

Effects of light smoking on salivary levels of alkaline phosphatase and osteocalcin in chronic periodontitis patients

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ABSTRACT

Background: Chronic periodontitis is an inflammatory disease that affects the supporting tissues of the teeth and it's common among adults. Smoking is an important risk factor for periodontitis induces alveolar bone loss. Alkaline phosphatase enzyme is involved in the destruction of the human periodontium. It is produced by many cells such as polymorphonuclear leukocytes, osteoblasts, macrophages and fibroblasts within the area of the periodontium and gingival crevice. Osteocalcin is one of the most abundant matrix proteins found in bones and the only matrix protein synthesized exclusively there. Smaller Osteocalcin fragments are found in areas of bone remodeling and are actually degradation products of the bone matrix. The purpose of this study was to evaluate the effect of smoking on the salivary alkaline phosphatase and Osteocalcin in subjects with chronic periodontitis compared to control subjects.

Materials and Methods: Five ml of unstimulated whole saliva samples and full-mouth clinical periodontal recordings (plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment level) were obtained from study groups (25 light smokers and 33 non-smokers subjects, both with chronic periodontitis) and control groups (8 light smokers and 13 non-smokers subjects, both with healthy periodontium). All subjects were systemically healthy males, with age range (30-50) years. Salivary Alkaline phosphatase and Osteocalcin levels were determined by Colorimetric and Enzyme-linked Immunosorbent Assays, respectively.

Results: Smoker chronic periodontitis patients revealed non-significant differences in clinical periodontal parameters with non-smoker counterparts ($P > 0.05$) in terms of Plaque index, Probing pocket depth and Clinical attachment loss, with slight increase in plaque index value in smoker chronic periodontitis group (1.42 ± 0.46) than non-smoker chronic periodontitis group, while there were highly significant differences in terms of Gingival index and Bleeding on probing ($P \leq 0.01$). Osteocalcin levels were lower in smoker chronic periodontitis group (0.13 ± 0.20) than non-smoker chronic periodontitis group (1.09 ± 2.26) with significant difference ($0.05 \geq P > 0.01$). Mean of Alkaline phosphatase level was lower in smoker chronic periodontitis (11.14 ± 4.53) than non-smoker chronic periodontitis (11.45 ± 4.17) with a non-significant difference, while there was a significant difference in Alkaline phosphatase concentrations between smoker and non-smoker control groups. There were non-significant differences between smoker chronic periodontitis and smoker control groups in terms of Osteocalcin and Alkaline phosphatase concentrations. There were non-significant differences between non-smoker chronic periodontitis and non-smoker control groups in terms of Osteocalcin and Alkaline phosphatase concentrations.

Conclusion: Within the limits of this study, it may be suggested that suppression of salivary Osteocalcin levels by smoking and slight increase in alkaline phosphatase in smokers groups, may explain the deleterious effects of smoking on periodontal health status.

Key words: Chronic periodontitis, smoking, osteocalcin and alkaline phosphatase. (J Bagh Coll Dentistry 2015; 27(2):110-114).

INTRODUCTION

The Chronic Periodontitis (CP) has been defined as "an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss"⁽¹⁾

Although the microbial components of the subgingival plaque biofilm are the aetiological factors, the pathogenesis of periodontitis is a complex interaction between the microbiota and host tissues modified by environmental factors in particular smoking, age, systemic disease, and genetic susceptibility⁽²⁾.

Alkaline Phosphatase (ALP) is intracellular enzyme produced by many cells such as Polymorphonuclear Leukocytes (PMNLs), osteoblasts, macrophages, and fibroblasts within the area of the periodontium and gingival crevice⁽³⁾.

When a periodontal tissue becomes diseased or its cells become damaged due to edema or destruction of a cellular membrane, i.e. of a cell as a whole, this intracellular enzyme is increasingly released into the GCF and saliva where its activity can be measured⁽⁴⁾.

Osteocalcin (OC) is the most abundant non-collagenous protein in bone, comprising almost 2% of total protein in the human body. It is important in bone metabolism and is used as a clinical marker for bone turnover⁽⁵⁾. It is a product of differentiated osteoblasts⁽⁶⁾. Its main physiological functions are calcium ion homeostasis, maintain the normal bone mineralization rate, inhibit the abnormal formation of hydroxy appetites crystal, and to be involved in bone remodeling through a negative feedback mechanism⁽⁷⁾.

Khiste et al⁽⁸⁾ found that OC levels in oral fluids increased during an increased PD activity.

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On the other hand, Özçaka Öet al⁽⁹⁾ found that the Smoker periodontitis patients revealed lower salivary OC levels than non-smoker counterparts.

Thus the aim of the present study was to evaluate the level of Osteocalcin and Alkaline phosphatase in saliva of patients with chronic periodontitis smokers compared to non-smokers. Light smoking was chosen in this study.

There is no consensus on how to best define "light smoking". Light smokers have been classified as smoking <1 pack per day, <15 cigarettes per day, <10 cigarettes per day, and 1 to 39 cigarettes per week⁽¹⁰⁾.

SUBJECTS AND METHODS

Subjects

Sample population consisted of seventy nine individuals; systemically healthy males with an age ranged between 30 to 50 years were involved in this study. They were from attendants seeking periodontal treatment in the Department of Periodontics at Teaching Hospital of Baghdad College of Dentistry.

The sample population was divided into two main groups study and control. The study groups consisted of fifty eight subjects with chronic periodontitis, twenty five of them were light smokers (G1), thirty three were non-smoker (G2), based on clinical examination. Chronic periodontitis was defined according to The Periodontal Disease Classification System of the American Academy of Periodontology⁽¹¹⁾. The control groups consisted of twenty one subjects with healthy periodontium, eight of them were light smokers subjects (G3), thirteen were non-smokers (G4), based on clinical examination. Clinically healthy gingiva was defined by Gingival Index scores (GI)⁽¹²⁾. This group represent as a base line data for the level of salivary OC and ALP.

Method

Saliva collection

The samples were collected between 10 a.m. and 1 p.m., at least 1 hour after the last meal. Each Subject was asked to rinse his mouth thoroughly with water to insure the removal of any debris, then waiting for 1-2 min for water clearance. Five ml of unstimulated whole saliva (resting) was collected from each subject before clinical examination. Then the collected saliva was divided into 2 parts: first part was subjected to cold centrifuge at 4000 rpm for 30 minutes in the Teaching Laboratories of Baghdad Medical City, and then the clear supernatant saliva was collected by micropipette into eppendorf tubes and store at -80°C (deep freeze) until biochemical analysis of

OC, which was done by Enzyme Linked Immuno-Sorbent Assay (ELISA) technique by using kit manufactured by (CUSABIO, China). The second part was subjected to centrifuge at 3000 rpm for 10 minutes in the Poisoning Center in Ghazy Alharery Hospital, and then the clear supernatant saliva was collected by micropipette into eppendorf tubes and store at -20 °C until biochemical analysis of ALP, which was done by Colorimetric method by using kit manufactured by (Biomare, France). Samples containing blood were discarded. All samples were allowed to stand at room temperature before their analysis.

Clinical Assessment

Then clinical examination was done for each subject on a dental chair; by using Michigan O periodontal probe, all periodontal variables were recorded on four surfaces for all teeth except third molars. The clinical periodontal parameters for study groups include: (PLI, GI, BOP, PPD and CAL), for control groups: (PLI & GI).

The assessment of dental plaque was made according to the Plaque Index (PLI) by Sillness and Loe⁽¹³⁾. The gingival condition was assessed by using the criteria of Gingival Index (GI) by Loe⁽¹²⁾.

Assessment of BOP was made by inserted periodontal probe to the bottom of the gingival pocket and is moved gently along the tooth (root) surface. If bleeding occurs within 30 seconds after probing, the site was given a positive score or (1), and a negative score or (0) for the non-bleeding site⁽¹⁴⁾.

Probing pocket depth (PPD) defined as the distance between the base of the pocket and the gingival margin. CAL the distance from the CEJ to the location of the inserted probe tip (bottom of gingival crevice or periodontal pocket).⁽¹⁾

Statistical Analysis

The study variables were statistically analyzed using Statistical Process for Social Science (SPSS version 19) by using Mean, Median, Standard Deviation (SD), Percentage, Mann-Whitney U Test.

RESULTS

Table (1) shows the mean \pm SD (standard deviation) for the clinical parameters, while table (2) shows the mean \pm SD (standard deviation) for the biochemical parameters which have been measured in this study. Table (3) demonstrates statistically significance differences among the groups.

The mean of Plaque index was slightly higher in G1 (**1.42 \pm 0.46**) than G2 (**1.38 \pm 0.50**), while

there were decreases in means of GI, BOP, PPD and CAL in G1 than G2.

Mean of OC was lower in G1 (0.13 ± 0.20) than G2 (1.09 ± 2.26), and lower in G3 (0.09 ± 0.05) than G4 (0.11 ± 0.05). Mean of ALP was lower in G1 (11.14 ± 4.53) than G2 (11.45 ± 4.17), while mean of ALP was higher in G3 (10.31 ± 2.51) than G4 (8.70 ± 2.75).

There were no significant differences between G1 and G2 in terms of PLI, PPD and CAL, but there was a highly significant difference in GI and BOP ($P \leq 0.01$). There was a significant difference in the concentration of salivary OC between G1 and G2 ($0.05 \geq P > 0.01$), while smoking had a

non-significant effect on OC concentration compared to non-smokers in control group.

There was a non-significant difference in ALP concentrations between G1 and G2 ($p = 0.05$). There was a significant difference in ALP concentrations between G3 and G4 ($0.05 \geq P > 0.01$). There was a non-significant difference between G1 and G3 in terms of OC and ALP concentration. There was a non-significant difference between G2 and G4 in terms of OC and ALP concentration.

Table 1: Descriptive statistics (mean \pm SD) of the clinical parameters in all groups

Variables	G1	G2	G3	G4
PLI	1.42 \pm 0.46	1.38 \pm 0.50	0.54 \pm 0.25	0.64 \pm 0.17
GI	0.92 \pm 0.33	1.12 \pm 0.14	0.31 \pm 0.13	0.51 \pm 0.06
BOP%	3.92 \pm 5.65	10.88 \pm 11.02	-	-
PPD	3.90 \pm 1.78	4.27 \pm 1.98	-	-
CAL	3.22 \pm 0.93	3.74 \pm 0.85	-	-

Table 2: Descriptive statistics (mean \pm SD) of the biochemical parameters in all groups

Variables	G1	G2	G3	G4
OC (ng/ml)	0.13 \pm 0.20	1.09 \pm 2.26	0.09 \pm 0.05	0.11 \pm 0.05
ALP (IU/L)	11.14 \pm 4.53	11.45 \pm 4.17	10.31 \pm 2.51	8.70 \pm 2.75

Table 3: inter group comparison for the clinical & biochemical parameters with significant difference

Variables	G1-G2	G1-G3	G2-G4	G3-G4
PLI	0.556			0.238
GI	0.000**			0.000**
BOP%	0.000**		-	-
PPD	0.151		-	-
CAL	0.064		-	-
OC	0.019*	0.704	0.359	0.210
ALP	0.700	0.737	0.051	0.050*

*Significant difference $0.05 \geq P > 0.01$, **Highly significant difference $P \leq 0.01$

DISCUSSION

In the present study, it appears that smoking has a non-significant effect on the level of PLI, PPD and CAL, although PLI seems to be slightly higher in smoker chronic periodontitis than non-smoker counterpart, this finding is in agreement with the other previous studies Haffajee and Socransky⁽¹⁵⁾; Maddipati et al⁽¹⁶⁾.

This increased level of plaque which has been observed in smokers' chronic periodontitis subjects has been tentatively attributed to personality traits as the educational level, leading to decreased oral hygiene habits in smokers. Also could be due to heat and accumulated product of combustion that result in tobacco stain as well as calculus are particular undesirable local irritants that increased with smoking⁽¹⁷⁾.

One study concluded that poorer cleanliness found in smokers both before and after tooth brushing may be explained, in part at least, by their shorter tooth brushing time⁽¹⁸⁾.

Results of the GI and BOP in the present study showed highly significant differences between smoker and non-smoker chronic periodontitis.

These results are in the agreement with the other previous study Ali and Ali⁽¹⁹⁾. This may be due to tobacco smoke products interfering with the vascular inflammatory response. It is generally accepted that smoking causes vasoconstriction of peripheral vessels. It is therefore conceivable that such a constrictive action on gingival vessels would result in the suppression of vascular properties of inflammation such as bleeding, redness, and

exudation. It is possible that substances in tobacco smoke can reduce the capacity of microorganisms in plaque to produce irritants⁽²⁰⁾.

Maddipati et al⁽¹⁶⁾, showed decrease in blood vessels density in smokers CP group.

Regarding the PPD and CAL, the present study clarified that the values of these parameters were slightly lower in smoker chronic periodontitis than in non-smoker counterpart and results illustrated non-significant differences between them. Number of previous studies agreed with the present results Hanioka et al⁽²¹⁾; Zcaka et al⁽⁹⁾.

The present study results disagreed with Haffajee and Socransky⁽¹⁵⁾ whom found there was general increase in PPD and CAL in smoker than non-smoker chronic Periodontitis groups. The possible cause of this result is reduced in sample size.

In the present study, the saliva OC concentrations were higher in the chronic Periodontitis groups than in control groups; this result is in agreement with Zcaka et al⁽⁹⁾. This increase may indicate an increase in the cellular activities of osteoblasts to repair the damaged alveolar bone.

Moreover, the present study indicated significantly lower salivary OC concentrations in the smoker chronic periodontitis than non-smoker counterpart; this result is in agreement with Önder et al⁽²²⁾; Zcaka et al⁽⁹⁾.

A non-significant difference of OC concentrations between control smoker and non-smoker counterpart with slight decrease in OC concentration in control smoker than non-smoker counterpart, this result is disagree with Zcaka et al⁽⁹⁾ who found a significantly lower OC concentrations in smoker than the non-smoker control groups. This difference in the results belongs to the differences in the number of subjects between two studies and differences in the statistical methods that were applied. These results suggest that smoking may induce osteoblast depression, either directly or via hormonal changes.

In this study the comparison between smoker chronic periodontitis and smoker control group, revealed a non-significant difference in OC concentration, also there was a non-significant differences between non-smoker chronic periodontitis and non-smoker control group with slight increase in the concentration of OC in non-smoker chronic periodontitis which disagree with Zcaka et al⁽⁹⁾, who found a significant differences in their results. The differences in patient numbers and/or the possible differences in the disease activity states may explain the

differences in findings of the present study and the previous ones.

The findings of this study revealed a non-significant difference in the salivary ALP concentrations between smoker and non-smoker chronic periodontitis groups with slightly increase in the concentration of ALP in non-smoker than smoker chronic periodontitis groups; this result was in disagreement with Ban & Leka'a⁽²³⁾, who found that the level of ALP in the smoker group was significantly higher than the non-smoker group. The general explanation is the different sample size between the two groups.

In this study, there was a significant difference in ALP concentration between smoker and non-smoker control groups. The presence of ALP in the saliva is usually indicating the pathological changes occurring in the underlying periodontal tissues.

In this study, there were non-significant differences between smoker chronic periodontitis and smoker control groups, and between non-smoker chronic periodontitis and non-smoker control groups in terms of ALP concentration, with slight increase in the ALP concentration in CP groups compared to control groups, the increased activity of ALP indicates that the pathological destructive process has affected the alveolar bone, which means that the periodontal disease has significantly advanced. Furthermore, among the various periodontopathogenic bacteria *P. intermedia* and *P. gingivalis* are known to have high ALP activity⁽²⁴⁾ and in this study the smoker group was shown to have higher PLI which mean higher number of bacteria and this also adding to the total ALP level.

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الخلاصة

الخلفية: التهاب اللثة المزمن هو مرض التهابي يصيب الأنسجة الداعمة للأسنان وهي حالة أكثر شيوعاً لدى البالغين. التدخين هو عامل خطر ومهم لتقدم وتطور التهاب اللثة المزمن ويعمل على تحفيز فقدان العظم المحيط بالأسنان. ينفرد أنزيم الفوسفاتيز القلوي في حالة التهاب الأنسجة المحيطة بالأسنان. ويتم إنتاجه من قبل العديد من الخلايا مثل الكريات البيض متعددة النوى، الخلايا البانية للعظم، والخلايا الليفية الضامة في منطقة الأربطة الداعمة للسن وشق اللثة. الأوستيوكالسين هو واحد من بروتينات المصفوفة العظمية الأكثر وفرة في العظام ويصنع حصراً هناك. تم العثور على قطع صغيرة من تكسر الأوستيوكالسين في مناطق إعادة تشكيل العظام وهي في الواقع نواتج التحلل من مصفوفة العظام. الهدف من الدراسة للتحقيق في ما إذا كان المرضى المدخنين تدخيناً خفيفاً من ذوي التهاب اللثة المزمن يظهر تراكيز مختلفة من أنزيم الفوسفاتيز القلوي للعاب والأوستيوكالسين ومقارنة النتائج مع مجموعة السيطرة.

المواد وطرق العمل: الحصول على خمسة مل من عينات اللعاب غير المحفز، وأجراء قياسات المؤشرات ماحول الأسنان السريرية وتتضمن: (مؤشر الصفيحة الجرثومية، مؤشر التهاب اللثة، مؤشر النزف عند التسبير، مؤشر عمق الجيوب بالإضافة إلى فقدان الأنسجة الرابطة سريريا) من مجموعتي الدراسة (25 من المدخنين تدخيناً خفيفاً، 33 من غير المدخنين ذوي مرض التهاب اللثة المزمن) ومجموعتي السيطرة (8 من المدخنين تدخيناً خفيفاً و 13 من غير المدخنين اللذين يملكون أنسجة ماحول الأسنان صحية). وجميع المشاركين في الدراسة كانوا من الذكور، أصحاء سريريا، و الفئة العمرية تتراوح ما بين (30-50) سنة. تم تحديد أنزيم الفوسفاتيز القلوي بواسطة مقياس الطيف الضوئي ومستويات الأوستيوكالسين للعابية باستخدام تقنية مقايصة الأنزيم المرتبط المتميز المناعي.

النتائج: أظهرت النتائج أن المدخنين تدخيناً خفيفاً من ذوي التهاب اللثة المزمن لديهم نتائج مماثلة لمؤشرات ماحول الأسنان السريرية (مؤشر الصفيحة الجرثومية، مؤشر عمق الجيوب بالإضافة إلى فقدان الأنسجة الرابطة سريريا) مع نظرائهم من غير المدخنين. مع ارتفاع طفيف في قيمة مؤشر الصفيحة الجرثومية لدى المدخنين تدخيناً خفيفاً من ذوي التهاب اللثة المزمن. بينما كانت هناك فروقات معنوية كبيرة للغاية من حيث مؤشر التهاب اللثة ومؤشر النزف عند التسبير. وكانت المستويات الدنيا للأوستيوكالسين لدى المدخنين تدخيناً خفيفاً من مرضى التهاب اللثة المزمن مقارنة مع نظيرهم من غير المدخنين مع وجود فروقات معنوية بالنسبة لتركيز أنزيم الفوسفاتيز القلوي. كان هناك فرق غير ملحوظ في تركيزه بين المدخنين تدخيناً خفيفاً وغير المدخنين لمرضى التهاب اللثة المزمن، في حين كان هناك اختلاف كبير في تركيز الفوسفاتيز القلوي بين المدخنين تدخيناً خفيفاً وغير المدخنين من مجموعة السيطرة. أظهرت النتائج عدم وجود فروقات معنوية كبيرة بين المدخنين تدخيناً خفيفاً من مرضى التهاب اللثة المزمن والمدخنين تدخيناً خفيفاً من مجموعة السيطرة من حيث الأوستيوكالسين وأنزيم الفوسفاتيز القلوي. وكذلك عدم وجود فروقات معنوية كبيرة بين غير المدخنين من مرضى التهاب اللثة المزمن وغير المدخنين من مجموعة السيطرة من حيث تراكيز الأوستيوكالسين وأنزيم الفوسفاتيز القلوي.

الاستنتاج: في حدود هذه الدراسة، فإنه يمكن استنتاج أن قلة مستوى الأوستيوكالسين للعابي والزيادة الضعيفة لأنزيم الفوسفاتيز القلوي نتيجة التدخين قد يفسر الآثار الضارة للتدخين على وضع الأنسجة المحيطة بالأسنان.