Original Article

Salivary antioxidant enzymes and lipid peroxidation product malondialdehyde and sialic acid levels among smokers and non-smokers with chronic periodontitis—A clinico-biochemical study

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Abstract

Background: Pathogenesis of most of the inflammatory process are associated with reactive oxygen species (ROS), derived from various metabolic sources and which may lead to direct or indirect tissue damage due to oxidative stress, resulting in periodontal diseases. Usually antioxidant systems are capable of removing free radicals, thereby preventing tissue damage from free radical. ROS can result in tissue damage, involving lipid peroxidation. The aim of this study was to evaluate and compare the levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), and sialic acid (SA) in periodontally healthy and chronic periodontitis among nonsmokers and smokers and to determine their value as diagnostic markers for chronic periodontitis. **Materials and Methods:** A total of 90 male patients aged 20--60 years were recruited and grouped as Group 1: 30 Healthy nonsmokers, who had never smoked. Group 2: 30 nonsmokers with chronic periodontitis. Group 3: 30 smokers with chronic periodontitis. Unstimulated saliva was collected for at least 5 min and clinical measurements; SOD, GPx, MDA and SA were assessed using a spectrophotometric method. **Results:** Data showed a significant correlation between salivary SOD, GPx, MDA, and SA in group 1, group 2, and group 3. SOD and GPx were found to be lower and MDA and SA levels were found to be higher among smokers with chronic periodontitis. **Conclusion:** Reduced levels of antioxidant enzymes SOD and GPx and elevated levels of lipid peroxidation product MDA as well as increased levels of SA could be used as diagnostic markers to measure oxidative stress in periodontal disease associated with risk factor such as smoking.

Keywords: Antioxidants, biomarkers, chronic periodontitis, saliva, smoking

Introduction

Chronic periodontitis is an infectious disease characterized by inflammation of the surrounding tissues of teeth and enlightened by destruction of the alveolar bone and connective tissues.[1] The etiology of periodontal disease is multifactorial in nature

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Received: 04-06-2019 **Revised:** 06-06-2019 **Accepted:** 12-07-2019

Access this article online

Quick Response Code:

Website: www.jfmpc.com

DOI: 10.4103/jfmpc.jfmpc_438_19

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How to cite this article: Naresh KC, Subramaniam MR, Prashanth RS, Ranganath V, Abhilasha SP, Anu AJ. Salivary antioxidant enzymes and lipid peroxidation product malondialdehyde and sialic acid levels among smokers and non-smokers with chronic periodontitis—A clinico-biochemical study. J Family Med Prim Care 2019;8:2960-4.

and a variety of risk factors are associated with periodontitis. Among the risk factors associated, smoking is one of the most predominant. It has been reported that cigarette smokers are associated with 3--6 folds of prevalence of periodontitis when compared with nonsmokers.[2] Thus, cigarette smoking is profusely the most responsible environmental factor for high prevalence of periodontal disease in the population.[3] Reactive oxygen species (ROS) are toxic substances that attack and damage biologic molecules.[4] Under physiological conditions, ROS are effectively neutralized by antioxidants, which prevent ROS-mediated tissue damage. In general, there is an equilibrium between ROS and antioxidants that may be disturbed by a multiplicity of factors, one of which being smoking. This dysregulation may bring about damage to cells either by lipid peroxidation (LPO), protein inactivation, and initiation of DNA damage.[5] Polymorphonuclear leukocytes are a major source of ROS which are essential for bacterial killing and may induce oxidative stress in periodontitis.[4,6] Smoking results in altered neutrophil function, reduction of blood flow, imprecise cytokine and growth factor production, inhibition of fibroblast growth, and reduced collagen production and vascularity and promotes oxidative burst in neutrophils.[7] Body's reserve of antioxidants (AOs) to clear the excess free radicals in smokers results in altered levels of AO. Periodontal disease is associated with a reduced total AO capacity and increased oxidative injury within the oral cavity.[8] Moreover, lipid peroxidation is seemingly high in patients with periodontitis.[4] The principal radical in the tar phase of cigarette smoke is a quinine--hydroquinone complicated which will cut back molecular oxygen to superoxide radicals. Polymorphonuclear leukocytes and macrophages initiate superoxide as an antibacterial agent in case of a bacterial challenge to the periodontium.[2] Superoxide is detached from tissues by dismutation to hydrogen peroxide spontaneously or catalyzed by superoxide dismutase (SOD). The hydrogen peroxide formed is removed by catalase in intracellular environment or by glutathione peroxidase (GPx) in extracellular environment. Increased GPx activity in the saliva, GCF, plasma, erythrocytes, and gingival tissues of the patients with periodontitis has also been reported.[4] Lipid peroxidation is one of the most substantial reactions of free radicals. Tissue destruction by oxidative stress can be determined by the final end products of lipid peroxidation, such as malondialdehyde (MDA).[2] Studies have suggested that increased MDA and GPx levels correlate with the presence of periodontal disease, and both parameters are oxidative stress markers in patients with periodontal disease.[9,10] Sialic acid (SA) is the common name for compounds of N-acetylated derivatives of neuraminic acid, which are primarily required for stabilizing the conformation of glycoproteins (GPs) and cellular membranes, help in cell--cell interaction and recognition and acting as chemical messengers in tissue and body fluids. Sialic acid in glycoproteins is an important component to scavenge hydroxyl free radical. SA is also an important constituent of salivary IgA and certain acute phase proteins.[11] This study was undertaken to ascertain the levels of salivary antioxidants such as SOD and GPx, lipid peroxidation product MDA and SA among nonsmokers and smokers with chronic periodontitis as diagnostic biomarkers.

Materials and Methods

A total of 90 male patients aged 20--60 years were selected from Outpatient Department of Periodontology, AECS Maaruti College of Dental Sciences and Research Centre, Bangalore, Karnataka, India. The study design was approved by the Institutional Ethical Committee and all the subjects were explained about the study and based on their approval, were asked to read carefully and sign the informed consent form. Patients who had received periodontal therapy or used antibiotics, anti-inflammatory agents, regular mouth wash, vitamin supplements, and any special dietary supplements in the last 3 months. Patients with systemic diseases like diabetes mellitus, cancer, coronary heart disease, hepatitis, cardiovascular disease, HIV infection, and epilepsy were excluded from the study. The subjects were grouped as follows: Group 1 consisted of 30 healthy with no history of periodontal disease nor tooth loss caused by periodontitis and had no clinical signs of periodontitis (clinical attachment level [CAL] ≤1 mm, probing pocket depth [PPD] ≤3 mm, and gingival index [GI] ≤1). Group 2 consisted 30 nonsmokers, who had never smoked, with chronic periodontitis having more than 20 residual teeth, having more than one teeth with sites of pocket probing depth (PPD) ≥4 mm and CAL ≥4 mm in all four quadrants.[12] Group 3 consisted of 30 smokers with chronic periodontitis who claimed to have smoked at least 10 cigarettes per day for the past 5 years at the minimum.[13]

Clinical parameters

Periodontal parameters such as plaque index (PI),[14] gingival index (GI),[15] PPD, and CAL were measured at six sites and recorded on each tooth, except third molars. All clinical periodontal measurements were performed using William's graduated periodontal probe by a single examiner.

Saliva sample collection and processing

The participants were instructed not to smoke 1 h prior and refrain from eat or drink 2 h prior to saliva collection. Samples were obtained in a quiet room between 9 am and 12 pm to prevent any variation which may be attributable to circadian rhythm. Participants were instructed to rinse their mouth using distilled water and unstimulated whole saliva was collected for at least 5--10 min in a 15 ml sterile polypropylene tubes. Samples were centrifuged to remove cells and debris for 5 min at 2,000--2,500 rpm. Supernatant obtained was stored at 4°C for the analysis of SOD and GPx and for analysis of MDA and SA supernatant was stored −20°C frozen till further analysis. Salivary samples of SOD, GPx, MDA, and SA concentrations were measured spectrophotometrically. SOD and GPx were estimated using a photometric RX Daytona plus (Randox Laboratories Ltd® Crumlin, United Kingdom) in a fully automated analyzer. MDA and SA (BioVisions laboratories kit® California, United States of America) was estimated using a spectrophotometer in a semiauto analyzer after adjusting a wavelength of 532 nm for MDA and at 570 nm wavelength for SA, respectively, and values were recorded.

Statistical analysis

Results on continuous measurements are presented on Mean ± SD (Min--Max) and results on categorical measurements are presented as number (*n*) and percentage (%). Statistical significance is assessed at $P < 0.05$. Kruskal--Wallis one-way analysis of variance (ANOVA) has been used to find the significance of study parameters in comparison of three groups of subjects. Multiple comparisons of each parameter were carried out between the groups by using Mann--Whitney U test. The correlations between PI, GI, PPD, CAL, and other parameters were analyzed using the Spearman's rank correlation method. SPSS 21.0 was used for data processing and analysis.

Results

The mean levels of SOD and GPx in the saliva of smokers and nonsmokers with chronic periodontitis were decreased compared with the healthy group ($P < 0.05$). The mean levels of MDA and SA in saliva of smokers and nonsmokers were increased compared with the control group ($P < 0.05$). PI, GI, PPD, and CAL were significantly higher in smokers with chronic periodontitis when compared with nonsmokers with chronic periodontitis and healthy subjects ($P < 0.05$). There was a statistical significant difference between all pair wise comparisons except for GI, PPD, and CAL between group 2 and 3 [Table 1]. Table 2 shows the correlation between PI with salivary antioxidants among different groups by Spearman's rank method. There was a negative correlation between PI and all salivary antioxidants among smokers with periodontitis (group 3) and exhibited a positive correlation among nonsmokers (group 2) except for SOD levels. Table 3 showed a positive correlation between GI and all salivary antioxidants except for SOD levels in group 1, 2, and 3. In Table 4, all correlations between PPD and salivary antioxidants were positive except for MDA in group 1, SOD in group 2, and GPx in group 3. Table 5 shows the correlation between CAL and salivary antioxidants in all three groups. There was a negative correlation between CAL and GPx in group 1, MDA in group 2 and SOD, GPx in group 3 ($P < 0.05$).

Discussion

Periodontitis is a multifactorial inflammatory disease affecting the supporting structures of the dentition. The severity of periodontitis can be modified by a variety of factors, the most important of which is smoking. Cigarette consumption and duration of smoking is related with the severity of periodontal disease. Smoking influences oxidative stress in the body and causes an imbalance between antioxidants and ROS.[6] Our study found lower levels of SOD in smokers with chronic periodontitis. This finding is in agreement with previous studies by Ali *et al.*,[16] Reddy *et al.*,[17] and Agnihotri *et al.*[6] The reduction of this antioxidative enzyme might be due to the excessive release of oxidative free radicals produced because of cigarette smoke, which consumes the enzymes and is utilized in cellular process. This reduction in the levels of SOD may also be related to an increased concentration of cadmium in cigarette smoke.

Table 1: Comparison of three groups (group 1, group 2, and group 3) Kruskal-Wallis ANOVA Followed by Mann-Whitney

	Mean±Std Dev			*P*
	Group 1 (*n*=30)	Group 2 (*n*=30)	Group 3 (*n*=30)	
Superoxide dismutase (U/ml)	67.13±6.64	50.41±4.25	34.96±4.8	0.00001*
Glutathione peroxidase (U/l)	147.57±5.54	124.41±4.74	111.39±6.79	0.00001*
Malondialdehyde (nmol/ml)	0.22±0.05	0.47±0.11	0.69±0.13	0.00001*
Sialic acid (nmol/μl)	0.07±0.02	0.14±0.02	0.22±0.04	0.00001*
Plaque index	0.25±0.09	1.17±0.18	1.55±0.21	0.00001*
Gingival index	0.21±0.08	0.93±0.17	1.00±0.39	0.00001*
Pocket probing depth (mm)	1.90±0.12	3.29±0.33	3.73±0.35	0.00001*
Clinical attachment level (mm)	1.90±0.12	3.68±0.37	3.88±0.39	0.00001*

Pair wise comparison of three groups by Mann-Whitney *U* test

	Superoxide dismutase *P*	Glutathione peroxidase *P*	Malondialdehyde *P*	Sialic acid *P*	PI *P*	GI *P*	PPD *P*	CAL *P*
Group 1 vs Group 2	0.00001*	0.00001*	0.00001*	0.00001*	0.00001*	0.00001*	0.00001*	0.00001*
Group 1 vs Group 3	0.00001*	0.00001*	0.00001*	0.00001*	0.00001*	0.00001*	0.00001*	0.00001*
Group 2 vs Group 3	0.00001*	0.00001*	0.00001*	0.00001*	0.00001*	0.3478	0.6709	0.7945

*$P<0.05$ denotes a significant factor

Table 2: Correlation between Plaque index (PI) scores with other parameters by Spearman's rank correlation method

Variables	Group 1		Group 2		Group 3	
	r	*P*-level	*r*	*P*-level	*r*	*P*-level
Superoxide Dismutase (U/ml)	-0.4301	0.0577	-0.1191	0.5309	-0.0597	0.05*
Glutathione Peroxide (U/l)	-0.0235	0.9020	0.0383	0.8408	-0.1092	0.05*
Malondialdehyde (nmol/ml)	0.1211	0.5237	0.0605	0.7506	-0.0304	0.8732
Sialic acid (nmol/μl)	0.0169	0.9294	0.2365	0.0483*	-0.0219	0.9087

*$P<0.05$ denotes a significant factor, *r*=coefficient

Table 3: Correlation between Gingival index (GI) scores with other parameters by Spearman's rank correlation method

Variables	Group 1		Group 2		Group 3	
	r	*P*-level	*r*	*P*-level	*r*	*P*-level
Superoxide Dismutase (U/ml)	-0.4484	0.0129*	-0.0191	0.9200	0.2812	0.1322
Glutathione Peroxide (U/l)	0.1029	0.5886	0.1422	0.4536	-0.3331	0.05*
Malondialdehyde (nmol/ml)	0.0476	0.8026	0.1374	0.4690	0.4773	0.0077*
Sialic acid (nmol/μl)	0.1097	0.5639	0.0130	0.9454	0.0070	0.9706

*$P<0.05$ denotes a significant factor. *r*=coefficient

Table 4: Correlation between Probing pocket depth (PPD) scores with other parameters by Spearman's rank correlation method

Variables	Group 1		Group 2		Group 3	
	r	*P*-level	*r*	*P*-level	*r*	*P*-level
Superoxide Dismutase (U/ml)	0.2117	0.2615	-0.1623	0.0391*	0.1998	0.2897
Glutathione Peroxide (U/l)	0.0446	0.8149	0.0262	0.8909	-0.2345	0.021*
Malondialdehyde (nmol/ml)	-0.0591	0.7563	0.1112	0.5586	0.1392	0.043*
Sialic acid (nmol/μl)	0.1412	0.7548	0.2134	0.2576	0.1048	0.05*

*$P<0.05$ denotes a significant factor. *r*=coefficient

Table 5: Correlation between Clinical attachment level (CAL) scores with other parameters by Spearman's rank correlation method

Variables	Group 1		Group 2		Group 3	
	r	*P*-level	*r*	*P*-level	*r*	*P*-level
Superoxide Dismutase (U/ml)	0.0870	0.6476	0.0205	0.9145	−0.2070	0.0225*
Glutathione Peroxide (U/l)	−0.1064	0.0475*	0.1409	0.4576	−0.2410	0.0195*
Malondialdehyde (nmol/ml)	−0.1562	0.4098	0.1549	0.0433*	0.1535	0.4617
Sialic acid (nmol/μl)	0.0098	0.9589	0.1647	0.3845	0.0999	0.05*

*$P<0.05$ denotes a significant factor. *r*=coefficient

Cadmium substitutes the bivalent metals in SOD, such as zinc, copper, and manganese, resulting in its inactivation. Increased accumulation of cadmium in blood and a decrease in the levels of SOD enhance the destructive process.[16] Mean GPx levels were lowest in smokers with chronic periodontitis. Our findings are in agreement with Greabu *et al.*,[18] Zappacosta *et al.*,[19] and Kanehira *et al.*[20] In a study by Zappacosta *et al.*,[19] results demonstrate that even one cigarette decreases the concentration of GPx in saliva, which, however, returns to the presmoking value after 1 or 2 h. Our results indicate that exposure to cigarette smoke can cause a statistically significant decrease in salivary GPx. Lipid peroxidation is one of the most significant reactions of free radicals. Tissue destruction by oxidative stress can be measured by the final end products of lipid peroxidation, such as MDA, one of many aldehydes produced during lipid peroxidation. It has been shown that increased MDA levels correlate with the presence of periodontal disease.[2] In the present study, mean MDA levels were highest in smokers with chronic periodontitis when compared with chronic periodontitis with nonsmoker and healthy subjects, respectively. This is in agreement with Garg *et al.*[3] who reported higher MDA levels in periodontitis patients especially in smokers. MDA as a biomarker is the principal and most studied product of lipid peroxidation, signifying that levels of oxidative stress are higher in pathological conditions than in healthy. Tsai *et al.*[9] also reported that lipid peroxidation in GCF and saliva was higher in diseased sites than in healthy sites and concluded that an imbalance exist between antioxidant and oxidative stress in periodontitis, ensuing an increased tissue damage by ROS. MDA levels measured were higher in chronic periodontitis nonsmokers group compared with healthy subjects. Thus, smoking may increase the effect of ROS in periodontitis, thereby increasing the tissue destruction resulting from oxidative stress.[21,22] In a recent study, Kurku *et al.*[23] have also demonstrated increased salivary MDA, decreased SOD, and decreased GPx levels in smokers as compared with periodontally healthy subjects. In the present study, mean SA levels were highest in smokers with chronic periodontitis. This is in agreement with AlSada[24] who suggested the salivary SA in periodontitis were significantly higher compared with normal levels in healthy. This elevation in SA may be due to the bacterial infection in case of periodontal disease. Kurtul *et al.*[11] have demonstrated that salivary total sialic acid (TSA) and MDA levels were significantly increased in smokers and maras powder users (MPU). Rathod *et al.*[25] indicated that serum and salivary TSA levels were higher in chronic periodontitis subjects compared with gingivitis and healthy subjects. Increased salivary TSA levels in smokers might be associated with several diseases, for example, cancer and cardiovascular diseases (CVD) which are also often associated with smoking.[11] Despite of epidemiological evidence between cigarette smoking with periodontal disease is overwhelming, the specific components of cigarette smoke responsible for this relationship, and the mechanisms by which they exert their effects

has not yet been clearly elucidated.[3] One of the limitation of our study was that we could not evaluate the female population because of their low prevalence rate of smoking habits. However, further studies incorporating a larger sample size, and longitudinal studies including female smokers for the estimation of these markers in chronic periodontitis are merited.

Conclusion

The present investigation revealed significantly reduced levels of antioxidant enzymes like SOD, GPx in smokers with chronic periodontitis as compared with healthy subjects and increased levels of lipid peroxidation product MDA and SA in smokers with chronic periodontitis. The induction of oxidative stress in the body by smoking and the subsequent depletion of antioxidants may be one of the mechanisms for the tissue damage. The delicate equilibrium between the ROS and antioxidants may be unbalanced by various factors, which include smoking. Levels of antioxidant enzymes, as well as lipid peroxidation product (MDA) and SA can be used as diagnostic markers to measure oxidative stress in periodontal disease associated with risk factor such as smoking.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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