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Gingival crevicular fluid CSF-1 and IL-34 levels in patients with stage III grade C periodontitis and uncontrolled type 2 diabetes mellitus

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Conflict of Interest
No potential conflict of interest relevant to this article was reported.

ABSTRACT

Purpose: Periodontal diseases are inflammatory conditions that alter the host's response to microbial pathogens. Type 2 diabetes mellitus (T2DM) is a complex disease that affects the incidence and severity of periodontal diseases. This study investigated the gingival crevicular fluid (GCF) levels of colony-stimulating factor-1 (CSF-1) and interleukin-34 (IL-34) in patients with stage III grade C periodontitis (SIII-GC-P) and stage III grade C periodontitis with uncontrolled type 2 diabetes (SIII-GC-PD).

Methods: In total, 72 individuals, including 24 periodontally healthy (PH), 24 SIII-GC-P, and 24 SIII-GC-PD patients, were recruited for this study. Periodontitis patients (stage III) had interdental attachment loss (AL) of 5 mm or more, probing depth (PD) of 6 mm or more, radiographic bone loss advancing to the middle or apical part of the root, and tooth loss (<5) due to periodontal disease. Radiographic bone loss in the teeth was also evaluated; grade C periodontitis was defined as a ratio of the percentage of root bone loss to age greater than 1.0. The plaque index (PI), gingival index (GI), presence of bleeding on probing (BOP), PD, and clinical AL were used for clinical periodontal assessments. GCF samples were obtained and analyzed using an enzyme-linked immunosorbent assay.

Results: All clinical parameters—PD, AL, GI, BOP, and PI—were significantly higher in the SIII-GC-PD group than in the PH and SIII-GC-P groups for both the full mouth and each sampling site (P<0.05). The total IL-34 and CSF-1 levels were significantly higher in the SIII-GC-PD group than in the PH and SIII-GC-P groups (P<0.05), and there were significant differences between the periodontitis groups (P<0.05).

Conclusions: These findings suggest that IL-34 and CSF-1 expression increases in patients with SIII-GC-PD. CSF-1 was associated with the inflammatory status of periodontal tissues and T2DM, while IL-34 was associated only with T2DM.

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Keywords: Diabetes mellitus; Gingival crevicular fluid; Interleukins; Periodontitis

Author Contributions

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a metabolic disorder that occurs as a result of disrupted insulin metabolism. This chronic metabolic disorder is concurrent with insulin resistance and the progressive deterioration of beta cells in the pancreas [1]. Periodontitis, which has been accepted as a risk factor for chronic oral inflammation, can lead to the development of T2DM by altering insulin resistance. Periodontitis is a chronic inflammatory disease that begins with the accumulation of dental plaque. As a result of microbial dysbiosis, a destructive host inflammatory response occurs and the loss of periodontal support tissue is observed. Periodontitis can be treated via professional treatment, with patient motivation, and by eliminating risk factors such as smoking and diabetes [2]. It has been reported that the risk of periodontitis is 2–3 times higher in individuals with diabetes mellitus (DM) [3]. Poor glycemic control increases the susceptibility to periodontal disease; this indicates that there is a bidirectional association between periodontal disease and DM [2]. Dysregulation of the inflammatory response appears to be a common feature of both of these diseases. They share similar pathophysiological mechanisms, with increased production of various inflammatory mediators, such as matrix metalloproteases, prostaglandin E2, cytokines, and chemokines [4].

Colony-stimulating factor-1 (CSF-1) is a cytokine that regulates the vital functions of myeloid cells, as well as monocytes, macrophages, and osteoclast precursor cells [5]. Interleukin-34 (IL-34) utilizes the same receptor (CSF1) as CSF-1; thus, IL-34 compensates for deficiencies in CSF-1 and plays a complementary role in this relationship. IL-34 is a cytokine that regulates the vital activities of macrophages and is mostly found in the spleen [6]. Clinical findings have shown that CSF-1 and IL-34 are associated with various inflammatory diseases, such as rheumatoid arthritis, lupus, and inflammatory bowel disease, and that a strong correlation exists between insulin resistance and IL-34 release [7,8]. IL-34, which replaces CSF-1 in RANKL-induced osteoclastogenesis, is secreted by gingival fibroblasts, similar to CSF-1 [9].

DM is a crucial factor in the pathogenesis of periodontitis, and periodontitis can have a negative effect on DM control [10]. However, no data exist to support the existence of a relationship between CSF-1 and IL-34 regarding periodontal disease and diabetes. Cytokines are implicated in the emergence of insulin resistance and the formation of hyperlipidemia [1,4]. Measurements of hemoglobin A1c (HbA1c) levels provide information about patients' mean blood glucose levels over a 3-month period [10]. Analyses of gingival crevicular fluid (GCF) samples provide valuable information about the pathophysiological status of periodontitis [11]. We hypothesized that the GCF levels of CSF-1 and IL-34 may be higher in stage III grade C periodontitis (SIII-GC-P) patients with T2DM than in those without T2DM. The objectives of this study were to determine the GCF levels of CSF-1 and IL-34 in SIII-GC-P patients with and without T2DM; to compare these levels with those in systemically and periodontally healthy (PH) individuals; and to investigate the correlations between biochemical and clinical parameters.

MATERIALS AND METHODS

Ethical considerations

Between January and May 2021, 72 volunteers (38 men and 34 women) aged between 28 and 60 years participated in a study conducted at the Department of Periodontology, Faculty

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of Dentistry of Usak University. Patients were selected among individuals who visited the oral and maxillofacial radiology clinic for routine examinations and were referred to the periodontology clinic to obtain clinical measurements. In total, 24 T2DM patients with periodontal disease (SIII-GC-PD group; 11 women and 13 men), 24 systemically healthy participants with periodontal disease (SIII-GC-P group; 10 women and 14 men), and 24 systemically and periodontally healthy volunteers (PH control group; 13 women and 11 men) were included.

This study was approved by the Ethics Committee of the Usak University Faculty of Medicine (protocol number: 84-84-11), conducted in compliance with the Helsinki Declaration, and recorded in the clinical studies registry (clinical registry number: NCT04891627). Written informed consent was received from all volunteers after they received a full explanation of the aims and design of the study.

Eligibility criteria

Individuals who had any systemic disease other than diabetes, who were breastfeeding or pregnant, who used drugs that affected the periodontium, or who had received non-surgical or surgical periodontal treatment in the last 6 months were excluded from the study. Patients in the age range of 20–60 years who had at least 20 permanent teeth in their mouth, did not smoke, and did not have systemic diseases or conditions other than diabetes were included. Based on these criteria, 3 smokers, 5 patients who had used antibiotics in the last 6 months, and 2 patients with systemic diseases other than diabetes were excluded; finally, 72 individuals participated in the study.

Assessment of clinical periodontal parameters

The degree of periodontitis is assessed based on the stage and grade system (16). Four stages (stages I, II, III, and IV) were defined according to the region with the highest attachment loss (AL). In the presence of certain complexity factors, the stage can be shifted to a higher level. Three grades (grades A, B, and C) were defined based on radiographic bone loss (according to the root length/age ratio) of the most affected tooth. In the presence of certain risk factors such as diabetes and smoking, the grade can be shifted to a higher level. Patients with stage III and grade C periodontitis were included in the study.

The individuals were categorized into 3 groups according to the new classification made in 2017:

1. The PH group consisted of individuals with at least 20 teeth in their mouth, without any systemic disease, without a history of periodontitis and with clinically healthy gingiva, without attachment and bone loss, and with bleeding on probing (BOP) <10% and probing depth (PD) of 3 mm or less [12].
2. The SIII-GC-P group consisted of patients with interdental AL of 5 mm or more, PD of 6 mm or more, radiographic bone loss advancing to the middle or apical part of the root, and tooth loss (<5) due to periodontal disease. Patients were excluded from the study if they had AL due to nonperiodontal reasons, such as root caries or gingival recession due to trauma [13,14]. Radiographic bone loss in the teeth was also evaluated, and grade C periodontitis was defined as rapid bone loss compared to the dental plaque accumulation and a ratio of the percentage of root bone loss to age greater than 1.0.
3. The SIII-GC-PD group consisted of patients who were diagnosed with T2DM by their physicians, based on their daily plasma glucose (200 mg/dL) and HbA1c (≥6.5%) levels, according to the report of the American Diabetes Association published in 2010 [15].

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The plaque index (PI), gingival index (GI) and the presence of BOP were measured by an experienced and calibrated periodontist (AD) who was unaware of the groups, using a manual probe (Williams; Hu-Friedy, Chicago, IL, USA), at 4 regions of each tooth except for the third molars [16-18]. The measurements of PD and AL were made at 6 regions of each tooth except for the third molars. Participants were questioned about whether tooth loss due to periodontitis had occurred.

A calibration exercise was performed in 10 periodontitis patients who were not included in this study, and the intra-examiner reliability of the parameters analyzed in the study was obtained. The intra-class correlation coefficients for PD and AL were 0.86 and 0.84, respectively.

Collection of GCF samples

First, the clinical periodontal measurements were performed; then, 24–48 hours later, the GCF samples were collected in the morning using strips of filter paper (PerioPaper; ProFlow, North Haven, CT, USA). The GCF samples were collected from the interproximal region of the buccal portion of 2 nonadjacent, single-rooted teeth. For the healthy control group, the GCF was taken from areas without inflammation and BOP. For the periodontitis group, the GCF was obtained from the areas with the highest PD and bone loss. After the plaque was removed from the teeth, the site was isolated using cotton rolls and slightly dried with an air freshener. The paper strips were then placed into the pocket until slight resistance was felt and left there for 30 s to absorb the GCF; next, the paper strips were placed in sterile tubes. Samples contaminated with oral fluids were excluded from the study. All samples were frozen at −40°C until further use.

Measurements of cytokine levels in GCF

Phosphate-buffered saline (300 mL, pH 7.4) was added to each Eppendorf tube containing paper strips. All tubes were shaken on an orbital shaker for 20 minutes at 240 rpm and centrifuged at 13,000 rpm for 5 minutes at 4°C. CSF-1 and IL-34 levels in the GCF were calculated using enzyme-linked immunosorbent assay (ELISA) kits (Human CSF-1 ELISA kit and Human IL-34 ELISA kit; Elabscience, Houston, TX, USA) according to the manufacturer's guidelines. The standards in the kits were diluted according to the manufacturer's instructions, and then the serum specimens were inserted into wells coated with antibodies specific to CSF-1 and IL-34. A stop solution was added to each well, and the absorbance was measured using a microplate reader (Microplate Reader; BioTek Instruments, Winooski, VT, USA). The total levels of CSF-1 (pg) and IL-34 (pg) collected in 30 seconds were determined; the lowest determining limits were reported to be 0.44 and 6.39 pg/mL for CSF-1 and IL-34, respectively.

Statistical analysis

In this study, the G*Power 3.1 program was used to establish the adequate example volumes and the sample size that would provide a type I error of 0.05, for each group in the study. With an effect size of 0.65, it was determined that the inclusion of at least 20 individuals would yield a test power of 80%. A *post hoc* power calculation was also performed, resulting in an observed power of 91%.

The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine whether the variables showed a normal distribution, and a critical value of P=0.05 was used. As the data did not show a normal distribution (P<0.05), comparisons between groups were performed using the Kruskal-Wallis nonparametric test followed by the Dunn-Bonferroni *post hoc* test. Frequency data were evaluated using the chi-square test. The relationships between clinical parameters

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and CSF-1 and IL-34 levels were evaluated via Spearman rank correlation analysis. Analysis of covariance was performed to examine the relationships between different periodontal conditions (healthy/low AL group vs. high/severe AL group) and biomarker levels after adjusting for age, sex, and the number of teeth. Data were analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA), and the threshold for statistical significance was set at $P<0.05$.

RESULTS

The demographic information of the groups is shown in **Table 1**. In total, 72 individuals participated in the study (34 women and 38 men), and the mean age was significantly higher in the SIII-GC-PD group than in the PH and SIII-GC-P groups ($P<0.05$). The number of teeth was significantly higher in the PH group than in the periodontitis group ($P<0.05$; **Table 1**).

Table 2 displays the clinical periodontal parameters, HbA1c levels, and total GCF levels of the biomarkers in the participants according to study group. All clinical parameters (PD, AL, GI, BOP, and PI) were significantly higher in the SIII-GC-PD group than in the PH and SIII-GC-P groups for both the full mouth and each sampling site ($P<0.05$; **Table 2**). The HbA1c levels were significantly higher in the SIII-GC-PD group than in the PH and SIII-GC-P groups ($P<0.05$; **Table 2**). The total CSF-1 levels were significantly higher in the SIII-GC-PD group

Table 1. The demographic characteristics of the study groups

Demographic variables	PH (n=26)	SIII-GC-P (n=26)	SIII-GC-PD (n=24)
Age (yr)	34 (28–44)	40 (36–46)[a]	52 (45–60)[a,b]
Gender (women/men)	14/12	10/16	11/13
Number of teeth	27 (25–28)	25 (24–28)[a]	25 (24–27)[a]

Comparisons between groups were performed using the Kruskal-Wallis/Dun-Bonferroni *post hoc* test. All data (except gender) are given as median (min–max).
PH: periodontally healthy, SIII-GC-P: stage III grade C periodontitis, SIII-GC-PD: stage III grade C periodontitis with diabetes.
[a]Significantly different from PH controls ($P<0.05$). [b]Significantly different from SIII-GC-P group ($P<0.05$).

Table 2. Clinical periodontal parameters, HbA1c and GCF total amounts of biomarkers of study groups

Parameters	PH (n=26)	SIII-GC-P (n=26)	SIII-GC-PD (n=24)
Full mouth			
PD (mm)	1.8 (1.3–2.3)	6.5 (6–6.8)[a]	6.8 (6–7.5)[a,b]
AL (mm)	0 (0–0)	5 (5–8)[a]	5.6 (5.2–6.2)[a,b]
GI	0.1 (0–0.4)	2.2 (1.2–3.1)[a]	2.5 (2.2–2.8)[a,b]
BOP (%)	1.7 (1–2)	80.2 (71.6–88.3)[a]	84.6 (81.1–86.9)[a,b]
PI	0.3 (0–0.7)	3 (2.2–3.2)[a]	3.1 (2.9–3.4)[a,b]
Sampling site			
PD (mm)	1.9 (1.7–2.3)	6.6 (6–7.5)[a]	7.1 (6–7.4)[a,b]
AL (mm)	0 (0–0)	5.4 (5–6)[a]	5.8 (5.5–6.4)[a,b]
GI	0 (0–0)	2.2 (1.9–3.1)[a]	3 (2.2–3.1)[a,b]
BOP (%)	0 (0–0)	100 (100–100)[a]	100 (100–100)[a]
PI	0.2 (0.1–0.6)	3 (2.3–3.2)[a]	3.2 (2.9–3.3)[a,b]
Total amounts of biomarkers			
CSF-1 (pg/30s)	0.54 (0.4–1)	1.56 (0.6–5.3)[a]	2.59 (1.5–5.6)[a,b]
IL-34 (pg/30s)	8.44 (6.3–11.8)	8.98 (6.3–18.3)	14.7 (6.5–21.3)[a,b]
HbA1c	4.2 (4.1–4.5)	4.8 (4.2–5.1)[a]	8 (7.4–8.8)[a,b]

Comparisons between groups were performed using the Kruskal-Wallis/Dun-Bonferroni post hoc test. All data are given as median (min–max).
HbA1c: hemoglobin A1c, GCF: gingival crevicular fluid, PH: periodontally healthy, SIII-GC-P: stage III grade C periodontitis, SIII-GC-PD: stage III grade C periodontitis with diabetes, PD: probing depth, AL: attachment loss, GI: gingival index, BOP: bleeding on probing, PI: plaque index, CSF-1: colony stimulating factor-1, IL-34: interleukin-34,.
[a]Significantly different from PH controls ($P<0.05$). [b]Significantly different from periodontal SIII-GC-P group ($P<0.05$).

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than in the PH and SIII-GC-P groups (P<0.05). The total IL-34 levels were significantly higher in the SIII-GC-PD group than in the PH and SIII-GC-P groups (P<0.05), and there were significant differences between the periodontitis groups (P<0.05; **Table 2**).

Positive correlations were observed among the full-mouth and sampling-site parameters, the total CSF-1 and IL-34 levels in the GCF, and the HbA1c levels (P<0.001 for all; **Table 3**).

The mean biomarker levels before and after adjustment for the effects of age, sex, number of teeth, and HbA1c levels for patients with different severities of AL (healthy/low vs. high/severe) are shown in **Table 4**. The differences in total CSF-1 levels were significant, and the CSF-1 levels were significantly lower in the group with a healthy/low AL of 0–2 mm than in the other groups (P<0.05).

Logistic regression analysis was used to examine the effects of independent variables (CSF-1 and IL-34 levels) on the dependent variables in the SIII-GC-P and SIII-GC-PD groups. The effects of the independent variables in the model were statistically significant. Most prominently, the probability of identifying CSF-1 in the SIII-GC-PD group was 1.684 times higher (0.99–2.85) than that in the other groups (SIII-GC-P) (P<0.05, **Table 5**).

Table 3. Correlations between CSF-1, IL-34 and HbA1c levels with clinical parameters of study groups

Parameters	CSF-1 (pg/30s)	IL-34 (pg/30s)	HbA1c (%)
Clinical parameters			
Whole mouth			
PI	0.648[a)]	0.393[a)]	0.637[a)]
GI	0.634[a)]	0.441[a)]	0.682[a)]
BOP	0.635[a)]	0.376[a)]	0.618[a)]
PD	0.641[a)]	0.553[a)]	0.405[a)]
AL	0.645[a)]	0.382[a)]	0.637[a)]
Sampling site			
PI	0.656[a)]	0.395[a)]	0.652[a)]
GI	0.657[a)]	0.411[a)]	0.676[a)]
BOP	0.632[a)]	0.357[a)]	0.588[a)]
PD	0.378[a)]	0.631[a)]	0.651[a)]
AL	0.643[a)]	0.393[a)]	0.637[a)]
Age	0.726[a)]	0.492[a)]	0.810[a)]
Biomarker levels			
CSF-1		0.613	0.606
IL-34	0.379		0.613

CSF-1: Colony stimulating factor-1, IL-34: interleukin-34, HbA1c: hemoglobin A1c, PI: plaque index, GI: gingival index, BOP: bleeding on probing, PD: probing depth, AL: attachment loss.
Spearman's rank correlation test: [a)]P<0.01.

Table 4. Unadjusted and adjusted scores of biomarker levels by severity of clinical AL

Biomarker levels	Clinical AL[a)]				Statistics	
	Unadjusted scores		Adjusted scores			
	Healthy/Low (n=26)	High/Severe (n=50)	Healthy/Low (n=26)	High/Severe (n=50)	F[b)]	P
CSF-1	0.59±0.14	2.54±1.41	1.05±0.30	2.30±0.19	8.842	0.004[c)]
IL-34	8.39±1.36	11.44±4.61	10.50±0.95	10.35±0.59	0.012	0.912

All data are given as mean±standard deviation. Healthy/Low groups: PH group. High/Severe groups: SIII-GC-P and SIII-GC-PD groups.
AL: attachment loss, CSF-1: colony stimulating factor-1, IL-34: interleukin-34, PH: periodontally healthy, SIII-GC-P: stage III grade C periodontitis, SIII-GC-PD: stage III grade C periodontitis with diabetes, HbA1c: hemoglobin A1c.
[a)]AL categories (mean full mounth AL): healthy/low: 0 to 2 mm; high/severe >3.0 mm[38]; [b)]Adjusted for age, gender, number of teeth and HbA1c values; [c)]Analysis of covariance P<0.05.

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Table 5. Results of logistic regression analysis on CSF-1 and IL-34 levels

Variables	Odds ratio (95% CI)[a]	*P* value
CSF-1	1.684 (0.99–2.85)	0.002[a]
IL-34	1.362 (1.11–1.66)	0.050[a]

Dependent variables: SIII-GC-P and SIII-GC-PD groups, independent variables: CSF-1 and IL-34 levels; logistic regression analysis.
CSF-1: colony stimulating factor-1, IL-34: interleukin-34, CI: confidence interval, SIII-GC-P: stage III grade C periodontitis, SIII-GC-PD: stage III grade C periodontitis with diabetes.
[a]$P<0.05$.

DISCUSSION

In this study, we evaluated the GCF levels of CSF-1 and IL-34 in SIII-GC-P patients with and without T2DM. T2DM is associated with chronic inflammation, cellular stress, and metabolic dysfunction and is a well-known risk factor for the severity and progression of periodontal disease [1,19]. T2DM accelerates the progression of periodontal disease by increasing the release of proinflammatory mediators, such as cytokines and prostaglandins, in periodontal tissues. This provides a scientific basis for the increased susceptibility of patients with diabetes to periodontal diseases [10,20]. It is important to understand the immunoinflammatory mechanisms that predispose patients with DM to periodontitis to a greater extent than individuals without DM. CSF-1 is a cytokine that plays a critical role in the differentiation and function of monocytes/macrophages and myeloid dendritic cells [21]. The discovery of IL-34, which has the same receptor as and plays a complementary role to CSF-1, has offered a new perspective on leukocyte functionality [9]. CSF-1 and IL-34 stimulate osteoclastogenesis and increase osteoclast proliferation. The roles of CSF-1 and IL-34 in joint inflammatory diseases have also been investigated, and the results indicate that CSF-1 and IL-34 secretion is increased in the serum and synovial fluids of rheumatoid arthritis patients, with a pathogenesis similar to that of periodontitis [7,22]. Given the similarity of the inflammatory processes of these 2 diseases, we hypothesized that CSF-1 and IL-34 might play a crucial role in the pathogenesis of periodontitis in patients with T2DM. To our knowledge, this is the first study to analyze the GCF levels of CSF-1, and IL-34 in SIII-GC-PD patients with diabetes. Our results showed that the GCF CSF-1 and IL-34 levels were significantly higher in DM patients with periodontitis. Furthermore, there were positive correlations among the clinical parameters, CSF-1 and IL-34 levels, and HbA1c levels.

In this study, GCF was chosen because it is a region-specific fluid that indicates an individual's inflammatory state of periodontal tissues; our results showed that CSF-1 and IL-34 levels in the GCF were representative of the levels observed in SIII-GC-PD patients. Additionally, the GCF-related data in our study were assessed as a total, which is a more reliable and convenient method than using concentration calculations for determining levels of periodontal disease markers [11,23-25].

In accordance with our hypothesis, we observed higher CSF-1 and IL-34 levels in patients with periodontitis than in healthy patients. Lira-Junior et al. [24] reported that patients with peri-implantitis had high levels of CSF-1 in their peri-implant crevicular fluid, indicating that CSF-1 could be of diagnostic value to distinguish peri-implantitis from mucositis [25]. In another study, significantly higher CSF-1 levels were observed in the saliva of patients with periodontitis than in the saliva of PH individuals. [26]. According to our results, the GCF CSF-1 levels were found to be significantly higher in SIII-GC-P patients than in the PH control group. These findings point to a role of CSF-1 in periodontal inflammation [21].

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Moreover, this result supports the hypothesis that CSF-1 increases bone loss, which is one of the differential consequences of periodontitis. Tissue destruction in periodontitis indicates an insufficient resolution of inflammation as a result of immune cell dysfunction, which is another potential indicator of the role of CSF-1 in periodontal diseases [27,28]. Ma et al. [29] concluded that IL-34 might be associated with inflammation in periapical lesions in chronic apical periodontitis. Batra et al. [30] evaluated IL-34 levels in patients with chronic and aggressive periodontitis. They found that the patients with periodontitis had higher IL-34 levels than healthy controls [30]. Similarly, a study by Guruprasad and Paradeep [31] revealed that IL-34 levels were significantly higher in individuals with periodontitis than in healthy controls. In contrast, in a study by Martinez et al. [26], lower IL-34 levels were observed in the saliva of patients with periodontitis than in the saliva of healthy controls. In this study, although GCF IL-34 levels were higher in SIII-GC-P patients than in healthy controls, no significant difference was found between them. Our differing results can be attributed to the differences in the number of patients, examination of markers from different fluids, and differences in the degree of periodontitis.

In the present study, the GCF CSF-1 and IL-34 levels were found to be significantly higher in patients with DM and periodontitis than in patients with periodontitis without DM. The results of this study indicate that DM increases the proinflammatory state in periodontal regions, as shown in previous studies [4,20,32]. Furthermore, glycation end products stimulate the production of proinflammatory proteins by interacting with specific receptors in the serum and gingival tissues of patients with diabetes, resulting in tissue damage [32]. IL-34-associated inflammation plays a critical role in various metabolic diseases [33]. Previous studies have reported an association between increased IL-34 levels and insulin resistance in obese patients [8,33-35]. In a study by Guruprasad and Pradeep [31], 175 individuals who were PH or had gingivitis or periodontitis were evaluated. IL-34 levels in patients with chronic periodontitis patients and T2DM were found to be significantly higher than those in the other groups. That study proposed IL-34 as a biomarker for determining disease activity in patients with diabetes and periodontal disease [31]. Although a few studies have examined the relationship between IL-34 and periodontitis and diabetes, to our knowledge, no studies have explored the role of CSF-1 in patients with periodontitis and diabetes.

In this study, significant correlations were observed between the GCF levels of CSF-1 and IL-34, clinical periodontal measurements (PI, GI, BOP, PD, and AL), and HbA1c levels. In particular, a strong correlation was observed among CSF-1 levels, HbA1c levels, and the GI in the sample area. In a study conducted by Gokhale et al. [36], both patients with periodontitis with and without DM had higher GI, PD, and periodontal index values than controls. Similarly, a study by Lu and Yang [37] compared the periodontal status of patients with and without diabetes and reported that those with diabetes had higher GI and AL, which were associated with HbA1c levels. Our results indicate that increases in GCF IL-34 and CSF-1 levels are associated with both T2DM and clinical periodontal parameters; notably, an increase in CSF-1 levels is more strongly associated with T2DM and gingival inflammation.

We found that CSF-1 levels were significantly higher in the SIII-GC-P and SIII-GC-PD groups (high/severe AL group) than in the PH group (healthy/low AL group) [38]. CSF-1 was associated with the severity of AL after adjusting for age, sex, and the number of teeth. However, no significant association was observed between IL-34 and AL severity. Based on the regression analysis conducted in this study, the patients in the SIII-GC-PD group were more likely to have higher CSF-1 and IL-34 levels. A significant correlation was observed

between the SIII-GC-PD group and CSF-1 levels (P=0.002); in particular, the contribution of the CSF-1 variable to the logistic regression model was found to be higher. Although diabetes was associated with higher cytokine levels in the periodontal tissues, it did not lead to a significant increase in IL-34 levels.

A major limitation of this study was the age difference among the individuals in the groups. The fact that individuals with type 2 diabetes with periodontitis were seen at an advanced age and the PH individuals in the control group were relatively young caused this difference. Another main limitation is the fact that the Periotron device was not used to calculate the GCF volume. Regarding the relationship between GCF components and periodontal diseases, it has been reported that the total amount of biomarkers may be a more valid and reliable diagnostic indicator than the concentration [39,40]. Therefore, in this study, the levels of CSF-1 and IL-34 in the GCF were calculated as the total amount, not the concentration. Another limitation of this study is the exclusion of patients with periodontitis of other stages. Additionally, the small sample size might be another limitation, as a larger cohort could increase the statistical power of the study.

Consistent with our hypothesis, the GCF IL-34 and CSF-1 levels were elevated in the SIII-GC-PD group and correlated with the clinical parameters. Furthermore, CSF-1 was found to be more strongly associated with periodontitis and diabetes. These data suggest that IL-34 is associated only with the hyperglycemic status of periodontal tissues; in contrast, CSF-1 is associated with both inflammation and the hyperglycemic status of periodontal tissues. Considering the results regarding the GCF levels of IL-34 and CSF-1, which are produced locally from inflammatory sites, these markers may help identify and monitor diseased tooth sites in the periodontal tissues of patients with diabetes. To our knowledge, this study provides the first data regarding the association between CSF-1 and IL-34 levels, SIII-GC-P, and diabetes; no other studies have evaluated these cytokines in individuals with SIII-GC-P and diabetes.

In conclusion, CSF-1 release is associated with the hyperglycemic and inflammatory status of periodontal tissues. In contrast, the release of IL-34, which binds to the same receptor as CSF-1 but plays a different role, is only associated with the hyperglycemic status of the periodontal tissues. Inflammation, which is enhanced in periodontitis, is exacerbated by diabetes. CSF-1 and IL-34 are likely important inflammatory status markers in SIII-GC-P patients with T2DM.

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