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The influence of smoking on oral neutrophils and matrix metalloproteinase-8 in periodontitis patients before and after nonsurgical treatment

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ABSTRACT

Objective: To evaluate and compare the oral neutrophil numbers (ONN) in saliva, the level of matrix metalloproteinase-8 (MMP-8) in gingival crevicular fluid (GCF) and the periodontal parameters in smokers versus non-smokers with periodontitis, before and after nonsurgical periodontal treatment (NSPT).
Materials and method: 40 chronic periodontitis patients including 20 smokers and 20 non-smokers were enrolled in this quasi-experimental study. All patients were received the NSPT included instructing oral hygiene, scaling and root planing. At baseline (T0) and after NSPT 1 month (T1) and 3 months (T3), all patients were assessed for salivary ONN, GCF MMP-8, and clinical parameters like plaque index (PlI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment loss (CAL). The differences between the two groups were analyzed using the independent sample *t*-test and the Mann-Whitney *U* test; and the differences between T0, T1 and T3 of each group were analyzed with paired-samples *t*-test and Wilcoxon signed-rank test. The level of significance was set at 0.05.
Results: The ONN was significantly less in smokers than in non-smokers although there was no significant difference in other parameters between the two groups at baseline ($p > 0.05$). All clinical periodontal parameters reduced significantly after 1 month and 3 months of NSPT in both groups ($p < 0.01$). PPD of non-smokers was significantly lower than those of smokers at T1 and T3. ONN and MMP-8 level showed a significant decrease in non-smoking subjects, while there was no significant difference in smoking ones after NSPT (T1 and T3). At 1 month after treatment, ONN tended to reduce in non-smokers whereas to increase in smokers significantly.
Conclusion: Smoking reduced ONN, impaired treatment effect in reducing PPD, and changed the MMP-8 level in gingival crevicular fluid to NSPT.
Trial registration: Identifier NCT04974502 in CLinicalTrials.gov

1. Introduction

Periodontitis is an infection that occurs due to an imbalance between the body's response to a bacterial attack, causing destruction of periodontal tissue and alveolar bone resorption.[1] If not treated appropriately, prolonged periodontal infection leads to tooth loss, affecting masticatory function, pronunciation and aesthetics.[2] Smoking has been identified as a risk factor for periodontal disease, affecting not only the frequency and severity of the disease but also leading to poor response to treatment.[3] Many studies have showed that smokers have a worse clinical response to nonsurgical periodontal treatment (NSPT) than non-smokers. For periodontal pockets with a depth of 5–7 mm, the average reduction in pocket depth and increase in clinical attachment after treatment for the non-smoker group were 1.7 mm and 0.8 mm, respectively; while those in the smoker group were 1 mm and 0.5 mm, respectively.[4,5]

In the pathogenesis of periodontitis, neutrophils are the first line of defense of the periodontal tissues in the response to bacteria. Periodontal neutrophils from the gingival blood flow through the junctional epithelium into the gingival crevice, where they form a barrier against

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infection and the formation of bacterial plaque.[6] Their presence is necessary to protect oral health, but their excess number may lead to damage in periodontal tissues. The majority of neutrophils in the oral cavity enter via the gingival crevice,[7] and the amount of these granulocytes migrating into the oral cavity increases with the degree of periodontitis.[8–10] The number of neutrophils in saliva and gingival fluid varies with respect to the severity of periodontal disease.[9] Based on the association between neutrophils and periodontal inflammation, Bender JS et al.[8] have developed a simple, non-invasive and rapid test to measure the oral neutrophil number (ONN) in saliva to assess the infection of periodontal tissue. But up to now, the use of this technique to assess the periodontal status and treatment effectiveness has not been spread, because the development of this test for clinical application is still quite new.

A review of the literature has showed that studies are inconsistent in the effect of smoking on the response of neutrophils to periodontitis. Some authors suggested that cigarettes had no influence on the neutrophil number in periodontal tissues,[11] whereas others found that the ONN in smoker patients was less than that in non-smoker patients.[12] Besides, many authors found that the clinical improvement after NSPT in smokers was worse than in non-smokers.[5,13,14] However, there has been no study assessing the effects of smoking on the response of oral neutrophils to NSPT.

Matrix metalloproteinases (MMPs) in neutrophils are related to the destruction of periodontal tissues.[15,16] In periodontitis, MMP expression increased with periodontal disease severity, with a positive association between their collagenase and gelatinase activity in gingival tissue, gingival fluid and periodontal pocket depth (PPD), clinical attachment loss (CAL).[17] Among these, MMP-8, which is mainly derived from neutrophils,[18] has been recommended as a potential biomarker for the diagnosis and management of the periodontal disease.[16] In the gingival crevicular fluid (GCF) of patients with periodontitis, MMP-8 accounts for 94–96% of all collagenase, and 90–95% of collagen destruction activity in GCF derived from MMP-8.[19] The concentration of GCF MMP-8 elevated at periodontal pockets in periodontitis patients and decreased to a nearly healthy level after treatment.[20–22] Smoking appeared to be associated with decreased MMP-8 in GCF in periodontitis patients[21]; however, there has been no consensus on the reduction of this marker in smokers after periodontal treatment.[16,21]

In view of these facts, we performed this study to evaluate and compare the ONN in saliva, the level of MMP-8 in GCF and the periodontal parameters in smokers and non-smokers with periodontitis, before and after NSPT.

2. Materials and method

2.1. Patient selection

Participants who came for periodontitis treatment, were randomizedly recruited at the Department of Periodontology, Faculty of Odonto-Stomatology, from June 2016 to December 2018.

The study was approved by the Institutional Review Board and the Ethics Committee of the University. Signed informed consent was obtained from all participants before enrollment.

2.2. Study design

This was a quasi-experimental study. The patients were divided into two groups: group 1: non-smoker patients and group 2: smoker patients. The intervention NSPT was applied in both groups. A periodontist performing the NSPT and an independent doctor collecting testing samples were blind about the patients' smoking status. We aimed to recruit an equal number of patients in each group to minimize confounding factors. A similar protocol was employed in previous studies,[13,23] to evaluate the influence of smoking on the changes in periodontal conditions.

2.3. Eligibility criteria

Inclusion criteria were male patients, aged from 30 to 60 years old, systemically healthy, having at least 20 teeth, diagnosed with moderate to severe periodontitis according to American Academy of Periodontology (AAP) 2015[24]: gingival bleeding on probing at examination, ≥5 mm PPD, bone resorption on panoramic dental X-ray films ≥16% or >3 mm root length; having at least two sites at anterior teeth with PPD from 5 to 7 mm. For group 1, subjects who never smoked. For group 2, subjects who had smoked more than 10 cigarettes per day for at least the past 10 years.

Exclusion criteria were patients with acute or chronic medical disorders, patients under any medication for the past 3 months, patients who had undergone periodontal therapy in the last 12 months. We also excluded patients with oral lesions such as ulcers, glossitis or multi-tooth decays.

2.4. Sample size

Sample size was calculated based on the percentage of oral neutrophils decreased after nonsurgical periodontal treatment, using the following formula:

$$p = \frac{p_1(1-p_2)}{p_2(1-p_1)}$$

$$p_{Discordant} = p_1(1-p_2) + p_2(1-p_1)$$

$$n_{pair} \geq \frac{\left(Z_{1-\alpha/2}(p+1) + Z_{1-\beta}\sqrt{(p+1)^2 - (p-1)^2 p_{Discordant}}\right)^2}{(p-1)^2 p_{Discordant}}$$

After our pilot study, we chose $p_1 = 12.5\%$, $p_2 = 62.5\%$.

With $\alpha = 0.05$ and $\beta = 0.1$, the sample size for each group was 18 patients. To compensate for sample loss, 20 subjects for each group were enrolled in this study.

53 patients were assessed for eligibility, 13 patients were excluded, resulting in 40 patients.

2.5. Research procedure

Patients who came to our hospital were given an oral examination and orthopantomography to screen for periodontitis before referral to the Department of Periodontology. Then, an experienced periodontist performed full-mouth periodontal examinations for all participants. Tooth sites which diagnosed as periodontitis according to AAP 2015 were taken periapical radiographs.

Patients were assessed for their smoking history and then allotted to respective groups. After periodontal examination and medical history evaluation, all patients received oral hygiene instructions. The modified-Bass tooth brushing technique,[25] as well as the use of dental floss and interproximal brushes (if needed) were showed to the patients. They were educated to brush their teeth at least twice a day. All patients were given a dentifrice and toothbrush. Patient motivation to quit smoking was also provided.

At the first visit, supragingival scaling with ultrasonic scalers (Cavitron Jet Plus (Densply Sirona, Mississauga, Canada) was provided to all patients. Occlusion adjustments were also performed in the case of indications. Supragingival scaling was provided to patients before baseline examination to remove all tobacco stains, ensuring the blindness of investigators about smoking status. After one week, the baseline (T0) parameters were recorded. The following samples were collected: (1) saliva, (2) gingival cervical fluid (GCF), and (3) clinical periodontal parameters. A doctor who collected the samples was blinded about the smoking status.

Subsequently, scaling and root planing (SRP) by ultrasonic scalers (Cavitron Jet Plus (Densply Sirona, Mississauga, Canada) and Gracey

curettes (Hu-Friedy, Chicago, USA), under local anesthesia, were performed by a periodontist, who was also blinded about the smoking status. The number of SRP visits was determined by patient disease conditions.

Following the completion of NSPT, all subjects were recalled after one month and three months for re-evaluation. The collection of (1) saliva, (2) GCF, and (3) clinical periodontal parameters were performed. Based on the patients' periodontal status, supragingival scaling and polishing were given.

2.6. Saliva collection for oral neutrophil evaluation

Patients were asked not to eat or drink at least 1 h prior to the examination. They rinsed their mouth gently with water to remove food debris 10 min before saliva collection. Then, the patients sucked 15 ml saline solution in their mouth, moving it back and forth for 30 s and spitting all solution out into a plastic cup. Subsequently, the sample was transferred to a sterile polypropylene tube, stored in an icebox at 4 °C and quickly brought to the laboratory to quantify oral neutrophils.

The saliva collection was performed at baseline (T0), after NSPT one month (T1) and three months (T3).

2.7. Salivary neutrophil quantification procedure

ONN was assessed according to the technique of Bender JS et al.,[8] with centrifugal speed and time being determined after a series of experiments in our laboratory to choose the most appropriate ones. In the laboratory, samples were centrifuged at 3000 rounds per minute (rpm) for 10 min. Then, the supernatant was discarded, and 10 ml saline was added. The second centrifugation was performed at 3000 rpm for 10 min. Again, the supernatant was discarded, 500 μl saline was added, and the samples were stored at 4 °C. The samples then were stained with Acrydin orange (AO), and the number of neutrophils was counted under the fluorescence microscope. The resulting sample consisted mostly of neutrophils, a few of epithelial cells and food debris.

The Kappa index for the investigator who counted the neutrophil number was 0.9.

2.8. GCF collection for MMP-8 evaluation

GCF samples were collected randomizedly from two anterior teeth with PPD from 5 to 7 mm, bleeding on probing. The supragingival plaque was removed from the sampling sites, and the sites were isolated with sterile cotton rolls and gently dried with air.

A periodontal paper strip was inserted 2 mm into the crevice, left in place for 30 s. Then, the strip was placed into a 2 ml Eppendorf tube containing 80 μl of buffer (including 50 mM Tris-HCl pH 7.5, 0.2 M NaCl, 5 mM $CaCl_2$ and 0.01% Triton X-100), stored in a cabinet at −80 °C until being analyzed.

Paper strips contaminated with blood were discarded.

The GCF collection to evaluate MMP-8 level was conducted at baseline (T0) and after NSPT three months (T3).

2.9. Analysis of GCF MMP-8 levels

MMP-8 levels were analyzed with an enzyme-linked immunosorbent assay (ELISA) kit (ab100609 Human MMP8 ELISA, Abcam, USA). Procedures were performed according to the manufacturer's instructions in the kit. The coefficient of variation was 3.8%.

2.10. Clinical periodontal parameters

Periodontal disease status of all patients was evaluated by the measurement of gingival index[26] (GI), plaque index[26] (PlI), bleeding on probing (BOP), PPD, and CAL, using periodontal probes (University of North Carolina-15 probe (UNC-15), Hu- Freidy's, USA) and conducted by the same periodontist. The investigator was trained and calibrated. The Kappa indices for PlI, GI, BOP were 0.82, 0.74, 0.91, respectively; and the intraclass correlation (ICC) for PPD, CAL were 0.96, 0.98, respectively.

CAL was measured at T0 and T3, while other periodontal parameters were measured at T0, T1 and T3.

2.11. Statistical analysis

The normal distribution of data was tested using the Shapiro-Wilk test. Nonparametric tests were applied in the case of ordinal variables or nonparametric data. The differences between the two groups were analyzed using the independent sample *t*-test (for age, PPD, and CAL), and the Mann-Whitney *U* test (for PlI, GI, BOP, ONN, and MMP-8 level). The differences between T0, T1 and T3 of each group were analyzed with paired-samples *t*-test (for PPD and CAL) and Wilcoxon signed-rank test (for PlI, GI, BOP, ONN, and MMP-8 level). The level of significance was set at 0.05. To correct for multiple comparisons, the Bonferroni method was applied. Statistical analysis was performed using the software IBM SPSS Statistics (SPSS, Chicago, IL, USA), version 22.

4. Results

20 male subjects for each group were included in this study. The mean age of groups 1 and 2 were 44.65 ± 8.31 and 47.65 ± 8.38 years old, respectively; there was no significant difference in age between the two groups ($p > 0.05$).

4.1. Clinical periodontal parameters

Table 1 demonstrates the whole mouth periodontal parameters of the non-smoker group and smoker group, at baseline (T0), after NSPT one month (T1) and three months (T3). All periodontal parameters showed a significant decrease at T1 and T3, in both groups.

Fig. 1 demonstrates the difference in clinical indices between the two groups. At baseline, there was no significant difference in any periodontal parameters between the two groups ($p > 0.05$).

The PlI, GI, and BOP also showed no significant difference between the two groups at T1 and T3 ($p > 0.05$). The PPD of the non-smoker group was significantly lower than those of the smoker group after NSPT ($p < 0.05$). The CAL of the non-smoker group was lower than that of the smoker group at T3; however, there was no significant difference in the CAL between the two groups ($p > 0.05$).

4.2. Oral neutrophil number (ONN)

Table 2 shows the salivary neutrophil number of the non-smoker group and smoker group, at baseline (T0), after NSPT one month (T1) and three months (T3).

The ONN of non-smokers experienced a significant decrease after NSPT ($p < 0.05$), whereas ONN of smokers showed no significant difference before and after NSPT 1 month and 3 months ($p > 0.05$). In contrast, the ONN of this group increased at T1; however, the difference was not significant ($p > 0.05$).

At baseline, the ONN of the non-smoker group was significantly lower than that of the smoker group ($p < 0.05$). There was no significant difference between the two groups at T1 and T3 ($p > 0.05$).

4.3. -8 levels in GCF

Table 3 shows the MMP-8 levels in GCF of the non-smoker group and smoker group, at baseline (T0), and after NSPT three months (T3).

MMP-8 was not detected in 6 and 8 samples in the non-smoker and smoker groups, respectively, due to low concentration.

After NSPT, in non-smokers, the MMP-8 level decreased significantly ($p < 0.01$), while that in smokers showed no significant decrease ($p >$

Table 1
Whole mouth periodontal parameters of the non-smoker group (Group 1) and smoker group (Group 2), at baseline (T0), after NSPT one month (T1) and three months (T3).

Parameters		T_0	T_1	T_3	p T_0/T_1 [(a)]	p T_0/T_3 [(a)]
PlI	Group 1	0.83 (0.48–1.37)	0.47 (0.30–0.80)	0.47 (0.22–0.91)	<0.001**	0.003**
	Group 2	1.24 (0.72–1.47)	0.78 (0.51–0.93)	0.58 (0.34–1.00)	0.003**	0.001**
	p	0.065	0.065	0.336		
	G1/G2 [(b)]					
GI	Group 1	0.90 (0.52–1.41)	0.46 (0.36–0.77)	0.48 (0.34–0.64)	<0.001**	<0.001**
	Group 2	1.07 (0.65–1.60)	0.53 (0.33–0.66)	0.34 (0.24–0.61)	<0.001**	<0.001**
	p	0.496	0.728	0.258		
	G1/G2 [(b)]					
BOP	Group 1	0.36 (0.24–0.49)	0.19 (0.12–0.31)	0.11 (0.08–0.21)	0.001**	<0.001**
	Group 2	0.44 (0.27–0.58)	0.29 (0.11–0.40)	0.10 (0.08–0.34)	0.001**	0.001**
	p	0.309	0.365	0.214		
	G1/G2 [(2)]					
PPD (mm)	Group 1	3.38 ± 0.41	2.75 ± 0.33	2.54 ± 0.39	<0.001**	<0.001**
	Group 2	3.67 ± 0.67	3.06 ± 0.50	2.97 ± 0.45	<0.001**	<0.001**
	p	0.169	0.044*	0.004**		
	G1/G2 [(b)]					
CAL (mm)	Group 1	3.90 ± 1.10	–	3.18 ± 0.97	–	<0.001**
	Group 2	4.10 ± 0.77	–	3.67 ± 0.80	–	<0.001**
	p	0.585		0.124		
	G1/G2 [(b)]					

Data of PlI, GI, BOP are expressed in median (interquartile range); data of PPD, CAL are expressed in mean ± standard deviation.
PlI: Plaque index; GI: Gingival index; BOP: Bleeding on probing; PPD: Probing pocket depth; CAL: Clinical attachment loss.
*: $p < 0.05$, **: $p < 0.01$.
[a] Wilcoxon signed-rank test for PlI, GI, BOP; Paired-sample *t*-test for PPD, CAL.
[b] Mann-Whitney *U* test for PlI, GI, BOP; Independent sample *t*-test for PPD and CAL.

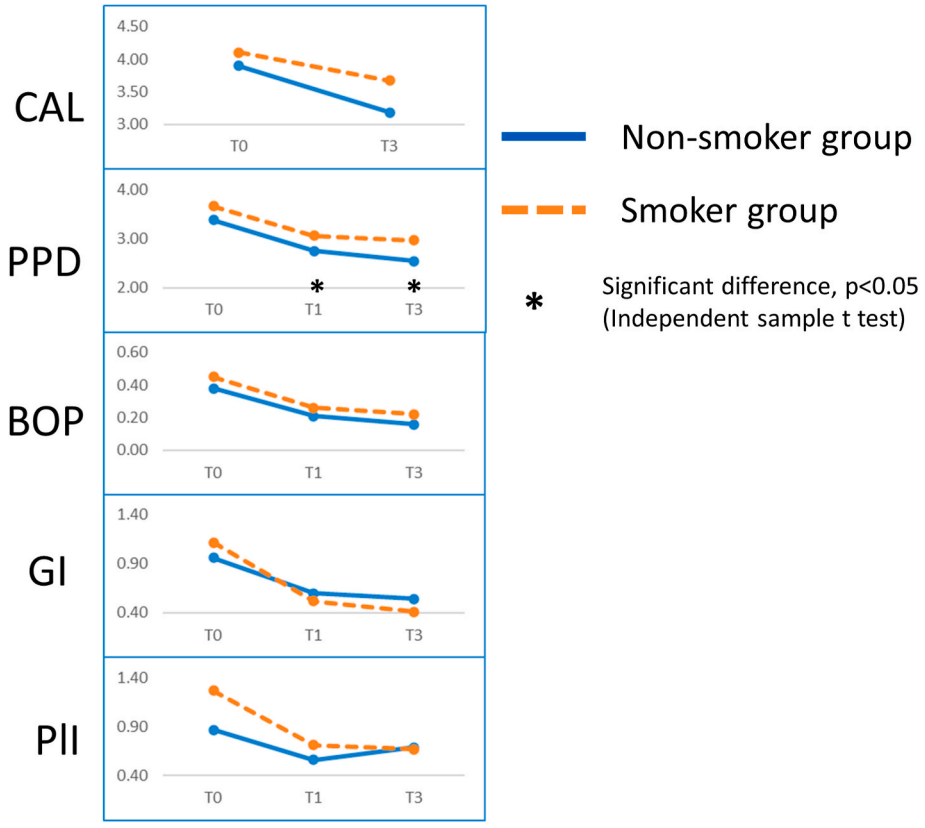

Fig. 1. The difference in periodontal parameters between the non-smoker group and smoker group, at baseline (T0), after NSPT one month (T1) and three months (T3).PlI: Plaque index; GI: Gingival index; BOP: Bleeding on probing; PPD: Probing pocket depth; CAL: Clinical attachment loss.

0.05).

There was no significant difference in MMP-8 levels between the two groups at any point of time ($p > 0.05$).

5. Discussion

The two groups in our study were identical in number and there was

Table 2
Salivary neutrophil number of the non-smoker group (Group 1) and smoker group (Group 2), at baseline (T0), after NSPT one month (T1) and three months (T3).

		T_0	T_1	T_3	p T_0/T_1[(a)]	p T_0/T_3[(a)]
Neutrophil (10^6 cells/ml)	Group 1	2.89 (1.73–12.96)	1.57 (0.49–3.18)	1.11 (0.37–1.69)	0.01*	<0.01*
	Group 2	1.78 (1.00–2.51)	1.98 (0.63–4.89)	1.29 (0.71–2.33)	0.13	0.05
	p G1/G2[(b)]	0.01*	0.167	0.519		

Data are expressed in median (interquartile range).
*: $p < 0.05$, **: $p < 0.01$.
[a] Wilcoxon signed-rank test.
[b] Mann-Whitney *U* test.

Table 3
Matrix metalloproteinase-8 (MMP-8) levels in gingival crevicular fluid of the non-smoker group (Group 1) and smoker group (Group 2), at baseline (T0), and after NSPT three months (T3).

		T_0	T_3	p T_0/T_3[(a)]
MMP-8 level (pg/ml)	Group 1	50,513 (18,383–76370)	20,064 (10,632–30,486)	<0.01**
	Group 2	44,336 (29,790–76220)	24,063 (15,686–57420)	0.13
	p G1/G2[(b)]	1	0.08	

Data are expressed in median (interquartile range).
**: $p < 0.01$.
[a] Wilcoxon signed-rank test.
[b] Mann-Whitney U test.

no significant difference in their mean age. All subjects were in good health, did not suffer from any systemic diseases and did not take any medications such as antibiotics, anti-inflammatory drugs in the past 3 months. To facilitate the homogeneity in the two groups, only males were included in this study, because the women's smoking rate in our country is as low as 4%. Only anterior teeth were used for GCF collection in this study for two reasons: 1) nonsurgical therapy has significantly different effectiveness on anterior and posterior teeth,[27] and 2) anterior teeth area facilitated isolation process, expediting and plaque collection procedure.

At baseline, non-smokers and smokers presented similar clinical periodontal conditions. After NSPT, PlI, GI and BOP of both groups decreased significantly, with no difference between the two groups. The results were concurrent with other studies.[4,5] The NSPT showed effectiveness in both non-smoking and smoking subjects.

At T1 and T3, the PPD of non-smoking patients was significantly lower than those of smoking ones, although there was no significant difference at baseline. This finding concurred with the results of other studies, which reported that non-smokers experienced better clinical periodontal improvement after NSPT, compared to smokers.[4,23,28]

At baseline, the ONN of smoking patients was significantly lower than that of the non-smoking ones. Previous studies have all concluded that there is a positive correlation between the concentration of salivary neutrophils and the level of periodontal infection.[8,9] With the same periodontal condition, the number of neutrophils in the oral cavity of smokers is lower than that of non-smokers. This may be because smoking reduces the immune system responses, reducing the neutrophil number to protect against infection.[12]

The ONN in saliva in the non-smoker group decreased significantly after 1 month and 3 months of treatment. This result was similar to the conclusions of Bender JS, Landberg M[8,10] that there was a positive correlation between ONN and periodontal disease. Reducing the infection of the periodontal tissue was coincident with salivary neutrophil count decrease,[29] because the infection of the periodontal tissue corresponded to the speed of neutrophils moving to the oral cavity.[29]

In this study, we found an increase in the salivary ONN in the smoking group after 1 month of NSPT. Although this change was not statistically significant ($p = 0.079$), but the increasing trend was opposite to that of the non-smoking group. This finding was in contrast to the study of Bender JS et al.[8] which showed that the ONN decreased after periodontal treatment. The study was performed on 42 periodontitis patients, with the major of them being non-smokers (38/42 patients); the results might have a tendency for non-smoking patients. In addition, the increase in ONN suggested that the inflammation phase after NSPT was in progress. After SRP, in the inflammatory phase of wound healing, neutrophils are enhanced to the lesion to fight against bacteria, present antigens, produce pro-inflammatory cytokines. In a normal wound, after performing functions, the neutrophils are phagocytized and also act as a signal to end the inflammatory phase, allowing healing to continue. At this time, the number of neutrophils will decrease. If the neutrophils continue to be recruited to the wound, or the phagocytosis function of macrophage is impaired, the inflammatory phase will be prolonged, leading to delayed healing.[30] The increase in the ONN after 1 month of treatment in the smoking group suggested that the inflammation phase is longer in smoker patients than non-smoker ones. This was a new finding in our study that no other study has reported before. After 1 month, the ONN in the smoking group tended to decrease gradually at 3-month after treatment, but there was no significant difference.

The non-smoker group experienced significant MMP-8 level reduction after 3 months of NSPT. The result of this study concurred with most of the studies, the concentration of MMP-8 in GCF significantly decreased after NSPT.[16,21] Even after 45 days of treatment, Mastromatteo-Alberga et al.[31] found that the GCF MMP-8 level in periodontitis patients decreased significantly. This finding proved that the risk of destruction in periodontal tissue has reduced after treatment, and the non-smoking patients responds well to the treatment.[31]

The GCF MMP-8 level in the smoking group decreased after NSPT 3 months, but the difference was not statistically significant. This suggested that the risk of periodontal tissue destruction in smokers may remain high after treatment. Our results were similar to the study of Mantyla et al.,[21] which showed that the concentration of MMP-8 in GCF at pocket depth ≥4 mm (mean 4.9 mm) decreased non-significantly from 1268 ± 2126 ng/ml to 0975 ± 1171 ng/ml. In contrast, Akbari et al.[16] reported that the concentration of GCF MMP-8 at the pockets with a depth of ≥5 mm (mean 6.64 mm) decreased significantly from 2008 ± 861 ng/ml to 1314 ± 676 ng/ml after 3 months of treatment with the ELISA technique. The inconsistency in the change in the concentration of MMP-8 GCF after treatment may be due to the characteristics of the study sample (pocket depth, race, …), different sampling and quantification techniques. When comparing the decrease in the concentration of MMP-8 gingival fluid, the non-smoker group showed a more decrease than the smoker group, but the difference was not significant. Although there was no significant difference between the two groups at baseline and after treatment (which might be because the sample of GCF in our study was not large enough), the MMP-8 level in the non-smoker group decreased significantly ($p < 0.001$) while the

decrease was not significant in the smoker group ($p = 0.126$). A similar change occurred after surgical periodontal therapy when the study found that MMP-8 in GCF showed no reduction in smoking patients, whereas significantly decreased in non-smoking patients.[32] This may indicate a poor response to treatment in smoking subjects.[32]

This study has showed differences in the response of oral neutrophils and MMP-8 levels between non-smokers and smokers to periodontal treatment. Biomarkers such as ONN and GCF MMP-8 concentration are valuable tools to diagnose periodontal disease in the early stages and support during the treatment procedure. The early different changes in ONN of the two groups at 1 month after NSPT, which was a new finding of this study, may help detect smokers who have a poor response to treatment, and hence an appropriate treatment plan may be indicated.

There were some limitations of this study. The sample size was calculated based on the ONN, which might be not large enough to demonstrate the significant differences in other parameters between the two groups. In addition, although the use of mediations such as antibiotics, anti-inflammatory or other drugs, was investigated in patient selection and during the study, it was not possible to completely control patient cooperation.

Therefore, future studies with a larger sample size, evaluating the response of oral neutrophils to periodontal treatment after one, two and three weeks, should be implemented, in order to detect the earliest stage that ONN shows difference between smoker and non-smoker patients. The changes in ONN and MMP-8 level in periodontitis patients with systemic diseases such as diabetes, obesity … should be investigated in well-designed long-term prospective studies, for comprehensive periodontal therapy.

6. Conclusion

Within the limitations of this study, smoking showed to reduce ONN, impair treatment effect in reducing PPD, and change MMP-8 level in gingival crevicular fluid to NSPT.

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