



ORIGINAL ARTICLE

# Salivary 8-Hydroxydeoxyguanosine – a valuable indicator for oxidative DNA damage in periodontal disease



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## KEYWORDS

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**Abstract** *Background:* 8-Hydroxydeoxyguanosine [8-OHdG] is the most common stable product of oxidative DNA damage caused from reactive oxygen species and has been reported to increase its levels in body fluids and tissues during inflammatory conditions.

*Materials and methods:* This case control study evaluates the salivary levels of 8-Hydroxydeoxyguanosine [8-OHdG] in thirty individuals with clinically healthy periodontium and thirty chronic periodontitis patients. Salivary 8-OHdG levels were evaluated at baseline and one month following initial periodontal therapy. 8-OHdG levels in saliva were investigated by using an enzyme linked immunosorbent assay [ELISA].

*Results:* A significant decrease of 8-OHdG levels after initial periodontal therapy were determined in chronic periodontitis group. The mean 8-OHdG level in the saliva of the CP group was significantly higher than H and CG groups ( $p < 0.001$ ). Statistically significant correlation was only observed between the salivary levels of 8-OHdG and gingival index ( $p < 0.05$ ) in CP group.

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**Conclusion:** A higher salivary 8-OHdG level seems to depict an increased oxidative stress during periodontal disease. Our study indicated that 8-OHdG levels in saliva appear to reflect status of periodontal health.

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## 1. Introduction

Humans live in an oxygen rich environment, and their survival depends on a subtle mitochondrial electric 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 [H<sub>2</sub>O<sub>2</sub>], oxygen superoxide [O<sub>2</sub><sup>-</sup>] or the hydroxyl radical [OH<sup>-</sup>]. These are highly reactive oxygen radicals that are responsible for most oxidative stress on cells.<sup>1</sup> Oxidative stress, occurring as a consequence of imbalance between the oxidant factors and protective antioxidant defense system.<sup>2</sup> Oxidative stress has been implicated as a major contributor in over 100 disorders and more recently periodontitis.<sup>3</sup>

Periodontal disease is an inflammatory disorder in which tissue damage occurs through the complex interactions between periodontal pathogens and components of the host defense mechanism. In periodontitis, neutrophils play a central role in the initial host inflammatory response to the periodontal pathogens.<sup>4</sup>

Moseley et al. has demonstrated that polymorphonuclear neutrophils [PMNs] produce a range of antimicrobial factors which include ROS during phagocytosis of periodontopathic bacteria which can accelerate the oxidative stress during periodontitis.<sup>5</sup> ROS not only play an important role in cell signaling and metabolic processes but are also active in depolymerization of extracellular matrix components, lipid peroxidation, oxidation of enzymes such as antiproteases, induction of proinflammatory cytokines and DNA damage.<sup>6</sup>

Inflammatory periodontal disease resulting in tissue damage are mediated by ROS which are formed during the phagocytosis of periodontopathic bacteria by polymorphonuclear leukocytes. ROS generation can occur through a number of mechanisms such as protein disruption,<sup>33</sup> lipid peroxidation,<sup>34,35</sup> induction of proinflammatory cytokines<sup>36</sup> and DNA damage.<sup>37</sup> Nucleoside derivatives are repair enzymes which remove damaged products in case of DNA damage. Amount of damaged products are remaining are determined by the balance of the rate of damage and repair. 8-Hydroxydeoxyguanosine (8-OHdG) is an oxidized nucleoside that is excreted in the bodily fluids with DNA repair.<sup>38,39</sup> 8-OHdG is a most common stable product of oxidative DNA damage following enzymatic cleavage after ROS induced 8-hydroxylation of guanine base on mitochondrial and nuclear DNA. Studies have demonstrated that the 8-OHdG in body fluids can act as biomarker of oxidative stress and is implicated in the pathogenesis of malignancy, inflammatory and autoimmune disorders.<sup>7</sup> Salivary 8-OHdG levels were intensively studied in several oral pathologies like Sjogren syndrome,<sup>8</sup> oral cancers<sup>9</sup> and periodontitis.<sup>10</sup> Correlation between 8-OHdG and periodontopathic bacteria has been reported and early determination of 8-OHdG in saliva was reported to be a useful biomarkers for assessing periodontal status accurately and for

evaluating the efficacy of periodontal treatment. Possible relationship between salivary levels of 8-OHdG and diseased periodontium were shown in previous studies<sup>27,28,40,21,41,42</sup> but the significance of elevated salivary 8-OHdG in patients with chronic periodontitis was not clear. Hence forth the present study evaluated the 8-OHdG concentrations in saliva of patients with and without chronic periodontitis as well as the values were compared along with changes in clinical parameters following initial periodontal treatment to ascertain the relationship between the same.

## 2. Materials and methods

A total of 60 subjects participated in this case-control study. The study was conducted from September 2011 to November 2011. (Initial screening and phase 1 therapy-subgingival scaling and root planning followed by reevaluation after one month.) Thirty subjects with clinical evidence of chronic periodontitis (cases) and thirty periodontally healthy subjects (control) were recruited from a private dental clinic Trivandrum. The study was approved by Institutional Ethics Committee, Kerala University, India. The study protocol was explained, and written informed consent was obtained from each individual before clinical periodontal examinations and saliva sampling were carried out. Subjects who fulfilled the following inclusion/exclusion criteria were included in the study.

### 2.1. Inclusion criteria

The diagnosis was based on the clinical and radiographic criteria stated and described on the 1999 Consensus Classification of Periodontal Diseases.<sup>43</sup> The CP group was divided into three subgroups according to the classification of disease severity of periodontitis described by Armitage<sup>43</sup> according to the amount of clinical attachment loss (CAL) [slight = 1 or 2 mm CAL, moderate = 3 or 4 mm CAL, and severe = 5 mm CAL].

Chronic periodontitis [CP] for the present study group only consist of patients with clinical attachment level (CAL 1–3 mm) slight to moderate disease severity ( $n = 30$ ).

The healthy control group  $n = 30$  was composed of individuals with no history of periodontal disease had at least 20 teeth, probing depth of < 3 mm and clinical attachment level of ≤ 1 mm in all the teeth examined.

### 2.2. Exclusion criteria

1. History of smoking in the past 6 months (self reported).
2. Subject with known systemic diseases such as diabetes mellitus, rheumatoid arthritis, and renal diseases.
3. Pregnant and lactating women.

4. History of periodontal therapy within the past 6 months.
5. The use of antibiotics, steroids or non-steroidal anti-inflammatory agents in the past 6 months.

### 2.3. Clinical measurements

Periodontal status was identified by measuring plaque index, gingival index, probing depth, clinical attachment level. Probing depth and clinical attachments were performed at six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) on each tooth present except for the third molars, using a Williams's periodontal probe (Hu-Friedy, Chicago, IL, USA). The clinical attachment level was assessed from the cement–enamel junction to the base of the probable pocket. Gingival index were by Loe and Sillness<sup>14</sup> and Plaque accumulation were recorded by Sillness and Loe plaque index.<sup>15</sup> After the periodontal measurements were taken and radiologically supported with oral pantomographs, the patients were divided into two sub groups: Group I – Control (Periodontal healthy (H): the mean of GI < 1, but with no sites of attachment loss.) and Group II -Case (Chronic periodontitis (CP): at least four teeth with a PPD  $\geq$  5 mm, with CAL 1–3 mm (mild to moderate). Dental examinations were conducted by the two experienced clinician throughout the study.

### 2.4. Saliva sampling

Before clinical measurements whole saliva samples were obtained in the morning after an overnight fast, during which subjects were requested not to drink (except water) or chew-gum. Parafin wax stimulated saliva samples were collected by expectoration into polypropylene tubes. The time period for sample collection was recorded in minutes. The collection time was five minutes. The samples were stored at  $-80^{\circ}\text{C}$  until analyzed. Single freeze process was performed.

### 2.5. Biochemical assay

Saliva samples were centrifuged at  $10,000\times g$  for 10 min and the supernatant was used to determine the 8-OHdG levels with a competitive ELISA kit – Cayman Chemical, USA. Levels ranged from 0.125 to 225 ng/ml.

### 2.6. Periodontal treatment

The Group II [Chronic periodontitis] patients received periodontal therapy, which included repeated oral hygiene instructions, full mouth supragingival scaling and subgingival scaling and root planning [SRP]. The instructions included demonstration of appropriate brushing technique and usage of chlorhexidine mouthwash twice daily after brushing. The Supragingival scaling was performed by qualified periodontologist using ultrasonic instrument and SRP using manual Gracey curettes (Hu-Friedy, Avco). The patients were monitored periodically, were followed up after 1 month after SRP and then three months and their clinical parameters were evaluated and saliva samples were collected at baseline prior to full mouth supragingival scaling and one month after SRP.

Subjects in group I received no periodontal treatment during the course of the study and were evaluated only once for their clinical parameters and biochemically to assess salivary 8-OHdG levels.

### 2.7. Statistical analysis

The data was analysed by using SPSS (16.0 version) software. Student's *t* test applied to find the significant between cases and controls. Paired sample *t*-test used to find the significant difference with in the cases. Probability (*P* value) less than 0.05 (<0.05) considered statically significant. The data is expresses in mean  $\pm$  SD. The Pearson rank correlation coefficient was used to determine the relationship between clinical periodontal parameters and salivary8-OHdG levels.

## 3. Results

The clinical parameters of the groups are shown in Table 1. Probing pocket depth [PPD], plaque index [PI], gingival index [GI], clinical attachment level [CAL] of the chronic periodontitis [CP ]were significantly higher than the control group ( $p < 0.001$ ) at baseline. The clinical periodontal measurements before and after periodontal treatment for Group II are presented on Table 2. All clinical parameters were significantly higher in the chronic Periodontitis group [Group II] compared to the control group [Group I] [ $p < 0.05$ ; Table 1]. The mean PI-GI, PD (mm), CAL (mm) values were significantly decreased after initial periodontal treatment.

### 3.1. Levels of salivary 8-OHdG

The mean  $\pm$  SD salivary levels of 8-OHdG were significantly higher in diseased sites [Group-I (Control)  $527.23 \pm 62.19$ , Group-II (Case)  $645.18 \pm 84.91$ ,  $p < 0.05$ ]. Salivary levels of 8-OHdG levels decreased significantly in Chronic Periodontitis group after the initial periodontal treatment [ $532.18 \pm 91.37$ ,  $p < 0.05$ , Table 3 and Fig. 1].

Among the clinical parameters statistically significant positive correlation was obtained only between salivary8-OHdG and gingival index [ $p < 0.05$ ] [Table 4].

## 4. Discussion

A large body of evidence implicates Oxidative Stress in the pathogenesis of periodontal tissue destruction.<sup>1,3,6</sup> Numerous studies indicated that an excess of ROS and depletion of anti-oxidant level in gingival crevicular fluid are responsible for the local activation of periodontal inflammation and tissue destruction.<sup>11–13</sup>

Recruitment of neutrophils at the gingival site and release of proteolytic enzymes and ROS are today considered the two main aspects of the host response upon bacterial antigen stimulation in periodontitis-susceptible individuals. It is widely agreed that, increased generation of ROS may cause toxic effects by oxidative damage of macromolecules, such as proteins, lipids and DNA.<sup>6</sup> Oxidative DNA damage is reported to be involved in the pathogenesis of many chronic conditions, including neurodegenerative disease,<sup>16</sup> diabetes,<sup>17</sup> cancer<sup>18</sup> and chronic inflammatory conditions.<sup>19</sup> However, information on

**Table 1** Comparison of clinical measurements between cases and controls.

Groups	PI (Mean ± SD)	GI (Mean ± SD)	PD (Mean ± SD)	CAL (Mean ± SD)
Group-I (Control)	0.52 ± 0.56	0.41 ± 0.15	1.38 ± 0.46	0.00 ± 0.00
Group-II (Case)	1.47 ± 0.24*	1.39 ± 0.18*	2.47 ± 0.78*	2.59 ± 0.95*

\*  $p < 0.05$  significant compared control group with case group.

**Table 2** Comparison of clinical measurements after one month of treatment in case group.

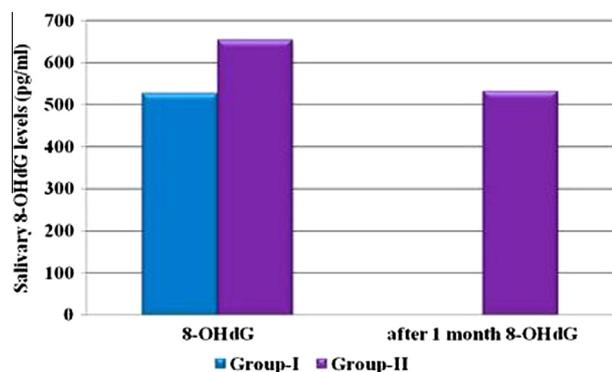
Groups	Base line values (Mean ± SD)				After 1 month of treatment (Mean ± SD)			
	PI	GI	PD (mm)	CAL (mm)	PI	GI	PD (mm)	CAL (mm)
Group-II	1.47 ± 0.24	1.39 ± 0.18	2.47 ± 0.78	2.59 ± 0.95	0.29 ± 0.17*	0.32 ± 0.15*	1.56 ± 0.34*	1.63 ± 0.45*

\*  $p < 0.05$  significant compared clinical parameters before and after one month of treatment.

**Table 3** Comparison of salivary 8-OHdG values between cases and controls.

Groups	Salivary 8-OHdG values (pg/ml) (Mean ± SD)	1 month after initial periodontal treatment salivary 8-OHdG values (pg/ml) (Mean ± SD)
Group-I (Control)	527.23 ± 62.19	527.23 ± 62.19
Group-II (Case)	645.18 ± 84.91*	532.18 ± 91.37*

\*  $p < 0.05$  significant compared salivary 8-OHdG levels between group-I and group-II.

**Figure 1** Comparison of salivary 8-OHdG values between cases and controls.

markers of ROS reactions with DNA in periodontitis is limited. 8-OHdG is currently used as a recognized biomarkers of oxidative-induced DNA damage mainly because of its reliable detectability. When DNA is attacked by oxidative stress such as ROS, UV light genotoxic agents, guanine is easily oxidized into 8-oxo-7,8-dihydroguanine (8-oxo-Gua).<sup>7</sup>

The existence of this oxidized guanine in genomic DNA can cause transversion mutation such as G-T or G-A binding, accumulation of which can lead to detrimental consequences.<sup>29,30</sup> Fortunately, mammalian cells have multiple repair systems such as base excision repair enzymes or nucleotide excision repair (NER) systems, which counteract the hazardous effects of 8-oxo-Gua. Consequently, 8-OHdG, a nucleoside form of 8-oxo-Gua is generated from either damaged oligomer which contains 8-oxo-Gua by nucleotide excision repair (NER) or from cytoplasmic oxidized nucleotides like 8-hydroxy-deoxyguanosine triphosphate.<sup>20</sup>

8-OHdG can cross the cell membrane unlike any other species that contains oxidized guanine, thus it is usually detected in saliva of patients who have diseases associated with oxidative stress.<sup>21</sup> Elevated levels of 8-OHdG from cancer patients compared with healthy subjects have been observed in lung cancer,<sup>22</sup> basal cell carcinoma,<sup>23</sup> colorectal cancer,<sup>24</sup> bladder cancer,<sup>25</sup> renal cell carcinoma.<sup>26</sup> Evidence from these studies suggests that elevated 8-OHdG levels in these malignant or premalignant diseases compared with healthy individuals would be a sign of oxidative stress, impaired antioxidant defense or inadequate repair of oxidatively damaged DNA. However, there are also some reports that have found no

**Table 4** Correlation between the salivary 8-OHdG levels and clinical parameters of periodontitis in Cases at different time periods.

Groups	Baseline				After 1 month of treatment			
	PI	GI	PD	CAL	PI	GI	PD	CAL
8-OHdG-baseline	-0.24	0.13	0.09	0.09	-0.1	0.47	0.28	0.30
$p$ Value	0.18	0.47	0.60	0.62	0.58	0.01*	0.13	0.09

\* Correlation is significant ( $p < 0.05$ ).

difference in the 8-OHdG levels between cancer patients and healthy subjects.<sup>14</sup> Regards to periodontium, majority of published data on oxidative damage to DNA has been reported by a Japanese group who investigated 8-OHdG levels in the saliva of periodontitis patients.<sup>27,28</sup> These studies demonstrated that salivary levels of 8-OHdG in samples from periodontitis patients were significantly higher than those from periodontally healthy controls. Saliva plays a major part in the maintenance of oral mucosal and dental health and changes in the amount and the quality of saliva may alter the oral health status. Saliva is a fluid that can be easily collected, contains locally and systemically derived markers of periodontal disease.<sup>28</sup> Hence forth the present study evaluated the 8-OHdG concentrations in saliva of patients with and without chronic periodontitis as well as the values were compared following periodontal treatment. The clinical measurements and their relationship with 8-OHdG were also examined before and after initial periodontal therapy.

In the present study we found a positive correlation between 8-OHdG levels in saliva of periodontal patients as compared with healthy subjects ( $p < 0.05$ ). Our findings are similar with those reported in the literature.<sup>10,28</sup> Mechanical removal of plaque helps dissolve inflammation and remove periodontal pathogens, which results in reduction of oxidative stress.<sup>10,32</sup> Interestingly, we observed a representative decreased level of 8-OHdG levels in saliva of periodontal patients after treatment and it was positively correlating with the clinical measurements ( $p < 0.05$ ). This result showed that initial periodontal therapy was effective in preventing and decreasing the oxidative DNA damage in periodontal pocket. This study is in agreement with previous studies where salivary 8-OHdG levels were decreased after initial periodontal therapy in chronic periodontitis group.<sup>10,31</sup> In a previous study undertaken by Takane et al.,<sup>28</sup> 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. Even though the disease activity and severity are different terms from each other; they have close relationship for disease progression. If so, 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.

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 according to disease severity. A positive correlation was obtained only with gingival index which can be possibly explained as mild to moderate cp cases were chosen were inflammatory burden was not sufficient enough increase the oxidative stress levels in the selected case group.

The present study suggests the involvement of oxidative stress in the periodontal environment which was reflected in the biological fluid is as a result of inflammatory reaction. Therefore, it has to be concluded that elevated 8-OHdG levels should be taken as evidence of impaired DNA repair as a result of reactive oxygen species. The mechanism behind these results offers an attractive topic for future studies.

## 5. Conclusion

Our studies suggest that for the quantification of oxidative stress biomarkers such as 8-OHdG biological product such as saliva can be worthwhile. Further elevated salivary levels of 8-OHdG may be a marker for disease activity which may reflect indirectly disease severity parameters which aids in the diagnosis and monitorisation of the treatment in periodontal disease. However, further investigations are needed to clarify the role of 8-OHdG for periodontal diagnosis as it can prove to be a cost effective method for screening the population.

## Conflict of interest

The authors have no conflict of interest to declare.

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