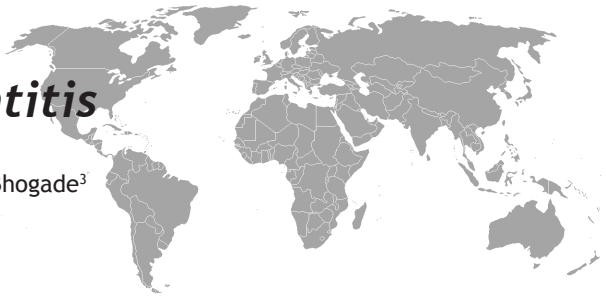
Oxidative Stress in Periodontitis



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ABSTRACT

Periodontitis is one of the most common oral infections induced by bacteria and bacterial products of dental plaque. Cigarette smoking is considered to be a risk factor for periodontitis. However, the exact mechanism by which smoking exerts its deleterious effects on periodontium remains unclear. Therefore the present study was planned to evaluate the relationship between cigarette smoking and periodontal damage in terms of the levels of free radicals and antioxidants. A total of 75 subjects were included in the study. Out of these, 25 were healthy controls, 25 were nonsmoker periodontitis patients and 25 were smoker periodontitis patients. All subjects were screened for serum lipid peroxide, nitric oxide and antioxidants such as superoxide dismutase, glutathione peroxidase along with total antioxidant capacity. A significant increase in serum lipid peroxide and nitric oxide with a corresponding decrease in serum superoxide dismutase, glutathione peroxidase and total antioxidant capacity was observed in both groups of periodontitis patients. Further, it was noticed that the oxidant levels were significantly higher and antioxidants were significantly lower in smoker patients than non-smoker patients. Thus, smoking plays a pivotal role in enhancing oxidative burden in periodontitis.

Key words: *Periodontitis, oxidative stress, smoking*

Periyodontisde Oksidatif Stres

ÖZET

Periodontitis diş plaklarından köken alan bakteri ve ürünlerinin oluşturduğu sık görülen oral infeksiyonlardan bir tanesidir. Sigara içimi periodontitis için bir risk faktörü olduğu düşünülmektedir. Bununla birlikte sigara içiminin periodontitis gelişimi üzerindeki olumsuz etkileri tam olarak açıklığa kavuşturulamamıştır. Bu nedenle bu çalışmada sigara içimi ile serbest radikaller ve antioksidan düzeyleri açısından periodontitis arasındaki ilişkiyi araştırmak amacıyla planlandı. Çalışmaya toplam olarak 75 olgu dahil edildi. Bunlar, 25 sağlıklı kontrol, 25 sigara içmeyen periodontitisli hasta ve 25 sigara içen periodontitisli hastalardı. Tüm olgular serum lipid peroksit, nitrik oksit ve süperoksit dismutaz, toplam antioksidan kapasite ile glutatyon peroksidaz gibi antioksidanlar açısından tarandı. Periodontitisi bulunan her iki hasta grubunda serum lipid peroksit ve nitrik oksit önemli artış saptanırken serum süperoksit dismutaz, glutatyon peroksidaz ve toplam antioksidan kapasitesi azalma gözlenmiştir. Bununda ötesinde, oksidan düzeyleri anlamlı düzeyde yüksek bulundu ve antioksidan düzeyleri sigara içen hastalarda içmeyenlere olgulara göre anlamlı olarak düşük olduğu fark edildi. Böylece, sigara periodontitis oksidatif yükünü artırmada önemli bir rol oynar.

Key words: *Periyodontis, oksidatif stres, sigara*

INTRODUCTION

Periodontitis is an oral inflammatory disorder that gives rise to tissue damage and loss, as a result of the complex interaction between pathogenic bacteria and the host's immune response (1). Evidence is accumulating which suggest that oxygen derived free radicals and their products play an important role in pathogenesis of chronic inflammatory disorder like periodontitis. Free radical may be defined as "any species capable of independent

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existence that contains one or more unpaired electrons." Prime targets of reactive oxygen species (ROS) are polyunsaturated fatty acids (PUFA) in membrane lipids causing lipid peroxidation. Malondialdehyde (MDA) is formed by peroxidation of PUFA and is used as a measure of lipid peroxidation (2). Nitric oxide (NO•), a short lived free radical is a unique biological messenger molecule involved in neurotransmission, vasodilatation and immune regulation. Altered NO• production has been reported in the pathogenesis of a number of disease processes including periodontitis (3).

The living organism has adapted itself to an existence under a continuous efflux of free radicals. Among the different adaptive mechanisms, the antioxidant defense mechanisms are of major importance. Antioxidants are "those substances which when present in lower concentration compared to that of an oxidisable substrate, will significantly delay or inhibit oxidation of that substance." The antioxidants like vitamin-E, vitamin-C, ceruloplasmin, glutathione peroxidase and superoxide dismutase protect tissue damage induced by free radicals (4). Smoking is an escalating public health problem which is implicated in the pathogenesis of respiratory disease, cardiovascular disease etc. Evidence suggests that smoking is consistently associated with a variety of deleterious changes in the oral cavity and consequently smokers have increased accumulations of plaque and calculus. Cigarette smoke contains a large number of free radicals and it has been suggested that it may increase the susceptibility to periodontal pathogens (5). As smoking may increase risk of periodontitis, the present study was aimed to evaluate effect of smoking on oxidative stress in these patients.

MATERIALS AND METHODS

The present study was carried out in the Department of Biochemistry, Dr. V. M. Govt. Medical College and S.C.S.M. General Hospital, Solapur. A total of 75 subjects were included in the study. Out of these, 25 were healthy controls (group 1) who were non smokers and 50 were periodontitis patients, which were further subgrouped as smokers and nonsmokers.

Inclusion criteria

A.Healthy controls: 25 non smoking healthy volunteers were selected and matched for age and sex. None of them was suffering from any acute / chronic disease/s.

B.Study group subjects: Periodontitis patients were selected who had; a.Clinical attachment loss ≥ 4mm. b.Periodontal pocket depth ≥ 4mm. c.Bleeding on probing. d.Not undergone any periodontal treatment for at least six month prior to sampling. Of the 50 subjects, 25 were smokers (group 2) (smoking a minimum of 10 cigarettes per day for more than 5 years) and 25 were nonsmokers (group 3).

Exclusion criteria

1.Subjects who require antibiotic or anti-inflammatory drug therapy. 2.Having history of alcoholism & diseases which induce oxidative stress such as diabetes mellitus, cardiovascular disease, oral cancer etc. 3.Subjects with vitamin supplements. 4.Pregnant or pre-eclamptic women. 5.Tobacco chewers.

The study was approved by institutional ethical committee. The purpose of our study was explained to all subjects and their consent was taken. A total of 5 ml venous blood was collected. Out of that 2 ml was collected in heparinized bulb and the remaining was allowed to clot. Plasma and serum were separated by centrifugation at 3000 rpm for 10 minutes at room temperature and was analyzed on the same day. Serum malondialdehyde levels were measured by Kei Satoh method (6). Nitric oxide levels were determined by N. Cortas and N. Wakid method (7). Superoxide dismutase activity was measured by the method of Kajari Das (8) and glutathione peroxidase was estimated by using Ransel kits (U.K.). Total antioxidant capacity was estimated by IFF Benzie et. al method (9) in which non enzymatic antioxidants were measured.

Statistical analysis

Statistical analysis was done by using students't-test'. The data was expressed as mean ± standard deviation. P value of <0.05 was considered to be statistically significant.

RESULTS

Table 1 shows the levels of serum total lipid peroxide (MDA), nitric oxide (NO•), superoxide dismutase (SOD), glutathione peroxidase (GPx) and total antioxidant capacity in healthy controls and periodontitis patients. Both groups of periodontitis patients i.e. group 2 and group 3 exhibited a significant increase ($p<0.001$) in serum MDA as well as NO• when compared to healthy controls. The levels of serum SOD, GPx and plasma

***Table 1**. The levels of serum total lipid peroxide, nitric oxide, superoxide dismutase, glutathione peroxidase and total antioxidant capacity in healthy controls and periodontitis patients*

Parameters	*Healthy controls (Group 1)*	*Non-smoker Periodontitis (Group 2)*	*Smoker Periodontitis (Group 3)*
Serum MDA (nmol/ml)	*3.71 ± 0.40*	*6.44 ± 1.06**	*7.5 ± 1.0*#*
Serum NO• (µmol/L)	*31.80 ± 3.83*	*54.8 ± 2.93**	*62 ± 2.35*#*
Serum SOD (U/L)	*5.62 ± 0.62*	*3.43 ± 0.23**	*2.55 ± 0.06*#*
Serum GPx (U/ml)	*4.61 ± 0.26*	*2.50 ± 0.19**	*1.30± 0.16*#*
Plasma total antioxidant capacity (mmol/l)	*2.32 ± 0.24*	*1.30 ± 0.11**	*0.95 ± 0.15*#*

** p<0.001 as compared to group 1, # p< 0.001 as compared to group 2*

total antioxidant capacity were significantly diminished in group 2 and group 3 periodontitis patients when compared with healthy controls (p< 0.001). Further, the oxidant levels (MDA and NO•) were found to be significantly higher in group 3 patients when compared with group 2 patients along with a concomitant significant decrease in antioxidant levels (SOD, GPx and total antioxidant capacity) (p<0.001).

DISCUSSION

Some of the studies support the adverse relationship between smoking and periodontitis (10,11). Smokers are almost four times more likely to have severe periodontitis than non-smokers (12). However, the exact mechanism by which smoking exerts its deleterious effects on periodontium remains unclear. One potential mechanism is through tissue damage mediated by oxidative species originating from cigarette smoke (10). ROS cause toxic effects by oxidative damage to macromolecules such as proteins, lipids and nucleic acids. The present study revealed extensive increase in total lipid peroxide in both smoker and nonsmoker groups of periodontitis which was a resultant of concomitant increase in ROS production.

Periodontitis is a chronic inflammatory condition; where neutrophils are predominant inflammatory cells, which are implicated in the disease pathogenesis because of resultant oxidative burst during phagocytosis. This interaction between pathogenic bacteria and the host immune response is accompanied by an increase in cytokine expression and immunological activity in gingival tissues (13). Thus, large amounts of pro-oxidants are produced in prolonged inflammatory response, as seen in periodontitis. Nitric oxide is the known bronchodilator and a potent inhibitor of platelet adhesion and aggregation and has got a multifaceted role in periodontitis. Present study revealed significantly elevated NO• levels in periodontitis patients. The increased nitric oxide production could be due to stimulation of inducible nitric oxide synthase (iNOS) by lipopolysaccharide of Gram negative bacteria of periodontal lesion.

Group III periodontitis patients showed higher MDA as well as NO• levels than Group II patients (p<0.001). Smoking habit is associated with a variety of deleterious changes in the oral cavity such as compromised vasodilatation, decreased blood flow to gingiva due to the vasoconstricting actions of nicotine. Also, due to the presence of a wide variety of ROS, smokers are presented with high level of oxidant stress. Exposure of cigarette smoke in periodontitis is associated with increased lipid peroxidation, as evidenced in the present study. Therefore, it can be suggested that oxidative damage in periodontitis is aggravated by the effect of smoking (14,15).

Antioxidants by counteracting the harmful effect of free radicals protect structural and tissue integrity. Imbalances between free radicals and antioxidants have been suggested to play an important role in the onset and development of several inflammatory oral diseases like periodontitis. Antioxidant enzymes like SOD and GPx provide protection against oxidative injury from oxygen free radicals. The function of SOD is to remove damaging ROS from the cellular environment by catalyzing the dismutation of superoxide radicals to H2O2, where as glutathione peroxidase reduces hydrogen peroxide and/or lipid hydrogen peroxides by the oxidation of reduced glutathione or s-nitrosoglutathione (16). The total antioxidative potential of the plasma reflects the ability of an individual to resist the oxidative stress .

Ferric reducing ability of plasma (FRAP) evaluates the plasma total antioxidant capacity (TAC) due to known and unknown antioxidants in the plasma.TAC have the advantage that they analyze the combined effectiveness of contributing species, which may be greater than the sum of the individual antioxidant.

In the present study, we found significantly decreased activities of SOD, GPx as well as total antioxidant capacity in periodontitis patients when compared to healthy controls. Further, it was also noticed that smoker patients had significantly diminished levels of SOD, GPx and total antioxidant capacity than non-smoker patients. This depleted antioxidant status in periodontitis could be due to the result of their overconsumption in response to increased oxidative burden which in turn is due to periodontal pathogens along with cigarette smoke. The study results reveal that smoking increases the level of free radicals in periodontal tissues, which in turn may be responsible for the destruction seen in periodontal diseases.

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