ORIGINALRESEARCH

REACTIVE ACTIVE METABOLITES IN CHRONIC PERIODONTITIS PATIENTS -A COMPARISON BETWEEN SMOKERS AND NON SMOKERS

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ABSTRACT

Background: Reactive oxygen species [ROS] if produced in large amounts, have destructive effects because of high reactivity. During oxidative stress there is an imbalance between the formation of free radicals and antioxidant defence mechanisms through damaging cellular macromolecules. Smoking is one of the major risk factor that affects the prevalence, degree and severity of periodontal disease.
AIM: . The purpose of the present study is to evaluate and compare the levels of ROM in GCF, saliva and plasma in smokers and non-smokers with periodontitis
MATERIALS & METHODS: 30 Participants (males) were selected and were divided into two groups Group I - Smokers with Chronic Periodontitis and Group II - Non-Smokers with Chronic Periodontitis with 15 each . GCF, Saliva and Plasma were collected from both the groups and were tested in the laboratory through the principle of Fenton's reaction.
RESULTS: One way ANOVA analysis and paired t test were used for analysis .On comparison of ROM levels in smokers and non-smokers, level of ROM was increased in smokers and it was highly significant with p value ≤ 0.05.
CONCLUSION: Free radical production mediated by cigarette smoke is highly toxic and impaired oxidant-antioxidant balance is a risk factor for periodontal disease, as the results from the present study suggested increased levels of ROM in smokers

Keywords ; Reactive Oxygen Spieces (ROS), GCF, Smokers, Periodontal Diseases.

INTRODUCTION

Reactive oxygen species [ROS] are produced as a resultof normal metabolism of oxygen and play major roles in cell signaling. If produced in large amounts, they have destructive effects because of high reactivity.
During oxidative stress which occurs when there is disequilibrium between the formation of free radicals and antioxidant defense mechanisms through damaging cellular macromolecules. The formation of oxidative stress depends on the activity of ROS and has played a role in the pathogenesis of chronic degenerative disease such as periodontal disease [1].
Smoking is one of the major risk factor that affects the prevalence, degree and severity of periodontal disease [2, 3]. Cigarette smoking induces the production of endogen oxidant and reactive species in the inflammatory response. Cigarette smoke containing volatile aldehydes, phenols,hydrocarbons,nitricoxideandsemiquinonechemicalstructureswhichcreatesasourceofox idative stress leading to formation of oxygen derived free radicals [4, 7]. Cigarette smoke has two major phases, tar and gas, consisting of a mixture of 7000 chemical compounds. Both phases are very rich in terms of ROS and reactive nitrogenspecies [5, 6].

AIM OF THE STUDY

The aim of the present studyis to evaluate and compare the levels of reactive oxygen species in GCF, saliva and plasma in smokers and non-smokers with periodontitis.

METHODOLOGY

Thirty subjects (males) were selected from the outpatient pool in the Department of Periodontics at Adhiparasakthi Dental College.The patients were divided into two groups of fifteen subjects each. Group I was comprised of smokers with chronic periodontitis, and Group II comprised of non-smokers with chronic periodontitis. The patients who were clinically and radiographically
confirmed to have chronic periodontitis were chosen. The subjects who had other systemic diseases, and who were taking long term antibiotics or other vitamin supplements were excluded from the study. All patients were briefly informed about the study and consent was obtained and ethical committee clearance was also obtained from the University.

ChronicPeriodontitis

- Visibleplaque
- Bleedingonprobing
- Atleastfourteethwithoneormoresitesexhibitingprobingdepth≥4mm
- Clinicalattachmentlevel≥ 4mm
- Chronicperiodontitiswithcurrentsmokers

ExclusionCriteria

- dH15teeth
- Presence of any systemic condition
- Subjects under antioxidants (vitamin C, vitamin E, & coenzyme Q), antibiotics, oranti-inflammatory drugs in the past 3 months.

Collection of GCF

Detailed case history, clinical examination and supragingival scaling were done one day before the collection of GCF. On the subsequent day after drying the area with a blast of air, supragingival plaque was removed without touching the marginal gingiva and the GCF was collected. A standardized volume of 1µl was collected from each site with an extracrevicular approach, using volumetric capillarypipettes that were caliberated from 1-5 µl. The collected GCF was transferred immediately to ependroff tubes and stored at -70° C until the time of assay.

CollectionofPlasma

Blood (3 ml) was drawn from antecubital vein, under aseptic precautations and collected in a colour coded herparinised testtube (greencolour) and centrifuged immediately at 3000 xg for 5 minutes. If not immediately assayed plasma aliquots were stored at -80° C until the analysis.

Collection of Saliva:Draining/SpittingMethod

The subject is asked to accumulate saliva in the floor of mouth and then spit into a pre-weighed or graduated test tube.

LaboratoryMethodforDetectionofReactiveOxygenMetabolites(ROM)

The d-ROM test, developed by world renowned Italian biochemist is a photometric test for measurement of the concentration of hydroperoxides (ROOH) in biological samples. The presence of ROOH in cells indicates oxidative attack of ROS on various organic substrates such as carbohydrates, lipids, amino acids, proteins, or nucleotides.

TestPrinciple

The d-ROM test uses the principle of Fenton’s reaction: by mixing a biological sample with an acidic buffer (Reagent R1), the newly created transition metal ion (iron or copper) catalyzes the breakdown of hydroperoxide, generating new radical species such as hydroxyperroxyl (ROO+) and alkoxyl (RO+). By adding a chromogen (N, N-diethyl-paraphenylen-diamine, Reagent R2) having the ability to donate an electron and changecolour when oxidized by free radicals , and using photometric reading available with the FRAS 4 dedicated analytical equipment, it becomes possible to quantify the level of hydroperoxides available in the sample.

Table1.ComparisonofROMlevelsinsmokersandnon-smokers (using Oneway ANOVA)

		N	Mean	Std.deviation	F value	P value
Non smoker	PLASMA	10	285.50	58.74663		
	SALIVA	10	253.30	32.90745	3.696	0.038*
	GCF	10	237.60	17.50682		
Smoker	PLASMA	10	490.80	65.79564		
	SALIVA	10	439.80	67.72789	11.532	<0.001*
	GCF	10	357.60	53.23783		

* P <0.05 isconsidered significant.

Table2.ComparisonofROMlevelsinGCF,saliva,andplasmainsmokers and non-smokers with chronic periodontitis

	Group	N	Mean	Std.deviation	Mean difference	T value	P value
Plasma	Smoker	10	490.80	65.79564	205.3	7.360	<0.001*
	Non smoker	10	285.50	58.74663			
Saliva	Smoker	10	439.80	67.72789	186.5	7.832	<0.001*
	Non smoker	10	253.30	32.90745			
GCF	Smoker	10	357.60	53.23783	120.0	6.771	<0.001*
	Non smoker	10	237.60	17.50682			

P <0.05 isconsidered significant

StatisticalAnalyses

One way ANOVA analysis and paired t test were used for analysis. Statisticalsignificance level were considered at $P \leq 0.05$.

RESULTS

ROM level was analysed in saliva, GCF and plasma in Smokers andNon-Smokers. Level of ROM was more in plasma when compared to GCF and saliva. While comparing the levels in smokers and non-smokers, level of ROM was increased in smokers and it was highly significant. The results were depicted in Table 1 and Table 2.

DISCUSSION

There is an abundance of literature supporting the relationship between smoking and periodontal diseases. A number of mechanisms by which smoking exerts its deleteriouseffects on the periodontium has been suggested. The induction of oxidative stress

in the body by nicotine and the subsequent depletion of antioxidants may be one of the mechanisms for the tissue damage.

The ROS and their corresponding antioxidants may be identified in the GCF, saliva and plasma[1]. In the present study, ROS was increased in plasma when compared to saliva and GCF in smokers. This could be due to the involvement of other systemic factors, whereas GCF is more site specific.

ROS are generated as a physiologic process, such as mitochondrial oxidation, oxygen transportation by haemoglobins and cytochrome P450 activity. The delicate balance between the ROS and tissue concentrations of antioxidants may be, by various factors, including smoking [7]. Elevated level of ROS stimulate the neutrophils to upregulate the adhesion integrins, leading to their increased accumulation in tissues and local sealing of antioxidant enzymes [8]. There is degradation and collagenolysis of ground substances (or) increased stimulation of excessive proinflammatory cytokines through nuclear transcription factorkappa B activation or an increased production of prostaglandin E2 through lipid peroxidation and superoxide release all are linked to bone resorption [9]. Smoking may also result in the reduction of antioxidant enzymes like Superoxide Dismutase (SOD) enzyme due to inactivation by increased production of hydrogen peroxide [10].

In the present study, the oxidative stress induced by smoking was reflected by increased levels of ROM in all the body fluids [Saliva, GCF & Plasma]. Similar results were reflectedin earlier studies.

A comparison of controls and smokers with periodontitis showed a substantial increaseof ROM levels in the GCF, saliva and plasma. In the present study, only heavy smokers were considered and comparisons were not done between mild and moderate smokers. This could be the reason for the highly significant value. This could also be one of the reason in worsening the already existing periodontal disease [4].

Increase in ROM level could be due to a reduction in SOD level, which could be due to the increased concentration of Cadmiumin smokers.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 levels of SOD enhance the destructive process [11].

In the present study, female smokers were not evaluated because of their low prevalence in India (≤4%)[12, 13].Anotherlimitation of the studywas thatsmoking status wasbased on self-report by the subjects. It has been suggested that the estimation of serum cotinine assays is more reliable for evaluation of smoking status. Therefore, further studies incorporating larger sample size, including female patients, coupled with estimation of serum cotinine assays is warranted.

CONCLUSION

The results of the present study suggest that cigarette smoking is associated with a significantincrease in the levels ofROM. Free radialproduction mediated bycigarette smoke is highly toxic and impaired oxidant-antioxidant balance is a risk factor for periodontal disease. A reduction in smoking exposure might be helpful in improving the antioxidant levels. Further studies must be undertaken in large sample size in various type of smokers which would overcome the limitations of the present study.

ETHICALCOMPLIANCE

The authors have stated all possible conflicts of interest within this work. The authors have stated all sources of funding for this work. If this work involved human participants, informed consent was received from each individual. If this work involved human participants, it was conducted in accordance with the 1964 Declaration of Helsinki. If this work involved experiments with humans or animals, it was conducted in accordance with the related institutions' research ethics guidelines.

REFERENCES

[1] Chapple IL. Reactive oxgen species and antioxidants in inflammatory diseases. *J. Clin. Periodontol*1997; 24:287-296.
[2] Burt B. Research, science and therapy committee of the American Academy of Periodontology. Position paper: Epidemiology of Periodontal diseases. *J. Periodontal* 2005;76:1406-1419.
[3] Luzzi LI, Greghi SL, Passanezi E, Saantlana AC. Evaluation of clinical periodontal conditions in smokers and non-smokers. *J. Appl. Oral Sci* 2007;15:512-517.
[4] Palmer RM, Wilson RF, Hasan AS, Scott DA. Mechanisms of action of environmental factors-Tobacco smoking. *J. Clin Periodontal* 2005; 32(supple 6):180-195.
[5] Lux, Cai J, Kong H et al. Analysis of cigarette smoke. Condensate by comprehensive twodimensionalgaschromatography/timeofflightMassspectrometryIacidicfraction. *Anal Chem* 2003;75:4441-4451.
[6] Pryor WA, Stone K. Oxidants in cigarette smoke Radicals, hydrogen peroxide, peroxynitrate and peroxynitrite. *Ann NV Acad Sci* 1993;686:12-27. Discussion 27-28.
[7] Garg N, Singh R, Dixit J, Jain A, Tewari V. Levels of lipid peroxides and antioxidants in smokers and non-smokers. *J. Periodontal Res* 2006; 41:405-410.
[8] 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. Periodontal* 1978;3:76-87.
[9] AlfanoMc.Theoriginofgingivalfluid.*J.TheorBiol*1974;47:127-136.
[10] Bray RC, Cockle SA, Fielden EM, Roberts PB, Rotelio G, Calabrese L. Reduction and inactivation of superoxide dismutase by H2O2. *Biochem J,* 1974;139:43-48.
[11] Raju P, George R, Ramesh VS, Arvind H. Influence of tobacco use on cataract development. *Br. J Ophthalmol*2006;90:1374-1371.
[12] Roni. M, Bonu S, Jha P, Nguyen SN. Tobaccon use in India: Prevalence and Predictors of smoking and chewing in a national cross sectional household survey. *Tab. Control* 2003;12:51-60.
[13] Jindal SR, Aggarwal AN, Chaudhry et al. Asthma epidemiology study group. Tobacco smoking in India: Prevalence, Quit. rates and respiratory morbidity. *Indian J Chest Dis Allied Scie*. 2006;4