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GCF Levels of Osteoclastogenesis-Related Cytokines in Periodontitis in Relation to Smoking During Non-Surgical Periodontal Therapy

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Abstract

Objective: Interleukins (IL) -1β, -34, receptor activator of nuclear factor-kB ligand (RANKL), and osteoprotegerin (OPG) play a crucial role in osteoclastogenesis and bone resorption through modulating inflammatory processes and osteoclastogenesis. Smoking is the major risk factor in the initiation and progression of the periodontitis, and adversely affects the outcomes of non-surgical periodontal therapy. To date, there is no study investigating both gingival crevicular fluid (GCF) IL-1β, IL-34, RANKL, and OPG levels before and after non-surgical periodontal therapy in smoking and non-smoking patients with periodontitis stage 3, grade B and C. The aim of current research was to examine the GCF levels of some osteoclastogenesis-related cytokines in periodontitis in relation to smoking before and after periodontal therapy.

Methods: At baseline, full-mouth periodontal status together with GCF samples were collected from 116 individuals, including 60 periodontitis patients (30 smokers and 30 nonsmokers) and 56 periodontally healthy controls (28 smokers and 28 nonsmokers). Non-surgical periodontal therapy, consisting of instruction for daily plaque control and scaling and root planing (SRP), was performed. GCF sampling and full-mouth periodontal measurements were repeated 6 weeks after completion of SRP. The GCF levels of biomarkers were measured by enzyme-linked immunosorbent assay.

Results: The periodontitis groups exhibited significant improvement in clinical parameters. At baseline, the GCF IL-1β levels in periodontitis groups were significantly higher than periodontally healthy controls ($p<0.05$) and it was significantly decreased in periodontitis groups after non-surgical periodontal therapy. At baseline, the GCF IL-34 levels in periodontitis groups were significantly higher than periodontal healthy controls($p<0.05$) and the GCF IL-34 level was significantly decreased in non-smoking periodontitis patients. At baseline and after periodontal therapy, the GCF RANKL levels were similar in all groups. The GCF OPG level was significantly lowest in non-smoking periodontitis patients at baseline and the GCF OPG level was significantly increased in smoking and non-smoking periodontitis patients after non-surgical periodontal therapy.

Conclusion: In the periodontal inflammation process, GCF IL-34 level followed a similar pathway to GCF IL-1β, suggesting that IL-34 may be a marker in the pathogenesis of periodontal disease. The significant decrease in GCF IL-34 and a significant increase in GCF OPG level in the non-smoker periodontitis group after periodontal therapy suggest the negative effect of smoking on the response to periodontal therapy. More comprehensive studies are needed by increasing the number of samples included in the study groups in order to better understand the pathogenesis of periodontitis.

Key words: Periodontitis, cytokine, non-surgical periodontal therapy, smoking

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INTRODUCTION

Periodontitis is an inflammatory and microbial disease defined by pathologic damage of the periodontium and alveolar bone (1). Systemic immune response, genetic and environmental risk determinants contribute to the establishment and improvement of periodontitis (2). Periodontal disease is under the influence of environmental factors and smoking is one of the most important of these. The prevalence and severity of periodontal destruction boost due to smoking. In addition, smoking negatively affects the response to periodontal therapy (3).

Osteoclasts, which are produced through a differentiation process called osteoclastogenesis, are regulated by numerous cytokines. Osteoprotegerin (OPG) inhibits osteoclast differentiation whereas osteoclastogenic cytokines, like receptor activator of nuclear factor-kB ligand (RANKL), interleukin-1beta (IL-1 β), and interleukin-34 (IL-34) induces this process (4). These cytokines have been related to bone loss in patients with periodontal diseases (5-7). In patients with periodontitis, it is identified that the gingival crevicular fluid (GCF) contains reduced OPG levels and elevated levels of RANKLwith which RANKL interacts during the inflammatory process. IL-1β plays a potent key role by inducing the expression of RANKL in some cells. In the results of previous studies, it was reported that the RANKL/OPG ratio showed significant increases in individuals with periodontitis compared to the control group (8, 9). There is no study evaluating the response after initial periodontal treatment in GCF IL-1β, IL-34, RANKL, OPG levels in Stage III, grade B and C periodontitis with smoking status. Our hypothesis in this clinical trial is GCF IL-1β, IL-34, and RANKL levels reduction at 6-week. The objectives of our clinical research were to determine the association of GCF levels of cytokines IL-1β, IL-34, RANKL, and OPG with the clinical substantiation of the periodontium and also to compare the cytokines for the estimation of the pathophysiological status of Stage III grade B and C periodontitis and improved answer of non-surgical periodontal therapy in relation to smoking.

METHODS

Design of the clinical trial

The study procedure was approved by the Medical Research Ethics Committee of Sivas Cumhuriyet University with regard to the Declaration of Helsinki (2018-07/02). All patients accepted and signed the detailed informed consent form. A total of 116 participants were concerned, including 60 periodontitis patients (30 smokers, 30

nonsmokers) and 56 periodontally healthy controls (28 smokers, 28 nonsmokers) (Figure 1). Patients with certain exclusion criteria were not included in the study. These exclusion criteria are listed below.

Patients who have conservative or prosthetic restorations in the sample site, patients who were not systemically healthy, in pregnancy or lactation process, patients who require antibiotic prophylaxis, patients who used anti-inflammatory or antibiotic drug in the last six months, patients who were treated periodontally within six months.

The patients were categorized according to the 2017 classification. The H group took place of participants with at least twenty teeth in their mouths. They mustn't have a history of periodontitis. With these criteria, bleeding on probing (BOP) <10% and probing pocket depth (PPD) of 3 mm or less, and besides, they have healthy gingiva without clinical inflammation, no loss of alveolar bone, and attachment. In addition, the smoking habits of the individuals were examined. Those who smoke 10 or more cigarettes a day were considered active cigarette users. The H group was divided into two groups smoking 10 < or non-smoking 10 > status.

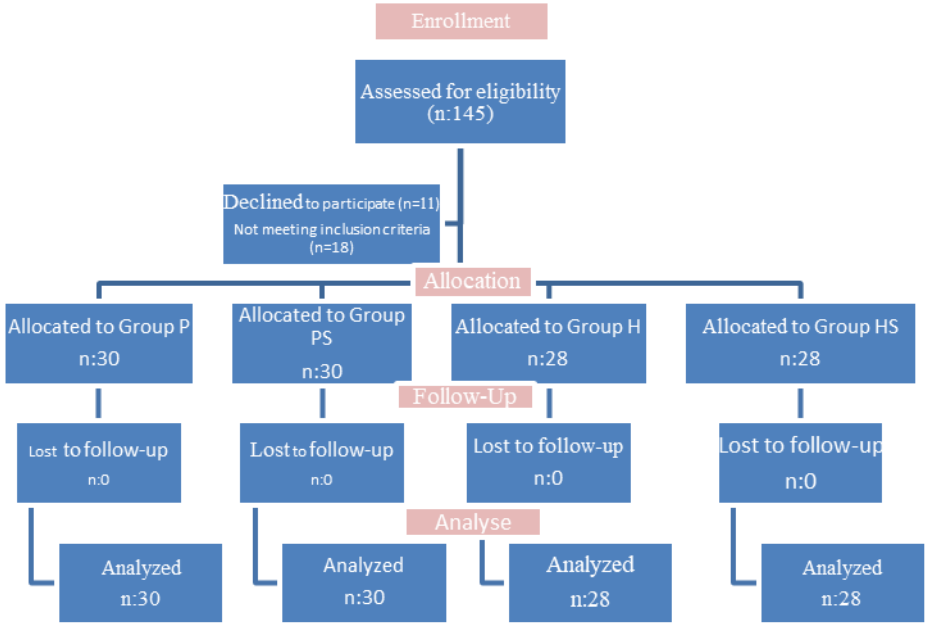

Figure 1. Flow diagram of patients' recruitment and follow-up

The Stage III -grade B periodontitis patients indicated clinical attachment loss (CAL) ≥5 mm and PPD ≥6 mm on at least two non-adjacent teeth. When we evaluated the bone loss radiographically, patients with not exceeding ≥30% of the teeth and up to the

middle or apical third of the root were included in this group. Since we do not have long-term follow-up radiographs of the patients, the grade of periodontitis patients has been defined by radiographic bone loss/age. The percentage of root length was determined by reference to the tooth with the highest radiographic bone loss. Grade B was determined the ratio of bone loss/age was 0.25–1.00 and the smoking status 10 >. The Stage III -grade C periodontitis group was similar to Stage III-grade B except for tooth loss (<5) due to periodontal disease. Destruction inconsistent with biofilm, a ratio of the percentage of root bone loss to age bigger than one, and smokers more than 10 cigarettes are other factors that determine the grade C periodontitis.

In periodontal measurements, we used a periodontal probe with indicator lines up to 15 mm (William's probe, Hu-Friedy, Chicago, USA). In order to optimize the CAL measurement, the individual acrylic stents, for which we determined the reference points in all periodontitis patients, were made of cold acrylic. Accordingly, the group definitions are as follows:

Group P: Periodontitis; Stage III, Grade B; Non-smoker

Group PS: Periodontitis; Stage III, Grade C; Smoker

Group H: Periodontal Healthy; Non-smoker

Group HS: Periodontal Healthy; Smoker

Clinical measurement and non-surgical periodontal therapy

The four quadrant clinical measurements of GI, PI, PD, and CAL were taken by all study participants. PD and CAL were measured at six points per tooth. The PI was based on the supragingival plaque, while the GI was based on gingival inflammation (10). A single expert (NA) performed the collection of clinical parameters and samples to ensure standardization. Clinicians' measurements are proven to be reliable on repeated measurements (≥98%). All clinical measurements of whole participants incorporated in the research were recorded before proceeding to the treatment phase. Firstly, periodontitis patients in stage III, grade B and C groups were given oral hygiene instructions within the scope of Phase 1 treatment. Then scaling and root planning (SRP) was applied within two weeks by using Gracey curettes with different forms (Hu-Friedy, Chicago, USA). Antibiotics and mouthwash were not prescribed to any of the patients. The patients were recalled 6 weeks later and whole-mouth clinical measurements were taken. GCF samples were retrieved from sample sites.

GCF sampling and ELISA analysis

GCF exemplification was performed by using the absorbent paper strip method in mesial or distal sites of the sampled tooth (OraFlow Inc., Amityville, NY, USA). For the collection of GCF specimens, three non-

abutting proximal sampling sites with a 5-6 mm probing pocket depth were preferred in the upper anterior region, which is easier to isolate and access. The periopaper was placed into pathologic periodontal pocket in periodontitis patients and gingival sulcus in periodontal healthy groups until a slight resistance was felt. We preferred to keep the periopaper in the pocket for 30 seconds. We have criteria for the exclusion of samples. These are blood and saliva contamination due to isolation, irritation, or excessive inflammation. All periopaper strips were placed into eppendorf tubes which were encoded with numbers.

The GCF samples, which were kept at -80 until the analysis time, were first left at room temperature, then 250 µL of phosphate buffer solution was annexed into the eppendorf tubes and mixed with vortex for 2 minutes to transfer the contents of the strip. Cytokine levels in GCF were analyzed via ELISA kits(FineTest, Wuhan, China) according to the prospectus. After the steps suggested by the company, the plates were read at 450 nm wavelength in the spectrometer, and the standard curves were used for GCF cytokine levels calculation. The data were presented as the total amount (picogram/site). As a result of the analysis, it was seen that there were regions with low cytokine levels than the detectability limits of the test, and these regions, which were relatively few, were given a score of 0.

Statistical Analysis

The SPSS program (IBM SPSS v22.0, IBM, USA) was preferred for statistical analysis. The Kolmogorov-Smirnov test was preferred to determine the distribution of data. While the Mann-Whitney test was preferred for pairwise comparisons, the numerical data were compared in groups by the Kruskal-Wallis ANOVA. Wilcoxon test was used to compare data during the treatment process. The chi-square test was preferred for evaluating frequencies. The p-value was set as <0.05. The computation of the sample size was performed under a 5% error considering a required sample size of 30 in each group, with a statistical power of 80% .

RESULTS

Demographic features and clinical periodontal measurements

The mean age P, PS, H, and HS groups ranged 39.5±8.8, 41.6±7.5, 36.2±10.9 and 37.9±8.1 years, respectively. The female/male ratio was 16/14 in P, 19/11 in PS, 17/11 in H, and 18/10 in the HS group. In the study groups, the mean age and gender distribution were homogeneous (p>0.05). Our primary outcome measure was changed in GCF biomarker levels from baseline to post-treatment assessment in groups based on smoking status. The secondary outcome measure was the changes in clinical parameters caused by the non-surgical therapy process.

Clinical Parameters

PI, PPD, and CAL values of clinical parameters before non-surgical periodontal therapy were higher in group PS than in group P, but only PPD values were statistically significant ($p<0.05$). GI values were decreased in group PS compared to group P but they were not statistically significant ($p>0.05$). All clinical parameters in 6th week were significantly decreased than at baseline levels.($p<0.05$)(Table 1).

Biochemical Parameters

At baseline GCF IL-1β levels in periodontitis groups were significantly higher than periodontal healthy groups ($p<0.05$). However, GCF IL-1β levels were higher in group PS than in group P, but the difference was not statistically significant ($p>0.05$). Significant reductions were observed in periodontitis groups in 6th week after non-surgical periodontal therapy ($p<0.05$). GCF IL-1β levels in the HS group were significantly higher than the H group ($p<0.05$).

At baseline GCF IL-34 levels in periodontitis groups were significantly higher than in periodontally healthy groups ($p<0.05$). However, the periodontally healthy group's GCF IL-34 levels were higher in group HS than in group H, but the difference was not statistically insignificant ($p>0.05$). Reductions were observed in periodontitis groups at the 6th week after non-surgical periodontal therapy but the difference was statistically significant in the P group only ($p<0.05$).

At baseline and 6-week after non-surgical periodontal therapy, GCF RANKL levels differences were insignificant between all groups ($p>0.05$).

At baseline GCF OPG levels in the PS group were significantly higher than in the P group ($p<0.05$). Increases were observed in periodontitis groups at the 6th week however they were insignificant ($p>0,05$) (Table 2).

Correlations

At baseline GCF IL-34 levels were negatively correlated with GCF OPG levels($r = -0.533$, $p <0.05$) in the PS group. There was a negative correlation between GCF RANKL and OPG levels($r =-0.642$, $p <0.05$) in the H group at baseline. There was a positive correlation between GCF IL-34 and RANKL levels ($r=0.617$, $p <0.05$) in the HS group at baseline.

Table 1. Comparison of the clinical data of the sample tooth region of the patients participating in the study before and after periodontal treatment and between groups

			Stage 3 Grade C	Stage 3 Grade B	Control Smoking	Control	*p*
GI	Baseline	mean ± SD	1.86±0.50[c,b]	2±0.74[b,c]	0.32±0.27	0.17±0.24	0.001*
		Med	2.00	2.00	0.50	0.00	
		Min	1.00	1.00	0	0.00	
		Max	3.00	3.00	1.00	0.50	
	6-week	mean ± SD	1.34±0.41[a,c,b]	1.46±0.57[a,b,c]			
		Med	1.10	1.00	0.50	0.00	
		Min	1.00	1.00	0.00	0.00	
		Max	2.00	3.00	1.00	0.50	
PI	Baseline	mean ± SD	2.25±0.25 [c,b]	2.10±0.40 [c,b]	0.39±0.41[b]	0.17±0.27	0.001*
		Med	2.25	2.00	0.50	0.00	
		Min	2.00	1.00	0.00	0.00	
		Max	2.50	3.00	1.00	1.00	
	6-week	mean ± SD	1.25±0.25[a,c,b]	1.11±0.21[a,c,b]			
		Med	1.25	1.00	0.50	0.00	
		Min	1.00	1.00	0.00	0.00	
		Max	1.50	1.50	1.00	1.00	
PD	Baseline	mean ± SD	5.15±0.26 [c,b]	4,61±0,58 [d,c,b]	1,73±0,56	1,57±0,48	0.001*
		Med	5.00	4.50	2.00	1.50	
		Min	5.00	4.00	1.00	1.00	
		Max	6.00	5.50	2.50	2.50	
	6-week	mean ± SD	2.23±0.25[a,c,b]	2.95±0.42[a,d,c,b]			
		Med	2.00	3.00	2.00	1.50	
		Min	2.00	2.00	1.00	1.00	
		Max	2.50	3.50	2.50	2.50	
CAL	Baseline	mean ± SD	10.06±0.34	9.31±1.13			0.001*
		Med	10.00	9.25			
		Min	9.50	5.00			
		Max	11.00	11.00			
	6-week	mean ± SD	8.83±0.44[a]	8.50±1.11[a]			0.191
		Med	9.00	8.50			
		Min	8.00	4.00			
		Max	9.50	10.00			

[a]: different from baseline
[b]: different from control non-smoking
[c]: different from control smoking
[d]: different from stage 3 grade C periodontitis group
mean±SD(standard deviation), med: median, min: minimum, max: maximum
* $p<0.05$
Wilcoxon test was used to compare data during the treatment process (baseline-6th week).
Comparisons of numeric variables of the study groups were evaluated by the Kruskal-Wallis ANOVA test with post hoc Mann-Whitney test for pairwise comparisons.

Table 2. Comparison of cytokine levels of patients participating in the study before and after periodontal treatment and between groups

			Stage 3 Grade C	Stage 3 Grade B	Control Smoking	Control	*p*
IL-1β	Baseline	mean ±SD	147.79±70.83[c,b]	140.94±70.36[c,b]	79.52±58.82[b]	36.19±25.92[c]	0.001*
		Med	134.727	122.415	66.354	37.000	
		Min	33.168	11.310	12.372	5.030	
		Max	244.053	244.053	232.018	97.680	
	6-week	mean± SD	85.09±52.99[a,b]	88.09±65.62[a,b]			
		Med	82.725	73.619			
		Min	5.912	11.664			
		Max	222.549	228.566			
IL-34	Baseline	mean± SD	0.98±0.06 [c,b]	1.03±0.08 [c,b]	0.88±0.031	-0.89±0.07	0.001*
		Med	0.958	1.003	-0.959	-0.914	
		Min	0.936	0.943	-1.029	-0.984	
		Max	1.151	1.268	0.673	-0.672	
	6-week	mean± SD	0.96±0.005 [c,b]	0.98±0.005 [a,c,b]			0.108
		Med	0.946	0.955			
		Min	0.932	0.934			
		Max	1.223	1.200			
RANKL	Baseline	mean± SD	1.46±0.13	1.44±0.006	1.43±0.45	1.44±0.06	0.108
		Med	1.405	1.422	1.422	1.424	
		Min	1.397	1.400	1.398	1.390	
		Max	1.830	1.675	1.598	1.636	
	6-week	mean± SD	1.43±0.007	1.43±0.003			0.347
		Med	1.408	1.413			
		Min	1.397	1.398			
		Max	1.726	1.561			
OPG	Baseline	mean± SD	168.45±15.11	141.42±15.33[c,b,d]	178.14±56.95	177.54±14.62	0.001*
		Med	169.889	137.228	170.181	182.347	
		Min	134.544	121.254	121.416	128.542	
		Max	191.295	174.395	394.299	187.729	
	6-week	mean± SD	175.02±56.60	171.27±12.24[a]			0.086
		Med	173.416	175.384			
		Min	121.720	133.578			
		Max	449.430	186.852			

[a]: different from baseline
[b]: different from control non-smoking
[c]: different from control smoking
[d]: different from stage 3 grade C periodontitis group
mean±SD(**standard deviation**), **med: median, min: minimum, max: maximum**
*** *p*<0,05**
Wilcoxon test was used to compare data during the treatment process(baseline-6th week).
Comparisons of numeric variables of the study groups were evaluated by the Kruskal-Wallis ANOVA test with post hoc Mann-Whitney test for pairwise comparisons.

DISCUSSION

We evaluated the predictive value of biomarkers IL-1 β, IL-34, RANKL, and OPG detected in the GCF of smoking and non-smoking stage 3 grade B and C periodontitis patients during the appraisal of the severity of periodontal diseases and the effect of non-surgical periodontal therapy. Considering the literature on periodontitis, it is a pioneering study that evaluated the success of periodontal treatment via full-mouth clinical measurements and biomarkers of GCF IL-1, IL-34, RANKL, and OPG in patients with smoking habits. As expected, the mean PI, GI, PD, and CAL values in the pre-treatment periodontitis groups were found to be statistically highly significant when

compared with the control groups. Again, as a predictable outcome, significant healing in clinical parameters was observed after treatment. In our study, the fact that the GI was lower in the smoking periodontitis group compared to the non-smoker periodontitis group is a result that supports the suppression of inflammation by smoking.

Periodontal disease is a multidirectional chronic disease that grounds the destruction of the periodontium (11). Periodontopathogens are necessary for the formation and progression of periodontitis and genetic predispositions, acquired diseases, and environmental factors determine the severity of periodontal destruction (12). The cytokine network is of great importance in revealing the molecular mechanisms of inflammatory diseases accompanied by active and passive periods, such as periodontal diseases. In periodontitis, pro-inflammatory cytokines are considered potential inflammatory markers and play a pioneering role in the inception and enhancement of the inflammatory response (13). Thus, the current study evaluated the role of pro-inflammatory cytokines as markers of bone resorption in different stages and grades of periodontitis patients.

Cytokines which are produced locally in the periodontal tissues are included in gingival crevicular fluid. The GCF is a non-invasive approach that consists of components of molecules involved in the host response network in the inflammatory process and is a reflection of the oral ecology located in the gingival sulcus. GCF is a unique model for the measurement of various potential inflammatory biomarkers, and investigation of periodontal immunoinflammatory and we can see the reflection of the host response (14). In the method of our study, it is among our priorities to evaluate the total amount of cytokine changes in GCF before and after periodontal treatment with smoking. In our study, 2 samples were taken from each patient and stored in the same eppendorf tube to support the detection rate in the analysis of biomarkers. Since many parameters affect the concentration in the presentation of GCF samples, the presentation of total cytokine activity is considered to be a good indicator (15).

Smoking may be at the top of the environmental risk factors for periodontitis, and a recent study reported that smoking is responsible for more than %50 of periodontitis cases (16). Studies have emphasized that there is a linear relationship between the inflammatory process of periodontitis and smoking habits (17, 18). While symptoms such as bleeding can be masked with smoking, an increase in plaque accumulation and the progression of the disease can accelerate. When smokers and non-smokers with similar plaque accumulation rates were compared, it was observed that the bleeding areas, the percentage of gingival discoloration, and the volume of

GCF were lower in the smokers (19, 20). According to our study findings, in smoker periodontitis patients PI, PPD, and CAL were higher and GI was lower than in non-smoker periodontitis patients. Our results support that is consistent with studies advocating the efficacy of smoking on increasing the amount of periodontal destruction (21). It is assumed that the destruction of periodontal tissues and alveolar bone loss is more common in actively smoking patients than in non-smokers. As a matter of fact, in our study, the level of GCF IL-1 β was found to be higher in all smoking groups.

IL-1β is a potent biomarker most studied in periodontal pathogenesis. Studies have shown that GCF IL-1β levels are dependent on the severity of inflammation, that the GCF IL-1β levels of periodontally healthy individuals are lower than those with periodontitis, and that these levels decrease in individuals with gingivitis and periodontitis after nonsurgical periodontal therapy (22, 23). Opposite to our result, Rawlinson et al. (24) announced lower GCF IL-1β levels in smokers. Differences in GCF IL-1β levels among smokers vary due to differences in host response, smoking-related variables, and the use of different techniques in GCF collection and analysis (25). Consistent with the outcomes of the studies introduced down there, in our study, GCF IL-1β level decreased after non-surgical periodontal treatment of P patients. IL-1β induces bone resorption and suppresses bone formation. Although it is a powerful pro-inflammatory cytokine involved in the pathophysiology of periodontitis, also it is comprised of inflammatory processes such as cell proliferation, differentiation, and apoptosis (26). Many studies have shown an interrelation between GCF and periodontal tissue IL-1β levels and the inflammatory state of periodontitis. In a study of periodontitis patients, it was reported that the IL-1β level decreased in the 2nd and 4th-month measurements compared to the initial measurements in shallow and deep pockets (22). Toker et al. reported that clinical complaints improved significantly with successful periodontal treatment and this clinical improvement was consistent with the reduced IL-1β in GCF (27).

IL-34 can completely substitute macrophage-colony stimulating factor in RANKL-induced osteoclastogenesis and this makes known the essential factor of IL-34 in inflammation-driven alveolar bone loss (28). In this research, before non-surgical periodontal therapy, the GCF IL-34 level was noticed to be higher in stage 3, grade B and C periodontitis groups compared to the smokers and non-smokers periodontally healthy groups. After non-surgical periodontal therapy, the GCF level of IL-34 in the periodontitis groups was reduced. Even after the non-surgical periodontal therapy, higher values were

observed in stage 3 grade B and C periodontitis groups compared to the controls. In support of our findings, in three different studies in which chronic periodontitis and healthy groups were evaluated together with obesity, smoking, and type-2 diabetes, they revealed that IL-34 level was uppermost in obese chronic periodontitis patients, followed by chronic periodontitis patients who smoked and then chronic periodontitis with type 2 diabetes mellitus (29). The IL-34 level was the lowest in systemically healthy periodontitis patients. (29,30). Guruprasad et al. assessed the GCF and plasma IL-34 levels in patients with chronic periodontitis and the efficacy of non-surgical periodontal treatment on GCF and plasma IL-34 levels. They informed that IL-34 levels decreased in measurements after non-surgical periodontal treatment (6). While there was no difference between GCF IL-34 levels between smoking and non-smoking control groups, the significant decrease in GCF IL-34 levels after 6-week in the stage 3 grade B periodontitis group can be the indication that smoking suppressed the response to treatment. In osteoclastogenesis, IL-34 plays an active role in the association of RANKL and ensures the adhesion and proliferation of osteoclast precursor cells. (34). In our previous study, we evaluated GCF IL-34 in chronic and aggressive periodontitis. We were informed that GCF IL-34 level was increased in the aggressive periodontitis group after initial periodontal therapy while this was the opposite in the chronic periodontitis group (31). When we look at the results of the current study, GCF IL-34 levels were significantly higher in the periodontitis groups compared with the periodontal healthy groups at the beginning. GCF IL-34 levels decreased in all periodontitis groups at 6-week. This is consistent with the results of other studies with IL-34 (5,32-33).

Osteoclastogenesis proceeds through the in vitro binding of RANK (reseptör of RANKL) with RANKL, resulting in a series of cellular events such as monocyte/macrophage progenitor differentiation and activation of mature osteoclasts. OPG inhibits osteoclast differentiation and bone resorption as a result of winning the race with the receptor of RANKL (34). In the literature, although the findings of studies examining RANKL and OPG levels in periodontitis patients differ, generally the results were that; the gingival tissues and GCF RANKL levels of periodontitis patients were statistically highly significant and OPG levels were decreased (8, 35-37). In a previous study, it was reported that smoking and increased periodontal inflammation did not affect the GCF RANKL level in the periodontitis group without any systemic disease (38). Similarly, in our study when the GCF RANKL levels before periodontal therapy were compared between the groups, the difference was insignificant.

Before periodontal therapy, GCF OPG levels were lower in Stage 3 groups against

periodontal healthy groups. However, the difference was significant only in non-smoking periodontitis patients. In addition, GCF OPG levels were statistically significantly higher in the smoker periodontitis patients than the non-smoker periodontitis patients. In the smoker periodontitis group, GCF IL-34 and OPG (r = 0.533) levels were negatively correlated. This correlation may indicate that cytokines simultaneously take an active role in the etiopathogenesis of periodontitis and the maintenance of periodontal health.

CONCLUSION

In the periodontal inflammation process, GCF IL-34 level followed a similar pathway to GCF IL-1β, suggesting that IL-34 may be a marker in the manner of development of periodontitis. The changes in IL-34 and OPG levels in the non-smoker periodontitis group after periodontal treatment suggest the negative effect of smoking on the response to periodontal treatment. More comprehensive studies are needed by increasing the number of samples included in the study groups to recognize the role of these bone destruction markers and smoking in the pathogenesis of periodontitis.

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Ethics Committee Approval: Ethics committee approval was received for this study from local ethics committee at Cumhuriyet University with file number 2021/39

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