Periodontal health status of heavy and light smokers and its correlation with salivary superoxide dismutase enzyme (A comparative study)

Banaz J. Ali, B.D.S. (1)
Leka'a M. Ibrahim, B.D.S., M.Sc. (2)
Ali Y. Majid, M.B.Ch.B., M.Sc., F.I.C.M.S. (3)

ABSTRACT

Background: Periodontal disease is a chronic bacterial infection that affects the gingiva and bone supporting the teeth. Smoking, which is an important risk factor for periodontitis, induce oxidative stress in the body and cause an imbalance between reactive oxygen species (ROS) and antioxidants, such as superoxide dismutase (SOD). This study aimed to evaluate the influence of smoking on periodontal health status by estimating the levels of salivary SOD level in non-smokers (controls) and light and heavy smokers and to test the correlation between the SOD enzyme level and the clinical periodontal parameters in each group.

Materials and Methods: The study sample consisted of 75 male, with age ranged from 35 to 50 years. Clinically, the periodontal parameters used in this study were Plaque index (PLI). Gingival index (GI), probing pocket depth (PPD), Bleeding on probing (BOP) and clinical attachment level (CAL), unstimulated saliva sample were collected from all subjects and the levels of superoxide dismutase enzyme was analyzed for each group , and correlate the mean of salivary enzyme levels with the clinical periodontal parameters.

Results: Highly significant differences in PLI between (non smokers/heavy smokers) and (light smokers/heavy smokers).On the other hand no significant difference in gingival index between groups.

There were a high association between severity of smoking & probing pocket depth and there is association between severity of smoking and clinical attachment loss. There were a significant difference in the level of salivary superoxide dismutase enzyme between the (non smokers/light smokers) groups & between (heavy smokers/light smokers) &there were highly significant differences between (non smokers/heavy smokers) groups. There is no correlation between the activities of the salivary superoxide dismutase enzyme and the clinical periodontal parameters except in SOD with (BOP score 0 and PPD score 1&score 3) in heavy smokers group.

Conclusions: Superoxide dismutase enzyme can be used as biomarker for estimating the level of oxidative stress on smoking habits.

Key words: Periodontal health status, superoxide dismutase, heavy and light smoking. (J Bagh Coll Dentistry 2013; 25(3):97-102).

INTRODUCTION

Periodontal disease is a chronic disease of the oral cavity comprising a group of inflammatory conditions affecting the supporting structures of the dentition [1, 2].

Saliva is an aqueous fluid found in the oral cavity, composed of a complex mixture of secretory products (organic and inorganic products from the salivary glands and other substances coming from the oropharynx, upper airway, gastrointestinal reflux, gingival sulcus fluid, food deposits, and blood-derived compounds [3,4] . For the local inflammatory process of periodontitis, salivary diagnostics may promote early diagnosis and aid in the monitoring of treatment [5]. For some diagnostic purposes, salivary biomarkers may prove more useful than serum analysis [6].

The severity of the periodontitis process can be modified by a variety of factors, the most important risk factor markedly affected the initiation and progression of periodontitis was smoking [7,8].

(1) M.Sc. student, department of Periodontics, College of Dentistry, Baghdad University.
(2) Professor, department of Periodontics, College of dentistry, Baghdad University.
(3) Consultant.

Cigarette consumption and duration of smoking are associated with the severity of periodontal disease. The more tobacco is smoked the more periodontal attachment loss has been observed [9]. Heavy smokers were more likely to suffer from periodontitis than non-smokers, with light smokers less likely to have this problem [10].

Smoking induces oxidative stress in the body and causes an imbalance between reactive oxygen species (ROS) and antioxidants, such as superoxide dismutase (SOD), the role of reactive oxygen species (ROS) has been established in the pathogenesis of periodontitis, in healthy individuals, ROS are produced during various physiologic processes. Normally there is a balance between ROS and antioxidants that may be disturbed by a variety of factors, including smoking [11]. This dysregulation may damage the cells by variant mechanisms, such as peroxidation of lipid membranes, protein inactivation, and induction of (Deoxyribonucleic acid) DNA damage. [12]

Superoxide ($O_2^{\bullet-}$) is biologically quite toxic and is deployed by the immune system to kill invading microorganisms in phagocytes, superoxide is produced in large quantities by the enzyme Nicotinamide adenine dinucleotide

phosphate (NADPH) oxidase for use in oxygen-dependent killing mechanisms of invading pathogens .Because superoxide is toxic, nearly all organisms living in the presence of oxygen contain isoforms of the superoxide scavenging enzyme, superoxide dismutase (SOD) [13].

SOD is an antioxidant enzyme that acts against superoxide, oxygen radical that is released in inflammatory pathways and causes connective tissue breakdown. This enzyme is released as a homeostatic mechanism to protect the tissues, and it can be detected in extra- and intracellular compartments [14,15]. Measurement of SOD in human saliva might be useful for estimating the level of oxidative stress on smoking habits.

MATERIALS AND METHODS

Human samples:- consist of 75 Subjects ,male only with age range (35-50) years old , attending the oral diagnosis department in the College of Dentistry / Al-Mustansiria University and divided into 3 groups:-

Group 1 (G1):- composed of 25 non smokers
Group2 (G2):- composed of 25 light smokers (who smoke ≤10 cig/day) for the last five years [16]. Group 3 (G3):- composed of 25 heavy smokers (who smoke ≥10cig/day) for the last five years [16]

All the subjects were in a good health, with no history of systemic disease, no a history of regular use of mouth washes, no special dietary requirements and did not take vitamins or minerals supplements or medication of any type.

The periodontal examination includes:

1-Assessment of dental plaque by PLI of Sillenes and Loe [17]

2- Assessment of gingival condition by GI of Loe and Silness [18]

3- Probing pocket depth (PPD)

A scale was designed for ease of estimation and as follows:

Scale 1 = 0-3 mm
Scale 2 = >3-5 mm
Scale 3 =>5-7mm
Scale 4 =>7mm

4- Assessment of clinical attachment level (CAL).

A scale was designed for ease of assessment as follows:

Scale 1= 1-3 mm
Scale 2= >3-5 mm
Scale 3= >5-7 mm
Scale 4= >7mm

Biochemical analysis: The biochemical analysis includes measuring the concentrations of superoxide dismutase enzyme in saliva. (spectrophotometric)

Principle of reaction: The O2- substrate for SOD is generated indirectly in the oxidation of epinephrine at alkaline pH by the action of oxygen on epinephrine. As O2- builds in the solution, the formation of adrenochrome accelerates because O2- also reacts with epinephrine to form adrenochrome. Toward the end of the reaction, when the epinephrine is consumed, the adrenochrome formation slows down. Super oxide dismutase enzyme reacts with the O2- formed during the epinephrine oxidation and therefore slows down the rate of formation of the adrenochrome as well as the amount that is formed. Because of this slowing process, SOD is said to inhibit the oxidation of epinephrine.

Statistical analysis: Data were analyzed through the use of SPSS (Statistical Process for Social Science).The following statistical data analysis approaches were used in order to analyze and assess the results of the study:

I. Descriptive data analysis: Arithmetic mean, standard deviation, standard error, two extreme values (min. and max.) of the calculated.

II. Inferential data analysis: These were used to accept or reject the statistical hypotheses, which included the Student t-test for equality of means of two independent groups. Also, Pearson's Correlation Coefficient was used for testing the correlation between the two independent variables; the clinical and biochemical parameters

RESULTS

Clinical parameters: The mean and standard deviation of PLI&GI for the groups are (0.936 ±0.415) (0.940 ±0.355) respectively for group 1 , (0.942 ±0.504) (0.9563±0.1680) respectively for group 2 and (1.333 ±0.407) (1.005±0.204) respectively for group 3 . As shown in table (1). Statistical analysis using the t-test to compare the mean of plaque index & gingival index between each two groups ,regarding PLI there was no significant difference between G1 and G2 while a highly Significant difference were found between G1and G3, G2and G3 as shown in the table (2). Regarding gingival index, there was no significant difference between the groups. There was a high association between severity of smoking & probing pocket depth and there is association between severity of smoking and clinical attachment loss. as shown in the table (4).

Biochemical parameters: The mean and standard deviation of SOD level in G1 (52.128±9.421)U/ml was higher than the other group, the mean and standard deviation of superoxid dismutase for G2 was(45.976 ±11.85)U/ml and for G3 was(37.244±15.657) U/ml. as shown in the table (5).

Statistical analysis using the t-test to compare mean between each groups revealed that there were a significant difference between the G1 andG2, G3and G2 and there were a highly significant difference between G1and G3 as shown in the table (6).

Correlation between clinical and biochemical Parameters: There is no correlation between the activities of the salivary superoxide dismutase enzyme and the clinical periodontal parameters except in SOD with BOP in score 0, there is significant positive strong correlation in heavy smokers group and with PPD in (score 1, there was significant positive week correlation & in score 3, there was significant negative week correlation) both in heavy smokers as shown in the table (7).

DISCUSSION

Clinical Periodontal health parameters:-

Dental plaque (PLI)

Significant difference was found between light smokers and heavy smokers group i.e. more plaque accumulation in heavy smokers group than non smokers group. This increased level of plaque which have been observed in smokers have been tentatively attributed to personality traits leading to decreased oral hygiene habits in smokers, this agree with Muller *et al,*Sreedhar & Shobha [19,20] who showed smokers have a higher prevalence of dental plaque than non-smokers and disagree with Jayashree & Vandana [21] who found that plaque level was similar in smokers and non-smokers. This indicated that smoking did not appear to increase the amount of plaque when controlling for other factors. Besides, heat and accumulated product of combustion that result in tobacco stain as well as calculus are particular undesirable local irritants that increased with smoking [22]. Non significant difference was found between the non smokers and light smokers groups and this might be explained from the case sheet data which shows a high level of education in majority of light smoker groups

Gingival index (GI)

The results showed that the gingival index in heavy smokers group was slightly elevated compared with non-smokers and light smoker, with non significant differences between them. According to the results, it has been found that smokers had slightly elevated gingival index than non-smokers, the explanation for the result that these alterations of gingival index follow physiologic changes related to the disease process (more plaque accumulation in smokers group lead to more gingival inflammation). This disagrees with Darby *et al* [23] who showed that smokers had a decreased expression of clinical inflammation in the presence of plaque accumulation when compared with non-smokers.

Probing pocket depth (PPD) and Clinical attachment loss (CAL)

According to the results, there were increased PPD with its different scores in smokers group compared with non-smokers group. This general increase in PPD in smokers group compared with non-smokers group was in a agreement with Haffajee & Socransky, Calcina *et al* [24,25]

There were an increased in CAL with its different scales in smokers group compared with non-smokers group, and this came in agreement with Susin *et al* & Bajloon [26,27]

It was shown that deleterious effects of smoking on periodontium resulted not only from plaque amount and poor oral hygiene, but also from the effect of direct tissue destruction of smoking in homogen groups. It has been suggested that smoking may also be a risk factor for gingival recession in adults with minimal periodontal destruction ([28].

Regarding the duration of smoking, a significant association was noted between gingival other recession and duration of smoking in the present study. This finding is consistent with other observations [19].

All scores were less in light smokers compared with non smokers group may be because most of the light smokers group was found to be educated patient from the record obtained from the case sheet.

Biochemical Finding

The mean of SOD level in control group was higher than the other groups, and the mean of SOD level in light smokers group was higher than heavy smokers group.

According to the results, the mean level of salivary SOD activity was significantly lower in the smokers group than non-smokers. This finding is agreed with Reddy *et al*, Agnihotri *et al* [11,29] and in disagreement with Kanehira *et al*, Baharvand [15,30] who showed that cigarette smoke leads to an elevation in salivary superoxide dismutase activity.

The result showed elevated level of SOD in light smokers compared with heavy smokers and this agree with Agnihotri *et al* [11] who showed that mean levels of SOD in the GCF and saliva of heavy smokers were lower than those in light smokers. The reduction of the antioxidative enzyme might be due to the excessive release of oxidative free radicals caused by cigarette smoke, which consumes the enzymes and are more utilized in the cellular process [31-33].

This reduction in the levels of SOD may be related to an increased concentration of cadmium in cigarette smoke. Cadmium replaces the bivalent metals in SOD, such as zinc, copper, and manganese, resulting in its inactivation. An increased accumulation of cadmium in blood and a decrease in the levels of SOD enhance the destructive process [34].

The saturation of already present SOD by the increased concentration of free radicals in cigarette smoke is another possible mechanism for the increased destruction of the periodontium, especially in heavy smokers [35]. A dose-related reduction of salivary and gingival crevicular fluid superoxide dismutase levels was found in both light and heavy smokers compared to non-smokers [11]

Correlation of SOD levels with clinical periodontal parameters:

In this study, there is negatively non significant relation between salivary superoxide dismutase with plaque index and gingival index. There is no statistically significant correlation between pocket depth and SOD except with score one and three in heavy smoker and no statistical significant correlation between SOD and clinical attachment loss. The delicate balance between the ROS and tissue concentrations of antioxidants may be disturbed by various factors, including smoking. [36] Elevated levels of ROS stimulate the neutrophils to upregulate the adhesion integrins, leading to their increased accumulation in tissues and a local sealing off of antioxidant enzymes, such as SOD, catalase, and protease inhibitors [37], consequent to this, there is degradation and collagenolysis of ground substance or increased stimulation of excessive proinflammatory cytokines through nuclear transcription factor-kappa B activation or an increased production of prostaglandin E2 via lipid peroxidation and superoxide release; all are linked to bone resorption [38]. There was a decrease in the levels of SOD as CAL and PPD increased. These findings are in accordance with [39]

REFERENCES

1. Giannobile WV, Beilker T, Kinney JS, Ramseier CA, Morelli T, Wong DT. Saliva as a diagnostic tool for periodontal disease: current state and future directions. Periodontol 2000 2009; 50: 52-64.
2. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999; 4:1-6
3. Douglas CR. Tratado de fisiologia aplicada à saúde. São Paulo: Robe Editorial; 2002
4. Dodds MWJ, Jonson DA, Yeh CK. Health benefits of saliva: a review. J Dent 2005; 33: 223–33.
5. Miller CS, King CP, et al. Salivary biomarkers of existing periodontal disease. JADA 2006; 137(3): 322-9.
6. Streckfus C, Bigler L. The use of soluble, salivary c-erbB-2 for the detection and post-operative follow-up of breast cancer in women: the results of a five-year translational research study. Adv Dent Res 2005;18:17-24
7. American Academy of Periodontology. Tobacco use and the periodontal patient, (position paper). J Periodontol 1999; 70:1419-27.
8. Jounson NW, Bain CA, Co-authers of the EU-working group on tobacco and oral health. Tobacco and oral disease. Br Dent J 2000; 189(4): 200-6
9. Martinez-Canut P, Lorca A, Magan R. Smoking and periodontal disease severity. J Clin Periodontol 1995; 22: 743-9.
10. Haber J, Wattles J, Crowley M, et al. Evidence for cigarettes smoking as a major risk factor for periodontitis. J Periodontol 1993; 64: 16–23.
11. Agnihotri R, Panduranq P, Kamath SU, et al. Association of cigarette smoking with superoxide dismutase enzyme levels in subjects with chronic periodontitis. J Periodontol 2009; 80(4): 657-62.
12. Chapple ILC. Reactive oxygen species and antioxidants in inflammatory disease. J Clin Periodontol 1997; 24: 287-96
13. Muller FL, Lustgarten MS, Jang Y, Richardson A, Van Remmen H. Trends in oxidative aging theories Free Radic Biol Med 2007; 43(4): 477–503.
14. Baltacioglu E, Akalin FA, Alver A, Balaban F, Unsal M, Karabulut E. Total antioxidant capacity and superoxide dismutase activity levels in serum and gingival crevicular fluid in post-menopausal women with chronic periodontitis. J Clin Periodontol 2006; 33: 385-92.
15. Kanehira T, Shibata K, Kashiwazaki H, Inoue N, Morita M .Comparison of antioxidant enzymes in saliva of elderly smokers and non-smokers. Gerodontol 2006; 23: 38-42
16. Maffei G, Brouwer N, Dolman KM, Van der Velden U, Roos D, Loos BG. Plasma levels of mannan-binding lectin in relation to periodontitis and smoking. J Periodontol 2005; 76:1881-9.
17. Silness J, Loe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964; 22: 121-35.
18. Loe H, Sillness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963; 21: 533.
19. Muller HP, Stadermann S, Heinecke A. Longitudinal association between plaque and gingival bleeding in smokers and non-smokers. J Clin Periodontol2002; 29: 287-294
20. Sreedhar A, Shobha P. Cigarette smoking as a risk factor for periodontal disease severity. NJIRM 2012; 3(2): 132-5.
21. Jayashree NV, Vandana KL. Gingival thickness among smokers and non-smokers. Indian J Dent Adv 2012; 4(1): 723-9.
22. Al-Bander JM, Stekfus GF, Adesanya MR, Winn DM. Cigar, pipe and cigarette smoking as risk factors for periodontal disease and tooth loss. J Periodontol 2000; 71: 1874-81.
23. Darby I, Hodge P, Riggio M, Kinane D. Microbial comparison of smoker and non-smoker adult and early

–onset periodontitis patients by polymerase chain reaction. J Clin Periodontol 2000; 27: 417-24.
24. Haffajee A, Socransky S. Relationship of cigarette smoking to the subgingival microflora. J Clin Periodontol 2001b; 28: 377-88.
25. Calcina G, Ramon J, Echeverria J. Effects of smoking on periodontal tissue. J Clin Periodontol 2002; 29: 771-6.
26. Susin C, Dalla Vechia CF, Oppermann RV, Haugejorden O, Albandar JM. Periodontal attachment loss in an urban population of Brazilian adults: effect of demographic, behavioral, and environmental risk indicators. J Periodontol 2004; 75:1033-1041
27. Bajloon M, Natto S, Bersgtrom J. Long-term effect of smoking on vertical periodontal loss. J Clin Periodontol 2005; 32:789-797.
28. Gunsolley JC, Quinn SM, Tew J, Gooss CM, Brooks CN, Schenkein HA. The effect of smoking on individuals with minimal periodontal destruction. J Periodontol 1998; 64:165-170.
29. Reddy S, et al. Influence of cigarette smoking on blood and salivary super oxide dismutase levels among smokers and non-smokers. J Investig Clin Dent 2012; 3(4): 298-303.
30. Baharvand M, Maghami AG, Azimi S, Bastani H, Ahmadieh A, Taghibakhsh M . Comparison of superoxide dismutase activity in saliva of smokers and nonsmokers. South Med J 2010 103(5): 425-7.
31. Scully DV, Langley-Evans SC. Salivary antioxidants and periodontal disease status. Proc Nutr Soc 2002; 61(1): 137-43.
32. Srivastava DSL, Mittal RD. Free radical injury and antioxidant status in patients with benign prostate hyperplasia and prostate cancer. Indian J Clin Biochem 2005; 20:162–5.
33. Rasheed MH, Beevi SS, Geetha A. Enhanced lipid peroxidation and nitric oxide Products with deranged antioxidant status in patients with head and neck squamous cell carcinoma. Oral Oncol 2007; 43: 333–8.
34. Raju P, George R, Ve Ramesh VS, Arvind H, Bhaskaran M, Vijaya L. Influence of tobacco use on cataract development. Br J Ophthalmol 2006; 90:1374-7.
35. Sharma SB, Dwivedi S, Prabhu KM, Singh G, Kumar N, Lal MK. Coronary risk variables in young asymptomatic smokers. Indian J Med Res 2005; 122: 205-10.
36. Garg N, Singh R, Dixit J, Jain A, Tewari V. Levels of lipid peroxides and antioxidants in smokers and nonsmokers. J Periodontal Res 2006; 41:405-10.
37. Ryder MI, Fujitaki R, Johnson G, Hyun W. Alterations of neutrophil oxidative burst by in vitro smoke exposure: Implications for oral and systemic diseases. Ann Periodontol 1998; 3:76-87.
38. Alfano MC. The origin of gingival fluid. J Theor Biol 1974; 47:127-36.
39. Ellis SD, Tucci MA, Serio FG, Johnson RB. Factors for progression of periodontal diseases. J Oral Pathol Med 1998; 27:101-5.

Table 1. Statistical description of PLI &GI findings (mean, standard deviation) for three groups

Clinical parameter	G1		G2		G3	
	Mean	SD	Mean	SD	Mean	SD
PLI	0.936	±0.415	0.942	± 0.504	1.333	± 0.407
GI	0.940	± 0.355	0.956	± 0.167	1.005	±0.205

Table 2. Inter groups Comparison of means of plaque index &gingival index for all groups.

Groups	Plaque index			Gingival index		
	t-test	P-value	Sig	t-test	P-value	Sig
G1-G2	0.5	0.961	NS	0.204	0.839	NS
G1-G3	3.41	0.001	HS	-0.791-	0.433	NS
G2-G3	3.007	0.004	HS	-0.922-	0.361	NS

Table 3. Numbers and Percentages of probing pocket depth &clinical attachment loss sites for three groups

Groups	PD								CAL							
	Score0		Score2		Score3		Score4		Score1		Score2		Score3		Score4	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
G1	2486	96.65	66	2.566	15	0.583	5	0.194	165	6.415	39	1.516	7	0.272	5	0.194
G2	2434	96.52	42	1.682	15	0.6	5	0.2	82	3.285	37	1.482	11	0.44	5	0.2
G3	2431	95.55	90	3.53	17	0.668	6	0.235	332	13.05	91	3.577	13	0.511	9	0.353

Table 4. Association between severity of smoking &clinical attachment loss, probing pocket depth by using Chi-square test

Group	Chi-square		DF		p-value		Sig	
	PPD	CAL	PPD	CAL	PPD	CAL	PPD	CAL
G1	17.477	15.704	6	6	0.00	0.015	HS	S
G2								
G3								

Table 5. Statistical description (mean level in U/mL, standard deviation) of SOD for each group

Group	G1	G2	G3
Mean U/ml	52.128	45.976	37.244
SD	± 9.421	11.85±	± 15.657

Table 6. Inter group comparison of mean of SOD level by using t-test

Group	t-test	P-value	Sig.
G1-G2	2.032	0.048	S*
G1-G3	4.072	0	HS**
G2-G3	2.223	0.031	S*

Table 7. The coefficients of Pearson correlation (r) of SOD levels with clinical periodontal parameters and their level of significant differences

Groups		PLI	GI	PPD				CAL			
				Score0	Score1	Score2	Score3	Score1	Score2	Score3	Score4
G1	r	0.039	0.127	-0.187	0.101	0.265	-0.017	-0.116	-0.331	-0.258	-0.150
	P-value	0.852	0.545	0.37	0.632	0.2	0.934	0.581	0.106	0.213	0.474
	Sig	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
G2	r	-0.055	0.099	0.069	0.098	-0.008	0.082	-0.215	0.17	0.117	-0.206
	P-value	0.779	0.637	0.744	0.642	0.97	0.695	0.301	0.417	0.578	0.324
	Sig	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
G3	r	-0.267	-0.351	0.439	-0.020	-0.429	-0.047	-0.175	-0.218	-0.205	0.082
	P-value	0.196	0.085	0.028	0.926	0.032	0.824	0.403	0.296	0.326	0.696
	Sig	NS	NS	S	NS	S	NS	NS	NS	NS	NS