Effect of passive smoking on the Periodontal Health Status and Salivary Enzymes Level : A comparative study

Original Research Article

ISSN : 2456-1045 (Online)
(ICV-MDS/Impact Value): 63.78
(GIF) Impact Factor: 4.126
Publishing Copyright @ International Journal Foundation
Journal Code: ARJMD/MDS/V-32.0/I-1/C-9/DEC-2018
Category : MEDICAL SCIENCE
Volume : 32.0 /**Chapter**- IX / **Issue** -1(DECEMBER-2018)
Journal Website: www.journalresearchijf.com
Paper Received: 25.12.2018
Paper Accepted: 03.01.2019
Date of Publication: 10-01-2019
Page: 47-51

Name of the Author (s):

Dr. Lekaa M. Ibraheem[1],
Dr.Ban Karem[2],
Dr.Ayat M. Dhafer[3]

[1,] Prof. Periodontology/Al-Israa University College, Dentistry Department,

[2] Assis. Lecturer,Periodontology/ Al Mustanseria University-College of Dentistry.

[3] B.D.S/ Baghdad University-College of Dentistry.

Citation of the Article

Ibraheem L.M. ; Karem B; Dhafer A.M. (2018) Effect of passive smoking on the periodontal health status and salivary enzymes level : A comparative study ; *Advance Research Journal of Multidisciplinary Discoveries.32(9)pp. 47-51*

ABSTRACT

Background: Environmental tobacco smoke (ETS) or passive smoke exposure is one of the most common preventable health hazards in the community. The risk of disease and death as a result of smoking is not limited to smokers; non-smokers are also at risk from exposure to SHS. Passive smoking has recently been implicated in the development of several systemic diseases.

Aims of the study: The purpose of this study was to evaluate the effects of passive smoking on periodontal health status and on the salivary levels of alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and creatine kinase (CK).

Materials and methods: The study comprised of 25 non-smokers and 22 passive smokers. Unstimulated saliva sample was collected from each subject 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 higher in smoker than the non smoker group and there was significant difference in the salivary LDH and CK levels between smokers and non-smokers groups. Probing pocket depth (PPD) and clinical attachment level (CAL) score 1 was higher in passive smokers compared with non smokers, while there was decrease in the number of bleeding sites.

Conclusions: Passive smoking has negative impact on periodontal health and can consider as risk factor for periodontal disease although further studies are needed. 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 passive smoking on periodontal health status.

KEYWORDS:

non smokers, passive smokers, salivaperiodontal health status.ry enzymes,

I. 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)] (Nunn, 2003).

Although periodontal disease occurs primarily due to bacteria within the gingival crevice or the periodontal pocket, it may be affected indirectly by many other risk factors occurring changes in the vascular system, severity of inflammatory reactions and systemic immunological responses [(1,2)] (Kinane, 2001; Nunn, 2003).

Environmental tobacco smoke (ETS) exposure is probably one of the most important public health hazards in the community. Scientific interest in potential adverse health effects of second-hand smoke expanded. Passive smoking or exposure to environmental tobacco smoke at home and/or workplace has recently been implicated in the development of several systemic diseases. It is etiologically related to many diseases, including cancer, cardiovascular diseases and many serious respiratory diseases [(3)] (US Surgeon General, 2006).

Nonsmokers exposed to secondhand smoke are recognized to be at increased risk of periodontitis. On reviewing updated evidence on involuntary exposure to tobacco smoke, the US Surgeon General concluded in 2006 that there is no risk-free level of exposure to secondhand smoke. A major review which also indicated a possible link between passive smoking and periodontal disease is by Johnson and Guthmiller in 2007. Persons exposed to passive smoking had 1.6 times the odds of having periodontal disease compared with those not exposed, after controlling for other covariates. [(4)]

Saliva has been used in the past few decades as a new diagnostic fluid [(5,6,7)] (Kaufman and Lamster, 2002; Wong, 2006; Lee *et al*, 2009). 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 [(8)] (Kaufman and Lamster, 2000).

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)] (Markus and Aaron, 2007).

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) [(8,10,11)](Kaufman and Lamster, 2000; Ozmeric, 2004; Todorovic *et al*, 2006)

II. MATERIALS AND METHODS

Human sample : The study comprised of 25 non-smokers and 22 passive smokers., 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 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 passive smokers group included 22 subjects who had been exposed to cigarette smoke at least 2 cigarette/day on ≥5 days/wk for at least 5 years [(12)] (Arinola *et al*, 2011).

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)](Silness and Loe in 1964), gingival index GI [(14)](Loe and Silness 1963), bleeding on probing BOP [(15)](Carrenza and Newman, 1996), 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 PD measurements

Scale	Score 0	Score 1	Score 2	Score 3
PD 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.

III. RESULT

A-Clinical periodontal parameters

The result of the study revealed non significant difference in the mean PLI between non smoker group with a mean value (1.062±0.349) and passive smokers with a mean value (1.036±0.260) as shown in Table 3.

Non significant difference was found in mean GI between non-smokers with a mean value (1.018±0.271) and passive smokers a mean value (1.008±0.184) as shown in Table 3.

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

Groups	PLI	T-test	P-value	Sig	GI	T- test	P-value	Sig.
Non smokers	1.062±0.349	0.293	0.771	NS	1.018±0.271	0.146	0.885	NS
Passive smokers	1.036±0.260				1.008±0.184			

The BOP results showed that passive smokers have less number of sites with bleeding on probing than non smoker group, Chi-square test was applied to BOP and revealed non significant difference between the two groups (P>0.05) (Table 4).

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

Score	Non smokers		Passive smokers		Chi-square	DF	p-value	Sig.
	No.	%	No.	%				
0	2275	88.31	1611	89.5	1.496	1	0.221	NS
1	301	11.68	189	10.5				

There was increase in the total number of PPD scores (1, 2 and 3) in passive smokers compared to non-smokers. Chi-square test revealed highly significant difference in PPD between the two groups (Table 5).

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

Score	Non smokers		Passive smokers		Chi-square	DF	p-value	Sig.
	No.	%	No.	%				
0	2561	99.417	1763	97.944	22.367	3	0	HS
1	15	0.582	30	1.666				
2	0	0	6	0.333				
3	0	0	1	0.055				

The results of the study revealed that passive smoker had increase in percentage of CAL only in score 1, while score 2 and 3 did not show marked change compared with non-smokers group. Chi-square test was applied to CAL and revealed highly significant difference between the two groups (P<0.01) as shown in Table 6.

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

Score	Non smokers		Passive smokers		Chi-square	DF	p-value	Sig.
	No.	%	No.	%				
1	139	5.395	134	7.444	9.931	2	0.007	HS
2	22	0.854	6	0.333				
3	5	0.194	1	0.055				
4	0	0	0	0				

B-Biochemical analysis

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

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

Enzymes	Non smokers group	Passive smokers group
ALP	29.673 ± 2.188	30.301 ± 3.902
LDH	63.937 ± 19.704	85.930 ± 39.177
CK	24.637 ± 11.615	32.803 ± 12.292

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	¯ 0.690	0.493	NS
LDH	¯2.476	0.017	S
CK	¯2.340	0.024	S

This study revealed non significant correlation between the activities of these enzymes with the clinical periodontal parameters (PLI, GI, BOP, PPD and CAL) in non smoker and passive smokers groups.

IV. DISCUSSION

The result of this study revealed non significant difference existed in the mean plaque index between non-smokers and passive smokers. The mean gingival index in non smokers and passive smokers was very close with slightly elevated gingival index in non smoker group, there was no statistically significant differences existed between them. This could be due to sample selection in which both groups selected randomly regardless periodontal health status.

The result showed that passive smoking had an effect on the number and percentage of bleeding sites among passive smoker group which was reduced when compared with non-smokers. This could be result from the vasoconstrictive actions of nicotine constituent on periodontal tissue. Secondhand tobacco smoke contains nicotine as well as carcinogens and toxins. Nicotine concentrations in the air in homes of smokers and in workplaces where smoking is permitted typically range on average from 2 to 10 micrograms/m3[(16)] (IARC, 2004).

In the present study there was increased PPD with its different scores in passive smokers group compared with non-smokers group (scores 1, 2 and 3) except for score 0 which was higher in non-smokers group compared with passive smokers group.

This result was in agreement with Rezaei and Sariri in 2011a [(17)] who show that probing pocket depth of > 3 < 6 mm is about 10 times more prevalence in passive smokers compared to control subjects. However, severe periodontitis status (≥ 6 mm) was only observed in two passive smokers, while the control group was almost healthy periodontically and only two cases (6.66%) of 4 mm pocket depths were recorded by the dentist who examined all subjects.

The result also agree with other studies which investigated the relationship between secondhand smoke and periodontal disease in nonsmokers and reported a possible link between passive smoking and periodontal disease [(18,19,4,20)] (Arbes *et al*, 2001; Yamamoto *et al*, 2005; Johnson and Guthmiller, 2007; Anne *et al*, 2011).

According to the results obtained from this research, there was increased PPD in passive smokers group compared with non-smokers group, suggesting that passive smoking adversely affects gingiva and dental health in a way very similar to direct smoking [(21,22,23)] (Olalekan, 2007; Avsar *et al*, 2008; Hajifattahi *et al*, 2010). It can be explained by considering the fact that cigarette smoke is operationally divided into gas-phase smoke and particulate matter (or tar). Tar is the material retained on a filter, whereas gas-phase smoke passes through the filter. Both the tar and gas-phase smoke are very rich sources of radicals [(24)] (Pryor and Stone, 1993). These free radicals could initiate the generation of various reactive oxygen species (ROS). ROS are then capable of increasing and promoting oxidative damage in the form of lipid peroxidation and tissue damage leading to various stages of periodontitis [(25)] (Koul *et al*, 2001).

According to the results, there was an increased in CAL score 1 in passive smoker group compared with non-smokers group. On the other hand CAL score 2 and 3 was decreased in passive smokers group compared with non-smokers group which could be resulting from sampling procedure in which both groups were selected in the study regardless the periodontal health status.

The results of the research showed that level of alkaline phosphatase (ALP) in the passive smoker group was higher than the non smoker group although the difference was not statistically significant.

Alkaline phosphatase is released from polymorphonuclear cells (PMNs) during inflammation [(26)] (Yan, 1995) and from osteoblasts [(27)] (Gibert *et al*, 2003) and periodontal ligament fibroblasts [(28)] (Taylor *et al*, 1994) 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(8,29,10) (Kaufman and Lamster, 2000; Numabe et al, 2004; Ozmeric, 2004).

There is reason to believe that passive smoking exerts similar systemic effects on the periodontal tissues as observed in active smokers, based on studies that have found that active and passive smoking have effects in the same direction, although not the same magnitude, on other health outcomes [(30,31,32)] (Howard *et al*, 1998 a,b ; Chen *et al*, 2010).

It was found that salivary alkaline phosphatase level increases with increase in periodontal destruction. Total amount of alkaline phosphatase levels were significantly higher in periodontitis as compared to healthy and gingivitis sites [(33)] (Randhir and Geeta, 2011) and scince the result of this study shown that passive smoker group had deeper periodontal pockets this may explain the increase in the salivary ALP in passive smokers. The increase in salivary ALP levels also may come from PMNs during inflammation within the periodontal pocket.

This indicate that although the passive smoking affected the periodontal tissue but the effect did not reach significant level as the active smoking and did not lead to sever distruction of the alveolar bone, yet the increased level show that some pathological process is affecting the underlying periodontal tissues.

The result of the study also revealed that the level of salivary LDH and CK in passive smoker group was significantly higher than the non smoker group.

This increase in salivary LDH and CK activities reflect the negative effect of the passive smoking on the periodontal tissue especially the soft tissue since the LDH and CK are intracellular enzymes included in metabolic processes of cells and it is mostly present in cells of soft tissues, also it is an indicator of a higher level of cellular damage and their increased activity in gingival crevicular fluid and 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 [(8,29,10)] (Kaufman and Lamster, 2000; Numabe *et al* 2004; Ozmeric, 2004).

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.

Passive smoking has deteriorating effect on periodontal health and can be considered as risk factor for periodontal disease although further studies are needed.

Salivary enzymes (ALP, LDH and CK) are considered as good biochemical markers of periodontal tissue destruction.

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