



## The association between a marker of oxidative stress in Saliva and severity of periodontal disease

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### Abstract

**Background:** The aim of this study is to determine the relationship between the level of 8-hydroxydeoxyguanosine (8-OHdG) in saliva and severity of periodontal disease

**Material and method:** Twenty five periodontitis patients were divided into two subgroups according to severity of periodontal disease (fifteen patients with probing pocket depth (PPD) <6mm and ten patients with PPD>6mm). Clinical examination which included measurement of both PPD and bleeding on probing (BOP) were carried out before and after initial periodontal treatment in addition to assessment of 8-OHdG in saliva and gingival crevicular fluid (GCF).

**Result:** Salivary level of 8-OHdG in those with PPD >6mm was higher than those with PPD<6mm. Also this study evaluated 8-OHdG levels in GCF, it was detected only in periodontitis patients with PPD>6mm (mean PPD = 7.34) and only in some cases (4 out of 10).

**Conclusion:** These data suggest that periodontally-involved teeth with deep pockets are a major source of salivary 8-OHdG levels may prove to be useful identifying patients with teeth of hopeless prognosis.

**Keyword:** 8-hydroxydeoxyguanosine, saliva, periodontitis.

### Introduction

Periodontal disease affects individuals at some points during life and it is a major cause of tooth loss in adult <sup>(1)</sup>. The distribution of periodontitis in the population suggests that a subset of individuals is highly susceptible to this infection, while the remaining majority exhibit varying degrees of resistance and moderate susceptibility <sup>(2)</sup>.

Bacterial plaque has been implicated as the primary etiological factor in inflammatory periodontal disease, but recently several studies have focused on the role of the immune system in the evaluation of periodontal disease. These studies including that bacterial antigens trigger an immunopathological reaction and that the susceptibility of the patient

determines the ultimate outcome of the disease processes <sup>(3-5)</sup>

In periodontitis, neutrophils play a central role in the initiation of the host inflammatory response to the periodontal pathogens <sup>(6)</sup>. As a result, oxidative stress is enhanced during periodontitis <sup>(6-11)</sup>. Oxidative stress can result in DNA damage, including oxidation of nucleosides. 8-hydroxydeoxyguanosine (8-OHdG) is an oxidized nucleoside that is excreted in the bodily fluid with DNA repair. Several studies have demonstrated that the 8-OHdG in bodily fluid can act as a biomarker of oxidative stress <sup>(12-15)</sup>, and 8-OHdG is commonly used as a marker to evaluate oxidative damage in disorders including chronic inflammatory disease.

Previously it was demonstrated that the mean level of 8-OHdG in saliva is

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useful marker to screen for periodontal disease <sup>(7)</sup>. Compared with healthy subjects, salivary 8-OHdG level was higher in the presence of periodontitis and they decreased significantly following periodontal treatment. There were no significant correlation between salivary 8-OHdG level and either age, probing pocket depth (PPD) or bleeding on probing (BOP) at base line.

The aim of this study is to determine relationship between 8-OHdG levels in saliva, gingival crevicular fluid (GCF) and severity of periodontal disease.

## Material and Method

The study group (no=25) comprised systemically healthy subjects with chronic periodontitis (i.e. at least 2 sites of PPD >4mm), while the control group (no=15) include individuals with healthy periodontium. The mean age was 48.1 (rang 35-55), they were non smoker and male only.

The clinical examination involved measurements of both PPD and BOP. These measurements were performed at the first appointment and after initial periodontal treatment which included oral hygiene instruction, scaling and root planning.

Before initial periodontal treatment, the study group with chronic periodontitis divided into two subgroups based on the depth of the pocket. Group I with PPD<6mm (no=15) and group II with PPD> 6 mm (no=10), the number of sites per subject were 20.3±0.18.

After the clinical examination paraffin wax stimulated whole saliva was collected and samples were stored at -20 0 c until analysis. A single freeze and thaw process showed no effect on 8-OHdG levels. Samples of GCF were obtained from periodontal pockets after drying the gingival

margin and gently insert a filter paper strip until resistant was felt <sup>(16, 17)</sup>. The strip was left for 30 seconds and immediately immersed in 100 µl of distilled water.

## Quantification of salivary 8-OHdG by enzyme linked immunosorbent assay (ELISA):

Saliva and GCF samples were centrifuged at 10.000 Å~ g for 10 minutes and levels of 8-OHdG in the supernatant were determined using competitive ELISA kit. The determination range was 0.125-200 ng/ml.

## Statistical analysis:

Difference in 8-OHdG levels in saliva samples, PPD and BOP before and after initial periodontal treatment were analyzed using student t-test. Statistical significance

## Results

The mean and standard deviation (SD) of salivary 8-OHdG level in subjects with control group was 1.56±0.1 ng/ml. In periodontitis subjects with pockets >6mm, mean and SD of was 4.42±0.88 ng/ml. This was significantly higher than that in periodontitis subjects with pockets <6mm (2.24±0.06) and control group as shown in table (1).

Table (2) showed that there was significant reduced in PPD and BOP (p<0.05), but no significant difference for the level of 8-OHdG in saliva sample for periodontitis subjects with pockets <6mm before and after periodontal treatment.

There was significant reduction in PPD and BOP before and after periodontal treatment for periodontitis subjects with pockets >6mm.as shown in table (3).Also this table showed that there was highly significant reduction in the level of salivary 8-OHdG before

and after periodontal treatment ( $4.42 \pm 0.03$  and  $1.88 \pm 0.07$  respectively) in those subjects with PPD  $>6$ mm

This study also examined 8-OHdG level in GCF, it has been found that only four out of ten samples were from subjects with advanced periodontal disease with pockets  $>6$ mm (mean PPD 7.34), but not in any sample from subjects with periodontitis with pockets  $<6$ mm (mean 4.87).

## Discussion

This study showed significant reduction in PPD and BOP in all subjects with periodontitis following initial periodontal treatment and this with other studies<sup>(18-20)</sup>

Numerous studies have evaluated the use of virus's host-derived factors in saliva for diagnosis of periodontal disease<sup>(21-24)</sup>. The correlation between 8-OHdG level in saliva and periodontal patients had been previously investigated in chronic periodontitis patients<sup>(7)</sup>. Periodontitis patients with pockets  $>6$ mm had an increased 8-OHdG concentration compared to control and following periodontal treatment, those levels highly significant declined and approached those observed in controls. However no significant difference in reduction of 8-OHdG before and after periodontal treatment for periodontitis patients with pockets  $<6$ mm. This may be due to that saliva antioxidants were significantly lower in the diseased patients. It was proposed that the reduction in the antioxidant capacity was either a direct causal factor in periodontal disease patients or that the reduction was due to reduction in scavenging antioxidants mediated through an increase oxidative stress<sup>(25)</sup>. Another factor may be leakage of GCF into saliva, which is more pronounced in the larger periodontal pockets associated with severe periodontal

disease<sup>(26)</sup>. Also in periodontitis patients with pockets  $>6$ mm the frequency and duration of distractive episodes induced by oxidative stress may be increased<sup>(27)</sup>.

8-OHdG in GCF was not noted in 6 out of 10 from periodontitis patients with through an increase in oxidative stress are likely to be a greater significance in the etiology of periodontitis and associated damage to gum and tooth<sup>(28)</sup>

Obtaining and analysis GCF sample is however a complex process requiring a degree of specialist. This suggests that saliva sampling may be preferable to GCF samples for detection of 8-OHdG. Saliva can be easily collected and therefore measurement of salivary 8-OHdG level may prove to be useful in identifying patients at risk of tooth loss. Moreover salivary analysis for periodontal diagnosis may prove cost-effective method for screening large population.

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Table 1: The level of saliva 8-OHdG in the control group and periodontitis patients

8-OHdG ng/ml	Control	PPD<6mm	PPD>6mm
	1.56±0.1	2.24±0.06	4.424±0.38*

\* P&lt;0.05

Table 2: Changes in the clinical measurement and saliva 8.OHdG in periodontitis patients with PPD &lt; 6mm

	Pre	Post
PPD Mean ±SD	3.12±0.12	2.34±0.16 *
BOP %	40 %	18 % *
8.OHdG ng/ml	2.24±0.06	1.65±0.12

\* P&lt;0.05

Table 3: Changes in the clinical measurement and saliva 8.OHdG in periodontal patients with PPD &gt;6mm

	Pre	Post
PPD Mean ±SD	<b>4.98±0.21</b>	<b>3.32±0.08 *</b>
BOP %	<b>52 %</b>	<b>23 % *</b>
8.OHdG ng/ml	<b>4.42±0.38</b>	<b>1.88±0.07 **</b>

\* P&lt;0.05

\*\* P&lt;0.001

Table 4: Levels of 8.OHdG in gingival crevicular fluid

	PPD>6mm (No. 10)	PPD<6mm (No. 15)
Detection of GCF 8.OHdG (%)	4/10 40%	0/15 0 %
Average of the deepest PPD (mm)	7.34±0.12	4.87±0.22
Average ng/ml	0.45±0.12	0