JO JOURNAL OF OSSEOINTEGRATION periodontics and prosthodontics

Evaluation of salivary osteocalcin level in smokers and non-smokers with dental implants: research article

V. FAKHRZADEH[1], H. ESLAMI[2], F. FARHADI SHABESTARI[2], M. ZOHDI[3], B. HASANALIZADEH[2], N. YASAMINEH[4]

[1]Tabriz University of Medical Sciences, Faculty of Dentistry, Department of Prosthodontics, Tabriz, Iran
[2]Department of Oral and Maxillofacial Surgery, School of Dentistry, Tabriz University of Medical Science, Tabriz, Iran
[3]Student's Research committee, Tabriz University of Medical Sciences, Tabriz, Iran
[4]Dental and Periodontal Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

TO CITE THIS ARTICLE
Fakhrzadeh V, Eslami H, Farhadi Shabestari F, Zohdi M, Hasanalizadeh B, Yasamineh N. Evaluation of salivary osteocalcin level in smokers and non-smokers with dental implants: research article. J Osseointegr 2022;14(4):237-240.

DOI 10.23805/JO.2022.14.32

KEYWORDS Dental implants, Osteocalcin, Smoking.

ABSTRACT

Aim The present study is designed to evaluate the salivary osteocalcin level of smokers and peer non-smokers to better understand the mechanisms involved in the effects of smoking on dental implants.

Methods The present clinical trial was conducted on saliva samples of patients with dental implants referring to the dentistry faculty of Tabriz University of Medical Sciences. Based on study inclusion and exclusion criteria, eligible patients were divided into two groups of smokers (case group) and non-smokers (control group), each including 35 patients. ELISA (enzyme-linked immune sorbent assay) kit was used to measure the salivary osteocalcin level. Results were analyzed using descriptive statistical methods (mean±SD, frequency, percentage) and SPSS version 17.

Results The salivary osteocalcin level is significantly lower in smokers compared with nonsmokers.

Conclusion Lower salivary osteocalcin level in smokers compared to nonsmokers may relate to bone resorption and high rate of dental implant failures in smokers.

INTRODUCTION

Advancement in surgical techniques and prosthesis, changing implant designing and topography, and modern radiography methods have significantly improved the success rate of implant treatment, and prosthesis-based implants are among the most common and successful dental treatments. Yet, in some cases, failure or complications occur in implant treatments, and the causes remain unknown in most cases. Failure or side effect of implant treatment may result from various biological and biomechanical factors such as surgical trauma, bacterial contamination, the thickness of alveolar bone, and systemic disease such as diabetes, smoking, immunoinflammatory response, genetic factors, etc. Identification and prevention of these factors affect the success of implant treatments (1, 2). Among the factors mentioned above, smoking is one of the most precarious , hence, several studies have reported a higher dental implant failure rate among smokers compared with nonsmokers (3-5). It has been proved that Probing Depth (PD) and Clinical Attachment Loss (CAL) and bone resorption occur more commonly in smokers, and tobacco consumption is involved in the resorption of implant-supporting bone and implant failure (6, 7). Moreover, smoking may affect immunoinflammatory systems, fibroblast function, chemotaxis, neutrophil phagocytosis, and immunoglobulin production (8).

Osteocalcin (OC), also known as bone γ-carboxy glutamic acid, is the marker of osteogenesis and the most important non-collagen protein of the bone, also found in teeth. OC is an important extracellular component of the bone and is mainly produced by osteoblasts. This protein may partly be released to Gingival Crevicular Fluid (GCF) (9). Joseph et al. reported a significant difference in salivary osteocalcin level in patients with periodontitis and healthy controls. Moreover, salivary osteocalcin level of patients with periodontitis was higher among smokers than nonsmokers (10, 11). Although many studies have shown the negative role of smoking on the healing process and success rate of implant treatments, review articles suggest that studies in this field should be continued pointing to the confounding factors (4). In this regard and considering the potential relationship between OC and implant failure, the present study was designed to assess salivary OC level in smokers and compare them with peer nonsmokers.

MATERIALS AND METHODS

This study received approval from the Human Research

Ethics Committee of Tabriz-Iran University of Medical Science under ethic number IR.TBZMED.REC.1396.93. Each subject in the project signed a detailed informed consent form.

Firstly, the medical history of patients referring to the dental faculty of Tabriz University of Medical Sciences for implant surgery (with a diameter above 3.7) was taken considering the 5-month healing time. Then, 35 patients with a history of smoking ten cigarettes a day for at least five years, who smoked during the study (case group), and 35 nonsmoker patients with a negative history of smoking (control group) were included in the study.

Inclusion criteria of group 1 were as follows.

1. Willingness to participate in the study.
2. Age 21-78 years with at least one implant.
3. Smoking.
4. No periodontitis.

Inclusion criteria of group 2 were as follows.

1. Willingness to participate in the study.
2. Aging 21-78 years with at least one implant.
3. No periodontitis.
4. Non-smoking.

Exclusion criteria for both groups were the following.

1. Unwillingness to participate in the study.
2. Any systemic disease.
3. Alcohol consumption.
4. Presence of orthodontic appliances.
5. Being pregnant or planning to get pregnant within the six months of the study.
6. Taking anti-inflammatory or antibiotic agents in the last three months.
7. Presence of periodontitis.
8. Any systemic disease or debilitating disease which may affect the oral hygiene.
9. A history of smoking more than ten cigarettes a day for less than five years.
10. A history of smoking less than ten cigarettes a day for more than five years.

Saliva specimens were collected in accordance with Navazeh-1993 protocol (12). Study participants were asked neither to brush their teeth for 12 hours before saliva collection nor eating and drinking for 1.5 hours before sampling. The participants rinsed their mouths with water 15 minutes before sampling, and then their oral cavity was examined for potentially left substances. They were asked to spit gently and put 5 ml of their saliva to dry in clean polyethylene vials without chewing. All samples were immediately stored at -80°C. Samples were refined by centrifuging, and osteocalcin level was measured by ELISA test using DRG kit (Germany) following their manufacturer instructions.

1. 100 µl of the standard solution with different concentrations and the saliva sample were put in the standard and serum wells, respectively.
2. 50 µl of anti-osteocalcin antibody was added to the wells.
3. The surface of the wells was covered, and they were incubated for 2 hours at room temperature (18-25 °C).
4. The solution of the wells was emptied and rinsed with buffer six times.
5. 100 µl of the Sreptavidin-HRP solution was added to the wells.
6. The surface of the wells was covered; they were incubated for 1 hour at room temperature (18-25 °C).
7. The solution of the wells was emptied and rinsed with buffer six times.
8. 100 µl of the Tetramethylbenzidine (TMB solution was added to the wells.
9. The microplate was incubated for 10 minutes in the dark and at room temperature (18-25 °C).
10. 100 µl of the Stop solution was added to the wells
11. The light absorbance of all wells was read at 450 wavelengths using an ELISA reader device.

Statistical analysis

The collected data was analyzed using descriptive statistical methods (mean±SD, frequency, percentage) using SPSS version 17. The P-value below 0.05 was considered statistically significant. Kolmogorov-Smirnov test was used to assess the normal distribution of salivary osteocalcin level among smokers and nonsmokers. In the case of normal distribution of the data (which was proved by Kolmogorov-Smirnov test), an independent t-test was used; otherwise, its non-parametric equivalent, Mann-Whitney U Test, would be used.

RESULTS

The present experimental study measured salivary osteocalcin level of 64 participants (31 nonsmokers and 33 smokers). First, normal data distribution was assessed using the Kolmogorov-Smirnov test, which showed the normal data distribution (P-value >0.05).

Results of the descriptive analysis are presented in Table 1. The mean osteocalcin level was 3.52±0.77 among nonsmokers and 2.69±0.55 among smokers. The intragroup difference was 0.83 (lower among smokers compared with nonsmokers), and the difference was statistically significant (P-value=0.001). The lowest and highest osteocalcin level among nonsmokers were 2.09 and 5.01, respectively. The lowest and highest osteocalcin level among smokers were 2.06 and 3.7, respectively

DISCUSSION

The cytotoxic effect of smoking occurs due to thousands of toxic gases and chemicals, such as nitrogen, carbon monoxide, carbon dioxide, ammonia, hydrogen cyanide, benzene, neuricotine, nicotine, anatabine, and anabasine. The cytotoxic effects of nicotine lead to impaired fibroblast proliferation and adhesion, as well as reduced blood flow,

	Osteocalcin level (Mean±SD)	Minimum	Maximum	P-value
Non-smokers	3.52±.77	2.09	5.01	0.001
Smokers	2.69±0.55	2.06	3.7	

TABLE 1 Comparison of salivary osteocalcin level between the two groups.

reduced collagen formation, increased level of plasma fibrinogen and carboxyhemoglobin, impaired neutrophil and macrophage function and platelet adhesion, reduced production of cellular protein, and thus lack of fibroblast adherence, impaired tissue repair, bone weakening and absorption in smokers (13, 14).

In 2015 Chrcanovic et al. investigated in a review article the effect of smoking on implant treatment and reported a higher failure rate among smokers. Moreover, their meta-analysis proved the effect of smoking on the increased rate of post-surgical infections and marginal bone resorption (4). Naseri et al.'s review article in 2020 examined the effect of the number of cigarettes smoked per day on implant treatment failure, which showed the higher risk of dental implant failure among patients who smoked more than 20 cigarettes per day compared with nonsmokers (5). Implant failure was reported twice as much in smokers compared with nonsmokers. Moreover, logistic regression proved the advancement of periodontal disease in smokers, which may be reduced by smoking cessation (15, 16).

Osteocalcin is a marker of osteogenesis and the most important non-collagen protein; the regulatory role of bone tissue on energy metabolism mainly occurs through osteocalcin (17, 18). Yet, other hormones such as parathormone play important roles in the regulation of blood calcium levels and are an important biomarker of bone metabolism in addition to osteocalcin (19). Serum osteocalcin level directly reflects the bone turnover trend and reveals different osteoblast function and osteogenesis aspects. Moreover, this protein is released into GCF and is present in the saliva. The serum osteocalcin level has recently been a bone turnover marker when bone resorption and formation occur simultaneously (17, 20-23).

In 2009, Gürlek et al. evaluated salivary OC and ICTP (pyridinoline cross-linked carboxyterminal telopeptide of type I collagen) levels in 67 patients with periodontitis. In this study, three groups of patients, including smokers (n=34), nonsmokers (n=33), and ex-smokers (n=11), underwent clinical and laboratory evaluation. Salivary OC level of smokers was significantly reduced, which is consistent with the findings of the present study. ICPT did not show a significant difference between the three groups (24). Kiyota et al. in 2020 showed that smoking cessation leads to increase of osteocalcin and slows down bone resorption (25).

Lappin et al. evaluated the plasma osteocalcin level and bone markers in patients with type 1 diabetes mellitus, which showed that plasma osteocalcin level in patients with periodontitis is lower than in healthy individuals (26). Another study by Yoshihara et al. showed the negative relationship between the osteocalcin level and the periodontitis extent, i.e., the level of osteocalcin reduces with the increased extent of periodontitis and increased bone resorption (27).

In 2020 Joseph et al. reported that salivary osteocalcin level of patients with periodontitis were lower among smokers compared with nonsmokers (10, 11). Considering the bone resorption in patients with periodontitis and the reported low osteocalcin level in these patients, which is consistent with the present study's findings, smoking may lead to implant failure through increased bone resorption.

CONCLUSION

Salivary osteocalcin level is lower among smokers compared with nonsmokers. On the other hand, previous studies suggest that osteocalcin level is closely related to bone resorption and that it increases in line with bone resorption. Considering the higher rate of implant failure among smokers than nonsmokers, bone resorption may be among the underlying mechanisms through which smoking leads to implant failure.

It is suggested to take a thorough history of smoker candidates of implant treatment regarding number, frequency, and history of smoking. Also, the adverse effects of smoking on the success rate of dental implants should be fully explained, with the beneficial effects of smoking cessation.

Disclaimers

All authors have contributed significantly, and are in agreement with the manuscript. The corresponding author is N. Yasamineh.

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Alghamdi HS, Jansen JA. The development and future of dental implants. Dent Mater J 2020;39(2):167-72.
2. Levin L, Kessler-Baruch O. Cigarette smoking and the alveolar bone around teeth and dental implants. N Y State Dent J 2013;79(5):53-9.
3. Levin L, Schwartz-Arad D. The effect of cigarette smoking on dental implants and related surgery. Implant Dent 2005;14(4):357-61.
4. Chrcanovic BR, Albrektsson T, Wennerberg A. Smoking and dental implants: A systematic review and meta-analysis. J Dent 2015;43(5):487-98.
5. Naseri R, Yaghini J, Feizi A. Levels of smoking and dental implants failure: A systematic review and meta-analysis. J Clin Periodontol 2020;47(4):518-28.
6. Figueredo CA, Abdelhay N, Figueredo CM, Catunda R, Gibson MP. The impact of vaping on periodontitis: A systematic review. Clin Exp Dent Res

2021;7(3):376-84.
7. Javed F, Abduljabbar T, Vohra F, Malmstrom H, Rahman I, Romanos GE. Comparison of periodontal parameters and self-perceived oral symptoms among cigarette smokers, individuals vaping electronic cigarettes, and never-smokers. J Periodontol 2017;88(10):1059-65.
8. Qiu F, Liang CL, Liu H, Zeng YQ, Hou S, Huang S, et al. Impacts of cigarette smoking on immune responsiveness: Up and down or upside down? Oncotarget 2017;8(1):268-84.
9. Ma L, Zwahlen RA, Zheng LW, Sham MH. Influence of nicotine on the biological activity of rabbit osteoblasts. Clin Oral Implants Res 2011;22(3):338-42.
10. Cakal OT, Efeoglu C, Bozkurt E. The evaluation of peri-implant sulcus fluid osteocalcin, osteopontin, and osteonectin levels in peri-implant diseases. J Periodontol 2018;89(4):418-23.
11. Joseph B, Javali MA, Khader MA, AlQahtani SM, Amanullah M. Salivary Osteocalcin as potential diagnostic marker of periodontal bone destruction among smokers. Biomolecules 2020;10(3).
12. Navazesh M. Methods for collecting saliva. Ann N Y Acad Sci 1993;694:72-7.
13. Holliday RS, Campbell J, Preshaw PM. Effect of nicotine on human gingival, periodontal ligament and oral epithelial cells. A systematic review of the literature. J Dent 2019;86:81-8.
14. Moore D. Is nicotine damaging to oral tissues? Evid Based Dent 2020;21(1):32-3.
15. Johnson GK, Hill M. Cigarette smoking and the periodontal patient. J Periodontol 2004;75(2):196-209.
16. Silva H. Tobacco use and periodontal disease-the role of microvascular dysfunction. Biology (Basel) 2021;10(5).
17. Komori T. What is the function of osteocalcin? J Oral Biosci 2020;62(3):223-7.
18. Guntur AR, Rosen CJ. Bone as an endocrine organ. Endocr Pract 2012;18(5):758-62.
19. Rahimi Z. Parathyroid hormone, glucose metabolism and diabetes mellitus. Mol Cell Endocrinol 2009;307:77-82.
20. Komori T. Functions of osteocalcin in bone, pancreas, testis, and muscle. Int J Mol Sci 2020;21(20).
21. Giannobile WV, Al-Shammari KF, Sarment DP. Matrix molecules and growth factors as indicators of periodontal disease activity. Periodontol 2000 2003;31(1):125-34.
22. Bullon P, Goberna B, Guerrero JM, Segura JJ, Perez-Cano R, Martinez-Sahuquillo A. Serum, saliva, and gingival crevicular fluid osteocalcin: their relation to periodontal status and bone mineral density in postmenopausal women. J Periodontol 2005;76(4):513-9.
23. Giannobile WV, Lynch SE, Denmark RG, Paquette DW, Fiorellini JP, Williams RC. Crevicular fluid osteocalcin and pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) as markers of rapid bone turnover in periodontitis: A pilot study in beagle dogs. J Clin Periodontol 1995;22(12):903-10.
24. Gürlek O, Lappin DF, Buduneli N. Effects of smoking on salivary C-telopeptide pyridinoline cross-links of type I collagen and osteocalcin levels. Arch Oral Biol 2009;54(12):1099-104.
25. Kiyota Y, Muramatsu H, Sato Y, Kobayashi T, Miyamoto K, Iwamoto T, et al. Smoking cessation increases levels of osteocalcin and uncarboxylated osteocalcin in human sera. Sci Rep 2020;10(1):16845.
26. Lappin DF, Eapen B, Robertson D, Young J, Hodge PJ. Markers of bone destruction and formation and periodontitis in type 1 diabetes mellitus. J Clin Periodontol 2009;36(8):634-41.
27. Yoshihara A, Deguchi T, Hanada N, Miyazaki H. Relation of bone turnover markers to periodontal disease and jaw bone morphology in elderly Japanese subjects. Oral Dis 2009;15(2):176-81.