial, distal, buccal, and palatal/lingual) of each tooth, excluding the third molars. The presence or absence of bleeding 10–15 s after probing was recorded as BOP. The measurements were conducted utilizing a Williams periodontal probe (Hu-Friedy, Chicago, Illinois, USA). Bone levels were designated via routine radiographic assessments (orthopantomograps).

BMI, abdominal circumference, and current glycated hemoglobin (HbA1c) were measured to evaluate the patients' clinical MetS parameters.

Sample collection

Both venous blood (serum) and gingival crevicular fluid (GCF) samples were obtained from every patient in the morning. In order to prevent the spread of contamination during the examination, samples of GCF were collected 24 h following the clinical periodontal measures. In the periodontally healthy group, the GCF samples were obtained from teeth with a PPD of less than 3 mm, and neither CAL nor BOP was seen. The GCF samples were obtained from locations in the periodontitis group where there was at least 30% bone loss, ≥ 6 mm PPD, and at ≥5 mm CAL. A total of 4 GCF samples were collected from the interproximal regions of the two teeth with the greatest pocket depth in each of the four quadrants. The sampling region was segregated using cotton rolls and thereafter subjected to gentle air drying. When collecting GCF samples, the intracrevicular approach was employed. This involved inserting specialized paper strips (Periopaper, Proflow, Inc., Amityville, NY) into the sulcus until a slight resistance was encountered, and then holding them in place for 30 s. The volume of the GCF was determined using calibrated Periotron equipment, specifically the Periotron 8010 model manufactured by Harco Electronics in Winnipeg, Canada. Strips contaminated with blood or saliva were excluded. The GCF samples were affixed to paper strips and inserted into Eppendorf tubes filled with 200 µL of phosphate-buffered saline at a pH of 7.4. Afterward, a 5 mL sample of serum was collected from the individuals. The serum samples were centrifuged at a rate of 4000 revolutions per minute for a period of 10 min. The resultant liquid fraction (serum) was subsequently transferred meticulously into Eppendorf tubes. The serum and GCF samples were kept in a freezer at a temperature of -80 °C until the day of biochemical analysis.

Biochemical analysis

The serum and GCF parameters of samples collected from all patients were assessed using enzyme-linked immunosorbent assay (ELISA) in the Medical Biochemistry Research and Application Laboratory of the Recep Tayyip Erdoğan University Faculty of Medicine using RANKL (BT Lab Kit, Cat. No. E0620Hu), OPG (BT Lab Kit, Cat. No. E1558Hu), IL-20 (Cat. No. E2163Hu), MMP-8 (Cat. No. E0903Hu), TAS (Cat. No. E4350Hu), and TOS (Cat. No. E1599Hu) Bioassay Technology Laboratory (BT LAB) commercial kits. GCF samples were not pooled, each sample was run separately. They were dissolved in 200 µl PBS. The procedure in the commercial kit used for the determination of RANKL, OPG, IL-20, MMP-8, TAS and TOS was followed. All reagents, standards, microplate and samples were brought to room temperature. Standards and samples were added to the wells of the microplate. Biotinylated test-specific antibody was added to the samples and incubated for 1 h, after which the microplate was washed. Streptavidin-HRP was added to all wells and incubated for 1 h, then the microplate was washed again. Substrate was added to all wells, the blue color after 10 min incubation turned yellow with the addition of stop solution. Optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. OPG, RANKL, IL-20, MMP-8, TAS and TOS concentrations were calculated by comparing the OD of the samples with the standard graphs. A standard graph was drawn with the absorbance values corresponding to the concentrations of the standards and the concentrations of the samples were calculated using this standard graph. OPG and MMP-8 as ng/mL, TAS and TOS as U/mL, IL-20 as ng/L, and RANKL as pg/mL were expressed. To calculate the oxidative stress index (OSI), the following formula was used: $OSI = TOS/(TAS \times 100)$. Not all parameters could be assayed in GCF due to the lack of sample fluid; RANKL, OPG, and IL-20 were evaluated in both GCF and serum, and MMP-8, TOS, and TAS were evaluated in serum.

Statistical analyses

The Shapiro-Wilk test, Q-Qplot, Skewness-Kurtosis value and Levene's test was utilized to determine if the continuous data in the study had a normal distribution. Descriptive statistics for continuous variables are determined by their median, minimum, maximum, percentage (%), and count (n). The Kruskal-Wallis H test was employed to compare measures across distinct groups. After doing the Kruskal-Wallis test, we used the post hoc test with the Bonferroni correction to identify the specific groups that were responsible for the observed differences. Spearman correlation coefficients were computed to ascertain the relationships between continuous variables. Chi-square tests were used to determine the associations between category variables. In addition, A significance level of $p<0.05$ was employed to ascertain statistical significance. The analyses were performed using the SPSS (IBM SPSS for Windows, version 26, USA) statistical software package.

Results

Demographic and anthropometric characteristics

The demographic and anthropometric data of the patients are shown in Table 1. Forty-one men and 39 women aged 29–64 years included in the study. There was no statistically significant disparity in age between the groups ($p>0.05$). Nevertheless, there were statistically significant disparities across the groups for BMI, abdominal

Table 1 Descriptive statistics of study population

Parameters	CG	PG	MG	MPG	*p* value
Age (years)	51.00(29.00–64.00)	56.00(37.00–64.00)	54.50(47.00–63.00)	55.50(47.00–62.00)	0.112*
Sex (females/males) (n)	9/11	10/10	10/10	10/10	0.985**
BMI (kg/m^2)	19.35(17.14–21.03)^b	19.60(17.95–21.37)^b	29.17(25.43–29.72)^a	28.34(25.61–29.40)^a	0.001*
Abdominal Circumference (cm)	88.50(71.00-101.00)^b	87.00(71.00-101.00)^b	102.00(89.00-141.00)^a	101.00(89.00-114.00)^a	0.001*
HbA1c (%)	5.10(4.70–5.50)^b	5.15(4.70–5.60)^b	7.10(6.50–8.30)^a	7.35(6.80–8.30)^a	0.001*
PI	0.93(0.20–1.99)^b	2.49(1.58–2.85)^a	0.78(0.49–1.42)^b	2.41(1.53–2.78)^a	0.001*
GI	0.66(0.22–1.86)^b	2.60(1.83–2.95)^a	0.56(0.23–0.88)^c	2.59(2.01–2.95)^a	0.001*
BOP (%)	2.60(1.20–8.70)^b	91.50(41.30–96.60)^a	2.71(1.50–4.70)^b	87.80(70.20–96.80)^a	0.001*
PPD (mm)	2.17(1.13–2.99)^d	3.33 (2.45–5.02)^b	2.43 (1.97–2.92)^c	3.77(3.37–5.48)^a	0.001*
CAL (mm)	0.00(0.00-0.34)^c	3.25(2.72–4.69)^b	0.00(0.00-0.36)^c	3.81(3.26–5.58)^a	0.001*
GCF volume (µl)	0.22(0.07–0.90)^b	0.52(0.22–0.66)^a	0.25(0.14–0.65)^b	0.55(0.24–0.67)^a	0.001*

Abbreviations BMI, Body mass index; HbA1c, Hemoglobin A1c; CG, Control group; PG, Periodontitis group; MG, Metabolic syndrome group; MPG, Metabolic syndrome-periodontitis group; SD, standard deviation; PI, plaque index; GI, gingival index; BOP, bleeding on probing; PPD, probing pocket depth; CAL, clinical attachment loss

* Significance level between groups according to Kruskal-Wallis H Test; a and b: Shows the difference between pairs according to Post-Hoc paired comparison test with Bonferroni correction. statistically significant at $p<0.05$; Note: All data are expressed as median (min-max), except for sex. min, minimum; max, maximum

** Significance level according to chi-square test results, statistically significant at $p<0.05$

Table 2 Biochemical findings (GCF) of the study groups

GCF		median (min-max)	*p-value
IL-20 (ng/L)	CG	796 (617–962)^b	**0.001***
	PG	893 (744–1123)^a	
	MG	839 (367–1001)^b	
	MPG	956.50 (895–1139)^a	
RANKL (pg/mL)	CG	236 (149–298)^b	**0.001***
	PG	348.50 (191–402)^a	
	MG	229 (200–355)^b	
	MPG	341 (228–435)^a	
OPG (ng/mL)	CG	5.98 (4.26–7.94)^a	**0.001***
	PG	5.19 (2.20–8.11)^b	
	MG	5.47 (4.83–6.19)^a	
	MPG	4.78 (3.74–6.63)^b	
RANKL/OPG	CG	40.33 (25.91–58.21)^b	**0.001***
	PG	67.63 (44.37–86.82)^a	
	MG	41.30 (33.89–60.48)^b	
	MPG	68.71 (50.34–93.95)^a	

Abbreviations IL-20, Interleukin 20; RANKL, Receptor activator of nuclear factor kappa B ligand; OPG, Osteoprotegerin; min, minimum; max, maximum; CG, Control group; PG, Periodontitis group; MG, Metabolic syndrome group; MPG, Metabolic syndrome-periodontitis group

* Significance level between groups according to Kruskal-Wallis H Test; a ve b: Shows the difference between pairs according to Post-Hoc paired comparison test with Bonferroni correction; statistically significant at $p<0.05$

circumference, and HbA1c readings ($p = 0.001$). The measurements in the MG and MPG groups were similar and significantly greater than those in the CG and PG groups. There was no statistically significant difference between the groups in terms of sex ($p > 0.05$). GCF volume value was statistically significantly higher in PG and MPG than in CG and MG ($p < 0001$).

Clinical parameters

Table 1 shows the periodontal clinical data of the patients, categorized into groups. Statistically significant variations were seen among the groups in relation to CAL, GI, PPD, PI, and BOP ($p = 0.001$). PI, GI, and BOP values were similar in the PG and MPG groups and significantly higher in these groups compared with the CG and MG groups. The PPD values of all groups were significantly different from each other. The highest CAL values were observed for the MPG group, followed by PG, MG, and CG, respectively.

Biochemical findings

GCF parameters

The results of intergroup comparisons of the GCF IL-20, RANKL, OPG, and RANKL/OPG values; significant differences were observed between the groups in terms of IL-20, RANKL, OPG and RANKL/OPG values (Table 2, p = *0.001*). The IL-20, RANKL, and RANKL/OPG were similar in the PG and MPG groups and significantly higher in these groups compared with the CG and MG groups. The OPG values were similar in the CG and MG groups and significantly higher in these groups compared with the PG and MPG groups (Table 2).

Serum parameters

The results of intergroup comparisons of the serum IL-20, OPG, MMP-8, TAS, TOS, OSI, RANK levels and RANKL/OPG ratio are shown in Table 3. When the IL-20 values were evaluated, a significant difference was observed between the groups ($p = 0.001$). The MPG group had the highest IL-20 values. However, no significant difference was observed between the groups in terms of OPG values ($p > 0.05$). The RANKL ($p = 0.002$) and RANKL/OPG ($p = 0.001$) values of the groups significantly differed from each other. The RANKL and RANKL/OPG of the PG and MPG groups considerably

Table 3 Biochemical findings (serum) of the study groups

Serum		Median (min-max)	*p-value
IL-20 (ng/L)	CG	141.50 (107–215)[d]	**0.001***
	PG	260.50 (183–413)[b]	
	MG	220.50 (112–333)[c]	
	MPG	305.00 (233–496)[a]	
RANKL (pg/mL)	CG	43 (18–82)[b]	**0.002***
	PG	59 (42–85)[a]	
	MG	47 (23–68)[b]	
	MPG	53.50 (45–151)[a]	
OPG (ng/mL)	CG	2.12 (1.03–3.23)	0.984
	PG	2.03 (1.37–3.19)	
	MG	2.02 (1.10–3.78)	
	MPG	1.97 (1.06–5.57)	
RANKL/OPG	CG	22.02 (13.94–28.73)[b]	**0.001***
	PG	28.66 (22.08–35.67)[a]	
	MG	22.79 (12.54–40.30)[b]	
	MPG	30.33 (21.43–42.61)[a]	
MMP-8 (ng/mL)	CG	1.50 (0.08–4.18)[b]	**0.001***
	PG	2.78 (0.24–6.42)[a]	
	MG	2.36 (1.35–7.14)[a]	
	MPG	2.73 (1.90–5.56)[a]	
TAS (U/mL)	CG	4.39 (2.59–6.67)[a]	**0.010***
	PG	2.64 (1.22–11.42)[a]	
	MG	2.72 (1.32–11.40)[b]	
	MPG	3.57 (2.13–5.83)[b]	
TOS (U/mL)	CG	3.10 (2.26–5.41)[b]	**0.012***
	PG	4.75 (2.49–8.53)[a]	
	MG	3.77 (1.11–16.70)[a]	
	MPG	5.55 (2.61–8.94)[a]	
OSI	CG	0.07 (0.04–0.12)[b]	**0.001***
	PG	0.14 (0.07–0.32)[a]	
	MG	0.13 (0.07–0.27)[a]	
	MPG	0.14 (0.10–0.28)[a]	

Abbreviations IL-20, Interleukin 20; RANKL, Receptor activator of nuclear factor kappa B ligand; OPG, Osteoprotegerin; MMP-8, Matrix metalloproteinase-8; TAS, Total antioxidant level; TOS, Total oxidant level; OSI, Oxidative stress index; min, minimum; max, maximum; CG, Control group; PG, Periodontitis group; MG, Metabolic syndrome group; MPG, Metabolic syndrome-periodontitis group

* Significance level between groups according to Kruskal-Wallis H Test; a, b, c, d: Shows the difference between pairs according to Post-Hoc paired comparison test with Bonferroni correction; statistically significant at $p<0.05$; min, minimum; max, maximum

higher compared with the other groups. MMP-8 levels were lowest in the CG group ($p = 0.001$).

The TAS values of the groups exhibited a significant difference from one another ($p=0.010$). The TAS levels of the CG and MG groups were comparable and significantly greater than those of the PG and MPG groups. There were no significant differences in TOS and OSI levels between the PG, MG, and MPG groups. However, there was a significant difference between these groups and the CG group ($p=0.012$ and $p=0.001$, respectively).

Correlational findings

A positive correlation was found between periodontal clinical parameters and serum and GCF IL-20 levels ($p < 0.01$). There was a positive correlation between GCF IL-20 value and GCF RANKL/OPG, RANKL and a negative correlation with GCF OPG ($p < 0.01$). There was a statistically positive correlation between serum IL-20 values and GCF IL-20, HbA1c, serum MMP-8, RANKL, RANKL/OPG ratio, TOS and OSI levels ($p < 0.01$) (Table 4).

Discussion

This study is the first to evaluate the levels of GCF and serum IL-20 in patients with MetS and periodontitis. It aims to examine the GCF and serum levels of IL-20 in individuals with Mets and periodontitis and to investigate its relationship with other molecules. The study findings demonstrated a positive correlation between GCF and serum IL-20 levels with periodontal clinical indicators. Additionally, GCF IL-20 was positively correlated with GCF RANKL, RANKL/OPG and negatively correlated with GCF OPG. Serum IL-20 was positively correlated with serum MMP-8, RANKL, RANKL/OPG, TOS and OSI. All findings suggest that IL-20 may be related with extracellular connective tissue matrix deterioration, osteoclastogenesis, and oxidative stress in the periodontal region.

Even though radiographic and clinical periodontal parameters provide knowledge about the severity of periodontal disease, these parameters are inadequate in determining presence of active disease. GCF measurements are frequently used in research because they can be collected easily, reproducibly, and non-invasively, as well as being found in the local destruction area of periodontal diseases and they are serum-derived [20]. Therefore, serum and GCF samples were collected from the patients of the present study. The study groups were constituted of male and female patients of similar age to avoid that the study results were not influenced by age and sex.

OPG, a receptor that traps RANKL, is a crucial element in protecting bones and maintaining their balance [21]. Behfarnia et al. [22] observed that there was no statistically significant disparity in the levels of serum OPG between their periodontitis group and their control group. In the current investigation, there was no notable disparity observed between the periodontitis and control groups for the levels of serum OPG. The GCF OPG value was observed to be markedly reduced in the periodontitis group in comparison to the control group [23–25]. Similarly, our investigation revealed that the GCF OPG levels were significantly lower in the group with periodontitis. Nabipour et al. [26] found that there was no statistically significant disparity in serum OPG levels between

Table 4 Correlation analysis

		PI	GI	BOP (%)	PPD	CAL	IL-20 (GCF)	RANKL (GCF)	OPG (GCF)	RANKL/ OPG (GCF)	IL-20 (Serum)	RANKL (Serum)	OPG (Serum)	RANKL/ OPG (Serum)	MMP-8 (Serum)	TAS (Serum)	TOS (Serum)	OSI (Serum)	Age	BMI	Abdominal Circumference
GI	r	0.942*																			
BOP (%)	r	0.871*	0.868*																		
PPD	r	0.802*	0.788*	0.850*																	
CAL	r	0.827*	0.839*	0.854*	0.926*																
IL-20 (ng/L)	r	0.485*	0.581*	0.513*	0.508*	0.593*															
RANKL (pg/mL)	r	0.557*	0.544*	0.523*	0.556*	0.580*	0.444*														
OPG (ng/mL)	r	-0.262*	-0.290*	-0.197	-0.284*	-0.378*	-0.317*	-0.029													
RANKL/ OPG	r	0.609*	0.620*	0.576*	0.633*	0.707*	0.548*	0.744*	-0.630*												
IL-20 (ng/L)	r	0.599*	0.634*	0.654*	0.725*	0.728*	0.491*	0.466*	-0.348*	0.566*											
RANKL (pg/mL)	r	0.472*	0.454*	0.416*	0.394*	0.428*	0.310*	0.239*	-0.428*	0.413*	0.449*										
OPG (ng/mL)	r	0.154	0.172	0.084	0.010	-0.038	0.118	-0.056	-0.102	-0.013	0.072	0.532*									
RANKL/ OPG	r	0.334*	0.330*	0.341*	0.418*	0.496*	0.220*	0.293*	-0.339*	0.442*	0.401*	0.374*	-0.494*								
MMP-8 (ng/mL)	r	0.365*	0.385*	0.424*	0.456*	0.471*	0.406*	0.250*	-0.275*	0.361*	0.429*	0.230*	-0.003	0.230*							
TAS (U/ mL)	r	0.119	0.088	0.00	0.022	0.028	0.011	-0.062	0.063	-0.110	0.184	0.183	0.390*	-0.302*	0.011						
TOS (U/ mL)	r	0.535*	0.480*	0.489*	0.555*	0.524*	0.339*	0.297*	-0.189	0.379*	0.681*	0.447*	0.260*	0.296	0.382*	0.446*					
OSI	r	0.304*	0.236*	0.378*	0.418*	0.368*	0.227*	0.298*	-0.171	0.390*	0.359*	0.152	-0.176	0.336*	0.227*	-0.573*	0.409*				
Age	r	0.010	0.019	0.052	0.037	0.075	0.054	0.056	-0.256*	0.261*	0.178	0.140	0.025	0.155	0.147	0.062	0.099	0.035			
BMI	r	-0.113	-0.137	-0.045	0.125	0.054	0.140	0.019	-0.018	0.031	0.187	0.007	-0.033	0.072	0.179	-0.186	0.174	0.351*	0.184		
Abdominal Circumference	r	-0.032	0.006	0.061	0.213	0.162	0.195	0.078	-0.015	0.062	0.155	-0.114	-0.123	-0.020	0.261*	-0.102	0.124	0.146	-0.016	0.524*	
HbA1c	r	-0.048	-0.027	0.022	0.253*	0.185	0.249*	0.156	-0.127	0.196	0.356*	0.121	-0.065	0.187	0.245*	-0.102	0.304*	0.355*	0.111	0.739*	0.548*

Abbreviations PI, plaque index; GI, gingival index; BOP, bleeding on probing; PPD, probing pocket depth; CAL, clinical attachment loss; IL-20, Interleukin 20; RANKL, Receptor activator of nuclear factor kappa B ligand; OPG, Osteoprotegerin; MMP-8, Matrix metalloproteinase-8; TAS, Total antioxidant level; TOS, Total oxidant level; OSI, Oxidative stress index; BMI, Body mass index; HbA1c, Hemoglobin A1c

*$p<0.05$; r: Spearman's rho Nonparametric Coefficients

individuals with and without MetS. In our investigation, we consistently found no significant difference in serum and GCF OPG levels between patients with MetS and individuals who were systemically healthy. In the present investigation, a statistically significant and negative correlation was detected between GCF OPG values and CAL, PPD, PI, and GI. Our study, like others, discovered a negative connection between OPG values and periodontal clinical indicators [23, 24].

RANKL is a main promoter of osteoclast function and differentiation. It has an important role in osteoclast-related diseases [21]. The present study found that GCF and serum RANKL levels were increased in the periodontitis group compared to the periodontally healthy group. In the literature, in addition to studies that found that individuals with periodontitis had higher GCF RANKL [23, 24] and serum RANKL [23] levels compared to those with periodontally healthy, Behfarnia et al. [22] found no difference. No statistically significant difference was observed in serum RANKL levels between patients with obesity, one of the components of MetS, and healthy individuals [27, 28]. This is thought to be due to kit sensitivity and the fact that serum RANKL is mainly cell-bound and thus only a small fraction of total RANKL, which cannot be detected in circulation. Similarly, in present study, no statistically significant difference was detected between participants with MetS and healthy controls. Concordant with previous studies, RANKL levels were found to have positive correlations with periodontal clinical parameters in present study [23–25].

Changes in the RANKL/OPG ratio can be defined as a predictor of bone resorption [24]. Similar to our study, in various studies, GCF [22–24] and serum [22, 23] RANKL/OPG values of patients with periodontitis were found to be higher than the control group. Likewise, it was reported that the RANKL/OPG ratio was elevated in rats with diabetes [29] and obesity [30], which are components of MetS. Some studies attributed this increased ratio to elevated RANKL and decreased OPG, whereas Perez de Ciriza et al. [31] suggested that OPG was elevated in MetS. Similar to the studies in the literature, in this study, RANKL/OPG ratio was detected to have a positive correlation with periodontal clinical parameters [23, 24].

MMPs are key proteases which play a role in periodontitis and are relateted with periodontal status. MMP-8 is the essential collagenase in periodontitis. Furthermore, 90–95% of the collagenolytic activity in GCF is due to MMP-8. Therefore, MMP-8 is recognised to be one of the most hopeful oral fluid biomarkers for periodontitis [32]. Some studies found that serum MMP-8 was statistically significantly higher in individuals with periodontitis than in periodontally healthy controls [33, 34]. Similar to these studies, serum MMP-8 values were increased in periodontitis in our study. Level of serum MMP-8 was reported to be higher in individuals with MetS [35]. Similarly, level of serum MMP-8 were increased in present study. In addition, MMP-8 levels were positively correlated with IL-20 levels and all clinical parameters measured in the present study.

TOS is a comprehensive measure used to evaluate the total effect of all oxidants in a biological system [36]. Previous research has indicated that individuals with periodontitis have increased serum TOS levels compared to control participants [23, 37–39]. Moreover, this study has demonstrated that the levels of serum TOS were increased in individuals suffering from periodontitis. Multiple investigations have demonstrated that patients with MetS exhibit elevated TOS values [40, 41]. The current investigation found that patients with MetS have elevated levels of serum TOS. However, Torumtay et al. [42] discovered that there was no significant statistical distinction in the serum TOS levels between patients with periodontitis and MetS and patients with periodontitis who were systemically healthy. Several studies have reported a positive correlation between GCF and serum TOS values and periodontal clinical indicators [23, 37]. In our study, a positive correlation was observed between serum TOS values and periodontal clinical parameters.

In our investigation, the serum TAS levels were shown to be greater in individuals with periodontally healthy compared to those with periodontitis. However, this difference was not statistically significant. In the literature, in contrast to studies reporting that individuals with periodontitis had statistically significantly lower TAS levels than periodontally healthy individuals [36, 38, 39], Chapple et al. [43] found no significant difference between individuals with periodontitis and individuals without periodontal disease. While there is an initial rise in antioxidants during the oxidative burst, the continuous overproduction of reactive oxygen species can lead to a decline in antioxidant defenses over time. This may elucidate the disparate findings documented in several studies regarding TAS levels in patients with periodontitis [36]. In addition, there are studies that found higher serum TAS in systemically healthy controls than in those with MetS [44, 45], which is consistent with the present study.

The serum OSI value was higher in the periodontitis group than without the periodontitis. Consistent with our research, previous studies have found that patients with periodontitis had higher serum OSI levels compared with those who are periodontally healthy [38, 39]. In a study including individuals with obesity, a condition associated with MetS, it was found that the levels of serum OSI were consistently greater in obese patients compared with healthy individuals. Similarly, the serum OSI levels were observed to be elevated in obese patients with periodontitis compared to systemically healthy

patients with periodontitis [38]. Our study demonstrated that patients with MetS have higher serum OSI values. In contrast, Torumtay et al. [42] identified no significant disparity in serum OSI values between the groups in their study involving patients with both MetS and periodontitis. The current investigation found a positive relationship between serum OSI levels and periodontal measures.

Our earlier research revealed that individuals with periodontitis had considerably elevated levels of IL-20 in both their serum and GCF compared to those who were periodontally healthy [13]. To our knowledge, this is the first study to examine IL-20 levels in patients with MetS with periodontitis. On the other hand, there are studies investigating the relationship of serum IL-20 levels with numerous inflammatory diseases such as diabetes mellitus, psoriasis and RA. Hence, the findings of this study were analyzed in relation to research on these illnesses, which share a similar etiology with periodontitis.

Research has shown that patients with RA have elevated levels of IL-20, and medications that target IL-20 can help alleviate RA symptoms [46, 47]. IL-20 has a significant impact on both the body's defensive mechanisms and the regulation of glucose metabolism [48]. Elevated levels of IL-20 were observed in patients with diabetes [49] and obesity [50] in comparison to healthy individuals, according to some investigations. In this investigation, similar to these studies, GCF and serum IL-20 levels were significantly higher in individuals with MetS compared to the control group. Conversely, Nikseresht et al. [51] proposed that there is a negative correlation between IL-20 and obesity. In addition, like our prior study, we observed elevated levels of IL-20 in both serum and GCF of patients with periodontitis. These levels were favorably associated with periodontal clinical indicators [13].

IL-20 regulates the osteo-immune system by altering RANK-RANKL signaling, which links synovial inflammation and bone destruction [47]. It has also been suggested that IL-20 contributes to osteoclastogenesis by promoting both the survival and development of osteoclast precursor cells and enhancing RANKL-induced activity [52]. A study of rats with arthritis found that anti-IL-20 antibodies prevented bone destruction and protected against bone loss [47]. IL-20 has been shown to inhibit OPG in bone mesenchymal stem cells and increase RANKL and RANKL/OPG [53]. Some studies have reported that IL-20 enhances the expression of MMP-1 and MMP-13 [54, 55]. Anti-IL-20 therapy has been shown to downregulates IL-20, MMPs, and RANKL in synovial tissues of rats [56]. The results suggest that anti-IL-20 both decreases arthritis symptoms and helps to the prevention of bone destruction. Similarly, in our study, levels of serum IL-20 were positively correlated with serum MMP-8, RANKL and RANKL/OPG values, and levels of GCF IL-20 were positively correlated with GCF RANKL and RANKL/OPG and negatively correlated with GCF OPG. It has been reported that IL-20 causes activation of Ca2+and the protein kinase C/NADPH oxidase pathway, which leads to increased oxidative stress. It has also been shown that the decrease in antioxidant superoxide dismutase (SOD) activity is prevented when IL-20 is blocked [57]. Tsai et al. [58] reported that IL-20 antibody injection significantly reversed MDA levels and upregulated SOD activity compared with the control group, and IL-20 antibody treatment protected tissues against oxidative stress by suppressing oxidative stress through modulation of NADPH oxidase activity. In present study, a positive correlation was found between levels of serum IL-20 and serum TOS and OSI values, in accordance with these studies.

This study has some limitations. First, due to limited sample fluid, all parameters investigated in this study (TAS, TOS and MMP-8) could not be evaluated in GCF. The second limitation is that the various potential molecules that may play a role in the effect mechanism of IL-20 in periodontal pathogenesis in patients with MetS were not evaluated. Third, we did not evaluate pre-treatment and post-treatment parameters with a larger sample size. One of the most important limitations of this study is that a cause-effect relationship cannot be established because it is a cross-sectional study.

Conclusions

IL-20 was found to be significantly elevated in both periodontitis and MetS patients, especially in GCF. This cross-sectional study suggests that IL-20 may contribute to MetS-related periodontal tissue destruction via RANKL-related osteoclastogenesis and oxidative stress increase pathways. Within this context, cohort studies to investigate periodontal therapy-related alterations in IL-20 and related biomarker levels in larger research groups which include MetS patients with both gingivitis and different staged and graded of periodontitis are needed.

Abbreviations

MetS	Metabolic syndrome
IL	Interleukin
GGF	Gingival crevicular fluid
CVD	Cardiovascular disease
DM	Diabetes mellitus
TAS	Total antioxidant status
TOS	Total oxidant status
TNF-α	Tumor necrosis factor-alpha
RA	Rheumatoid arthritis
MCP-1	Monocyte chemotactic protein-1
RANK	Receptor activator of nuclear factor kappa B
OPG	Osteoprotegerin
HDL	High-density lipoprotein
BMI	Body mass index
PPD	Probing pocket depth
CAL	Clinical attachment loss

BOP Bleeding on probing index
GI Gingival index
PI Plaque index

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Author contributions
All authors contributed to the study conception and design. Methodology: RS, HY, OK, SMK; Formal analysis and investigation: RS, HY, OK, SMK, AY; Writing - original draft preparation: RS, HY, SMK; Writing - review and editing: All authors. All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Human ethics and consent to participate
Ethical approval was obtained from the Non-Invasive Clinical Research Ethics Committee of Recep Tayyip Erdoğan University (Decision no: 2022/27). The study was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2013. The purpose and content of the research were explained to all individuals included in the study and voluntary consent forms were signed.

Constent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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