



YES! OXIDATIVE STRESS MAY BE A RISK FACTOR FOR CHRONIC PERIODONTITIS PATIENTS

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ABSTRACT

Oxidative stress is defined as the condition occurring when the physiological balance between oxidants and antioxidants is disrupted in favor of the former with potential damage for the organism. The pathological events leading to the destruction of the periodontium encompasses complex interactions involving an imbalance between oxidant and antioxidant enzymatic and non-enzymatic defense mechanisms. Periodontitis is an oral inflammatory disorder affecting alveolar bone, gingiva, and periodontal ligament initiated by the complex interaction between the presence of microorganisms (periodontopathic bacteria) and the host's immune response. Whole saliva samples were collected from 25 chronic periodontitis patients. Saliva samples were centrifuged and the supernatant was used to determine the 8-OHdG levels with a competitive ELISA kit. To determine the clinical condition of each subject, the Bleeding on Probing (BOP), Clinical attachment level (CAL), and Pocket probing depth (PPD) were measured. This study shows significant correlation between increased values of oxidative stress biomarker and clinical parameters in chronic periodontitis patients.

KEYWORDS : Periodontitis, Oxidative Stress, 8-OHdG

INTRODUCTION

Humans survive in an oxygen rich environment, and their survival depends on a subtle mitochondrial current that generates ATP, the common energy source for all cells. Electrons, however can escape from the mitochondrial transport chain and generate reactive oxygen species [ROS], such as hydrogen peroxide [H₂O₂], oxygen superoxide [O₂•] or the hydroxyl radical [OH•]. These are highly reactive oxygen radicals that are liable for most oxidative stress on cells. Oxidative stress, occurring as a consequence of imbalance between the oxidant actors and protective antioxidant defense system. Oxidative stress has been implicated as a significant contributor in over 100 disorders and more recently periodontitis

Several studies have demonstrated that 8-OHdG in bodily fluids can act as a biomarker of oxidative stress [1],[2] and 8-OHdG is commonly used as a marker to evaluate oxidative DNA damage in disorders including chronic inflammatory diseases.

Oxidative stress is induced by an imbalance between excessive ROS production and anti-oxidant mechanisms. Increased levels of ROS leading to a state of Oxidative Stress have been implicated in the pathogenesis of a large number of diseases, including cardiovascular diseases and diabetes [3].

A study done by Chapple et al in 2007 reported that oxidative Stress has been linked with both onset of periodontal tissue destruction⁴ and systemic inflammation [5].

In the present study we hypothesized that the level of 8-OHdG in saliva increases by the periodontal destruction severity determined by clinical parameters as clinical attachment level, Probing pocket depth, Bleeding on Probing.

SUBJECTS AND METHODS

Experimental design

This observational analytical study was carried out between June–December, 2019 in (Department of Periodontics, Sri Sankara dental college, Varkala). Twenty five subjects were included in the study with inclusion criteria ; Patients with moderate/severe chronic periodontitis, Subjects having ≥ 30% sites with CAL ≥ 3mm, at least 30% sites in with PD ≥ 5mm and bleeding on probing , age between 30-50 years, having at least 20 teeth excluding third molars, radiographic evidence of generalized bone loss, willing to give an informed consent .

Exclusion criteria for the study were history of periodontal therapy in the previous 6 months ,history of alcoholism and smoking ,anti-inflammatory, antimicrobial and immunosuppressive therapies during previous 6 months, Systemic conditions that could affect the progression of periodontitis (e.g. diabetes, immunologic disorders), pregnant, lactating, postmenopausal subjects Needing antibiotics for infective endocarditis prophylaxis during dental procedures; having acute illness symptoms (i.e., fever, sore throat, body aches, or Diarrhoea).

2.2 Sample collection

Unstimulated whole saliva (2 mL) was collected in the morning (10am-12 pm) from all the subjects. All the subjects were asked to refrain from eating, drinking and physical exercise for a minimum of two hours before saliva collection. Subjects were comfortably seated and asked to rinse their mouth using distilled water. After a few minutes of relaxation, they were asked to first swallow saliva and after 5 minutes remaining saliva in the mouth was spit into the sterile container collected saliva was placed in a nitrogen flask till transport to the lab, where it was stored at -80°C. Before analysis, samples were defrosted, centrifuged at 12,000 rpm for 10 min in a centrifuge

to remove the particulates and the supernatant was separated and analyzed using an ELISA test.

2.3 Assessment Of clinical Parameters

Probing pocket depth (PPD), clinical attachment loss (CAL) were recorded immediately before saliva collection.

- I. Probing pocket depth (PPD): All teeth will be probed at six sites (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, distolingual) using Williams Graduated periodontal probe, to the nearest millimeter. It is measured from crest of marginal gingival to the depth of the sulcus. For diagnosis of generalized moderate to severe chronic periodontitis ≥ 30% sites with PD ≥ 5mm.
- II. Bleeding on Probing (BOP): Full mouth bleeding on probing will be registered in conjunction with periodontal probing after 30 seconds. The bleeding will be recorded as present (1) or absent (0).
- III. Clinical Attachment Loss (CAL): It is measured from CEJ to the base of the sulcus using Williams Graduated Periodontal Probe, to the nearest millimeter. Individuals with periodontal disease should have ≥ 30% of sites with CAL at least 3mm.

2.4 Elisa Assay Procedure

Before adding saliva samples to wells, equilibrated the TMB substrate for at least 30 min at room temperature (37°C). When diluting samples and reagents, they must be mixed completely and evenly. It was recommend to plot a standard curve for each test. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, recorded their positions. Washed the plate 2 times before adding standard, sample and control (zero) wells then 50 L of Standard, Blank, or Sample were added per well. The blank well was added with Sample/Standard dilution buffer. Immediately 50 L of Biotin-detection antibody working solution was added to each well and covered with the Plate sealer provided and gently tapped the plate to ensure thorough mixing, incubated for 45minutes at 37°C, aspirated and washed 3 times by filling each well with Wash Buffer (approximately 350 L). Then 100 L of SABC working solution was added to each well again covered with a new Plate sealer and incubated for 30minutes at 37°C. Then repeatedly done the aspiration/wash process for five times and 90 L of TMB Substrate was added to each well, covered with a new Plate sealer, incubated for about 15-20 minutes at 37°C. When apparent gradient appeared in standard wells, terminated the reaction and added 50 L of Stop Solution to each well, the color turned to yellow immediately. Then stop solution was added, adding order of stop solution should be as the same as the substrate solution. Finally, determined the optical density (OD Value) of each well at once, using a microplate reader set to 450 nm, the concentration of the corresponding sample was calculated.

STATISTICAL ANALYSIS

The correlations between saliva 8-OHdG levels and clinical parameters were calculated using Pearson's correlation test in this study, where the value r = 1 means a perfect positive correlation and the value r = -1 means a perfect negative correlation. A p value ≤ 0.001 was considered statistically significant.

RESULTS

A total of 25subjects were included in the study based on inclusion and exclusion criteria. 8 females and 17 males were included in the study with general chronic periodontitis (AAP 1999).

The study showed significant increase in levels of 8- OHdG salivary biomarker as the severity of periodontal disease progress. The mean probing pocket depth in patients with chronic periodontitis were statistically significant in relation to

salivary 8-OHdG biomarker (p<0.001) (Table 1).Between mean clinical attachment level in patients with chronic periodontitis and salivary 8-OHdG biomarker showed statistically significant (p<0.001) (Table 2)

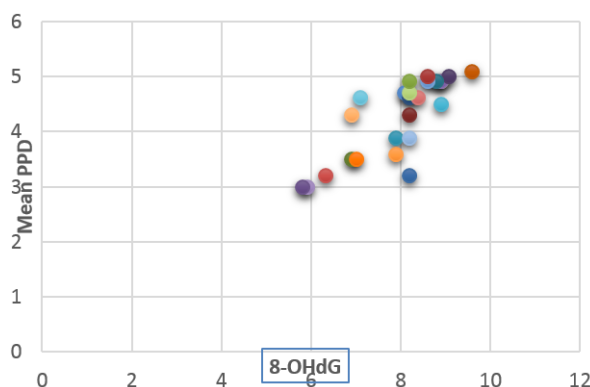
A strong positive correlation between mean probing pocket depth with salivary 8-OHdG levels was observed (graph 1). Significant increase in mean clinical attachment level with salivary 8-OHdG levels was also noted in the study (graph 2)

The mean salivary 8-OHdG level in 25 subjects with chronic periodontitis was 4.28 ± 0.1 ng/ml.

Table 1: Correlation Between mean probing pocket depth and salivary 8-OHdG biomarker

Mean PPD	n	r	p
7.952	25	0.8228	P < 0.01

(PPD: probing pocket depth, n: number of subjects, r: Pearson's correlation coefficient, P: probability value) **: Significant at 0.01 level

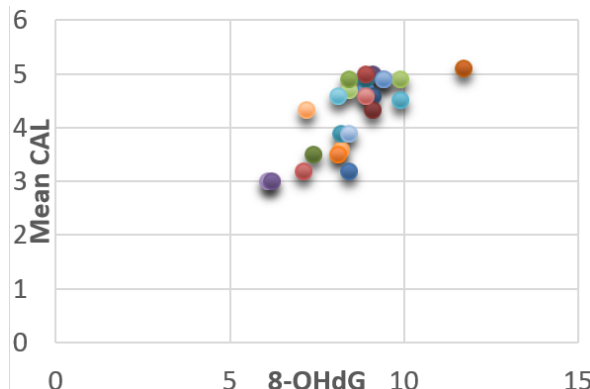


Graph 1: Scatter diagram for mean probing pocket depth and salivary 8-OHdG levels

Table 2: Correlation Between mean clinical attachment level and salivary 8-OHd biomarker

Mean CAL	n	r	p
8.536	25	0.7664	P < 0.01

(CAL: clinical attachment level , n: number of subjects, r: Pearson's correlation coefficient, P: probability value) **: Significant at 0.01 level



Graph 2: Scatter diagram for mean clinical attachment level and salivary 8-OHdG level

DISCUSSION

Periodontal diseases are one of the most widespread chronic conditions affecting the larger populations of the world. Periodontal disease is initiated by the colonization of bacterial pathogens, such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Tannerella*

forsythus. These microorganisms have the capacity to stimulate the host defense mechanisms to produce reactive oxygen species that damage the nearby host tissue in addition to destroying the pathogens [6].

Oxidative stress, is an imbalance between free oxygen radicals and antioxidant defense system, and is capable of causing damage to various cellular and extracellular components. 8-OHdG is one of the major products of nucleotide oxidation in DNA. Salivary 8-OHdG levels are often studied in oral pathology, including periodontitis and oral cancers. It was found that salivary 8-OHdG levels were very high in patients with periodontally hopeless teeth and teeth with advanced periodontal breakdown.

Saliva is a fluid that is easily available and contains locally produced microbial and host response mediators that may offer the basis for a patient specific diagnostic test for periodontitis. It includes constituents of non-salivary origin derived from gingival crevicular fluid (GCF), expectorated bronchial secretions, serum, blood cells from oral wounds, as well as bacteria and bacterial products, viruses and fungi, desquamated epithelial cells and food debris.

Oxidative damage to lipids, proteins, and nucleic acids can be monitored using established markers that are present in saliva. The indicators for oxidative stress are products obtained from oxidatively damaged macro-molecules. Proposed markers for periodontal disease include bacterial products, immunoglobulins and host enzymes [7]. Established salivary markers of oxidative stress include Malondialdehyde (MDA), thiobarbituric acid-reacting substances (TBARS) as a marker of lipid peroxidation, advanced oxidation protein products (AOPP) as a marker of protein oxidation, and advanced glycation end products (AGEs) as a marker of protein glycation and, thus, carbonyl stress that is tightly interconnected with oxidative stress. After exposure of mammalian chromatin to a free radical, various DNA-base adducts have been identified. Of these, 8-OHdG is one of the most common stable products of oxidative DNA damage following specific enzymatic cleavage and acts as one of the most sensitive biomarkers for oxidative stress [8].

In this study, we first examined the oxidative DNA damage in periodontitis patients by measuring the salivary 8-OHdG levels, and these levels were compared not only according to presence of disease but also but also according to disease severity. CAL which shows the disease severity may be affected by oxidative DNA damage, and as a result elevated salivary level of 8-OHdG, which shows disease activity, can be detected. This hypothesis coheres with the characteristic of chronic periodontitis.

Canakci et al. reported that high salivary 8-OHdG levels and low antioxidant activity increased oxygen radical activity [9].

In another study reporting the correlation between 8-OHdG and periodontopathogens, it was suggested that the determination of salivary 8-OHdG was a useful biomarker to evaluate periodontal condition and effectiveness of periodontal therapy [10].

Dede et al. evaluated the effects of initial periodontal treatment on GCF and salivary levels of 8-OHdG in periodontitis, and they revealed that 8-OHdG level in GCF was significantly decreased after initial periodontal therapy, whereas salivary levels of 8-OHdG did not change [11].

In a previous study undertaken by Takane et al, authors found significantly higher salivary 8-OHdG levels in subject with periodontally hopeless teeth (high level of attachment loss / severe periodontitis) than those in subject without periodontally hopeless teeth and those in clinically healthy controls [12].

All of these findings have supported that 8-OHdG levels in GCF and saliva reflect oxidative DNA damage in the presence of periodontal disease.

The present study suggests the involvement of oxidative stress in the periodontal environment which was reflected in the biological fluid as a result of inflammatory reaction. Saliva can be easily collected, and therefore measurement of salivary 8-OHdG levels may prove to be useful in identifying patients at risk of tooth loss. Moreover, salivary analysis for periodontal diagnosis may prove a cost effective method for screening large populations

CONCLUSION

We suggest that elevated salivary levels of 8-OHdG may be a marker for disease activity and it reflects on directly disease severity parameters such as CAL, PD. However, further investigations are needed to clarify the exact mechanism of oxidative DNA damage in diseased periodontium.

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