

Oxidative stress in periodontitis: A critical link to cardiovascular disease

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Abstract

Periodontitis is one of the most common oral infections induced by bacteria and bacterial products of dental plaque and is characterized by inflammatory destruction of tooth supporting connective tissues and alveolar bone. Recently, an association between periodontitis and cardiovascular disease has received considerable attention. The present study was carried out to assess the possible mechanisms which underlie the pathogenesis of periodontitis and cardiovascular disease. 100 periodontitis patients and 100 healthy controls were screened for periodontal pocket depth and clinical attachment loss as a measure of periodontal status along with serum and salivary oxidants (lipid peroxide & nitric oxide) and total antioxidant capacity. They were also screened for total lipid profile, which is an established risk marker of cardiovascular disease. Highly significant increase in periodontal pocket depth and clinical attachment loss was seen in periodontitis patients as compared to healthy controls. A significant increase in serum as well as salivary total lipid peroxide (MDA) and nitric oxide along with a decrease in total antioxidant capacity was observed in periodontitis patients when compared with healthy controls. A highly significant increase in total cholesterol, LDL-cholesterol and triglyceride levels with a concomitant decline in HDL-cholesterol level in periodontitis patients was observed as compared with control group. The increased oxidative stress and altered lipid profile in periodontitis patients could contribute towards the development of cardiovascular disease in these patients.

Key words: Periodontitis, cardiovascular disease, oxidants, total antioxidant capacity.

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Introduction

Periodontitis is mainly caused by bacteria and is characterized by inflammation of the tissues that support the teeth, which results in destruction of periodontal ligament and loss of the adjacent bone. It affects a large number of individuals, especially adults and promotes continuous exposure to bacteria, endotoxins (lipopolysaccharides) and other bacterial products in both the periodontal tissue and the blood stream. This can induce local and systemic inflammatory reactions in the host [1, 2] affecting lipid metabolism. Evidence suggests that chronic exposure to Gram negative microorganisms and /or their LPS can manifest a state of altered lipid metabolism; the main features of which are hypertriglyceridemia & lipid oxidation. The underlying mechanism for these alterations is the release of TNF- α , IL-1 β in response to Gram negative LPS exposure. These two cytokines exerts effects on lipid metabolism by influencing the production of other cytokines [3] altering hemodynamics /amino acid utilization of various tissues involved in this process. It is well known that there is a causal relationship between serum lipid levels and cardiovascular disease [4].

Interest has recently increased in the relationship between periodontitis and cardiovascular disease. Periodontitis and cardiovascular disease (CVD) have complex etiologies. The factors that place individuals at risk for periodontitis may also place them at risk for CVD and this means periodontitis and CVD may share common risk factors such as smoking, dietary habits, socio-economic status, diabetes as well as free radicals [5]. Ardita Aliko and Joshipura K J et al. [5, 6] referred to the possibility of an association between periodontitis and risk of CVD. Hujoel P.P. [7] suggested that chronic periodontitis increases the risk of CVD by 15%. However, some of the researchers such as Kinane [8], Seymour [9] and Armitage [10] found no significant association between periodontitis and CVD. Thus insufficient evidence is available to confirm the association between periodontitis and CVD.

Free radicals are highly reactive species characterized by an unpaired electron in their outer orbital. Free radical reactions, including lipid peroxidation contribute to pathogenic processes in a variety of inflammatory disorders and can damage proteins, lipids, carbohydrates and

nucleic acids. Plasma membranes are critical targets of free radical reactions. [11] Malondialdehyde (MDA) is formed by peroxidation of polyunsaturated fatty acids and is used as a measure of lipid peroxidation [12]. Recently, it has been claimed that the imbalances in the levels free radicals, reactive oxygen species and antioxidants in saliva may play an important role in the onset of periodontal diseases, therefore measurement of oxidative stress in saliva represents major intraoral condition and this would provide a more accurate account of the oral environment [13].

Cells have developed various antioxidant systems to defend against this free radical attack. Many enzymatic antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase as well as non-enzymatic antioxidants like vitamin - E, vitamin - C can detoxify free radicals. Oxidative stress results due to the disturbance of the pro-oxidant - antioxidant balance in favour of the former [12].

Some of the recent studies [12, 14] indicate that periodontitis is an oxidative stress state and because oxidative modification dominates current etiology concerning the pathogenesis of atherosclerosis, our study has focused on the role of oxidative stress as a probable underlying mechanism that relates periodontitis and CVD.

Materials and Methods

A total of 200 subjects were recruited in the study and out of these, 100 were healthy controls and 100 were periodontitis patients. Periodontitis group included 60 male & 40 female patients suffering for more than six months.

Inclusion criteria

1. Healthy controls: 100 healthy volunteers were selected and matched for age and sex. None of them was suffering from any chronic disease/s.
2. Study group subjects: 100 periodontitis patients were included who had –
3. Clinical attachment loss of ≥ 4 mm measured by using Williams's periodontal probe.
4. Periodontal pocket depth ≥ 4 mm.
5. Bleeding on probing.
6. Not undergone any periodontal treatment for at least six months prior to sampling.

Exclusion criteria:

7. Subjects who required antibiotic or anti-inflammatory drug therapy.

8. Having history of alcoholism, smoking and diseases which induce oxidative stress such as diabetes mellitus, cardiovascular disease etc.

9. Subjects who regularly use mouth washes like Chlorhexidine mouth wash etc.

The study was approved by institutional ethical committee. The purpose of our study was explained to all subjects and their consent was taken. 6 ml fasting venous blood was collected from the subjects under aseptic condition. Out of that 3 ml was collected in sterile heparinised bulb and rest of the blood was allowed to clot. Serum and plasma were separated by centrifugation at 3000 rpm for 10 minutes at room temperature. All the samples were analyzed on the same day of collection.

Saliva collection:

Unstimulated saliva was collected for the estimation of oxidants and total antioxidant capacity. The saliva was allowed to accumulate in patient's mouth for 2 minutes and obtained by expectorating into disposable tubes. It was then centrifuged at 3000 rpm for 10 minutes at room temperature and supernatant was analyzed on the same day.

Clinical Examination:

Body mass index (BMI) and waist circumference (WC) was used to assess overall adiposity and abdominal adiposity respectively. To obtain BMI, heights of all individuals were measured and they were accurately weighed with digital balance. BMI is defined as the individuals body weight divided by the square of their height and measured in Kg/m^2 . ($\text{BMI} = \text{weight (kg)} / \text{height (m}^2\text{)}$). Waist circumference was measured in centimeters at the level of umbilicus. The measurements were taken after participants exhaled.

Serum and salivary malondialdehyde (MDA) levels were measured by reacting it with thiobarbituric acid at high temperature to form pink coloured complex as in Kei Satoh method [15]. Nitric oxide was determined by Cortas N. and Wakid N. method [16], in which nitrate is reduced to nitrite by copper coated cadmium granules. This nitrite produced is determined by diazotization of sulphanylamine coupling to naphthylethylenediamine to form purple complex. Total antioxidant capacity was measured by the method of IFF Benzie et al. [17]. Antioxidant power convert ferric to ferrus ion reduction at low pH causes a coloured ferrus tripyridyltriazine complex. This ferric reducing ability of plasma was obtained by comparing the absorbance change at 593nm in test, with those containing ferrus ion in known concentration. Plasma

lipoproteins were measured by using kits of Span Diagnostics Limited, India.

Statistical analysis

Statistical analysis was done by using students 't'-test and the data was expressed as mean \pm standard deviation. Probability values of < 0.05 were considered to be statistically significant.

Results

Table 1 shows the levels of body mass index, waist circumference, clinical attachment loss and periodontal pocket depth in healthy controls and periodontitis patients. Body mass index and waist circumference were significantly higher in periodontitis patients when compared to healthy controls ($p < 0.001$). Periodontitis patients also demonstrated significantly higher clinical attachment

loss and periodontal pocket depth when compared to healthy controls ($p < 0.001$).

The levels of serum and salivary oxidants (MDA, nitric oxide) and total antioxidant capacity are given in Table 2. Periodontitis patients documented a significant increase ($p < 0.001$) in serum as well as salivary MDA and nitric oxide when compared to healthy controls. Total antioxidant capacity was significantly decreased in periodontitis patients ($p < 0.001$) when compared with healthy controls.

Table 3 depicts the levels of total cholesterol, LDL-cholesterol, HDL-cholesterol & triglyceride in healthy controls and periodontitis patients. Levels of total cholesterol, LDL-cholesterol and triglyceride were significantly higher in periodontitis patients ($p < 0.001$) when compared to healthy controls; whereas a significant decrease was observed in HDL-cholesterol in periodontitis patients than healthy controls ($p < 0.001$).

Table 1. Shows the levels of body mass index (BMI), waist circumference (WC), clinical attachment loss (CAL) & periodontal pocket depth (PD) in healthy controls and periodontitis patients

Sr. No.	Parameters	Healthy controls	Periodontitis patients
1.	Age (years)	50.3 \pm 9.39	52.7 \pm 9.27
2.	Body mass index (Kg/m ²)	24.1 \pm 3.88	27.7 \pm 2.38*
3.	Waist circumference (cm)	83 \pm 1.19	108 \pm 2.28*
4.	Clinical attachment loss (mm)	2.24 \pm 0.1	4.70 \pm 0.14*
5.	Periodontal pocket depth (mm)	1.40 \pm 0.11	4.27 \pm 0.11*

Compared to healthy controls * $P < 0.001$

Table 2. Depicts serum and salivary levels of total lipid peroxide (MDA), nitric oxide (NO) and plasma & salivary total antioxidant capacity (TAC) in healthy controls and periodontitis patients

Sr. No.	Parameters	Healthy controls	Periodontitis patients
1.	Serum MDA (nmol/ml)	3.71 \pm 0.40	6.55 \pm 1.21*
2.	Salivary MDA (nmol/ml)	5.21 \pm 0.60	7.36 \pm 0.72*
3.	Serum Nitric Oxide (Nitrite) (μ mol/L)	31.80 \pm 3.83	56.77 \pm 3.11*
4.	Salivary Nitric Oxide (Nitrite) (μ mol/L)	29.8 \pm 4.60	55.36 \pm 6.16*
5.	Plasma Total antioxidant capacity (mmol/L)	2.32 \pm 0.24	1.30 \pm 0.15*
6.	Salivary Total antioxidant capacity (mmol/L)	1.07 \pm 0.03	0.46 \pm 0.07*

Compared to healthy controls * $p < 0.001$

Table 3. Shows the levels of plasma total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) & triglyceride (TG) in healthy controls and periodontitis patients

Sr. No.	Parameters	Healthy controls	Periodontitis patients
1.	Total cholesterol (mg/dl)	190 \pm 19.10	222.7 \pm 35.46*
2.	LDL-cholesterol (mg/dl)	119.8 \pm 11.66	152.5 \pm 23.15*
3.	HDL-cholesterol (mg/dl)	46 \pm 7.82	40 \pm 7.28*
4.	Triglyceride (mg/dl)	111 \pm 17.81	145 \pm 29.23*

Compared to healthy controls * $p < 0.001$

Discussion

The focus of the relationship between periodontitis and cardiovascular disease is shifting from a purely epidemiological association towards biological understanding of the underlying mechanisms. Evidence is rapidly mounting indicating obesity as an independent or aggravating risk factor for CVD. Studying the relationship between obesity and periodontitis is, therefore important since this association could further contribute to increased morbidity of this disease in obese individuals. The present findings of increased BMI & WC in periodontitis patients are in accordance with previous findings supporting a positive correlation between obesity and periodontal damage [18, 19]. Obesity has been postulated to reduce blood flow to the periodontal tissues, promoting the development of periodontal disease [20]. Furthermore, obesity may enhance immunological or inflammatory disorders, which might be the reason obese subjects, tend to exhibit escalating poor periodontal status relative to non-obese individuals [21]. A proposed model linking obesity and periodontal infection suggested that insulin resistance mediates the relationship between them. Insulin resistance contributes to a generalized hyperinflammatory state, including periodontal tissue, especially when triggered by oral pathogens [22].

The present study revealed extensive increase in serum and salivary total lipid peroxide which was a resultant of concomitant increase in reactive oxygen species production in periodontitis. Periodontitis is a Gram negative bacterial infection. Key bacteria which include *Porphyromonas gingivalis*, *Treponema denticola* & *Bacteroides forsythus* have special enzymes and proteins that enable them to trigger host inflammation. Neutrophils are predominant inflammatory cells in gingival tissues, which are implicated in the disease pathogenesis because of resultant oxidative burst during phagocytosis [23]. Diseased sites are associated with increased levels of a variety of cytokines and chemokines produced by inflammatory cells and normal resident cell population within periodontal tissues. Thus reactive oxygen species (ROS) plays a pivotal role in periodontal tissue destruction. ROS generation in periodontitis causes bone resorption; degrade connective tissue and increases matrix metalloproteinase activity. [24]

Atherosclerosis is the root cause of CVD and is a multifactorial, multistep process that involves chronic inflammation at every stage, from initiation to progression and eventually plaque rupture [25]. Along with inflammation, free radical mediated injury to the vessel wall has been

implicated in the pathogenesis of CVD. The increased free radical activity in periodontitis patients as observed in present study may contribute to the development of CVD in these patients.

Nitric oxide is a known bronchodilator and a potent inhibitor of platelet adhesion and aggregation & has got a multifaceted role in periodontitis. Present study showed significantly increased nitric oxide levels in periodontitis patients. The increased nitric oxide production could be due to stimulation of inducible nitric oxide synthase (iNOS) by lipopolysaccharides of Gram-negative bacteria [26]. It has been shown that endothelial cell damage and vascular permeability may result from increased synthesis of nitric oxide. Besides, overexpression of iNOS is implicated as a mechanism of tissue dysfunction and damage in many chronic and acute diseases of cardiovascular system [27].

Mutual co-operation between different antioxidant pathways provides greater protection against attack by reactive oxygen species, compared to any single compound. Thus total antioxidant capacity may give more relevant biological information compared to that obtained by the measurement of individual biomarkers, as it considers the cumulative effect of all antioxidants present in plasma and body fluids [28]. In the present study, we found significantly lowered total antioxidant capacity because of increased generation of ROS, as oxidative damage lies at the heart of periodontitis.

Increased levels of total cholesterol, LDL-cholesterol & triglyceride in periodontitis patients are documented in the present study. One possible explanation for these findings could be that pro-inflammatory cytokines such as interleukin-1 β & tumor necrosis factor- α leaking from periodontal lesion into the circulation inhibits the lipoprotein lipase activity, causing disturbance in lipid metabolism [29]. In addition, periodontitis also diminishes antiatherogenic potency of HDL by impairing its efflux capacity and thus increases risk for CVD. [30]

Inflammation is strongly associated with dyslipidemia as well as oxidative stress in periodontitis. Thus, an uncontrolled, over-exuberant inflammatory response in periodontitis may serve as intermediate variable between these two [31].

In the present study, we observed a severe oxidative stress as well as altered lipid profile in periodontitis patients. When we consider these data together, with the results of the study, we hypothesize that periodontal disease may be a potential risk factor for the severity, progression and

even the initiation of cardiovascular disease because of reduced antioxidant capacity/ or increased oxidative stress and atherogenic lipoproteins. However studies of larger groups with the clinical endpoint and the analysis of oxidant/antioxidant status along with atherogenic lipid profile are required to address this hypothesis.

A clear understanding of this relationship may assist health care providers in their efforts to detect both of the diseases earlier. In addition, preventing strategies for example antioxidant treatment may be developed that impact the prevalence of both diseases. Increased dialogue among medical and dental professionals regarding this association will be increasingly important in achieving & maintaining the optimal health of patient.

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