# Estimation of 8-Hydroxy-deoxyguanosine (8-OHdG) in Saliva as a Marker of Oxidative Stress in Patients with Chronic Periodontitis: Preliminary Data

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**Abstract**

**Background**: This preliminary study assesses the effects of initial periodontal treatment on salivary levels of 8-hydroxy-deoxyguanosine (8-OHdG) as a marker of oxidative stress in patients with chronic periodontitis (CP).

**Methods**: At baseline, clinical parameters and saliva samples were obtained from 20 patients with CP and 10 patients with clinically healthy periodontium. Saliva samples were collected, and clinical periodontal measurements were repeated at 2, 4, and 8 weeks after initial periodontal therapy in patients with CP. An enzyme-linked immunosorbent assay was used to investigate 8-OHdG levels of saliva samples.

**Results**: Statistically significant higher 8-OHdG levels of saliva and a significant decrease after initial periodontal therapy were determined in the CP group ($p < 0.001$). A significant positive correlation was found between 8-OHdG levels of saliva and clinical periodontal measurements ($p < 0.001$)

**Conclusion**: Within the limits of this short-term preliminary study it can be concluded that the estimation of 8-OHdG levels in saliva may be used to evaluate the oxidative stress in periodontitis patients. It also appears that oxidative stress, which is reflected by salivary 8-OHdG levels, might reflect the present status of periodontal health.

*Keywords: 8-hydroxy-deoxyguanosine, chronic periodontitis, saliva, reactive oxygen species*

## Introduction

Periodontal disease is the result of complex interactions between mechanisms of host defence and periodontal pathogens leading to tissue damage. In periodontitis, polymorphonuclear neutrophils play a vital role in the initial host and inflammatory response to the periodontal pathogens. This interaction enhances oxidative stress during periodontitis (Takane *et al.*, 2002; Guentsch *et al.*, 2008). Usually the oxidative stress that includes DNA damage is the derivative of oxidation of the nucleosides. 8-Hydroxy-deoxyguanosine (8-OHdG) is one of the oxidised nucleosides that is excreted in body fluids during DNA repair (Takane *et al.,* 2005).

There is increasing evidence of implicating reactive oxygen species (ROS), derived predominantly from the polymorphonuclear leukocytes, in the pathogenesis of periodontal tissue destruction. These ROS cause tissue damage through an array of different mechanisms, such as DNA damage, lipid peroxidation, protein disruption and stimulation of inflammatory cytokine release (Battino *et al.*, 1999; Chapple, 1997; Waddington *et al.*, 2000).

8-OHdG levels have been implicated in neuro-degenerative disorders, cancers and chronic inflammatory conditions (Takane *et al.,* 2005). According to the literature, the product of oxidative DNA damage, *i.e.* 8-OHdG, becomes the guanine base after specific enzymatic cleav-

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age. Continuous reparation of the oxidized DNA occurs followed by excretion of excised deoxyribonucleosides in the serum and urine. Multiple studies have reported the presence of 8-OHdG as a biomarker of oxidative stress in the body fluids (Canakcxi *et al*., 2005; Chapple and Matthews, 2007; Rai and Anand, 2008). 8-OHdG is commonly used as a marker to evaluate oxidative damage in chronic inflammatory diseases. At present, 8-OHdG is one of the most commonly used markers for evaluating oxidative damage in the periodontal tissue (Ekuni *et al*., 2008; Canakcxi *et al*., 2009; Su *et al*., 2009).

Studies reported that in patients with chronic periodontitis [CP], there was an increase in gingival crevicular fluid [GCF] levels of 8-OHdG that reduced following initial periodontal treatment (Dede *et al*., 2013). Further, in patients with periodontitis, there is an increase in concentration of 8-OHdG in the gingival blood and reduction in the level of antioxidants (Konopka *et al*., 2007).

Whole saliva is a complex mixture of secretions from the major and minor salivary glands and gingival crevicular fluid, as well as mucosal, nasal and oral transudate (Humphrey and Williamson, 2001). Additionally, blood and desquamated epithelial cells, oral bacteria and their metabolites, various chemical products and food debris are present in the saliva (de Almeida *et al*., 2008). Saliva is often called "the mirror of health of the organism" because it may reflect the current physiological condition of the body (Farnaud *et al.,* 2010; Yoshizawa *et al*., 2013). Increased salivary 8-OHdG levels and decreased salivary antioxidant activities reflected higher oxygen radical activity during inflammation of periodontal tissue (Canakci *et al*., 2009). In order to evaluate periodontal status accurately and evaluate the efficacy of periodontal treatment, early determination of 8-OHdG in saliva was considered to be a useful biomarker (Sawamoto *et al*., 2005).

Currently it is still unclear whether oxidative stress is caused by the lack of antioxidants or by an overproduction of reactive oxygen species due to inflammation. Literature reveals that antioxidant treatments could slow down or prevent the progress of periodontitis, thus making the use of salivary markers of oxidative stress even more interesting (Tóthová *et al*., 2015). Infectious diseases such as *Helicobacter pylori* infection and papillomavirus infection demonstrate increased levels of 8-OHdG (Romano *et al.,* 2000; Hahm *et al.,* 1998). Furthermore, previous studies have demonstrated that the 8-OHdG in body fluids can act as a biomarker of oxidative stress in disorders, including chronic inflammatory diseases (Chiou *et al.,* 2003; Wu *et al*., 2004). These findings connote that 8-OHdG may be a useful marker for oxidative damage of periodontal tissue. As there are limited data demonstrating the changes of oxidative markers in saliva after initial periodontal therapy in patients with CP (Takane *et al*., 2002; Takane *et al.,* 2005), the objective of this study is to evaluate the marker for oxidative stress, *i.e*., 8-OHdG levels in the saliva, and also compare the salivary levels of 8-OHdG before and after initial periodontal treatment along with the changes in clinical periodontal measurements in patients with chronic periodontitis.

## Materials and methods

A total of 30 patients, including 20 males and 10 females, attending the outpatient section of Department of Periodontology, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore were enrolled in the study. The study protocol was reviewed and approved by the institutional ethical committee and review board. The design and nature of the clinical trial was explained to the patients and an informed written consent for their participation was procured from them.

### *Selection criteria*

Systemically healthy patients between the ages of 25 - 50 years were included in the study. The individuals in the control group had no history of periodontal disease, pocket probing depths (PPD) of ≤ 3 mm at all sites in the mouth, no gingival inflammation and no bleeding on probing (BOP). Participants were diagnosed with chronic periodontitis and included in the test group if a minimum of four teeth (six sites per tooth) had PPD ≥ 5 mm, CAL ≥ 5 mm, radiographic alveolar bone destruction ≥ 30% and presence of bleeding on gentle probing. Except for the third molars, all the teeth were present in the selected patients. In order to measure bleeding on probing, the sites that bled on gentle probing were recorded and the percentage of bleeding sites/ total sites was calculated for each individual (Ainamo and Bay, 1975). Medically compromised patients, smokers, patients who have received any periodontal therapy, surgical or non-surgical and antibiotic drugs within six months of baseline examination, patients having a history of alcohol or antioxidant vitamin consumption within six weeks or using either antibacterial mouthwash or medicated tooth paste within one month of baseline examination were excluded from the study.

### *Sampling technique*

This single center prospective short-term study was of 2 months duration and comprised of 10 patients with clinically healthy periodontium (control group) and 20 patients with a history of chronic periodontitis (test group) who received scaling and root planing. The same experienced examiner recorded all clinical parameters to ensure an unbiased evaluation measurement with a UNC-15 (Hu-Friedy, Chicago, Illinois, USA) manual probe.

The clinical examination included sites with PPD, CAL and BOP. Clinical recordings were done at baseline, 2, 4 and 8 weeks post-operatively for test group. The same parameters were recorded for the control group at the baseline examination to rule out any underlying periodontal disease. Patients were instructed to brush their teeth and use interdental cleansing aids.

### *Determination of salivary 8-OHdG by enzyme linked immunosorbent assay (ELISA)*

After clinical examination, paraffin wax-stimulated 2 mL of whole saliva was collected in a quiet room between 9:00 – 10 AM, at least 8 hours after intake of food, by expectorating into disposable containers from control group at baseline and from the test group at baseline and 2 months after scaling and root planing that were performed by using ultrasonic scalers and area-specific curettes. Saliva samples were immediately centrifuged at 10,000 × g for 10 minutes to remove cell debris and the supernatant (50 μL each) was stored at -80º C until analyzed. The supernatant was used to determine 8-OHdG levels with a commercially available ELISA kit (Highly Sensitive 8-OHdG Check; Japan Institute for the Control of Aging, Cosmo Bio Co. Ltd., Japan). The 8-OHdG ELISA kit is a competitive *in vitro* enzyme-linked immunosorbent assay for quantitative measurement of the oxidative DNA 8-OHdG. The determination range of 8-OHdG levels was 0.125 to 200 ng/mL. Salivary samples from different groups as well as different time points were tested at the same time on the same plate using the manufacturer's instructions. The operator performing the ELISA test was different from the operator who collected the salivary samples in order to eliminate bias.

### *Statistical analysis*

The Shapiro-Wilk test was done to assess the normality of data, and because the data were normally distributed and quantitative in nature, parametric tests were applied. Differences in 8-OHdG levels, PPD, BOP and CAL before and after initial periodontal treatment and between test and control groups at baseline were done by Student's *t*-test. The correlations between saliva 8-OHdG levels and clinical parameters were calculated using Pearson's correlation test. A $p$ value $\leq 0.001$ was considered statistically significant. An SPSS version 21 software program analyzed all the data.

## Results

### *Salivary 8-OHdG levels*

Mean salivary 8-OHdG levels in clinically healthy subjects (control group) were 1.686 ± 0.148 ng/mL and, significantly higher in the chronic periodontitis subjects (test group), were 5.227 ± 1.340 ng/mL at baseline (*Table 1*). Mean salivary 8-OHdG levels in chronic periodontitis patients after scaling and root planing decreased significantly to 1.909 ± 0.071 ng/mL (*Table 1*).

### *Bleeding on probing*

Mean BOP in clinically healthy subjects (control group) was 5.57 ± 2.75% and chronic periodontitis patients (test group) was 96.79 ± 4.16% at baseline. Thus, at baseline mean BOP was significantly higher in the test group ($p \leq 0.001$; *Table 1*). Mean BOP in the test group at 8 weeks after scaling and root planing was 15.70 ± 2.9, which was statistically lower than at baseline ($p \leq 0.001$).

### *Probing pocket depth*

Mean PPD in clinically healthy subjects (control group) was 0.87 ± 0.08 mm and in chronic periodontitis patients (test group) was 3.49 ± 0.28 mm at baseline.The difference between the two groups was statistically significant, with greater PPD observed in the test group ($p \leq 0.0001$; *Table 1*). Mean PPD in the test group following scaling and root planing at 8 weeks was 2.45 ± 0.35 mm. Therefore, a significant reduction in PPD was observed in the test group after scaling and root planing as compared to baseline ($p \leq 0.001$).

**Table 1.** Mean comparison of 8-OHdG (ng/mL), BOP, PPD, CAL between the study groups pre- and post-treatment.

| | | Pre-treatment | | | Post-treatment (8 weeks) | |
|---|---|---|---|---|---|---|
| **Parameters** | | **Control Group** | **Test Group** | ***p* value** | **Test Group** | ***p* value** |
| n | | 10 | 20 | | 20 | |
| Age (mean ± SD) | | 29.80 ± 5.63 | 33.90 ± 7.66 | 0.146 | | |
| Gender | M | 30% | 45% | 0.429 | | |
| (mean ± SD) | F | 70% | 55% | | | |
| BOP (mean ± SD) | | 5.57 ± 2.75 | 96.79 ± 4.16 | 0.001* | 15.70 ± 2.9 | 0.001* |
| PPD (mean ± SD) | | 0.879 ± 0.08 | 3.50 ± 0.28 | 0.001* | 2.45 ± 0.35 | 0.001* |
| CAL (mean ± SD) | | 0.879 ± 0.08 | 2.552 ± 0.31 | 0.002 | 2.45 ± 0.35 | 0.002 |
| 8-OHdG (ng/mL) (mean ± SD) | | 1.686 ± 0.14 | 5.227 ± 1.340 | 0.001* | 1.909 ± 0.07 | 0.001* |

*$p \leq 0.001$; BOP, bleeding on probing; PPD, pocket probing depth; CAL, clinical attachment level; M, male; F, female

### *Clinical attachment level*

Mean CAL in clinically healthy subjects (control group) was 0.87 ± 0.08 mm and in chronic periodontitis patients (test group) at baseline was 2.55 ± 0.31 mm, and the difference was statistically significant ($p \leq 0.002$; *Table 1*). Mean CAL in the test group at 8 weeks after scaling and root planing was 2.45 ± 0.35 mm. A significant reduction in CAL was observed in the test group after scaling and root planing as compared to baseline ($p \leq 0.001$).

The correlation between clinical parameters and the 8-OHdG in whole saliva from periodontally diseased subjects was assessed. A negative correlation was observed between 8-OHdG and pre-treatment bleeding on probing (r = -0.051, $p = 0.830$) and probing depth (r = -0.135, $p = 0.571$) whereas a positive correlation was seen with clinical attachment levels (r = 0.347, p = 0.134). Similarly, 8 weeks post-operatively, a negative correlation was observed between 8-OHdG and PPD (r = -0.305, $p = 0.192$) and CAL (r = -0.538, $p = 0.014$), whereas a positive correlation was seen with BOP (r = 0.309, $p = 0.185$).

## 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 (Kakimoto *et al.*, 2002). The lethal action of neutrophil-derived radicals is resisted by the antioxidant enzyme possessed by *P. gingivalis*. The ramifications of this process cause host tissue damage that may thus contribute to the destructive process in periodontal disease (Baik *et al.*, 1996; Lee *et al.*, 1999; Sculley and Langley–Evans, 2003).

Higher levels of ROS products are detected in the gingival crevicular fluid (GCF). Collection and analysis of GCF samples require time and effort even though the information is site-specific. Saliva is an easily available fluid that contains host response mediators and microbes and may offer a basis for diagnostic test for periodontitis that is specific to the patient. Saliva contains low molecular antioxidants such as glutathione, ascorbic acid, melatonin and uric acid (Moore *et al.,* 1994; Balaji *et al.,* 2015) along with antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidise (Battino *et al.,* 2002) thereby protecting the oral cavity against ROS. Furthermore, saliva also contains proteases as well as membrane and DNA repair enzymes and is considered to be the first line of defense against free radicals (Amerongen and Veerman, 2002; Battino *et al.,* 2002). Therefore, saliva may be considered as a potentially beneficial diagnostic tool to assess biomarkers for oxidative stress. However, its use as a diagnostic tool may be questioned, as in patients suffering from periodontitis there is a higher probability of blood leakage into saliva. This could lead to the occurrence of blood components in saliva (Schwartz and Granger, 2004) and interfere with various analytical methods, thereby hindering the diagnostic use of saliva. However, at least for some of the most commonly used markers of oxidative stress, it was found that blood contamination up to 1% does not affect even spectrophotometric methods, and samples with higher blood concentrations can easily be excluded (Kamodyová *et al.,* 2015).

Proposed markers for periodontal disease include bacterial products, immunoglobulins and host enzymes (Kaufman and Lamster, 2000). High specificity and sensitivity in periodontal tissue destruction should be exhibited by salivary markers. The indicators for oxidative stress are products obtained from oxidatively damaged macro-molecules. 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 (Kasai and Nishimura, 1984; Dizdaroglu, 1991; Thompson and Wilton, 1991).

Brushing teeth provides mechanical stimulation and helps to eliminate dental plaque, thereby decreasing inflammatory cell infiltration (Horiuchi *et al.*, 2002). Mechanical stimulation aids in resolving inflammation and removing periodontal pathogens, thereby resulting in oxidative stress reduction (Perry *et al.*, 1997). Ekuni *et al.* (2008) reported that oxidative DNA damage triggered by periodontal inflammation is cured by brushing teeth, thereby reducing 8-OHdG levels in the plasma. Therefore, after scaling and root planing, study participants were strongly motivated to brush their teeth and use interdental cleansing aids.

In the present study, the mean value of salivary 8-OHdG in the control group was 1.686 ± 0.148 ng/mL and in the test group was 5.227 ± 1.340 ng/mL. A statistically significant decrease in salivary 8-OHdG levels was seen after scaling and root planing ($p < 0.001$) in the test group with mild periodontitis. These results proved that periodontally diseased subjects had a higher significant level of salivary 8-OHdG than that of the control group. These results are in accordance with the study done by Takane *et al.* (2002). Also, the 8-OHdG levels were significantly higher in the whole saliva of subjects with periodontitis than in subjects with healthy periodontium.

The mean BOP and reduction in PPD showed a significant reduction from baseline to 2 weeks, after 4 weeks and after 8 weeks in the test group. These results were comparable to studies conducted in the past (Takane *et al*., 2002; Takane *et al.,* 2005). There was no significant gain in the CAL from baseline to 2 weeks in the test group. However at the end of 8 weeks, there was a statistically significant gain in CAL. Hence, it can be inferred that the greater gain in CAL in the test group with chronic periodontitis mainly depends upon the better healing at specific sites. These results are similar and consistent with the available literature in that most of the healing seems to be complete at 3 months following initial periodontal therapy (Badersten *et al*., 1984; Chapple, 1997; Takane *et al.,* 2005; Su *et al.,* 2009)

Thus, based on the limited data from this preliminary study, it seems that salivary 8-OHdG level might be used as a marker for assessing the effect of periodontal therapy in systemically healthy subjects. Lastly, since the present study is limited to a small group, further research with a much larger number of patients needs to be elucidated to reach more conclusive results with regard to the relationship between salivary 8-OHdG levels and progression of periodontal disease.

## Acknowledgments

The study was supported by the Indian Council of Medical Research

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