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Research Article

Impact of Nonsurgical Periodontal Therapy (NSPT) on Salivary Osteonectin and Osteopontin Levels in Smokers and Nonsmokers Periodontitis Patients: A Prospective Clinical Study

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Abstract

Background: Periodontitis is an inflammatory disease that destroys tooth-supporting tissue, with proteins like osteonectin and osteopontin playing roles in bone metabolism and inflammation, making them potential biomarkers for assessing periodontal healing after treatment. ***Objectives***: The effect of non-surgical periodontal treatment (NSPT) on concentrations of osteonectin and osteopontin within the saliva of periodontitis individuals who smoke and those who do not. ***Methods***: Twenty-eight periodontist patients were allocated into 14 nonsmokers and 14 smokers with probing depths of 4-6mm who were treated by root surface debridement (RSD). Plaque index, bleeding on probing, probing pocket depth, and clinical attachment level were measured. In addition, osteonectin (ON) and osteopontin (OPN) in saliva were measured at baseline and 1-month and 3-month follow-up visits by ELISA. ***Results***: Osteonectin levels in saliva increased in nonsmoker patients (4.80±3.52ng/ml and 21.47±1.92 ng/ml) and were significantly greater than in the smoker patients (1.30±1.26ng/ml and 4.02±3.29ng/ml) in 1 month and 3 months, respectively, while osteopontin levels in saliva decreased in both groups, the nonsmokers group (4.62±1.91ng/ml and 5.78±1.39 ng/ml) and the smokers group (5.46±2.30 ng/ml and 6.76±2.88 ng/ml) in 1 month and 3 months, respectively, but with no significant differences between them. ***Conclusions***: NSPT increases ON in both smoker and non-smoker unstable periodontitis individuals, with a higher increase observed in non-smokers. However, NSPT decreases OPN in these groups, with no significant difference post-treatment.

Keywords: Nonsurgical periodontal therapy, Osteonectin, Osteopontin, Smoking, Periodontitis.

تأثير علاج اللثة غير الجراحي على مستويات اللعاب العظمي والعظم لدى المدخنين وغير المدخنين مرضى التهاب دواعم السن: دراسة سريرية مستقبلية

الخلاصة

الخلفية: التهاب دواعم السن هو مرض التهابي يدمر الأنسجة الداعمة للأسنان، حيث تلعب البروتينات مثل استيونيكتين و اوستيوبونتين أدوارا في استقلاب العظام والالتهابات، مما يجعلها مؤشرات حيوية محتملة لتقييم شفاء اللثة بعد العلاج. **الأهداف**: تأثير علاج اللثة غير الجراحي على تراكيز استيونيكتين و اوستيوبونتين داخل لعاب الأفراد المصابين بالتهاب دواعم السن وأولئك الذين لا يدخنون. **الطرائق**: تم تخصيص ثمانية وعشرين مريضا لأمراض اللثة في 14 غير مدخن و 14 مدخنا بعمق فحص 4-6 مم تم علاجهم عن طريق تنضير سطح الجذر. تم قياس مؤشر البلاك والنزيف عند الفحص وعمق الجيب ومستوى التعلق السريري. بالإضافة إلى ذلك، تم قياس أوستيونكتين وأوستيوبونتين في اللعاب في خط الأساس وزيارات متابعة لمدة شهر و 3 أشهر بواسطة ELISA. **النتائج**: زادت مستويات الأستيونكتين في اللعاب لدى المرضى غير المدخنين (4.80±3.52 نانوغرام/مل و 21.47±1.92 نانوغرام/مل) كانت أكبر بكثير من المرضى المدخنين (1.30±1.26 نانوغرام/مل و 4.02±3.29 نانوغرام/مل) في شهر واحد و 3 أشهر، على التوالي، بينما انخفضت مستويات أوستيوبونتين في اللعاب في كلتا المجموعتين، مجموعة غير المدخنين (4.62±1.91 نانوغرام/ مل و 5.78±1.39 نانوغرام/ مل) ومجموعة المدخنين (5.46±2.30 نانوغرام/ مل و 6.76±2.88 نانوغرام/ مل) في شهر واحد و 3 أشهر، على التوالي، ولكن مع عدم وجود فروق ذات دلالة إحصائية بينهما. **الاستنتاجات**: يزيد العلاج غير الجراحي من اوستيونيكتين في كل من الأفراد المصابين بالتهاب دواعم السن غير المستقر المدخنين وغير المدخنين، مع ملاحظة زيادة أعلى في غير المدخنين. ومع ذلك ، فإن هذا العلاج يقلل من اوستيوبونتين في هذه المجموعات، مع عدم وجود فرق كبير بعد العلاج.

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INTRODUCTION

Periodontitis is an inflammatory disease resulting from bacterial biofilm buildup on the tooth surface. This bacterial biofilm causes an immune response that, in susceptible individuals, can damage the gingiva, periodontal ligament, cementum, and alveolar bone. The severity of periodontitis is assessed through symptoms including probing depth, clinical attachment loss, and bleeding on probing [1]. Diabetes and smoking are examples of acquired or environmental risk factors that can both allow and exacerbate the periodontitis change [2]. Nicotine constitutes a worldwide epidemic, impacting approximately 22.2% of the worldwide population [3]. Cigarettes adversely influence the body's immune and chronic mechanisms, leading to detrimental effects on systemic inflammatory conditions [4]. Periodontitis is

more common and severe in smokers, with increased depth of probing, gingival recession, and alveolar bone loss [5-7]. Non-surgical treatment of periodontal disease, including scaling and root surface debridement (RSD) and oral hygiene instructions [8], is the most effective therapy for periodontitis. The approach seeks to eliminate biofilm present on both supra- and subgingival tooth surfaces, along with postponing the eventual colonization of these areas by pathogens that cause [9]. Studies indicate that these treatments can halt the progression of periodontitis in pockets ranging from shallow to moderate [10-12]. Salivary biomarkers serve as valuable diagnostic and prognostic indicators for periodontal disease and markers for monitoring the healing process [13-15]. Osteonectin, or SPARC-produced protein, acidic and rich in cysteine, is produced through bone marrow cells, fibroblasts, and the RBCs, serving as a chemoattractant for calcium ions that reside in bone structure. Its concentrations in the structure of the bone environment are intricately linked to the bone-making processes through remodeling [16]. Osteonectin is widely expressed in mineralized tissues, including alveolar bone, crucial in bone regeneration in periodontal disease. It helps hydroxyapatite crystals form and controls osteoblast differentiation. This makes it easier for osteocytes to mature and brings osteoblasts to places where bone is being formed. Due to its role in bone formation, osteonectin is a key biomarker for bone formation [16]. Osteopontin (OPN), a protein that is prominently present in mineral structures like bones and teeth and that osteoclastic and osteoblastic cells produce in large quantities, contributes to the structural integrity of bones. However, it's noteworthy that OPN expression from osteoclasts has been observed to inhibit hydroxyapatite formation [17]. Osteopontin binds to integrins and CD44 receptors on osteoclasts, increasing differentiation and activity [17]. The OPN component was recently detected in the gingival crevicular fluid (GCF), with a significant increase in its concentration associated with the advancement of the periodontal condition [18,19]. This observation suggests that the levels of OPN may serve as an indicator of alveolar bone destruction [20]. The use of ON as a bone formation marker and OPN as a bone destruction marker in saliva for monitoring the healing response to non-surgical periodontal therapy needs to be investigated. A few studies on the effect of NSPT on bone remodeling markers like ON and OPN in saliva. Therefore, this study evaluated osteonectin and osteopontin levels within the saliva of individuals with unstable periodontitis before and after non-surgical periodontal treatment (NSPT) in smokers and non-smokers.

METHODS

Study design

The research represented a prospective clinical trial done at a multicenter in Baghdad, Iraq, between December 2023 and July 2024. Every individual supplied signed informed consent prior to involvement. The Committee on Ethics within the Dentistry College, Baghdad University, approved the study protocol (Approval No. 860623, dated 03/12/2023). The study was executed in compliance with the Helsinki Declaration (2013). This research was filed at ClinicalTrials.gov with the registration ID NCT06270004.

Inclusion criteria

The criteria for inclusion for the present study required participants to be systemically healthy, with no history of systemic diseases. Participants hadn't been given dental care in the preceding six months or consumed drugs in the three months preceding their study. They also needed to have at least 20 teeth and be diagnosed with moderate periodontitis, characterized by a probing pocket depth (PPD) of 4–6 mm and generalized, unstable periodontitis.

Exclusion criteria

Exclusion criteria included intraoral plaque-retentive factors such as crowns, bridges, partial dentures, orthodontic appliances, furcation-involved teeth, overhanging restorations, and tooth anomalies. Patients who had received antibiotic therapy within the previous three months were also excluded. For the smokers' group, only patients who smoked more than ten cigarettes per day for at least the past 3 years [21].

Sample size calculation

A pilot study was conducted to determine the collection measurement, revealing that salivary osteopontin levels decreased following nonsurgical periodontal treatment as the primary outcome. The estimated effect size from the pilot study was 1.13, with a total sample size of 28 participants, a power of 0.80, and a level of error of 5%. Thirty-two participants were considered to compensate for any potential dropouts, divided into two groups: non-smokers and smokers. The sample size was estimated using the G*Power software (v.3.1.9.4).

Baseline visit

Following the identification of suitable individuals, unstimulated saliva samples were obtained through morning visits before the clinical assessment, utilizing the drooling method outlined by Navazesh and Kumar [22]. Individuals were directed to abstain from food and beverage intake for 30 minutes before sampling. Saliva was gathered in round-bottom polypropylene tubes (50 mL Falcon® tubes, 30 mm in diameter) and centrifuged at 3000 rpm for 5 minutes. The supernatants were preserved at −20°C for subsequent analysis. After the saliva collection process, medical periodontal indicators such as Plaque Index (PI), Bleeding on Probing (BOP), Probing Pocket Depth (PPD), and Clinical Attachment Level (CAL) were

evaluated. Measurements were taken at six sites per tooth, except for PI, which was assessed at four sites across entire dentitions. An accredited examiner conducted all evaluations utilizing a UNC (University of North Carolina) 15 periodontal probe. Using five participants for measurement discussions, the technician attained kappa values exceeding 0.75 for PI and BOP and an intraclass correlation coefficient surpassing 90% for CAL and PPD measurements. Every patient obtained oral hygiene instructions following medical evaluations and underwent comprehensive supragingival scaling of the mouth using an ultrasonic scaler (WOODPECKER® UDS-K). Furthermore, they were directed to use dental floss and interproximal brushes as necessary and to brush their teeth bi-daily. Every individual received a toothbrush and toothpaste [23].

1st visit after one week

Each patient was treated with root surface debridement (RSD) using Gracey curettes, followed by irrigation with normal saline to remove all debris from periodontal pockets.

2nd and 3rd visits

The participants were scheduled to return to the clinic for 2nd visit (one month) and 3rd visit (three months) for saliva collection and periodontal parameter assessment. At these visits, the clinical periodontal parameters were reassessed by the same calibrated examiner who had recorded the periodontal parameters at baseline. Furthermore, the patients received reinforcement on self-oral hygiene practices to ensure ongoing adherence to proper oral care routines.

Outcome measurements

Salivary osteonectin (ON) and osteopontin (OPN) concentrations were quantified utilizing an enzyme-linked immunosorbent assay (ELISA) technique using human ON and OPN ELISA kits. Every measure was conducted following the company's specifications. Antibodies specific to human ON and OPN were pre-coated onto 96-well and 84-well plates, respectively. Samples and standards for ON and OPN were pipetted into the wells, allowing the immobilized antibodies to bind to the target proteins. After washing to remove unbound substances, a biotinylated antibody and streptavidin were added, followed by another wash.A one-step tetramethylbenzidine (TMB) substrate solution was added, which led to a color reaction that was related to the levels of ON and OPN. Finally, a stopping solution was added, and the optical density was measured at 450 nm using a spectrophotometer.

Statistical analysis

The data was analyzed using GraphPad Prism 8 and SPSS version 26. Data were presented as mean ± standard deviation. The normality test of the data was assessed using the Shapiro–Wilk test. We used the Friedman test and repeated measures ANOVA to compare clinical periodontal parameters and biomarkers between recall visits within the same group. The Mann-Whitney U and independent t-test were applied for intergroup by mean change comparisons to analyze differences in clinical periodontal parameters and biomarker levels.

RESULTS

The present study included 28 male subjects for both groups. Groups of non-smokers and smokers had mean ages of 45.75 ± 9.41 and 46.64 ± 9.58 years, respectively, with no statistically significant difference ($p > 0.05$) between them. After testing, normality showed in the osteonectin and osteopontin. BOP, PI, and CAL were parametric data, while PPD was nonparametric data. All patients in the non-smoker's group reached the end point of healing at which PPD ≤ 4 with no bleeding on probing, while two patients from the smoker's group could not reach the end point of healing. However, the statistical analysis was done for all patients without excluding anyone reaching the 3rd visit. The flow of the study is explained in Figure 1.

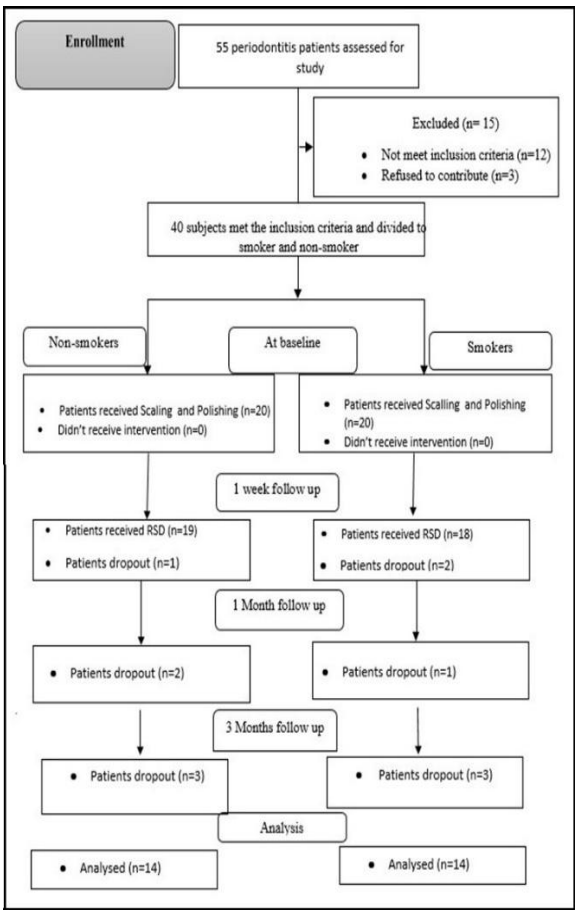

Figure 1: Flowchart of the study. n: number of participants; RSD: root surface debridement.

The comprehensive periodontal measurements for both nonsmoker and smoker groups exhibited

significant reductions at 1 and 3 months following NSPT, as shown in Table 1.

Table 1: Comparison of the clinical parameters among intragroup at baseline, 1 and 3 months after the NSPT

Clinical outcomes	Time	Non-smokers	Smokers
PI (%)	Baseline	52.8±6.41[a]	58.93±8.05[a]
	1 month	13.6±1.59[b]	19.36±3.27[b]
	3 months	9.14±1.65[c]	12.79±2.72[c]
BOP (%)	Baseline	51.7±6.98[a]	35.64±5.48[a]
	1 month	15.6±2.37[b]	19.64±3.71[b]
	3 months	9.21±1.42[c]	12.36±4.27[c]
PPD (mm)	Baseline	5.0±0.24[a]	5.02±0.27[a]
	1 month	4.24±0.14[b]	4.47±0.19[b]
	3 months	3.68±0.16[c]	4.16±0.19[c]
CAL (mm)	Baseline	5.08±0.23[a]	5.25±0.33[a]
	1 month	4.75±0.16[b]	4.82±0.33[b]
	3 months	4.37±0.15[c]	4.75±0.31[c]

Values were expressed as mean±SD. Values with different superscripts (a,b,c) among different followup periods of each parameter are significantly different (p<0.05). PI: Plaque index, BOP: Bleeding on probing, and CAL: clinical attachment loss.

Table 2 shows the comparison of mean changes in periodontal parameters between nonsmokers and smokers at baseline. The Plaque Index (PI) indicated no statistically significant difference comparing both groups for both 1 and 3 months (p > 0.05).

Table 2: Comparison of mean changes of periodontal clinical parameters between intergroups at one and three months after NSPT

Clinical outcomes	Time-BL	Non-smokers	Smokers	*p-value*
PI (%)	1 month	39.1±6.07	39.5±6.64	0.852
	3 months	43.7±5.5	46.1±7.24	0.328
BOP (%)	1 month	36.1±5.5	16.0±3.23	<0.001
	3 months	42.5±6.12	23.28±6.48	<0.001
PPD (mm)	1 month	0.7±0.28	0.55±0.18	0.026
	3 month	1.3±0.28	0.85±0.22	0.004
CAL (mm)	1 month	0.3±0.13	0.30±0.06	0.440
	3 month	0.7±0.17	0.5±0.15	0.002

Values were expressed as mean±SD. Unpaired t-test is used at p<0.05. PI: plaque index, BOP: bleeding on probing, CAL: clinical attachment loss, PPD: probing pocket depth, BL: baseline.

After NSPT, there was a significant difference between the groups in Bleeding on Probing (BOP), Probing Pocket Depth (PPD), and Clinical Attachment Level (CAL). The nonsmoker group had a higher mean difference from baseline for all three symptoms compared to the smoker group (p< 0.05). Salivary ON levels in non-smokers and smokers increased significantly after NSPT (p<0.05). In contrast, OPN levels in both groups decreased substantially following NSPT at one and three months (p<0.05), as shown in Table 3 and Figure 2.

Table 3: Comparison of the chemical biomarkers among intragroup at baseline, 1 and 3 months after NSPT

Biomarkers	Time	Non-smokers	Smokers
Osteonectin (ng/ml)	Baseline	3.6±2.26[a]	1.7±0.82[a]
	1 month	8.4±2.94[b]	3.01±1.64[b]
	3months	25.02±1.85[c]	5.72±3.25[c]
Osteopontin (ng/ml)	Baseline	7.01±2.01[a]	10.1±2.39[a]
	1 months	2.38±1.33[b]	4.63±1.53[b]
	3months	1.22±0.74[c]	3.34±2.16[c]

Values were expressed as mean±SD. Values with different superscripts (a,b,c) among different followup periods of each biomarker are significantly different (p<0.05).

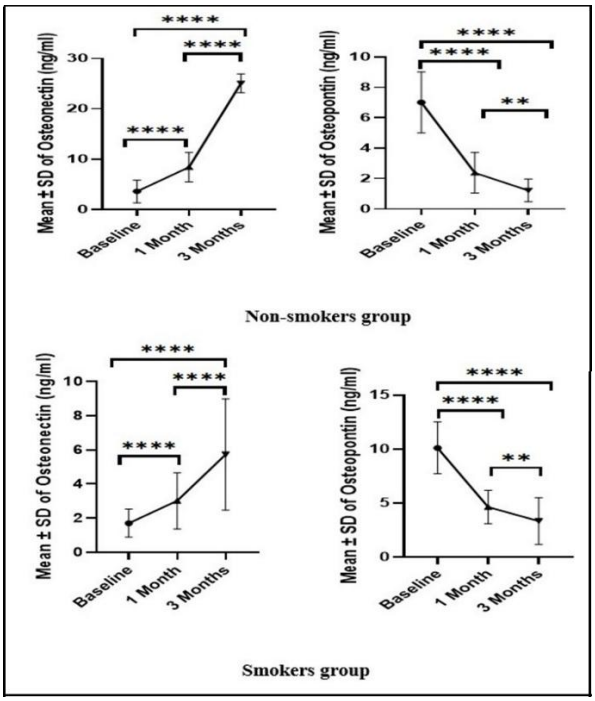

Figure 2: Non-smokers and smokers intra-group comparison among biomarkers using ANOVA test for Osteonectin and Osteopontin. ****: significance at p< 0.0001; ** significant at p< 0.01.

Furthermore, Table 4 and Figure 3 showed the comparison of salivary ON and OPN between nonsmokers and smokers, which were calculated as the mean change in a variable from baseline.

Table 4: Comparison of the chemical biomarkers by using mean change between intergroups at 1 and 3 months after NSPT

Biomarkers	Time-BL	Non-smokers	Smokers	*p*-value
Osteonectin (ng/ml)	1 month	4.80±3.52	1.30±1.26	0.001
	3 months	21.47±1.92	4.02±3.29	<0.001
Osteopontin (ng/ml)	1 month	4.62±1.91	5.46±2.30	0.302
	3 months	5.78±1.39	6.76±2.88	0.261

Values were expressed as mean±SD. BL: baseline. Unpaired t-test is used at p<0.05.

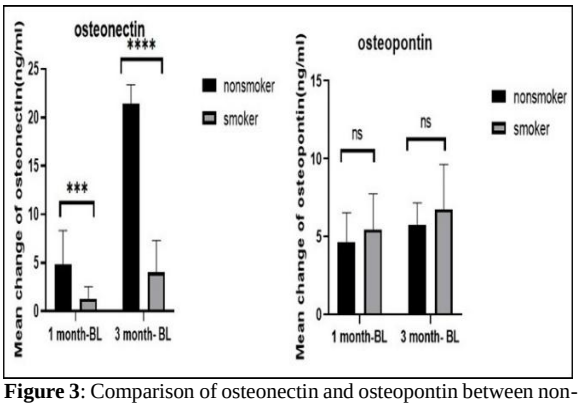

Figure 3: Comparison of osteonectin and osteopontin between non-smokers and smokers at different healing times following NSPT. **** Significant difference at p < 0.0001. *** p < 0.001. ns: non-significant.

DISCUSSION

The current study aimed to determine the impact of NSPT on bone remodeling biomarkers (Osteonectin and Osteopontin) in nonsmoker and smoker patients

with unstable periodontitis over one month and three months (healing times). Both groups in the current study were matched for sample size and mean age. To ensure homogeneity between the groups, only male participants were included in the study; according to the WHO report in 2023, Iraq has a relatively low smoking rate among women. Osteonectin is a glycoprotein essential for bone mineralization and cell adhesion, and it contributes to the structural integrity of bone and periodontal tissues. In periodontal disease, osteonectin levels can be a biomarker for assessing periodontal health and the efficacy of treatments like NSPT [24]. Osteopontin acts as a physical limiter, restricting crystal formation in bones and teeth, which may have implications for mineralization processes and pathological conditions associated with abnormal mineral deposition [25]. Non-surgical periodontal therapy (NSPT) is a common treatment for periodontal disease, aimed at reducing inflammation and improving clinical parameters such as probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP), and plaque index (PI) [26]. In the present study, all clinical parameters were significantly improved with nonsurgical periodontal treatment for both smokers and nonsmoker patients. At baseline, nonsmokers and smokers exhibited comparable clinical periodontal conditions. Following NSPT, the Plaque Index (PI) significantly decreased in both groups, with no significant difference. These findings align with previous studies [27]. The NSPT proved effective in nonsmokers and smokers [28]. The reasonable explanation for the reduction in plaque index is that, in the present study, both groups were treated by root surface debridement with heavy instruction of proper teeth brushing from the gingival margin to the occlusal and incisal surfaces of teeth and the use of interdental aids (dental flossing and interdental brush). Meanwhile, bleeding on probing is a highly reliable and sensitive indicator of gingival inflammation at the deepest point in periodontal pockets [29]. The mean percentage of BOP among intra-group significantly descended at one-month and three-month follow-up visits compared to the baseline level in both study groups, with statistically significant differences between them. This finding agrees with [30]. Due to ongoing exposure to tobacco smoke and its associated inflammatory effects, smokers are more likely to experience recurrent periodontal issues. This could result in higher rates of BOP recurrence over time compared to non-smokers [31]. Smokers exhibited a significantly lower reduction in Probing Pocket Depth (PPD) and a smaller gain in Clinical Attachment Level (CAL) compared to nonsmokers at three months following NSPT, despite no baseline differences between the groups. This result is consistent with previous studies, which have reported that nonsmokers experience greater clinical periodontal improvement following NSPT than smokers [32], maybe because smoking causes vasoconstriction (the narrowing of blood vessels), which reduces blood flow to periodontal tissues. Tobacco smoke contains chemicals such as nicotine, carbon monoxide, and hydrogen cyanide, which interfere with the natural healing process. Nicotine, for example, inhibits fibroblast function and collagen production, which are required for periodontal tissue regeneration [33]. With the same periodontal condition, smokers have a lower concentration of salivary osteonectin than nonsmokers. This reduction may be linked to the overall negative effects of smoking on bone health and periodontal tissues, as smoking inhibits the differentiation of mesenchymal stem cells and osteoblasts in bone formation and promotes osteoclast differentiation in bone resorption [34,35]. After one and three months of treatment, the ON in both nonsmokers and smokers increases significantly. This increase may indicate a periodontal healing response following root surface debridement in both nonsmokers and smokers with periodontitis. The current study's findings may suggest that periodontal healing is in progress, which agrees with Trombetta *et al.* [36]. While osteopontin plays a role in bone resorption, Kido *et al*., in their case-control study, reported that elevated levels of OPN in gingival crevicular fluid (GCF) were associated with increased probing pocket depth (PPD) [37]. This finding suggests that osteopontin may be a potential marker for bone resorption [38]. In the present study, values of osteopontin in saliva decreased after therapy, similar to P. Rajapriya *et al*. [39] and Sharma and Pradeep [40]; another study found that GCF osteopontin levels were significantly elevated in patients with severe periodontitis, and these levels decreased considerably after non-surgical periodontal therapy [41]. Furthermore, there is a limited amount of research investigating the levels of osteopontin in saliva. In the current study, the OPN levels decreased at one-month and three-month follow-ups, which indicates a reduction in inflammation and an improvement in periodontal healing. As the periodontal tissues heal and inflammation subsides, the need for OPN (associated with inflammation and tissue repair) diminishes, resulting in lower saliva levels. Consistent with previous findings of Missy Mercia *et al.* [42]. Although baseline osteopontin levels were found to be elevated in smokers relative to non-smokers, there was no statistically significant difference between the two groups.

Study limitations

The current study had limitations. The OPN was used to calculate the sample size, which may not be enough to detect any significant differences in clinical parameters and biomarkers between smokers and non-smokers. However, the main strength of this study lies in highlighting the impact of nonsurgical periodontal treatment on bone formation (ON) and bone resorption (OPN) biomarker levels in smokers and nonsmokers with periodontitis. Future studies of a larger sample size and a longer follow-up period are recommended.

Conclusion

The non-surgical treatment of periodontal disease significantly enhanced periodontal clinical variables.

It reduced OPN salivary concentrations in each group, and no statistically significant variations in OPN levels were observed compared to the two groups. In the two groups, ON salivary concentrations rose following NSPT, with statistically significant differences observed comparing smoking and nonsmoking.

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Conflict of interests

The authors declared no conflict of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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