The effect of smoking on salivary osteocalcin in patients with chronic periodontitis

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**ABSTRACT**

**Introduction:** Periodontitis is a multi-factorial inflammatory disease of periodontium. A precise mechanism in which smoking affects periodontium is not accurately understood. This study was planned to survey salivary osteocalcin (OC) level among smokers with chronic periodontitis and its comparison to non-smoker patients which may help achieve a better understanding of mechanisms being involved in beginning and developing of periodontal disease.

**Materials and Methods:** This case-control study was done among patients referred to Babol Dental School. The patients who had systemic disease or who had been taking intervening drugs, and also the patients who were under periodontal treatment at least 6 months before the start of the study, were excluded. Thirty-five patients with history of smoking 10 cigarettes/day during at least 5 years entered into case group and 35 patients who had not smoked, and did not have a smoking history in the past, entered the control group (both groups had periodontitis). Clinical parameters according to Ramfjord system, including clinical attachment loss (CAL) and probing pocket depth (PPD) were recorded. One milliliter of unstimulated saliva of patients was collected in tubes and stored at −80°C. Enzyme-linked immunosorbent assay test was done to evaluate the salivary OC levels. The data were analyzed with Mann–Whitney test.

**Result:** The mean age in case group was 43.03 (± 7.71) and 40.37 (± 5.73) years in the control group. There was no statistically significant difference in salivary OC levels between two groups (P > 0.050), but the average of PPD and CAL were significantly higher in case group and the difference was statistically significant (P < 0.050).

**Conclusion:** Our study showed that smoking and periodontal diseases are associated, but the present study did not prove the destructive effects of smoking on periodontal tissues by changing the salivary levels of OC.

**Keywords:** Salivary Osteocalcin, Chronic Periodontitis, Smoking, Enzyme-Linked Immunosorbent Assay

**Introduction**

Periodontitis is a chronic inflammatory disease of the supporting tissues of teeth which is caused by specific microorganisms or groups of microorganisms residing in plaque below the gum and lead to the loss of clinical periodontal ligaments, bone loss and eventually tooth loss [1, 2]. A series of interactions between microorganisms involving in periodontal disease with host’s immune system leads to an inflammatory response and destruction of periodontal tissue. Each factor, which can change the host’s response severity or create conditions to replace other
pathogens, may affect periodontal tissue diseases [3, 4].

Smoking is known as a risk factor for many diseases. Since mucosa and oral cavity are the first parts which are in contact with gases and particles from combustion of tobacco, so the role of this risk factor in periodontal disease cannot be ignored [5, 6]. In a study, Bergstrom [6] noted that there has not been any known factor found to this day which can be effective on periodontal tissues as much as smoking. In smoker patients suffering from periodontitis, gingival recession, periodontal pocket depth, alveolar bone loss, and tooth loss following periodontal disease are more severe than non-smoker patients [4, 7-9].

Smoking can even affect the diversity of microorganisms in dental plaque, as well as short- and long-term results of periodontitis treatments [6]. In their study, Kubota et al. [10] found the relationship between bleeding from the gums and Campylobacter rectus bacteria, Prevotella intermedia and smoking. They also found out that in smoker patients, the prevalence of Aggregatibacter actinomycetemcomitans bacteria in dental plaque was lower than non-smokers. However, some studies have not found any difference between the variety of living bacteria found in dental plaque of smokers and non-smokers [10]. Although the relationship between smoking and periodontitis is dose-response dependent [11], but the mechanism by which smoking may aggravate the periodontitis situation is still not well known [12].

Osteocalcin (OC) is a non-collagen protein bonded to bone calcium which is produced by osteoblasts and has a major proportion among non-collagenous proteins in mineralized tissues. Serum level of this protein is known as a marker for osteogenic activity [13]. Lower serum OC level in patients with periodontitis was reported compared to healthy persons, which reflects less osteoblastic activity and lower bone construction in periodontitis than in healthy persons [13, 14].

Saliva is a fluid which is composed of secretion of major salivary glands and in smaller scales of minor salivary glands and gingival crevicular fluid (GCF) and by examining it the systemic condition of body can be assessed. Several inflammatory mediators have been found in the saliva of patients with periodontitis [14]. Since the saliva is a kind of sample of secretion from different parts of periodontal tissues and is easy to be collected, maybe its assessment is the best way to show the periodontal disease status rather than analysis of GCF which is in fact site-specific [15]. The relation between smoking, chronic periodontitis and OC concentration is not well understood; therefore, the aim of the present study is to examine salivary OC levels in smoker and non-smoker patients suffering from chronic periodontitis.

**Material and Methods**

This is a case-control study on male patients referred to the Department of Periodontology, School of Dentistry of Babol Medical University. Subjects were enrolled with following conditions: (1) Lack of systemic diseases affecting the periodontal tissues, (2) disuse of antibiotics or any medication affecting the periodontal tissues during last month, (3) lack of inflammatory and non-inflammatory lesions inside the mouth, (4) disuse of alcohol, (5) lack of scaling, root filling, and periodontal surgeries during at least the last 6 months, (6) at least 5 years of smoking and mean number of 10 cigarettes daily, (7) with generalized mild chronic periodontitis [clinical attachment loss (CAL) ≥ 2 mm in 30% of the probing sites].

To eliminate the influence of age on periodontal status, the age of the control and case groups were matched. Non-stimulating saliva was collected by the spitting method. The participants in the study were asked to prevent eating, drinking, brushing, and flossing about 90 minutes before collecting their non-stimulating saliva samples. The participants poured 2 ml of their saliva into the clamshell pipe, then put it in a flask containing ice packs and transferred it to the laboratory. Using centrifuge (Spectra fuge 24D, Labnet International Inc., Germany) with 3000 rpm for 5 minutes, samples were centrifuged and supernatants were collected and poured into micro-tubes; samples have been kept at −80°C until testing time. On testing day, all samples were brought out of the freezer and then put into hot water bath (Bain-Marie). Evaluation of salivary OC was performed by the Sandwich enzyme-linked immunosorbent assay method using Salivary OC Kit (DRG, Germany).

Clinical evaluation was performed by CAL and probing pocket depth (PPD) indicators. Pocket depth measurements in Ramfjord teeth [16] (central and one of the left premolars and one of right maxillary molars, central and one of the left molars and one right premolar of mandible) was performed. William’s periodontal probe was used parallel with longitudinal axis of the tooth, from marginal gingiva to the pocket depth for assessing PPD. All measurements were performed by a single calibrated examiner.

Data were analyzed by using SPSS software (version 18; SPSS Inc., Chicago, IL, USA), the Kolmogorov-Smirnov test was used to evaluate the data distribution; then Mann–Whitney test was used to compare the salivary levels of OC, age, PPD, and CAL in two smokers and non-smokers groups.

The study protocol was approved by the ethics committee of the Babol University of Medical Sciences.

**Results**

Seventy male patients suffered from chronic periodontitis were enrolled in the study (35 in the case group, 35 in the control group). The mean age of participants of this study was 41.7 (± 1.88) years. Furthermore, the mean age of participants in case group was 43.03 (± 7.71) years and in the control group was 40.37 (± 5.37) years (P = 0.197). The mean PPD and CAL indices in smokers were significantly higher than non-smokers (P < 0.050). The mean salivary OC concentration was not significant between two different groups (P = 0.109) (Table 1).
Discussion

This study examines the salivary OC levels in smokers and non-smoker patients with chronic periodontitis. Few studies have examined the concentration of OC in smoker and non-smoker patients with chronic periodontitis. The mean PPD and CAL indices were significantly higher in smokers. Furthermore, there was no significant difference in salivary OC between these two groups.

The results of the study of Gurlek et al. [17] show that salivary OC in smokers was significantly lower than non-smokers or quit-smokers. Bleeding on probing (BOP) index in smokers group was significantly lower than non-smokers.

Ozcaka et al. [4] showed that the salivary OC level in smoker and non-smoker periodontal patients was significantly higher than healthy smoker and non-smoker groups. Moreover, the salivary OC level in healthy smokers and smokers with periodontitis was significantly lower than healthy and non-smoker periodontal patients.

In Ozcaka et al.’s study [4], BOP in smoker group with healthy periodontium was less than non-smoker group with healthy periodontium; however, in both groups it was significantly lower than group with periodontitis. Furthermore, CAL index in smoker group with periodontitis was lower than non-smokers with periodontitis. In other study, they investigate plasma level of OC in chronic periodontitis, in spite of higher level of plasma OC, no significant difference was reported in its level between periodontitis patients and healthy group [7].

Shi et al. [13] and Lappin et al. [3] reported no higher serum OC level in periodontitis patients than healthy group. In Lappin et al.’s study [3], patients suffering from chronic periodontitis have more than two areas with pocket depth and CAL more than 4 mm. They finally stated that OC concentration was negatively correlated with the development of periodontal disease. However, the most important issue in their study was the use of serum OC instead of salivary OC.

Gerits et al. studied 380 smokers (smoke 10 cigarettes/day) and non-smoker patients. They concluded that PPD in non-smoker patients was higher than smokers, and there was no significant difference between salivary OC [18].

Becerik et al. studied [19] investigate GCF OC and cross-linked N-terminal telopeptide (NTx) levels in health along with different periodontal diseases. They show that chronic periodontitis and generalized aggressive periodontitis groups had lower GCF OC total amount compared to gingivitis and healthy groups. Chronic periodontitis group had higher GCF NTx but lower OC total amount and OC/NTx ratio than the generalized aggressive periodontitis group. They suggest fluctuating GCF levels of OC and NTx might point out to the abnormal bone turnover in periodontitis.

Rai et al. [20] found that BOP index and depth of periodontal pockets in smoker group was significantly higher than in non-smokers group. However, in their study, there were 12 patients in smoker periodontitis group and 10 patients in non-smoker periodontitis group. In Haffajee and Socransky study [21], depth of periodontal pocket in smokers was significantly higher than non-smokers and quit-smoking people.

In Lafzi et al.’s study [22], depth of periodontal pocket in heavy smoker patients was significantly higher than non-smoker ones; while in light smokers there was no significant difference. Furthermore, BOP index in heavy smoker patients was significantly lower than non-smoker and light smoker patients. Studies of Haffajee and Socransky [21], Calsina et al. [23], and Bergstrom and Bostrom [8] have shown a significant decrease of BOP index in smoker patients in comparison with non-smoker patients.

In Tang study [24], the increase in period of smoking during life time results in stopping the production of osteoprotegerin (OPG) and increase of the ratio of PANKAL to OPG; this could partly explain the increase of bone erosion in periodontitis caused by smoking.

The results of the study of Buduneli et al. [25] showed that the difference in concentration of OPG and PANKAL between treated and untreated groups was significant. In smoker group compared to non-smoker group, OPG concentration in saliva was significantly lower and OPG/PANKAL ratio was higher. It was determined that in both treated and untreated smoker groups, OPG and PANKAL concentration in saliva is influenced by smoking; besides, in smokers PANKAL concentration is higher, and OPG concentration is lower than non-smokers.

Serum OC levels increase in several diseases such as osteoporosis where rapid bone turnover are seen and accepted as a valid marker of bone turnover when resorption and formation are coupled [26]. It has been previously shown that bone turnover profiles from periodontal bone surfaces and GCF differed from systemic bone turnover profiles [27]. In periodontitis OC has been suggested to be a marker of bone formation where bone resorption is greater than formation, and GCF OC levels are more revealing than serum

### Table 1. The mean periodontal health indicators in two smokers and non-smokers groups with chronic periodontitis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Smokers</th>
<th>Non-smoker</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>PPD (mm)</td>
<td>2.21 ± 0.59</td>
<td>1.12 ± 0.59</td>
<td>0.001*</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>1.04 ± 0.40</td>
<td>0.35 ± 0.33</td>
<td>0.001*</td>
</tr>
<tr>
<td>Salivary OC (pg/ml)</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.109</td>
</tr>
</tbody>
</table>

* Statistical significant difference at α = 0.05. CAL: Clinical attachment level; SD: Standard deviation; OC: Osteocalcin; PPD: Probing pocket depth.
or saliva levels regarding bone turnover in periodontium [28]. On the other hand, there is a controversy about the GCF OC levels in periodontal diseases. Kunimatsu et al. [29] did not find OC in GCF of patients with gingivitis while in periodontitis GCF OC was positively correlated with clinical parameters. Lee et al. [30] demonstrated similar GCF OC levels in diseased and healthy sites in patients with chronic periodontitis. Wilson et al. [27] could not detect OC in GCF of untreated periodontitis patients. On the other hand, Nakashima et al. [31] found elevated OC total amount in GCF from periodontitis sites compared to those found in healthy and gingivitis sites. Moreover, variations in OC levels among different studies as well as the current study might reflect inability to differentiate between sites undergoing attachment loss and others in a “bone loss arrest” state, where clinical signs of PD (CAL, increased PPD, BOP) are present, but no activity in present.

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Conflict of Interest: ‘None declared’.

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