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Salivary Lipid Peroxidation and Antioxidant Status in Nigerian Cigarette Smokers with or Without Periodontitis

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To provide information on the susceptibility of cigarette smokers to oral diseases. This was achieved by assessing the degree of salivary oxidative stress markers in smokers with or without periodontitis. We measured salivary concentrations of malondialdehyde (MDA) and hydrogen peroxide (H2O2), myeloperoxidase (MPx) activity, enzymatic antioxidants (superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and glutathione peroxidase (GPx activities) and reduced glutathione (GSH) concentration.
Materials and Methods: About 5 ml of unstimulated saliva was collected into plain bottles from 25

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newly diagnosed subjects with periodontitis, 24 smokers without periodontitis, 20 smokers with periodontitis compared with 21 sex/age-matched apparently healthy subjects who are non-smokers and without periodontitis. The samples were collected between 09:00 hours and 11:00 hours at least 1hour after eating or washing of mouth and concentrations of salivary MDA, H2O2 and GSH and salivary MPx, SOD, CAT, GST and GPx activites were determined spectrophotometrically.

Results: Reduced salivary MDA concentration and elevated CAT activity were observed in non-smokers with periodontitis compared with non-smokers without periodontitis. Salivary CAT activity was higher in smokers without periodontitis compared with non-smokers with periodontitis. In contrast, salivary CAT activity was reduced in smokers with periodontitis compared with smokers without periodontitis. Salivary GSH was significantly reduced in smokers with periodontitis compared with non-smokers without periodontitis.

Conclusion: Reduced CAT activity may explain susceptibility of cigarette smokers to oral diseases or progression of periodontitis.

Clinical Significance: Cigarette smoking contributes to the progression of periodontitis and oral diseases.

Keywords: Antioxidant enzymes; oxidative stress; oral diseases; cigarette.

1. INTRODUCTION

Lipid peroxidation has been shown to cause a profound alteration in the structural integrity and functions of cell membranes. Free radical-induced lipid peroxidation has been implicated in the pathogenesis of several pathological disorders, including cancer [1]. Moreover, it has been well established that over-production of reactive oxygen species (ROS) occurs at sites of chronic inflammation [2].

Saliva is a complex secretion whose components are involved in health and disease [3], possesses lubricant properties [4], soft-tissue repair and many antibacterial activities [5]. Furthermore, saliva contains various antioxidants which contribute almost 70% of the total radical-trapping antioxidant capacity [6]. The first contact with products of cigarette smoke is mouth. Cigarette smoking is reportedly associated with deleterious effects on oral tissues ranging from increase in periodontal disease and dental caries to oral carcinoma [7]. Many studies have reported that tobacco smoking reduces body's antioxidant content which induces tissue damage [8,9].

Cigarette smoking causes endogenous formation of oxidants, which affect the inflammatory-immune system [10]. Periodontitis is an inflammatory condition representing the response of the periodontal tissues to lipopolysaccharide derived from Gram-negative anaerobic bacteria [11]. The persistence of the bacterial stimulus results in the inflammatory process becoming chronic in nature. However, this is not associated with progressive damage to the periodontal tissues [12].

These previous studies did not consider the contribution of cigarette smoking in the progression of periodontitis. Also, most of the previous studies concentrated on serum oxidants and antioxidants neglecting the oral part of the body specifically affected by effects of cigarette smoking and periodontitis. This study assessed the degree of oxidative stress in cigarette smokers with or without periodontitis. Analysis of lipid peroxidation status (as the concentration of MDA) and antioxidant defense systems (as the concentrations of GSH and activities of GPx, GST, CAT, and SOD). The aim is to provide information for the susceptibility of smokers to periodontitis.

2. MATERIALS AND METHODS

2.1 Subjects

Ninety subjects, divided into four groups, were recruited for this study after obtaining informed consent from each subject and an ethical approval from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee. Group I- NS+P: consisted of 25 newly diagnosed subjects with periodontitis. Group II- S-P: 24 smokers without periodontitis. Group III- S+P: 20 smokers with periodontitis. Group IV- NS-P: 21 sex and age-matched apparently healthy subjects without periodontitis and are non-smokers. Periodontitis was diagnosed by a dentist using periodontal probing

radiographs (periodontal probing radiographs usually reveal the extent of bone loss between the roots of the tooth and the bony support for the tooth [the spongy bone] which may have receded due to infection).

Those excluded from the study were individuals with pregnancy, diabetes and human immunodeficiency virus (HIV) infection. Subjects with other forms of oral disease were also excluded.

A short-structured questionnaire was administered on each subject to obtain information on age, sex, occupation, cigarette smoking and drug consumption.

2.2 Samples

About 5ml of unstimulated saliva was collected from each subject into plain bottles. The samples were collected between 09:00 hours and 11:00 hours at least 1 hour after eating or washing of mouth. The samples were centrifuged at 3000 g for 5 minutes and the clear supernatant was gently pipetted out into another clean plain bottle and stored at -20°C until analyzed.

2.3 Malondialdehyde, Glutathione, Hydrogen Peroxide and Myeloperoxidase Assays

MDA concentrations were assayed for the products of lipid peroxidation by measuring thiobarbituric acid reactive substance (TBARS) formation as described by Varshney and Kale [13]. LPO (lipid peroxidation) was expressed in terms of MDA equivalents using an extinction coefficient of 1.56 x $10^5 M^{-1}$ and results were expressed as units MDA/g tissue. GSH concentration was assayed by measuring the rate of formation of chromophoric product in a reaction between 5', 5'-dithiobis-(2-nitrobenzoate) and free sulphydryl groups (such as reduced glutathione) at 412 nm as described by Jollow et al. [14] Hydrogen peroxide generated was determined based on Wolff's method [15]. Myeloperoxidase (MPx) activity was monitored spectrophotometrically at 470 nm through the oxidation of guaiacol to oxidized guaiacol by H_2O_2 according to the method of Desser et al. [16]. Total protein concentration was estimated by Biuret method using Bovine Serum Albumin (BSA) as standard [17].

2.4 Salivary Antioxidant Enzyme Assay

Superoxide dismutase (SOD) activity was measured by the nitro-blue tetrazolium reduction method of McCord and Fridovich [18]. It was based on the ability of SOD to inhibit the spontaneous oxidation of adrenaline to adrenochrome. Catalase activity (CAT) was assessed according to the method of Sinha [19] and the principle was based on the ability of CAT to induce the disappearance of H_2O_2, which was followed spectrophotometrically. Glutathione S-transferase (GST) activity was determined according to the method of Habig et al. [20]. The method is based on the rate of conjugate formation between reduced glutathione and 1-chloro-2,4-dinitrobenzene while glutathione peroxidase (GPx) activity was determined according to the method of Rotruck et al. [21] which involves the monitoring of glutathione utilization spectrophotometrically by H_2O_2 at 412 nm.

2.5 Statistical Analysis

The data were presented as mean and standard deviation. Student's t-test (unpaired) was used to determine significant difference between the means. Values of $p \leq 0.05$ were regarded as statistically significant.

3. RESULTS

As shown in table 1, the concentration of GSH in smokers with periodontitis was significantly lower compared with non-smokers without periodontitis ($p<0.05$). In Table 1, Fig. 2 and 4, the concentration of H_2O_2 and activities of GST, GPx, MPx, and SOD were not significantly different when all groups were compared. In Fig. 1, MDA concentrations in non-smokers with periodontitis was significantly lower compared with non-smokers without periodontitis ($p<0.05$). Also, MDA concentration in smokers with periodontitis was significantly higher compared with non-smokers with periodontitis ($p<0.05$) or smokers without periodontitis ($p<0.05$). In Fig. 3, CAT activity in non-smokers with periodontitis was significantly higher compared with non-smokers without periodontitis ($p<0.05$). A significant increase in CAT activity was observed in smokers without periodontitis compared with non-smokers with periodontitis ($p<0.05$) while a significant reduction in CAT activity was observed in smokers with periodontitis compared with smokers without periodontitis ($p<0.05$).

Table 1. Showing the mean GSH concentration, GST, GPX and MPX activities in the various groups

	MEAN GSH (µg/ml)	MEAN GST (µmol/min/mg protein)	MEAN GPX (GSH consumed/ mg protein)	MEAN MPX (units/mg protein)
NS-P	127±51	4.1±2.2	29.7±13	2.1±1.3
NS+P	117±53	3.7±2.0	26.8±11	1.7±1.4
S-P	112±52	3.86±2.1	30.8±17	2.1±1.6
S+P	87±28[a]	3.7±1.8	30.6±13	2.2±1.3

NS-P: Non-smokers without periodontitis,
NS+P: Non-smokers with periodontitis,
S-P: Smokers without periodontitis,
S+P: Smokers with periodontitis
a- significantly different from NS-P

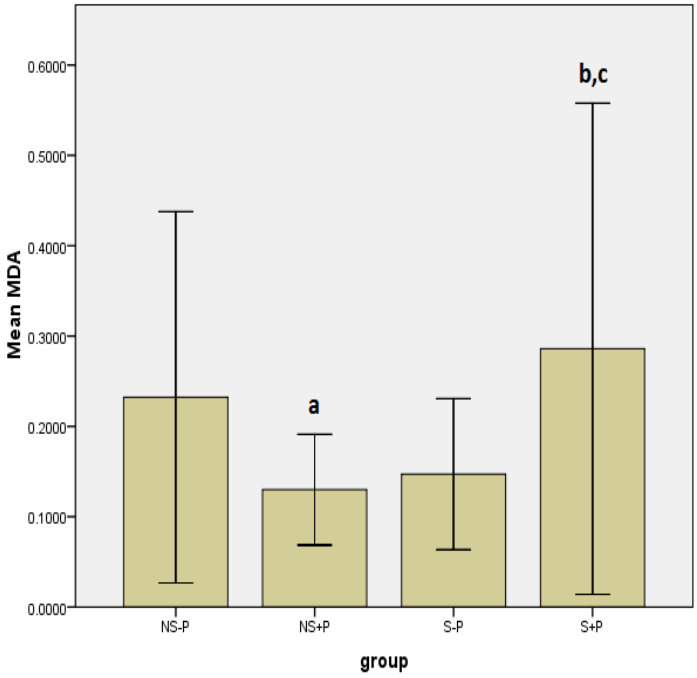

Fig. 1. Histogram showing the mean MDA (units/mg protein) level in the various groups

NS-P: Non-smokers without periodontitis,
NS+P: Non-smokers with periodontitis,
S-P: Smokers without periodontitis,
S+P: Smokers with periodontitis
a- significantly different from NS-P;
b- significantly different from NS+P;
c- significantly different from S-P

4. DISCUSSION

Cigarette smoke contains free radicals and free radical generators in both gaseous and particulate phases. They cause tissue damage by reacting with polyunsaturated fatty acids at cellular membranes and nucleotides at the DNA level [22]. Oral structures and tissues are susceptible to the noxious effects of many irritating compounds from active smoking, oral inflammatory and degenerative diseases that can lead to neoplastic transformation [23]. A few cigarettes induce increased concentrations of tobacco metabolites (nicotine and cotinine) in saliva, plasma and urine, and modify various biochemical and biological functions [24].

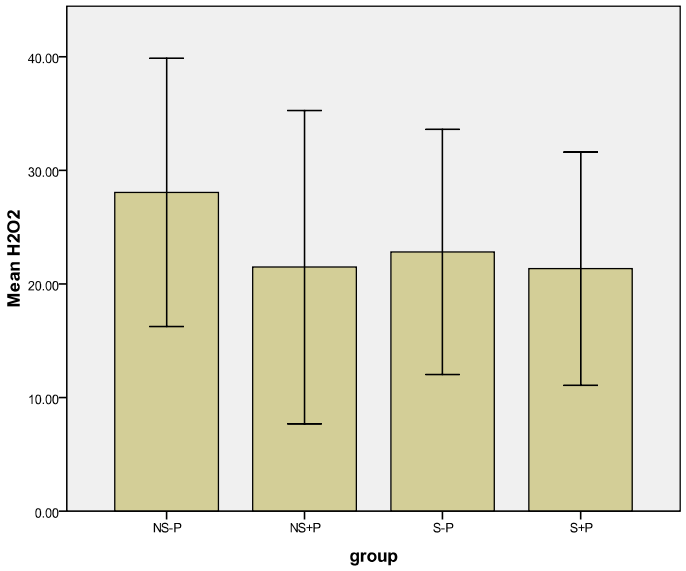

Fig. 2. Histogram showing the mean H_2O_2 (µmol/mg protein) level in the various groups

NS-P: Non-smokers without periodontitis,
NS+P: Non-smokers with periodontitis,
S-P: Smokers without periodontitis,
S+P: Smokers with periodontitis

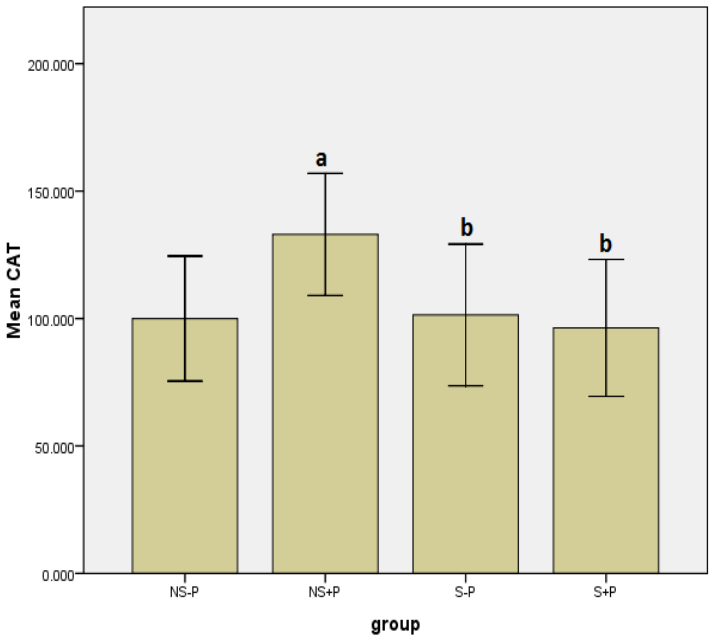

Fig. 3. Histogram showing the mean CAT (units/mg protein) activity in the various groups

NS-P: Non-smokers without periodontitis,
NS+P: Non-smokers with periodontitis,
S-P: Smokers without periodontitis,
S+P: Smokers with periodontitis
a- significantly different from NS-P;
b- significantly different from NS+P

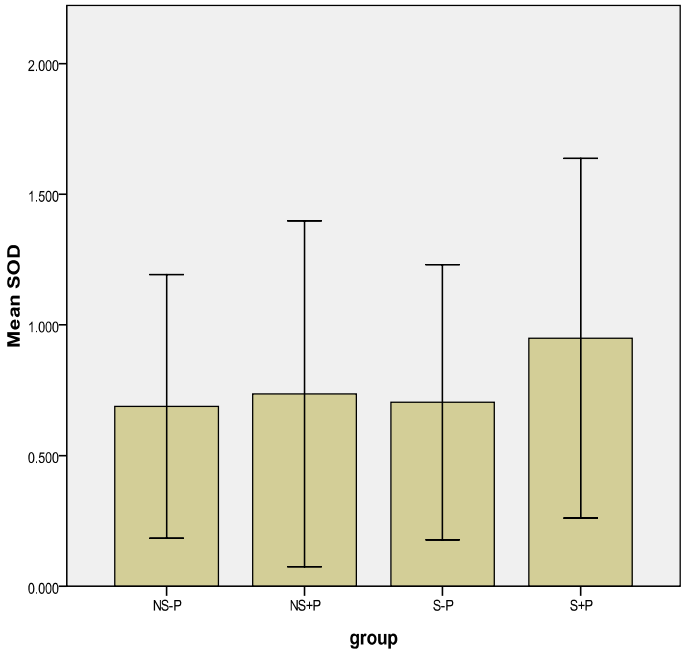

Fig. 4. Histogram showing the mean SOD (units/mg protein) activity in the various groups
NS-P: Non-smokers without periodontitis,
NS+P: Non-smokers with periodontitis,
S-P: Smokers without periodontitis,
S+P: Smokers with periodontitis

Malondialdehyde is a reactive end product of oxidative damage to membrane lipids (lipid peroxidation) or oxidative modification of proteins and DNA lesion. It is implicated in a wide variety of disease conditions (cancer, viral, protozoal, bacterial infections etc) and lifestyle such as (alcohol consumption and cigarette smoking) [1]. The presence of antioxidant enzymes (SOD, CAT, GST, GPx) and endogenous reduced glutathione (GSH) inhibits, terminates or limits the formation of toxic and reactive end products before their peroxidative damage to membrane proteins, lipids and DNA molecules by ROS and reactive nitrogen species (RNS) [25].

In this study, the significant decrease in endogenous GSH in smokers with periodontitis compared with non-smokers without periodontitis was observed. This implied increased generation of free radicals in smokers with periodontitis leading to utilization of GSH by GST and GPx to detoxify generated free radicals. It is also likely that there is formation of conjugates between sulphur atom of GSH and compounds from the cigarette smoke in periodontitis patients. This is in accord with the findings of Zappocosta et al. [26]. He reported a decrease in salivary GSH concentration of smokers. The present study adds to the existing data that tobacco smoking decreases the antioxidant defense mechanisms of the upper airways or mouth. Rai [27] also reported a decrease in GSH concentration and SOD activity in periodontitis subjects while Symone et al. [28] reported that tobacco smoke promotes ROS release and reduced GSH status resulting in heightened oxidative damage to periodontal tissue.

Studies on SOD, CAT, GST and GPx activities in relation to periodontal tissues or the oral cavity are limited and the results are conflicting. Ellis et al. [29] found a significant and progressive reduction in SOD, CAT and GPx activities in saliva and within gingiva adjacent to deeper pockets. Marton et al. [30] showed that SOD, GST and CAT activities were similar in periapical granuloma and healthy gingiva. The present study reveals an insignificant difference in the activities of GST, GPx and SOD when all groups were compared. Our result is consistent with the findings of Akalin et al. [31] who reported that SOD, CAT and GST activities in gingival crevicular fluid did not change significantly in the periodontal disease state compared with chronic

periodontitis subjects. Also, studies have indicated that total salivary antioxidant activities remain at the same level [32] (similar to our finding) or was reduced [33] in periodontal disease. It is known that these antioxidant enzymes are mainly found in cells and tissues and there is only a minor activity in extracellular fluids [34]. It might be one of the reasons for insignificant salivary SOD, CAT, GST and GPx activities in all our subjects. Another possibility might be that a suppressed production of these enzymes has occurred in gingival crevicular fluid because of the oxidative damage [35]. Thus, GST, GPx, MPx and SOD are not involved in the progression of periodontitis or oral damage caused by cigarette smoking.

SOD removes $O_2^{\cdot-}$, by greatly accelerating its conversion to H_2O_2. CAT converts H_2O_2 into water and oxygen and help to dispose off H_2O_2. GPx also removes H_2O_2 by using it to oxidize GSH to oxidized GSH (GSSG) [36]. This explains the significant decrease in MDA concentration and significant increase in CAT activity in non-smokers with periodontitis compared with healthy controls. This implies that lipid peroxidation/DNA adduct formation by MDA was significantly reduced in non-smokers with periodontitis. Contrary to our findings, Khalili and Biloklytska [37] reported increased MDA concentration in periodontal disease patients with clinically healthy periodontonium as controls. Also, Tonguc et al. [38] reported decreased SOD, CAT GPx activities and significantly elevated MDA concentrations in periodontally healthy non-smoking controls compared with chronic periodontal smokers. Cigarette smoking has been reported to increase lipid peroxidation through ROS/free radical generation [10,39] and the overwhelming of host antioxidant defense mechanisms due to increases in H_2O_2 and $O_2^{\cdot-}$ generation. This may favour increased lipid peroxidation and DNA adduct formation through the production of MDA [25]. This phenomenon was observed in smokers with periodontitis. The increased concentrations of lipid peroxidation may play a role in the inflammation and destruction of the periodontium in periodontitis. This observation is in accordance with findings of Tsai et al. [40] where significantly increased concentrations of MDA, reduced GSH and non-significant GPx and CAT activities were observed in gingival crevicular fluid and saliva of chronic periodontitis patients compared with healthy controls. Kuppusamy et al. [41] also observed significantly elevated concentrations of MDA with reduced plasma and red blood cell GSH in chronic periodontitis patients compared with healthy controls. Therefore, smoking will aggravates lipid peroxidation in the progression of periodontitis. The increased CAT activity observed in non-smokers with periodontitis compared with non-smokers with periodontitis points to enzymatic detoxification of H_2O_2 to water and oxygen. This may also supports the insignificant levels of H_2O_2 in all groups and non-involvement of H_2O_2 and $O_2^{\cdot-}$ in the pathogenesis and progression of periodontitis.

Reduced CAT activity observed in smoker with periodontitis smokers without periodontitis compared with non-smokers with periodontitis might be due to the increased production of free radicals species overwhelming the host antioxidant status. This is supported by the findings of Garg et al. [42]. He concluded that smoking increases the level of free radicals in periodontal tissues, which in turn might be responsible for the destruction seen in periodontal diseases.

5. CONCLUSION

Our study showed increased salivary CAT activity in non-smokers with periodontitis compared with non-smokers without periodontitis. Reduced salivary CAT activity was also observed in smokers with periodontitis compared with smokers without periodontitis. Therefore, reduced CAT activity is involved in the progression of periodontitis and mechanism of cigarette induced oral diseases. Monitoring CAT activity may be a useful tool in diagnosis and prognosis of periodontitis in clinical practice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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