Clinical and Experimental Health Sciences

Clinical and Biochemical Effects of Smoking on Non-Surgical Periodontal Treatment in Grade III Stage C Periodontitis Patients

Volkan Arıkan[1], Nimet Gül Görgülü[1], Başak Doğan[2]

[1] Marmara University, Institute of Health Science, Department of Periodontology, İstanbul, Türkiye.
[2] Marmara University, Faculty of Dentistry, Department of Periodontology, İstanbul, Türkiye.

Correspondence Author: Başak Doğan
E-mail: basakdogan@marmara.edu.tr
Received: 08.06.2022 **Accepted:** 07.08.2022

ABSTRACT

Objective: The purpose of this study was to evaluate the effect of smoking on clinical parameters and the serum and saliva levels of RANKL, OPG, and IL-34 in periodontitis stage III grade C (III-C) patients after non-surgical periodontal treatment (NSPT).

Methods: A total of 60 subjects, 40 periodontitis-III-C patients (20 smokers and 20 non-smokers) and 20 non-smoker periodontally healthy individuals, were included. All clinical periodontal parameters were recorded, and unstimulated saliva and serum samples were collected from all patients at baseline, but at 1 and 3 months only from periodontitis patients (N=40). Saliva and serum levels of RANKL, OPG, and IL-34 were analyzed by ELISA.

Results: At baseline only whole mouth probing depth (PD) and percent of sites with PD>5mm were higher in smokers than non-smoker periodontitis patients ($p<.05$). All periodontal measurements significantly improved in both periodontitis groups after NSPT ($p<.001$). After NSPT, the reduction in gingival index (GI), bleeding on probing (BOP), and mean PD in sites initially PD≥5mm were lower in smokers than non-smoker periodontitis patients ($p>.05$). Only saliva IL-34 levels were higher in all-periodontitis patients than healthy individuals ($p=.001$) and decreased in both periodontitis groups after NSPT ($p<.05$). Moreover, elevated serum RANKL level was detected in smokers compared to non-smoker periodontitis or healthy ones at baseline ($p<.05$). Serum RANKL levels exhibited no change after NSPT in either periodontitis groups.

Conclusions: The smokers are less responsive to NSPT, and saliva IL-34 can be a potential inflammatory marker of periodontitis-III-C. Moreover, high serum RANKL levels are associated with smoking.

Keywords: periodontitis, smoking, interleukin-34, receptor activator of nuclear factor-kappa B ligand, osteoprotegerin

1. INTRODUCTION

Periodontitis is caused by an inflammatory response to periopathogens in periodontal tissues that results in periodontal attachment loss and bone resorption around the teeth (1). Several risk factors, such as smoking, gender, obesity, metabolic syndrome, and genetic factors, play a crucial role in periodontal disease progression. Previous studies revealed that smokers are 2 to 8 times more prone to periodontal disease (2) and have more severe tissue destruction, insufficient wound healing due to reduced vascularization, and lesser response to periodontal treatments (3). Although some studies reported higher whole mouth PD reductions in non-smokers than smokers after non-surgical periodontal treatment (NSPT) (4,5), some studies reported similar results (6,7).

Most research supports that smoking affects the periodontal tissues by microcirculatory and host immune systems and bone metabolism (8,9). However, the exact mechanisms of alveolar bone are still unclear. Nicotine is the primary compound in tobacco, which inhibits osteogenesis and angiogenesis. Nicotine can bind to nicotinic receptors in osteoblasts. This binding promotes cell proliferation when nicotine levels are low; however, increased nicotine levels inhibit osteoblast formation, leading to cell death (10). In the osteoclastogenesis and bone turnover cycle, the balance of three molecules, receptor activator of nuclear factor kappa B (RANK), RANK ligand (RANKL), and osteoprotegerin (OPG) is essential (11). The proliferation and differentiation of osteoclasts is stimulated by the attachment of RANKL to RANK on osteoclast or osteoclast precursor cells (12,13). RANKL can be detected in two forms: a cell membrane-bound variant (mRANKL) and a primary soluble form (sRANKL) (12). As for OPG, a naturally soluble decoy receptor generated by osteoclasts has the opposite effect and suppresses osteoclast differentiation. RANKL is bound by OPG, which prevents it from interacting with the RANK receptor (14).

DOI: 10.33808/clinexphealthsci.1128101

Increased RANKL/OPG ratio caused by bone loss and due to increase in RANKL or decrease in OPG levels, or both. Therefore, the balance between OPG and RANKL activity can accelerate bone resorption or bone formation (13). Many studies investigate the relationship between smoking and RANKL–OPG system. However, the results of these studies were controversial. Some studies reported that smokers had a lower serum or gingival crevicular fluid (GCF) level of OPG and a higher RANKL/OPG ratio than non-smokers (15-17). On the other hand, some studies reported no statistical differences in GCF, saliva, and serum levels of OPG between smoker and non-smoker periodontitis patients (18,19). Regarding the effect of NSPT on OPG, studies showed that its level in GCF was decreased after NSPT, but in saliva or serum either increased or showed no difference (20-22).

Recently, functional screening of proteins secreted from an embryonic kidney cell has led to the recognition a new molecule called interleukin 34 (IL-34). It has been observed that this cytokine stimulates macrophages to create colonies from bone marrow cells (23). In RANKL-induced osteoclastogenesis, IL-34 can replace macrophage colony-stimulating factor (M-CSF) and promote osteoclast differentiation as M-CSF does. A recent study showed that serum and GCF IL-34 levels in smokers were higher than in non-smoker patients, which suggested that smoking may be an essential factor in releasing IL-34 (24). To our knowledge, no study that reported the relationship between smoking and the salivary IL-34 before and after NSPT. In the present study, we hypothesize that elevated salivary IL-34 levels are associated with smoking and periodontitis, and NSPT might explore a positive impact on salivary IL-34 levels.

Therefore, the purpose of this study is to analyze the impact of smoking on clinical periodontal parameters and the serum and saliva levels of RANKL, OPG, and IL-34 in periodontitis stage III grade C (III-C) patients after NSPT.

2. METHODS

2.1. Study Population

A total of 60 patients were involved in this study. All patients were selected from the Department of Periodontology, Faculty of Dentistry, Marmara University, Istanbul, Turkey. The study was approved by the Clinical Research Ethics Committee of Faculty of Medicine, Marmara University (No: 09.2018.513/ Date: 13.07.2018) and submitted to clinicaltrials.gov with the number NCT05262153. The study protocol was explained to the participants and each participant signed informed consent form.

Medical and dental histories were obtained. All volunteers were selected based on these criteria; (1) systemically healthy adults (aged>18 years), (2) having at least 24 teeth, (3) no periodontal treatment in the last 6 months, (4) no use of any antibiotics or anti-inflammatory drugs that could affect their periodontal status for 3 months, (5) no lactation, and (6) no pregnancy.

The selection of patients was made according to the clinical and radiological criteria proposed by the 2018 classification of periodontal disease (25). The periodontitis patients (N=40, 20 smokers and 20 non-smokers) were stage III in severity and grade C in the progression rate. These patients had at least 5 non-adjacent interdental sites with interdental PD≥ 6mm, clinical attachment level (CAL)≥5mm (due to periodontal causes), and BOP≥30%. They had not lost more than four teeth due to periodontitis. The grade was assessed according to the the indirect evidence of progression due to lack of direct evidence. To calculate the bone loss%/age, the worst affected tooth in the dentition was selected and radiographic bone loss was expressed as a percentage of root length, and divided by the patient's age. All patients were grade C, regardless of smoking risk factor, since bone loss%/age were higher than 1.0 (25). Inclusion criteria of the healthy group presented PD≤ 3mm with no alveolar bone loss or no interproximal attachment loss, BOP<10% (26), and no smoking. Smokers defined as smoking more than 10 cigarettes per day for at least 5 years, while non-smokers as never smoked.

2.2. Sample Size Calculation

In this study, the sample size required to obtain sufficient power was calculated based on the changes in probing depth. In a prior study, (27) the mean difference in PD reduction between treatment groups was 0.44 mm, with an estimated standard deviation of 0.29 mm. Hence, 12 subjects were needed in each group to detect a difference with a power of 95% and an α error of 0.05 error. Twenty individuals were recruited in each group to compensate for any potential dropouts during the trial.

2.3. Non-Surgical Periodontal Treatment

Before NSPT, oral hygiene instructions, including brushing, flossing, and interdental brushing, were given to all patients. In both treatment groups, scaling and root planning was performed by using ultrasonic instruments (Guilin Woodpecker Medicals Ins. Co., China) and Gracey curettes (Hu-Friedy, Chicago, IL, USA) under local anesthesia twice a week in 4 sessions.

2.4. Clinical Periodontal Measurements

Clinical periodontal examinations were performed by one calibrated clinician (V.A.). Five periodontitis patients, who were not included in the study, were subjected to intra-examiner calibration. PD and CAL parameters were recorded with one-day apart. The intraexaminer kappa score was 0.91 for PD and 0.90 for CAL. All clinical measurements were recorded at baseline, 1 and 3 months after NSPT, including plaque index (PI), (28) GI, (29) BOP, PD, and CAL. During these procedures, the UNC-15 periodontal probe (Hu-Friedy, Chicago, IL, USA) was used, and except the third molars, all teeth were measured from six points.

2.5. Saliva and Serum Sampling

Unstimulated saliva samples were obtained from all the patients in the morning after at least 8 hours of fasting to minimize diurnal variations. Oral hygiene, food, and drinking were not allowed 3 hours before the procedure (30). The patients were asked to accumulate their saliva in the oral cavity for 2 minutes and then transfer at least 3.0 ml of saliva directly to a sterile glass beaker. Collected samples were transferred to sterile propylene tubes (Safe-Lock Tubes 2.0 ml, Sigma, Hamburg) with an automatic pipette. Subsequently, samples were stored at – 80° C until the day of analysis.

Serum was collected from the right or left antecubital regions with a vacutainer (BD Vacutainer Safety-Lok Blood Collection Set, USA) into 8.5 ml vacuum tubes (BD Vacutainer SST-II Advance) and centrifuged at 5000 rpm for 10 minutes. Serum samples were transferred to sterile propylene tubes and preserved at – 80 °C till the analysis day.

2.6. Biochemical Analysis

At the day of analyses, all samples were thawed. The salivary samples were centrifuged at 5500 rpm for 15 min at room temperature. Salivary cotinine (BT Labsystems, Shangai, China), salivary and serum RANKL, OPG, and IL-34 (Elabscience, Beijing, China) levels were analyzed with ELISA technique. All samples were tested in duplicate. RANKL, OPG, and IL-34 were detectable in all saliva and serum samples. The limit of detection for the assay was 0.5 ng/ml for cotinine, 0.10 ng/ml OPG, 9.38 pg/ml for RANKL, and 56.25 pg/ml for IL-34.

2.7. Statistical Analysis

The findings of the study were evaluated using the statistical package program (SPSS v22.0 for Windows, IBM, Chicago, IL). Distribution of clinical and biochemical variables was measured by Shapiro Wilk test. Since the variables were not distributed normally nonparametric tests were performed. The Kruskal-Wallis test was used for inter-group multiple comparisons and Bonferroni-corrected Mann-Whitney U test or Mann-Whitney U test for inter-group pairwise comparisons. For multiple intra-group comparisons Friedman test were performed when significant Bonferroni-corrected Wilcoxon test for pairwise comparisons. The Chi-Square test was performed to compare the gender distributions among groups. The Spearman correlation test was used to determine the correlations between clinical and biochemical parameters. The association of periodontitis or smoking with biochemical parameters was assessed using multinomial logistic regression. Statistical significance was accepted as p <.05.

3. RESULTS

During the whole study period, there were no dropouts, and the individuals did not change their smoking habits.

3.1. Clinical Findings

All study groups were similar in gender distribution (p>.05). The periodontitis groups were similar in age distribution (p>.05), but the healthy group was significantly younger than both periodontitis groups (p<.001). The median cigarette consumption/day was 20 and smoking duration/year was 20 in the smoker periodontitis group (Table 1).

***Table 1**. Demographic findings of the study*

Demographic variables	GROUPS (A)Healthy N=20	(B) Non-smoker Periodontitis-III-C N=20	(C) Smoker Periodontitis-III-C N=20	(A-B-C) p[a]	(A-B) p[b]	(A-C) p[b]	(B-C) p[b]
Age (years) median (Q1–Q3)	30.50 (27–33.5)	38 (35–51)	42.5 (38–48)	**<.001**	**<.001**	**<.001**	1.000
Sex N (%) **Female**	12 (60)	10 (50)	7 (35)				
Male	8 (40)	10 (50)	13 (65)	.281			
Cigarette consumption/day median (Q1–Q3)	-	-	20 (20–20)	-			
Smoking duration (year) median (Q1–Q3)	-	-	20 (15–25)	-			

[a]For gender Chi square test, others Kruskal Wallis test [b]Bonferroni-corrected Mann-Whitney U-test, p<.05 Statistically significant differences are marked in bold.

All clinical periodontal parameters in the healthy individuals were lower than in the periodontitis patients at baseline (p<.001) (Table 2). Except for PD, all clinical periodontal parameters at baseline between the non-smoker and the smoker periodontitis patients were similar (p>.05). Mean PD was higher in the smoker periodontitis group than in the non-smoker periodontitis group (p=.030). At 1 month, except for PD≥5mm+BOP and PD sites (%) with PD>6mm, all periodontal parameters were higher in the smoker periodontitis patients than in non-smoker periodontitis ones (p<.05). At 3 months, PD, PD sites with initially PD≥5mm, PD sites (%) with PD≥5mm, and CAL were higher in smoker than non-smoker periodontitis (p<.05).

***Table 2**. Comparison of clinical periodontal parameters among treatment groups at baseline, 1 and 3 months.*

Clinical parameters	Time points	GROUPS (A) Healthy N=20	(B) Non-smoker Periodontitis-III-C N=20	(C) Smoker Periodontitis-III-C N=20	(A-B-C) p[a]	(A-B) p[b]	(A-C) p[b]	(B-C) p[b]
PI	Baseline	0.07 (0.04–0.10)	2.23 (2.06–2.43)	2.25 (2.13–2.43)	**<.001**	**<.001**	**<.001**	1.000
	1 month	-	0.16 (0.04–0.33)[d]	0.38 (0.23–0.55)[d]				**.002**
	3 months	-	0.18 (0.04–0.28)[d]	0.19 (0.14–0.25)[d,e]				.323
	p[c]	-	**<.001**	**<.001**				
	Δ 0-3	-	2.14 (1.95–2.29)	2.02(1.91–2.25)				.892
GI	Baseline	0.04 (0.02–0.07)	1.45 (0.72–1.99)	1.21(0.62–1.48)	**<.001**	**<.001**	**<.001**	.984
	1 month	-	0.17 (0.06–0.41)[d]	0.44 (0.24–0.60)[d]				**.005**
	3 months	-	0.13 (0.08–0.26)[d]	0.20 (0.14–0.36)[d,e]				.072
	p[c]	-	**<.001**	**<.001**				
	Δ 0-3	-	1.24 (0.61–1.84)	0.88 (0.51–1.12)				**.036**
BOP (%)	Baseline	2.38 (1.34–4.74)	72.24 (36.2–95.30)	58.38 (31.35–72.7)	**<.001**	**<.001**	**<.001**	.696
	1 month	-	10.66 (5.7–20.56)[d]	22.34 (12.05–29.73)[d]				**.022**
	3 months	-	12.08 (5.73–15.71)[d]	9.97 (7.08–17.85)[d,e]				.776
	p[c]	-	**<.001**	**<.001**				
	Δ 0-3	-	58.52 (30.80–82.22)	44.15 (25.75–54.39)				**.026**
PD (mm)	Baseline	1.99(1.85–2.07)	3.86 (3.41–4.15)	4.54 (4.12–4.95)	**<.001**	**<.001**	**<.001**	**.030**
	1 month		2.79 (2.51–2.93)[d]	3.47 (3.22–3.95)[d]				**<.001**
	3 months		2.54 (2.37–2.74)[d]	3.33 (3.07–3.71)[d,e]				**<.001**
	p[c]	-	**<.001**	**<.001**				
	Δ 0-3	-	1.34 (0.83–1.44)	1.18 (1.04–1.52)				.960
Mean PD (mm) sites initially PD≥5 mm	Baseline		5.71 (5.5–5.9)	5.66 (5.4–6)				.659
	1 month		3.54 (3.2–3.9)[d]	4.16 (3.8–4.5)[d]				**.006**
	3 months		3.44 (2.9–3.8)[d]	3.93 (3.5–4.3)[d,e]				**.010**
	p[c]		**<.001**	**<.001**				
	Δ 0-3		2.40 (2.2–2.7)	1.84 (1.6–2.1)				**<.001**
Sites with PD≥5 mm (%)	Baseline		35.37 (21.3–42.2)	49.97 (37.6–61.9)				**<.001**
	1 month		7.44 (4.2–12.8)[d]	20.20 (8.4–34)[d]				**.005**
	3 months		4.17 (3–9.4)[d]	14.20 (6.9–31)[d]				**.002**
	p[c]		**<.001**	**<.001**				
	Δ 0-3		23.36 (15.1–35.8)	30.45 (24.5–37.6)				.068
Sites with PD≥5 mm and BOP (%)	Baseline		25.74 (15.1–40.4)	31 (14.3–44.2)				.383
	1 month		3.12 (1.3–6.1)[d]	5.10 (1.6–8.1)[d]				.242
	3 months		1.65 (0.7–4.8)[d]	3.15 (0.9–5.6)[d]				.355
	p[c]		**<.001**	**<.001**				
	Δ 0-3		23.07 (12–35.7)	27.46 (11.9–37.6)				.369
Sites with PD>6 mm (%)	Baseline		6.45 (4–10.1)	9.92 (4.2–17.1)				.102
	1 month		0.70 (0–1.5)[d]	1.25 (0–3.6)				.242
	3 months		0.00 (0–0.7)[d]	0.00 (0–2.4)[d]				.414
	p[c]		**<.001**	**<.001**				
	Δ 0-3		5.60 (4–8.3)	9.20 (3.7–15.1)				.121
CAL (mm)	Baseline	2.01(1.9–2.1)	4.61 (3.9–5.5)	5.32 (4.7–5.9)	**<.001**	**<.001**	**<.001**	.498
	1 month	-	3.54 (3–4.0)[d]	4.28 (3.7–4.9)[d]				**.007**
	3 months	-	3.22 (2.8–3.7)[d]	4.06 (3.4–4.8)[d,e]				**.008**
	p[c]	-	**<.001**	**<.001**				
	Δ 0-3	-	1.14 (0.6–1.8)	1.15 (0.9–1.5)				.522

Data are presented as median (Q1–Q3) values. [a]Kruskal Wallis test. [b]Bonferroni-corrected Mann-Whitney U-test or Mann-Whitney U-test. [c]Friedman test. [d]Significant difference compared to baseline, [e]Significant difference compared to 1 month (posthoc Bonferroni-corrected Wilcoxon sign-rank test), p<.05. Statistically significant differences are marked in bold. Abbreviations: PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level.

***Table 3**. Comparison of biochemical parameters among groups at baseline, 1 and 3 months.* Data are presented as median (Q1–Q3) values.

Biochemical parameters	Time points	GROUPS (A) Healthy N=20	(B) Non-smoker Periodontitis-III-C N=20	(C) Smoker Periodontitis-III-C N=20	(A-B-C) p[a]	(A-B) p[b]	(A-C) p[b]	(B-C) p[b]
Saliva Cotinine (ng/ml)	Baseline	0.10 (0.05–0.59)	0.28 (0.08–0.60)	10.80 (9.14–19.81)	**<.001**	1.000	**<.001**	**<.001**
Saliva RANKL (pg/ml)	Baseline	729.05 (474.8–948.7)	736.60 (624–948.5)	787.50 (682.4–887.7)	.591	-	-	-
	1 month		607.66 (394–791.4)	806.25 (611.9–1268.3)				**.014**
	3 months		667.29 (458.7–1040.8)	830.81 (609.7–1042.2)				.211
	p[c]		.142	.861				
	Δ 0-3		-23 (-198.2–374.2)	-75.14 (-244.6–235.1)				.758
Saliva OPG (pg/ml)	Baseline	1200 (362.5–2175)	1835 (1037.5–2277.5)	1960 (947.5–3392.5)	.064	-	-	-
	1 month		870 (552.5–1492.5)[d]	1105 (455–1725)				.583
	3 months		875 (587.5–1262.5)[d]	635 (572.5–1080)[d]				.355
	p[c]		**.002**	**.001**				
	Δ 0-3		535 (52.5–1292.5)	850 (465–2572.5)				.091
Saliva RANKL/OPG (pg/ml)	Baseline	0.61 (0.44–1.41)	0.41 (0.4–0.6)	0.43 (0.3–0.7)	.079	-	-	-
	1 month		0.68 (0.3–1.1)	1.07 (0.4–1.4)[d]				.086
	3 months		0.95 (0.6–1.2)[d]	1.43 (0.6–2.0)[d]				.114
	p[c]		**.041**	**.002**				
	Δ 0-3		-0.39 (-0.8 – – 0.2)	-0.59 (-1.7– – 0.2)				.134
Saliva IL-34 (pg/ml)	Baseline	99.45 (17.8–346.4)	431.38 (132.3–1165)	340.38 (184.1–1543.3)	**.001**	**.001**	**.001**	.718
	1 month		299.36 (157.1–502.7)[d]	304.69 (191.4–495.1)[d]		**.018**	**.007**	.779
	3 months		266.87 (172.7–368)[d]	237.02 (169–416.4)[d]		**.024**	**.017**	.947
	p[c]		**.040**	**.047**				
	Δ 0-3		197.87 (79.1–785.5)	168.8 (-82.1–1106.2)				.820
Serum RANKL (pg/ml)	Baseline	13.85 (6.9–42.7)	11.25 (8.5–27.4)	34.56 (15.5–51.7)	**.012**	.989	**.026**	**.003**
	1 month		11.89 (5.7–29.6)	34.52 (11.4–58.1)				**.028**
	3 months		9.39 (4.9–15.1)	25.9 (14.9–48.5)				**.009**
	p[c]		.449	.861				
	Δ 0-3		1.97 (-4.6–21.6)	4.93 (-8.4–20.4)				.989
Serum OPG (pg/ml)	Baseline	195 (122.5–320)	220 (152.5– 965)	515 (257.5–777.5)	.076	-	-	-
	1 month		330 (137.5–660)	290 (157.5–777.5)				.947
	3 months		240 (172.5–330)	485 (152.5–605)				.108
	p[c]		.711	.091				
	Δ 0-3		20 (-50–362.5)	35 (-77.5–155)				.779
Serum RANKL/ OPG (pg/ml)	Baseline	0.07 (0.03–0.2)	0.05 (0.03–0.08)	0.07 (0.05–0.12)	.073	-	-	-
	1 month		0.04 (0.02–0.06)	0.06 (0.03–0.18)				**.043**
	3 months		0.04 (0.03–0.07)	0.05 (0.04–0.10)				.127
	p[c]		.522	.819				
	Δ 0-3		0.01 (-0.02–0.03)	0.01 (-0.04–0.06)				.947
Serum IL-34 (pg/ml)	Baseline	24.65 (22.73–26.9)	24.35 (23.3–25.4)	24.94 (23.9–26)	.519	-	-	-
	1 month		17.59 (15.7–24.4)[d]	15.99 (15.5–19.1)[d]				.134
	3 months		16.44 (15.9–18.2)[d]	17.36 (16–51.4)				.327
	p[c]		**<.001**	**<.001**				
	Δ 0-3		7.35 (5.2–9.4)	7.52 (-27–8.9)				.445

[a]Kruskal Wallis test. [b]Bonferroni-corrected Mann-Whitney U-test or Mann-Whitney U-test. [c]Friedman test. [d]Significant difference compared to baseline (posthoc Bonferroni-corrected Wilcoxon sign-rank test), p<.05 Statistically significant differences are marked in bold. Abbreviations: RANKL, nuclear factor kappa B ligand; OPG, osteoprotegerin; IL, interleukin; pg, picogram; ml, mililiter ; ng, nanogram

Significant improvements in all clinical periodontal parameters at 1 and 3 months compared to baseline (p<.05) were detected in both periodontitis groups. Although reduction in BOP and GI were significantly higher at baseline to 3 months in the non-smoker than smoker periodontitis group (p=.036 and p=.026, respectively), PD and CAL reduction were similar in non-smoker and smoker periodontitis patients (p>.05). The reduction in the mean PD sites with initially PD≥5mm was significantly higher at baseline to 3 months in non-smoker than smoker periodontitis (p<.001).

3.2. Biochemical Findings

All self-defined smoker subjects had cotinine levels ≥8 ng/ml and were confirmed as active smokers (31). The median value of salivary cotinine levels in smoker periodontitis was higher than in healthy or non-smoker periodontitis groups (p<.001) (Table 3).

Salivary levels of OPG, RANKL, RANKL/OPG were similar among groups at baseline (p>.05). In both periodontitis groups, salivary OPG levels decreased, and RANKL/OPG levels increased after NSPT (p<.05). Only IL-34 levels in saliva were lower in the non-smoker periodontally healthy group than both periodontitis groups at all time points (p<.05). Besides, in the non-smoker periodontitis group the salivary level of IL-34 decreased significantly both at 1 and 3 months after NSPT compared to baseline (p<.05) but in the smoker periodontitis group only at 3 months (p<.05).

In serum, OPG and IL-34 levels at baseline were similar between all groups (p>.05), but serum RANKL levels were higher in the smoker periodontitis group than both non-smoker periodontitis and healthy ones at all time points (p<.05). At baseline and 1-month elevated serum RANKL/OPG levels were detected in smoker compared to non-smoker periodontitis group. (p<.05). There were no changes in serum OPG, RANKL, and RANKL/OPG levels in either periodontitis groups after NSPT (p>.05). However, serum IL-34 levels decreased in both groups at all time points compared to baseline (p<.001).

Since only salivary IL-34 and serum RANKL caused significant differences between the study groups, further analyzes were performed on these two molecules. Salivary IL-34 levels were higher in periodontitis patients than in healthy group (p<.001) (Table 4). Salivary IL-34 and serum RANKL levels were lower in the non-smoker individuals than in the smoker individuals (p=.044 and p=.003, respectively).

Correlation analysis revealed that age was positively correlated with all clinical parameters and salivary IL-34 (p<.05) (Table 5). The salivary IL-34 levels were positively correlated with PI, GI, BOP, PD, CAL, cotinine level, smoking duration, and number of smoking (p<.05). Serum RANKL had weak positive correlations with PD and cotinine levels (p<.05) but strong positive correlations with smoking duration and the number of smoking (p<.001).

Significant associations between periodontitis and salivary IL-34 were found both before and after adjusting for age and smoking (p<.05) (Table 6). Moreover, salivary IL-34 levels were associated with smoking (p=.034). Smoking was associated with serum RANKL both before and after adjusting for age and periodontitis (p=.039 and p=.030, respectively).

Table 4. *Comparison of baseline biochemical parameters in relation to periodontal and smoking status.*

Biochemical Parameters	*Periodontitis N=40*	*Periodontally healthy N=20*	*p*[a]	*Smokers N=20*	*Nonsmokers N=40*	*p*[a]
Saliva IL-34 (pg/ml)	425.63 (184.16–1409.6)	99.45 (17.8–346.4)	**<.001**	340.38 (184.1–1543.3)	251.03 (73.5–559.3)	**.044**
Serum RANKL (pg/ml)	21.48 (10.9–43.66)	13.85 (6.94–42.69)	.196	34.55 (15.5–51.7)	12.65 (7.95–35.14)	**.003**

Data are presented as median (Q1-Q3) values. [a]Mann-Whitney U-test. p<.05. Statistically significant differences are marked in bold. Abbreviations: IL, interleukin; RANKL, nuclear factor kappa B ligand; pg, picogram; ml, mililiter

Table 5. *Correlations between selected clinical and biochemical parameters at baseline.*

All groups	Age	Saliva IL-34 (pg/ml)	Serum RANKL (pg/ml)	Cotinine (ng/ml)	Smoking Duration	Number of Smoking Cigarette
PI	**0.459****	**0.449****	0.178	**0.422****	**0.422****	**0.440****
GI	**0.446****	**0.522****	0.134	**0.361****	**0.296***	**0.293***
BOP (%)	**0.408****	**0.561****	0.101	**0.331***	**0.266***	**0.269***
PD (mm)	**0.535****	**0.503****	**0.270***	**0.554****	**0.713****	**0.708****
CAL (mm)	**0.614****	**0.429****	0.182	**0.497****	**0.569****	**0.587****
Saliva IL-34 (pg/ml)	**0.309***	-	-	**0.341****	**0.301***	**0.277***
Serum RANKL (pg/ml)	0.176	-	-	**0.273***	**0.376****	**0.374****

*Correlation coefficient values by Spearman-correlation test. *p<.05, **p<.01. Abbreviations: PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level; mm, millimeter; IL, interleukin; RANKL, nuclear factor kappa B ligand; pg, picogram; ml, millilitre*

Table 6. *Multinominal logistic regression analysis for unadjusted and adjusted associations between salivary IL-34, serum RANKL, periodontitis III-C and smoking.*

	Periodontitis III-C Unadjusted OR (95% CI), p	Adjusted (Smoking and Age) OR (95% CI), p	Smoking Unadjusted OR (95% CI), p	Adjusted (Periodontitis and Age) OR (95% CI), p
Saliva IL-34	1.003 (1.001-1.006), **.015**	1.004 (1.000-1.008), **.027**	1.001 (1.000-1.002), **.034**	1.000 (1.000-1.001), .412
Serum RANKL	1.005 (0.986-1.025), .593	0.994 (0.961-1.028), .738	1.022 (1.001-1.043), **.039**	1.042 (1.004-1.082), **.030**

p<.05. Statistical differences are marked in bold. Abbreviations: 95% CI, confidence interval of 95%; OR, odds ratio; IL, interleukin; RANKL, nuclear factor kappa B ligand.

4. DISCUSSION

Smoking is one of the most important risk factors in periodontal disease development and one of periodontitis grade determining factors in the latest periodontal disease classification. The main feature of periodontitis is the alveolar bone loss, which is regulated by the osteoclast and osteoblast activities. The important role of RANKL, OPG and, more recently, IL-34 in osteogenesis was demonstrated (32,33). In the present study, the effect of smoking on clinical parameters and RANKL, OPG, and IL-34 levels in saliva and serum after NSPT in periodontitis-III-C was investigated.

Previous studies suggested that smoking had a negative effect on BOP reduction (1,7). In line with that in the present study, the reductions from baseline to 3 months in GI and BOP were greater in non-smoker periodontitis than in smokers. Considering the reduction in PD and CAL gain, some studies reported that smokers were less responsive to NSPT(4,5), as well as others showed similar results between smoker and non-smoker periodontitis patients (6,7). In this study, smokers and non-smokers with periodontitis patients had similar PD decrease and CAL gain. A recent meta-analysis (34) reported a significant but modest negative effect of smoking in NSPT in periodontitis patients with mean difference 0.33 mm in PD reduction and 0.20 mm in CAL gain. Although these differences are statistically significant, their clinical significance is questionable. Since PD and CAL reflect the average of the whole mouth, including all of the shallow and deep PD, it is more accurate to examine the diseased areas to evaluate the response to NSPT. According to the same meta-analysis, when comparing PD reduction in initially deep pockets (≥5 mm), the difference in PD reduction between smokers and non-smokers periodontitis patients was more pronounced (0.50 mm) (34). Our results indicated that PD reduction baseline to 3-months in sites initially PD≥5 mm was higher in the non-smoker periodontitis patients than in the smoker ones, with a difference of 0.56 mm.

RANKL is the master regulator of osteoclastogenesis and plays an essential role in osteoclast-associated diseases, like periodontitis (35). OPG, on the other hand, is a natural inhibitor of osteoclast differentiation (14). Although the effect of smoking on alveolar bone destruction is well documented, the exact processes by which smoking impacts the periodontium remain unknown. Nicotine and LPS together induce the formation of osteoclast-like cells by increasing M-CSF and PGE2 and decreasing OPG production from osteoblasts. Regardless of the administration of nicotine or LPS, no RANKL expression was found (10).

Although the RANKL-OPG mechanism has been explained in cell and animal studies, the results of the clinical studies in which they were examined in different body fluids could not reach a consensus. Some studies showed elevated levels of RANKL and downregulation of OPG in GCF, saliva or periodontally diseased tissues in periodontitis patients, (20,21) yet others have presented similar RANKL (19,22,36) and OPG (19,37) levels or higher OPG levels (38) in saliva or GCF in periodontitis patients compared with their controls. In addition, the effect of smoking on the RANKL-OPG mechanism has not been clarified. Lappin et al.(16) and Tang et al.(17) reported increased RANKL/OPG ratios in serum and GCF in smokers than in non-smoker periodontitis ones. However, other studies showed no statistical differences in OPG and RANKL levels in serum and GCF between smoker and non-smoker periodontitis patients (15,18,19). While Behfarnia et al. (19) found the salivary RANKL/OPG ratio high in non-smokers, Buduneli et al. (37) found it higher in smokers. Furthermore, in a significant portion of the studies in the literature, salivary RANKL and OPG levels have frequently stayed below the detection limit (36). Although applied laboratory methodologies and selected study populations are often used as justifications for the contradictory results across different studies, RANKL and OPG results, especially in saliva levels, were not consistent.

The recent meta-analysis reported that elevated GCF RANKL levels were significantly detected periodontitis compared to healthy. Nevertheless, no differences in RANKL and OPG levels were found in saliva or serum (39). In line with that, in the present study, salivary RANKL and OPG levels were similar between the healthy and periodontitis groups. However, salivary OPG levels had a trend toward elevation in the periodontitis patients and decreased after NSPT in line with the previous studies (18,40). This could be explained by the increase of osteoclast precursor cells during the periodontal breakdown, and the decrease in release of OPG . However, after NSPT, the need for inhibition decreases, resulting in a reduction in OPG levels.

Another important issue to consider is which form of RANKL contributes to periodontal bone loss. RANKL is an agonistic ligand for RANK in both its soluble and membrane-bound forms. However, the mRANKL is more effective than sRANKL (41). This may be due to the presence of

mRANKL in cell-to-cell contact, but sRANKL is not. The cell-to-cell interaction between osteoclast precursor cells and mesenchymal cells in bone is required to activate osteoclastogenesis. As a result, it's thought that RANKL's cellular origins should be in direct contact with the alveolar bone (35). Indeed, the contribution of sRANKL in periodontal bone resorption has been shown to be negligible in animal studies (42). Thus, salivary and serum sRANKL and OPG levels may not be suitable for detecting active bone resorption in periodontitis.

Smoking may increase matrix metalloproteinase (MMP) expression (43). MMPs appear to be a responsible molecule cleaving the mRANKL to its soluble form, while tissue inhibitors of MMPs (TIMP) prevent this conversion (44). Previous research has indicated that smoker periodontitis patients have significantly higher levels of MMP-9 and lower levels of TIMP-1 in serum than non-smoker periodontitis patients (45,46). This could be the possible explanation for elevated serum RANKL levels in smoker patients in the present study. Moreover, serum RANKL was associated with smoking even adjusted for periodontitis and age. However, more studies are needed to clarify the possible effect of smoking on the relationship between sRANKL and MMPs.

In RANKL-induced osteoclastogenesis, IL-34 operates similarly to M-CSF in osteoclast differentiation. IL-34 in combination with RANKL stimulates osteoclast differentiation, causes bone destruction and decrease bone mass in M-CSF-deficient mouse bone marrow cells (32). The result of present study indicated that salivary IL-34 levels were higher in periodontitis groups than in the healthy group at baseline and associated with periodontitis-III-C in line with a previous study from our group (30). Salivary and serum IL-34 levels were decreased significantly in smoker and non-smoker periodontitis patients after NSPT. These findings were in accordance with previous reports of altered levels of these markers in GCF (21,24,47) and serum (24,47) in periodontitis and decreased after NSPT (21,47). However, some studies reported that healthy controls showed higher IL-34 levels in saliva (48,49) and elevated after NSPT (49). The reason for all these contradictory results may be that IL-34 has both an anti – and pro-inflammatory role (50). However, based on our results we may speculate that IL-34 can be a possible periodontitis and treatment response marker but failed to differentiate the smoking effect. As a result, it's possible to suggest that IL-34 has a pro-inflammatory effect and plays a critical function in the pathology of periodontitis. However, future studies should confirm these results to investigate the potential role of IL-34 in periodontal disease.

Well-characterized study groups, follow-up design, and evaluation of cotinine levels are strengths of the present study. Limitations of this study are the lack of GCF to confirm our results and lack of a systemically healthy smoker group and other stages or grades of periodontitis.

5. CONCLUSION

The clinical outcomes of the present study showed that smokers are less responsive to NSPT than non-smoker periodontitis-III-C. In addition, elevated serum RANKL levels are associated with smoking. Furthermore, salivary IL-34 can be a promising marker for periodontitis-III-C, but its role in other stages and grades of periodontitis urges further investigation.

Funding: *This study was supported by grants from Marmara University Scientific Research Projects Coordination Unit #SAG-C-DRP-241.018.0569*

Conflicts of interest: *The authors declare that they have no conflict of interest.*

Ethics Committee Approval: *This study was approved by Clinical Research Ethics Committee of Faculty of Medicine, Marmara University (Decision date / number: 13.07.2018 / 09.2018.513).*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: V.A., B.D.
Design of the study: B.D.
Acquisition of data for the study: V.A.
Analysis of data for the study: V.A., N.G.G.
Interpretation of data for the study: V.A., N.G.G., B.D.
Drafting the manuscript: V.A., N.G.G.
Revising it critically for important intellectual content: B.D.
Final approval of the version to be published: V.A., N.G.G., B.D.

REFERENCES

[1] Apatzidou D, Riggio M, Kinane D. Impact of smoking on the clinical, microbiological and immunological parameters of adult patients with periodontitis. J Clin Periodontol. 2005;32(9):973-983. DOI: 10.1111/j.1600-051X.2005.00788.x

[2] Johnson GK, Guthmiller JM. The impact of cigarette smoking on periodontal disease and treatment. Periodontol 2000. 2007;44(1):178-194. DOI: 10.1111/j.1600-0757.2007.00212.x

[3] Helal O, Göstemeyer G, Krois J, Fawzy El Sayed K, Graetz C, Schwendicke F. Predictors for tooth loss in periodontitis patients: Systematic review and meta-analysis. J Clin Periodontol. 2019;46(7):699-712. DOI: 10.1111/jcpe.13118

[4] Guru S, Sam SE, Rajan S, Padmanabhan S. Comparative evaluation of salivary hepatocyte growth factor in smokers and non-smokers with chronic periodontitis. J Investig Clin Dent. 2018;9(2):e12306. DOI: 10.1111/jicd.12306

[5] Preshaw PM, Lauffart B, Zak E, Jeffcoat MK, Barton I, Heasman PA. Progression and treatment of chronic adult periodontitis. J Periodontol. 1999;70(10):1209-1220. DOI: 10.1902/jop.1999.70.10.1209

[6] Preshaw P, Holliday R, Law H, Heasman P. Outcomes of non-surgical periodontal treatment by dental hygienists in training: impact of site-and patient-level factors. Int J Dent Hyg. 2013;11(4):273-279. DOI: 10.1111/idh.12032

[7] Ardais R, Mário Tde G, Boligon J, Kantorski KZ, Moreira CH. The effect of smoking on bleeding on probing after nonsurgical periodontal therapy: a quasi-experimental study. Braz Oral Res. 2014;28:1-7. DOI: 10.1590/1807-3107bor-2014.vol28.0058

[8] Buduneli N, Kardeşler L, Işık H, Willis III CS, Hawkins SI, Kinane DF, Scott DA. Effects of smoking and gingival inflammation on salivary antioxidant capacity. J Clin Periodontol. 2006;33(3):159-164. DOI: 10.1111/j.1600-051X.2006.00892.x

[9] Ojima M, Hanioka T. Destructive effects of smoking on molecular and genetic factors of periodontal disease. Tob Induc Dis. 2010;8(1):1-8. DOI: 10.1186/1617-9625-8-4

[10] Tanaka H, Tanabe N, Shoji M, Suzuki N, Katono T, Sato S, Motohashi M, Maeno M. Nicotine and lipopolysaccharide stimulate the formation of osteoclast-like cells by increasing macrophage colony-stimulating factor and prostaglandin E2 production by osteoblasts. Life Sci. 2006;78(15):1733-1740. DOI: 10.1016/j.lfs.2005.08.017

[11] Linden GJ, Lyons A, Scannapieco FA. Periodontal systemic associations: Review of the evidence. J Periodontol. 2013;84 (Suppl 4):8-19. DOI: 10.1902/jop.2013.134.0010.

[12] Kong Y-Y, Yoshida H, Sarosi I, Tan H-L, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature. 1999;397(6717):315-323. DOI: 10.1038/16852

[13] Lacey D, Timms E, Tan H-L, Kelley M, Dunstan C, Burgess T, Elliott R, Colombero A, Elliott G, Scully S. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell. 1998;93(2):165-176. DOI: 10.1016/S0092-8674(00)81569-X

[14] Simonet W, Lacey D, Dunstan C, Kelley M, Chang M-S, Lüthy R, Nguyen H, Wooden S, Bennett L, Boone T. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell. 1997;89(2):309-319. DOI: 10.1016/S0092-8674(00)80209-3

[15] Özçaka Ö, Nalbantsoy A, Köse T, Buduneli N. Plasma osteoprotegerin levels are decreased in smoker chronic periodontitis patients. Aust Dent J. 2010;55(4):405-410. DOI: 10.1111/j.1834-7819.2010.01261.x

[16] Lappin DF, Sherrabeh S, Jenkins WM, Macpherson LM. Effect of smoking on serum RANKL and OPG in sex, age and clinically matched supportive-therapy periodontitis patients. J Clin Periodontol. 2007;34(4):271-277. DOI: 10.1111/j.1600-051X.2007.01048.x

[17] Tang TH, Fitzsimmons TR, Bartold PM. Effect of smoking on concentrations of receptor activator of nuclear factor κ B ligand and osteoprotegerin in human gingival crevicular fluid. J Clin Periodontol. 2009;36(9):713-718. DOI: 10.1111/j.1600-051X.2009.01444.x

[18] Buduneli N, Buduneli E, Kütükçüler N. Interleukin-17, RANKL, and osteoprotegerin levels in gingival crevicular fluid from smoking and non-smoking patients with chronic periodontitis during initial periodontal treatment. J Clin Periodontol. 2009;80(8):1274-1280. DOI: 10.1902/jop.2009.090106

[19] Behfarnia P, Saied-Moallemi Z, Javanmard SH, Naseri R. Serum, saliva, and GCF concentration of RANKL and osteoprotegerin in smokers versus nonsmokers with chronic periodontitis. Adv Biomed Res. 2016;5:80. DOI: 10.4103/2277-9175.180992.

[20] Yuce HB, Gokturk O, Turkal HA, Inanir A, Benli I, Demir O. Assessment of local and systemic 25-hydroxy-vitamin D, RANKL, OPG, and TNF levels in patients with rheumatoid arthritis and periodontitis. J Oral Sci. 2017;59(3):397-404. DOI: 10.2334/josnusd.16-0677

[21] Doğan SB, Dede FÖ, Balli U, Sertoglu E. Emerging roles of Interleukin-34 together with receptor activator of nuclear factor-kB ligand and osteoprotegerin levels in periodontal disease. Cytokine. 2021;144:155584. DOI: 10.1016/j.cyto.2021.155584

[22] Shetty B, Divakar DD, Al-Kheraif AA, Alharbi AO, Almutairi MST, Alanazi MM. Role of PDT as an adjunct to SRP on whole salivary RANKL and OPG ratio in type-2 diabetic and normoglycemic individuals with chronic periodontitis. Cytokine. 2021;34:102220. DOI: 10.1016/j.pdpdt.2021.102220

[23] Lin H, Lee E, Hestir K, Leo C, Huang M, Bosch E, Halenbeck R, Wu G, Zhou A, Behrens D. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. Science. 2008;320(5877):807-811. DOI: 10.1126/science.1154370

[24] Guruprasad C, Pradeep A. Influence of smoking on interleukin-34 levels in gingival crevicular fluid and plasma in periodontal health and disease: A clinico-biochemical study. Bull Tokyo Dent Coll. 2018;59(4):247-255. DOI: 10.2209/tdcpublication.2017-0051

[25] Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Periodontol. 2018;89(Suppl 1):S159-S172. DOI: 10.1002/jper.18-0006

[26] Lang NP, Bartold PM. Periodontal health. J Periodontol. 2018;89 Suppl 1:S9-s16. DOI: 10.1002/jper.16-0517

[27] Türkoğlu O, Eren G, Emingil G, Azarsız E, Kutukculer N, Atilla G. Does smoking affect gingival crevicular fluid LL-37 levels following non-surgical periodontal treatment in chronic periodontitis? Arch Oral Biol. 2016;61:98-105. DOI: 10.1016/j.archoralbio.2015.10.018

[28] Silness J, Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand. 1964;22(1):121-135. DOI: 10.3109/000.163.56408993968.

[29] Löe H, Silness J. Periodontal disease in pregnancy I. Prevalence and severity. Acta Odontol Scand. 1963;21(6):533-551. DOI: 10.3109/000.163.56309011240

[30] Görgülü NG, Doğan B. Effect of non-surgical periodontal treatment on salivary and serum biomarkers in stage III grade B and C periodontitis. J Periodontol. 2022. DOI: 10.1002/JPER.21-0536

[31] Yamamoto Y, Nishida N, Tanaka M, Hayashi N, Matsuse R, Nakayama K, Morimoto K, Shizukuishi S. Association between passive and active smoking evaluated by salivary cotinine and periodontitis. J Clin Periodontol. 2005;32(10):1041-1046. DOI: 10.1111/j.1600-051X.2005.00819.x

[32] Chen Z, Buki K, Vääräniemi J, Gu G, Väänänen HK. The critical role of IL-34 in osteoclastogenesis. PloS one. 2011;6(4):e18689. DOI: 10.1371/journal.pone.0018689

[33] Yasuda H. Discovery of the RANKL/RANK/OPG system. J Bone Miner Metab. 2021;39(1):2-11. DOI: 10.1007/s00774.020.01175-1

[34] Chang J, Meng HW, Lalla E, Lee CT. The impact of smoking on non-surgical periodontal therapy: A systematic review and meta-analysis. J Clin Periodontol. 2021;48(1):60-75. DOI: 10.1111/jcpe.13384

[35] Tsukasaki M. RANKL and osteoimmunology in periodontitis. J Bone Miner Metab. 2021;39(1):82-90. DOI: 10.1007/s00774.020.01165-3

[36] Frodge BD, Ebersole JL, Kryscio RJ, Thomas MV, Miller CS. Bone remodeling biomarkers of periodontal disease in saliva. J Periodontol. 2008;79(10):1913-1919. DOI: 10.1902/jop.2008.080070

[37] Buduneli N, Bıyıkoğlu B, Sherrabeh S, Lappin DF. Saliva concentrations of RANKL and osteoprotegerin in smoker

versus non-smoker chronic periodontitis patients. J Clin Periodontol. 2008;35(10):846-852. DOI: 10.1111/j.1600-051X.2008.01310.x

[38] Al-Sabbagh M, Alladah A, Lin Y, Kryscio RJ, Thomas MV, Ebersole JL, Miller CS. Bone remodeling-associated salivary biomarker MIP-1α distinguishes periodontal disease from health. J Periodontal Res. 2012;47(3):389-395. DOI: 10.1111/j.1600-0765.2011.01445.x

[39] Caldeira FID, Hidalgo MAR, Dias MLDC, Scarel-Caminaga RM, Pigossi SC. Systematic review of ratios between disease/health periodontitis modulators and meta-analysis of their levels in gingival tissue and biological fluids. Arch Oral Biol. 2021;127:105147. DOI: 10.1016/j.archoralbio.2021.105147

[40] Tabari ZA, Azadmehr A, Tabrizi MAA, Hamissi J, Ghaedi FB. Salivary soluble receptor activator of nuclear factor kappa B ligand/osteoprotegerin ratio in periodontal disease and health. J Periodontal Implant Sci. 2013;43(5):227. DOI: 10.5051/jpis.2013.43.5.227

[41] Hikita A, Yana I, Wakeyama H, Nakamura M, Kadono Y, Oshima Y, Nakamura K, Seiki M, Tanaka S. Negative regulation of osteoclastogenesis by ectodomain shedding of receptor activator of NF-kappaB ligand. J Biol Chem. 2006;281(48):36846-36855. DOI: 10.1074/jbc.M606656200

[42] Tsukasaki M, Komatsu N, Nagashima K, Nitta T, Pluemsakunthai W, Shukunami C, Iwakura Y, Nakashima T, Okamoto K, Takayanagi H. Host defense against oral microbiota by bone-damaging T cells. Nat Commun. 2018;9(1):701. DOI: 10.1038/s41467.018.03147-6

[43] Perlstein TS, Lee RT. Smoking, metalloproteinases, and vascular disease. Arterioscler Thromb Vasc Biol. 2006;26(2):250-256. DOI: 10.1161/01.ATV.000.019.9268.27395.4f

[44] Lum L, Wong BR, Josien R, Becherer JD, Erdjument-Bromage H, Schlöndorff J, Tempst P, Choi Y, Blobel CP. Evidence for a role of a tumor necrosis factor-alpha (TNF-alpha)-converting enzyme-like protease in shedding of TRANCE, a TNF family member involved in osteoclastogenesis and dendritic cell survival. J Biol Chem. 1999;274(19):13613-13618. DOI: 10.1074/jbc.274.19.13613

[45] Ozçaka O, Biçakci N, Pussinen P, Sorsa T, Köse T, Buduneli N. Smoking and matrix metalloproteinases, neutrophil elastase and myeloperoxidase in chronic periodontitis. Oral Dis. 2011;17(1):68-76. DOI: 10.1111/j.1601-0825.2010.01705.x

[46] He C-Y, Gao X-Q, Jiang L-P. The impact of smoking on levels of chronic periodontitis-associated biomarkers. Exp Mol Pathol. 2016;101(1):110-115. DOI: 10.1016/j.yexmp.2016.07.004

[47] Guruprasad C, Pradeep A. Effect of nonsurgical periodontal therapy on interleukin-34 levels in periodontal health and disease. Indian J Dent Res. 2018;29(3):280-285. DOI: 10.4103/ijdr.IJDR_527_17

[48] Martinez G, Majster M, Bjurshammar N, Johannsen A, Figueredo C, Boström E. Salivary colony stimulating factor-1 and interleukin-34 in periodontal disease. J Periodontol. 2017;88(8):e140-e149. DOI: 10.1902/jop.2017.170081

[49] Lira-Junior R, Bissett SM, Preshaw PM, Taylor JJ, Boström EA. Levels of myeloid-related proteins in saliva for screening and monitoring of periodontal disease. J Clin Periodontol. 2021;48(11):1430-1440. DOI: 10.1111/jcpe.13534

[50] Muñoz-Garcia J, Cochonneau D, Télétchéa S, Moranton E, Lanoe D, Brion R, Lézot F, Heymann MF, Heymann D. The twin cytokines interleukin-34 and CSF-1: masterful conductors of macrophage homeostasis. Theranostics. 2021;11(4):1568-1593. DOI: 10.7150/thno.50683

How to cite this article: Arıkan V, Görgülü NG, Doğan B. Clinical and Biochemical Effects of Smoking on Non-Surgical Periodontal Treatment in Grade III Stage C Periodontitis Patients. Clin Exp Health Sci 2023; 13: 218-227. DOI: 10.33808/clinexphealthsci.1128101