Periodontal health status and salivary enzymes level in smokers and non-smokers (comparative, cross sectional study)

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ABSTRACT

Background: Smoking is considering a major risk factor for development and progression of periodontal disease. Investigations regarding the association between smoking and periodontal disease have consistently demonstrated negative periodontal effects and greater probabilities of established periodontal disease among smokers in comparison with non smokers. The purpose of this study was to evaluate the effects of smoking on periodontal health status and on the salivary levels of alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and creatine kinase (CK), and to correlate the clinical parameters of periodontal health with the biochemical findings in smokers and non-smokers.

Materials and methods: Unstimulated saliva sample was collected from 25 smokers and 25 non-smokers for biochemical analysis of salivary enzymes. Periodontal parameters including: plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment level were recorded.

Results: statistical analysis revealed that ALP in saliva was significantly higher in smoker than the non smoker group and there was a highly significant difference in the salivary LDH and CK levels between smokers and non-smokers groups. Plaque index (PLI), probing pocket depth (PPD) and clinical attachment level (CAL) were higher in smokers compared with non smokers, while there was decrease in the number of bleeding sites.

Conclusions: smokers group revealed more periodontal tissue destruction than non-smokers group represented by deeper pockets and more clinical attachment level. Salivary enzymes (ALP, LDH and CK) are considered as good biochemical markers of periodontal tissue destruction and can be used to evaluate the effect of smoking on periodontal health status.

Keywords: Non-smokers, smokers, salivary enzymes, periodontal health status. (J Bagh Coll Dentistry 2013; 25(3):91-96).

INTRODUCTION

Periodontal disease is defined as inflammatory destruction of periodontal tissue and alveolar bone supporting the teeth. Severe and prolonged periodontal inflammation leads to loss of teeth, thereby affecting oral functions (e.g., mastication, speech and facial esthetics). Progression and severity of the disease depends on complex interactions between several risk factors such as microbial, immunological, environmental and genetic factors as well as age, sex and race [1].

Tobacco smoking, mostly in the form of cigarette smoking, is recognized as the most important environmental risk factor in periodontitis [2]. The role of smoking in periodontal diseases has been extensively studied for many years. Clinical and epidemiological studies build up an increasing amount of scientific data which support the concept that tobacco use has a clear association with the prevalence and progression of periodontal disease [3].

Studies have indicated that the risk of having periodontitis among smokers varied from two to six times compared with non-smokers [4, 5].

Saliva has been used in the past few decades as a new diagnostic fluid [6-8].

Saliva has been extensively studied in relation to periodontal disease because it is easily collected and allows analysis of several local and/or systemic biological markers. Proposed salivary diagnostic markers for periodontal diseases have included serum and salivary molecules such as immunoglobulins, enzymes constituents of gingival crevicular fluid, bacterial components or products, volatile compounds and phenotypic markers, such as epithelial keratins [6].

Enzymes are biological catalysts that carry out tightly controlled biological reactions with high specificity. Like a chemical catalyst, an enzyme acts by lowering the activation energy of a reaction, thereby inducing the formation of the products from the substrates [9].

Intracellular enzymes are increasingly released from the damaged cells of periodontal tissues into the gingival crevicular fluid (GCF) and saliva. Several enzymes are evaluated for the early diagnosis of periodontal disease such as alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and creatine kinase (CK) [6, 10, 11].

MATERIALS AND METHODS

Human sample

Fifty subjects were enrolled in the study, the subjects with an age range (30-45) year's old males and females. Subjects included in the study were drawn randomly regardless the periodontal

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health status from patients attending the Department of Diagnosis in the College of Dentistry, University of Baghdad and Department of Diagnosis in Al-karama specialized center.

Each participant received complete medical and dental history to determine their suitability to the study and all of them had no history of systemic disease. All subjects were presenting at least 20 teeth.

The exclusion criteria were including: A course of anti-inflammatory or antimicrobial therapy within the previous three months, a history of regular use of mouth washes, use of any vitamin supplementation, mucosal lesions, chemotherapy, radiation therapy, medications that cause xerostomia.

Pregnant and lactating females, female patients during the menstrual cycle or suffering from any hormonal disturbance, post-menopausal females or others on estrogen therapy were excluded from this study. Former smokers were also excluded.

The subjects were divided into non-smokers group included 25 subjects who are not smoking and never smoked before and smokers group included 25 subjects regularly smoked at least 15 cigarettes on average per day for at least 5 years; current smoker and had not quit smoking [12].

Saliva samples collection

Un-stimulated whole saliva was collected before the clinical examination. A sample was collected after an individual was asked to rinse his/her mouth thoroughly with water to insure the removal of any possible debris or contaminating materials and waiting for 1-2 min for water clearance. The samples were collected at least 1 h after the last meal. Each one of the groups' subjects was asked to spit saliva into the polyethylene tubes until 5 ml was collected. The collected saliva was centrifuged and then the centrifuged clear supernatant saliva was collected by micropipette into eppendrof tubes and kept frozen and store at -20^{o}C until biochemical analysis of salivary enzymes.

Clinical examination

Clinical periodontal parameters included assessment of plaque index (PLI) [13], gingival index (GI) [14], bleeding on probing (BOP) [15], probing pocket depth PPD and clinical attachment level CAL. Collected data were recorded by using William's periodontal probe on four sites around each tooth (mesial, buccal, distal and lingual) excluding third molars. The probe was directed parallel to the long axis of the tooth.

The PPD measurement has been performed using a scale for ease of comparison between groups; it contains scores from (0-3) as shown in Table 1.

Table 1: Scale of PPD measurements

Scale	Score 0	Score 1	Score 2	Score 3
PPD in (mm)	0-3	>3-5	>5-7	>7

Clinical attachment level was obtained by measuring the distance from the cemento-enamel junction to the bottom of the pocket at each site. The CAL measurement performed using a scale that contains scores from (1-4) as shown in Table 2.

Table 2: Scale of CAL measurements

Scale	Score 1	Score 2	Score 3	Score 4
CAL in (mm)	1-3	>3-5	>5-7	>7

Biochemical analysis

For enzymes analysis we use kits manufactured by BIOLABO SA (ALP, CK), also we used kit manufactured by Human; German company, for LDH enzyme. The activity of ALP was determined by measuring its absorbance at 510 nm by the spectrophotometer, while the activities of LDH and CK were determined by measuring the absorbance at 340 nm by the spectrophotometer.

Statistical analysis

Descriptive statistics in the form of mean, standard deviation and Percentage and inferential statistics in the form of Student t-test, p-value and Pearson correlation were used in this study. The level of significance was accepted at $P< 0.05$, and highly significance when $P< 0.01$.

RESULTS

A-Clinical periodontal parameters

The result of the study revealed that PLI in smokers was higher that non smokes and the difference was statistically significant, while non significant difference was found in GI between smokers and non smokers as shown in Table 3.

The BOP results showed that smokers have less number of sites with bleeding on probing than non smoker group, Chi-square test was applied to BOP and revealed highly significant difference between the two groups ($P<0.01$) (Table 4).

There was increase in the total number of PPD scores (1and 2) in smokers compared to non-smokers except for score 0 which was decreased. Chi-square test revealed highly significant difference in PPD between the two groups (Table 5).

The results of the study revealed increase in CAL with its different scores in smokers group (score 1, 2 and 3) when compared with non-

smokers group. Chi-square test was applied to CAL and revealed non significant difference between the two groups (P>0.05) as shown in Table 6.

B-Biochemical analysis

The obtained results have shown that the activity of examined enzymes (ALP, LDH and CK) in saliva of smokers group was higher than non smokers group as shown in Table 7. Statistical analysis using the student t-test revealed the presence of significant difference in the activity of ALP and a highly significant difference in the activity of LDH and CK between non smokers and smokers groups (p-value < 0.01) as shown in Table 8.

C-Correlation of ALP, LDH and CK levels with clinical periodontal parameters

This study revealed non significant correlation between the activities of these enzymes with the clinical periodontal parameters (PLI, GI, BOP, PPD and CAL), except between LDH and PPD and between LDH and CAL**.**

There was positive significant correlation between PD score 1 and salivary LDH level in smoker group (r=0.487, P=0.014). There was positive strong highly significant correlation between CAL score 2 and LDH (r=0.589, P=0.002) and positive significant correlation between CAL score 3 and LDH (r=0.407, P=0.043) in smoker group.

DISCUSSION

The result of this study revealed more plaque accumulation in smokers than non-smokers, and this was in agreement with other studies [16, 17, 18], while its disagree with Giannopoulou *et al* [19].

It's possible that the increased level of plaque accumulation and debris observed in smokers may attributed to personality trait leading to decreased oral hygiene and / or increased rates of plaque formation [20].

The results showed that there was slight elevated gingival index in smokers group compared with non-smokers and the difference between them was non significant. This was in agreement with Hashim [21], Ustün and Alptekin [22] and Rosa *et al* [23], and in disagreement with Sreedevi *et al* [18] and Mohammad [24]. This could be due to sample selection in which both groups selected randomly regardless periodontal health status.

The result of this study revealed that the smokers have less number of sites with bleeding on probing than non smoker group and this was in agreement with Sreedevi *et al* [18], Hashim [21] and Thomas [25], while disagreement was recorded with Linden and Mullally [26].

This result may be explained by the fact that one of numerous tobacco smokes by-products, nicotine, exerts local vasoconstriction, reducing blood flow, edema and acts to inhibit what are normally early signs of periodontal problems by decreasing gingival inflammation, redness and bleeding [27]. Studies have suggested that nicotine increases rate of proliferation of gingival epithelium, thus increasing epithelial thickness among smokers [28]. Also tobacco use has been associated with reduced permeability of peripheral blood vessels [29].

According to results of this study, there was increased PPD with its different scores in smokers group compared with non-smokers group (scores 1 and 2) except for score 0 which represent normal gingival sulcus and it was higher in non-smokers group compared with smokers group.

This general increase in PPD in smokers group compared with non-smokers group was in agreement with many studies [17, 18, 21, 24], while it was disagree with Preber *et al* [30].

Pocket depth measurements are found to be greater in smokers due to increased alveolar bone loss [31, 32]. Alveolar bone is one of the tissues that is most affected by the progression of periodontal disease.

The mechanism of alveolar bone damage produced by smoking is related to the components of tobacco and nicotine metabolites which may act directly as local irritants on the gingival and alveolar bone or systemically because these components are absorbed in the lung, which affects the cellular host defense or bone turnover.

Smoking seems to disturb the balance between proteolytic and anti-proteolytic activities in periodontal tissue. Some in vitro studies provided other possible intimate mechanisms by which smoking may affect bone metabolism. Rosa *et al* [23] reported that nicotine increased the secretion of interleukin-6 and tumor necrosis factor alpha in osteoblasts, also nicotine increased the production of tissue-type plasminogen activator, prostaglandin E2 and matrix metalloproteinase, thereby tipping the balance between bone matrix formation and resorption toward the latter process, as reported by Katono *et al* [33].

According to the results, there was increased CAL with its different scores in smokers group (score 1, 2 and 3) when compared with non-smokers group.

This general increase in CAL in smokers group compared with non-smokers group was in agreement with Sreedevi *et al* [18] and Mohammad [24].

The general explanation for increase CAL in smokers when compared with non-smokers can

be derived from the same explanations of increased PPD in smokers which were mentioned previously as both of them represent the most important features of chronic periodontitis and reflect the progress and severity of periodontal tissue destruction.

Cigarette smoking is associated with periodontal disease and considered as major risk factor that lead to increased severity of periodontal disease [34, 35] and increased rates of disease progression [4] and this destruction of periodontal tissue in smokers could affect salivary ALP, LDH and CK levels.

Findings of this study revealed that the level of Alkaline Phosphatase ALP in the smoker group was significantly higher than the non smoker group.

Only one study was found about the effect of smoking on salivary ALP by Kibayashi *et al* [36]. In this study alkaline phosphatase were significantly lower in current smokers than in non-current smokers. In this study different enzyme analytical and statistical methods was used.

Alkaline phosphatase is released from polymorphonuclear cells (PMNs) during inflammation [37] and from osteoblasts [38] and periodontal ligament fibroblasts [39] during bone formation and periodontal regeneration respectively.

During the active stages of periodotitis, there will be destruction of alveolar bone osteoblasts and fibroblasts and their cell membrane will be ruptured releasing their intracellular contents outside. Therefore ALP will be released into GCF and saliva and the level of ALP will increase in saliva [6, 10, 40].

Further more, among the various periodontopathogenic bacteria *Prevotella intermedia* and *Porphyromaonas gingivalis* are known to have high ALP activity [17] and in this study the smoker group was shown to have higher plaque index which mean higher number of bacteria and this also adding to the total ALP level.

In this study, there was highly significant difference in salivary LDH level between smoker and non smoker groups. This result was in agreement with Ria *et al* [41], Leyva *et al* [42] and Mohammad [24], while it disagrees with Kibayashi *et al* [36].

The result of this study showed highly significant difference in salivary CK level between smoker and non smoker groups. In past literature, no study could be traced that assessed the effect of smoking on the CK level in saliva.

This increase in the LDH and CK levels in smoker group could be resulting from the destructive effect of smoking on periodontal tissue.

LDH and CK are intracellular enzymes included in metabolic processes of cells and they are mostly present in cells of soft tissues. These enzymes are indicators of a higher level of cellular damage and their increased activity in saliva is a consequence of their increased release from the damaged cells of soft tissues of periodontium and a reflection of metabolic changes in the inflamed gingiva [6, 10, 40].

Consequently, LDH and CK concentrations in saliva, as an expression of tissue breakdown, could be a specific indicator for periodontal disease that affects the integrity of the periodontium in smokers.

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Table 3: Statistical description (Mean ± SD) and T-test of PLI and GI between smoker and non smoker

Groups	PLI	T-test	P-value	Sig.	GI	T- test	P-value	Sig.
Non smokers	1.062±0.349	–2.615	0.012	S	1.018±0.271	- 0.919	0.363	NS
Smokers	1.347±0.417				1.071±0.090			

Table 4: Number and percentage (in sites) of bleeding on probing scores and Chi-square test for both groups

Score	Non smokers		smokers		Chi-square	DF	p-value	Sig.
	No.	%	No.	%				
0	2275	88.31	2347	92.40	24.481	1	0.000	HS
1	301	11.68	193	7.59				

Table 5: Number and percentage (in sites) of probing pocket depth scores and Chi-square test for both groups

Score	Non smokers		smokers		Chi-square	DF	p-value	Sig.
	No.	%	No.	%				
0	2561	99.417	2445	96.259	60.849	2	0.000	HS
1	15	0.582	92	3.622				
2	0	0	3	0.118				
3	0	0	0	0				

Table 6: Number and percentage (in sites) of clinical attachment level and Chi-square test for both groups

Score	Non smokers		smokers		Chi-square	DF	p-value	Sig.
	No.	%	No.	%				
1	139	5.395	409	16.102	4.516	2	0.105	NS
2	22	0.854	110	4.330				
3	5	0.194	14	0.551				
4	0	0	0	0				

Table 7: Statistical description (mean level in IU/L ± SD) of ALP, LDH and CK in both groups

Enzymes	Non smokers group	Smokers group
ALP	29.673 ± 2.188	31.333 ± 3.442
LDH	63.937 ± 19.704	151.712 ± 65.576
CK	24.637 ± 11.615	45.233 ± 19.491

Table 8: Inter group comparison between non smokers and smokers groups by using t-test for mean ALP, LDH and CK

Enzymes	t-test	p-value	significant
ALP	- 2.035	0.047	S
LDH	- 6.404	0.000	HS
CK	- 4.539	0.000	HS