

Increased salivary levels of 8-hydroxydeoxyguanosine may be a marker for disease activity for periodontitis

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Abstract. *Background:* 8-hydroxydeoxyguanosine (8-OHdG) is commonly used as a marker to evaluate oxidative DNA damage in disorders including chronic inflammatory diseases such as inflammatory periodontal pathologies. In the current 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 (CAL).

Materials and methods: A cross-sectional study was conducted on a sum of 60 age gender balanced; chronic periodontitis (CP) ($n = 20$), chronic gingivitis (CG) ($n = 20$) and healthy (H) ($n = 20$) individuals. Clinical periodontal parameters and salivary 8-OHdG levels were evaluated.

Results: 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 age ($p < 0.05$), probing depth (PD) and CAL ($p < 0.001$) in CP group. However, when CP patients were classified according to their CAL levels (CAL ≥ 3 mm ($n = 11$) and CAL < 3 mm ($n = 9$)) statistically significant correlation was only observed between the salivary levels of 8-OHdG and CAL ≥ 3 mm patients ($p < 0.001$).

Conclusion: We suggest that elevated salivary levels of 8-OHdG may be a marker for disease activity and it may reflect indirectly disease severity parameters such as CAL.

Keywords: Periodontitis, saliva, 8-hydroxydeoxyguanosine, clinical attachment level, disease activity, oxidative DNA damage

1. Introduction

Periodontitis is defined as an inflammation of the periodontium that extends beyond the gingival tissue and produces destruction of the connective tissue attachment of the teeth [1,2]. Development of periodontal diseases is originated through the complex interactions between periodontopathic bacteria and host defense systems. Exaggerated monocyte and neutrophil activity are consistently reported to be the biological features of the periodontitis phenotype, in harmony with a host response that is “hyper-inflammatory” in nature. Evidences exist for polymorphonuclear cells (PMNs) to be

the primary mediators of the host response against periodontopathic bacteria [3,4]. Thereby the generation of extracellular reactive oxygen species (ROS) predominantly from PMNs is thought to be one of the major factors in periodontal disease pathogenesis [5,6]. Tissue damage in inflammatory periodontal pathologies can be mediated by ROS resulting from the physiological activity of polymorphonuclear leukocytes during phagocytosis of periodontopathic bacteria, and can occur through a number of mechanisms such as protein disruption [7], lipid peroxidation [8,9], induction of proinflammatory cytokines [6] and DNA damage [10].

In cases of DNA damage; damaged products are removed by repair enzymes and they are identified as nucleoside derivatives. Balance of the rate of damage and repair establishes the level of these products. 8-hydroxydeoxyguanosine (8-OHdG) is an oxidized nucleoside that is excreted in the bodily fluids with DNA

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repair [11,12]. Several studies have demonstrated that 8-OHdG in bodily fluids can act as a biomarker of oxidative stress [13–15] and 8-OHdG is commonly used as a marker to evaluate oxidative DNA damage in disorders including chronic inflammatory diseases [16–19]. Previous studies [20–25] indicated a possible relationship between salivary levels of 8-OHdG and diseased periodontium. However, results of these studies did not clearly address an important question the significance of elevated salivary 8-OHdG in patients with periodontal disease.

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. We investigated the salivary 8-OHdG levels in periodontitis patients, gingivitis patients, and healthy controls and explored correlations between these oxidative DNA damage markers and clinical parameters of periodontitis and age.

2. Materials and methods

2.1. Study groups

Those patients who applied for treatment due to periodontal problems or for routine check-ups to the Ataturk University, Faculty of Dentistry, Department of Periodontology were included in this study. All individuals in this study signed an informed consent to a research protocol that had been reviewed and approved by the ethics committee of Ataturk University. A total of 60 patients in age-gender balanced three groups were included in this study. Twenty patients with chronic periodontitis (CP) (10 males and 10 females, age range: 29–58, mean age: $43,00 \pm 7,66$ years (mean \pm SD)), 20 patients with chronic gingivitis (CG) (10 males and 10 females, age range: 29–58, mean age: $42,35 \pm 8,29$ years (mean \pm SD)), and 20 periodontally healthy controls (H) (10 males and 10 females, age range: 29–55, mean age: $40,30 \pm 8,68$ years (mean \pm SD)). Dental examinations were conducted by the same clinician (U.S.). All dental variables were assessed at six different sites (mesio-buccal, mid-buccal, distobuccal, mesio-lingual, mid-lingual and disto-lingual) of each tooth present, excluding wisdom teeth. Clinical measurements of periodontal parameters included plaque index (PI) [26], gingival index (GI) [27], probing pocket depth (PD), clinical attachment level (CAL). All assessments were carried out using the Williams periodontal probe. After the periodontal measurements

were taken and radiologically supported with oral pantomographs, the patients were divided into three groups as periodontal healthy (H), gingivitis (CG) and chronic periodontitis (CP). The diagnosis was based on the clinical and radiographic criteria stated and described on the 1999 Consensus Classification of Periodontal Diseases [2] as follows: Periodontal healthy (H): the mean of $GI < 1$, but with no sites of attachment loss. Gingivitis (CG): at least two teeth with two or more sites with $GI < 1$, but with no sites of attachment loss related to chronic inflammatory periodontal disease. Chronic periodontitis (CP): at least four teeth with a $PPD \geq 5$ mm, with $CAL \geq 2$ mm at the same time [2]. The CP group was divided into two subgroups according to the classification of disease severity of periodontitis described by Armitage [2] according to the amount of clinical attachment loss (CAL) as follows: Slight = 1 or 2 mm CAL, moderate = 3 or 4 mm CAL, and severe = ≥ 5 mm CAL. CP1 group consisted of chronic periodontitis patients with clinical attachment level ($CAL \geq 3$ mm; moderate+severe) (n:11) where CP2 (n:9) group consisted of chronic periodontitis patients with $CAL < 3$ mm (slight). The CG group consisted of patients who had different degrees of inflammation in their gingiva but had no alveolar bone loss. The control group was composed of individuals with no history of any periodontal disease, with no gingival inflammation, and with good oral hygiene. The subjects who participated in the study had no history of systemic disease, and had received no periodontal therapy, taken no antibiotics, anti-inflammatory drugs or any other drugs for at least six months. Patients who had less than 18 teeth in the mouth and current smokers were excluded from the study.

2.2. Collection of saliva samples

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°C until analyzed. Single freeze process was performed.

2.3. Determination of salivary

8-hydroxydeoxyguanosine by enzyme-linked immunosorbent assay (ELISA)

Saliva samples were centrifuged at $10.000 \times g$ for 10 minutes and the supernatant was used to determine

Table 1
Clinical parameters of the groups (mean \pm SD)

| | Healthy group (H) n ^a : 20 | Chronic gingivitis group (CG) n ^b : 20 | Chronic periodontitis group (CP) n ^c : 20 | p |
|---------|--|--|---|---|
| Age | 40.30 \pm 8.68 | 42.35 \pm 8.29 | 43.00 \pm 7.66 | $p > 0.05^{ab}$ $p > 0.05^{ac}$ $p > 0.05^{bc}$ |
| PI | 0.59 \pm 0.19 | 2.00 \pm 0.28 | 2.02 \pm 0.26 | $p < 0.001^{ab*}$ $p < 0.001^{ac*}$ $p > 0.05^{bc}$ |
| GI | 0.57 \pm 0.18 | 2.00 \pm 0.23 | 2.05 \pm 0.17 | $p < 0.001^{ab*}$ $p < 0.001^{ac*}$ $p > 0.05^{bc}$ |
| PD(mm) | 1.96 \pm 0.10 ^a | 3.27 \pm 0.36 ^b | 5.20 \pm 0.29 ^c | $p < 0.001^{ab*}$ $p < 0.001^{ac*}$ $p < 0.001^{bc*}$ |
| CAL(mm) | 0.00 \pm 0.00 ^a | 0.00 \pm 0.00 ^a | 3.98 \pm 0.40 ^b | $p > 0.05^{ab}$ $p < 0.001^{ac*}$ $p < 0.001^{bc*}$ |

^{ab} comparison of mean values between healthy control group (H) and Chronic gingivitis group (CG); ^{ac} comparison of mean values between (H) and Chronic periodontitis (CP) group (CP); ^{bc} comparison of mean values between Chronic gingivitis group (CG); and Chronic periodontitis group (CP).

* $p < 0.001$ Difference is significant.

8-OHdG levels with a competitive ELISA kit (8-OHdG Check, Highly Sensitive 8-OHdG Check, Japan Institute for the Control of Aging, Shizuoka, Japan. The determination range was 0.125 to 200 ng/ml.

2.4. Statistical analyses

Mean \pm SD were used for descriptive statistics. Differences in clinical parameters between groups were analyzed by ANOVA test. The Pearson rank correlation coefficient was used to determine the relationship between clinical periodontal parameters and salivary 8-OHdG levels. All statistical analyses were performed using statistical software (SPSS 16.0 for Windows, SPSS, Chicago, IL, USA).

3. Results

3.1. Clinical findings

The clinical parameters of the groups are shown in Table 1. There was no significant difference between the mean ages of the three groups ($p > 0.05$). PI, GI scores of the CP and CG groups were significantly higher than the control group ($p < 0.001$). However, there was no significant difference between PI and GI scores between CG and CP group. PD scores were significantly different between three groups and there were significant difference between CAL scores of CP

group and the other two groups where there was no statistically significant difference between CAL scores of CG and H groups.

3.2. Laboratory findings

Table 2 shows the mean 8-OHdG levels in the saliva of three groups. The mean 8-OHdG level in the saliva of the periodontally diseased patients in the CP group (3.13 ± 0.22 ng/ml) was significantly higher ($p < 0.001$) than control (1.56 ± 0.12 ng/ml) and CG (1.58 ± 0.13 ng/ml) groups (Fig. 1 shows the distribution of 8-OHdG levels within patient groups). There was no statistically significant difference between CG and H groups ($p > 0.05$).

3.2.1. Correlation of the salivary 8-OHdG levels with clinical parameters in the CP group

We evaluated the relationships between the occurrence of salivary 8-OHdG levels with the clinical parameters (PI, GI, PD, CAL and age) in the CP group. Statistically significant positive correlations were observed between the salivary levels of 8-OHdG and age ($p < 0.05$), PD and CAL. ($p < 0.001$) (Table 3). Figure 2 shows the distribution of the salivary 8-OHdG levels and probing depth levels of periodontitis and gingivitis patients and Fig. 3 shows the distribution of the salivary 8-OHdG levels and clinical attachment levels of periodontitis patients.

Table 2
8-OHdG levels of the groups (mean \pm SD)

| | Healthy group (H) n ^a : 20 | Chronic gingivitis group (CG) n ^b : 20 | Chronic periodontitis group (CP) n ^c : 20 | p |
|----------------|--|--|---|--|
| 8-OHdG (ng/ml) | 1.56 \pm 0.12 | 1.58 \pm 0.13 | 3.13 \pm 0.22 | $p > 0.05$ ^{ab} $p < 0.001$ ^{ac*} $p < 0.001$ ^{bc*} |

^{ab} comparison of mean values between healthy control group (H) and Chronic gingivitis group (CG); ^{ac} comparison of mean values between (H) and Chronic periodontitis group (CP); ^{bc} comparison of mean values between Chronic gingivitis group (CG); and Chronic periodontitis group (CP).

* $p < 0.001$ Difference is significant.

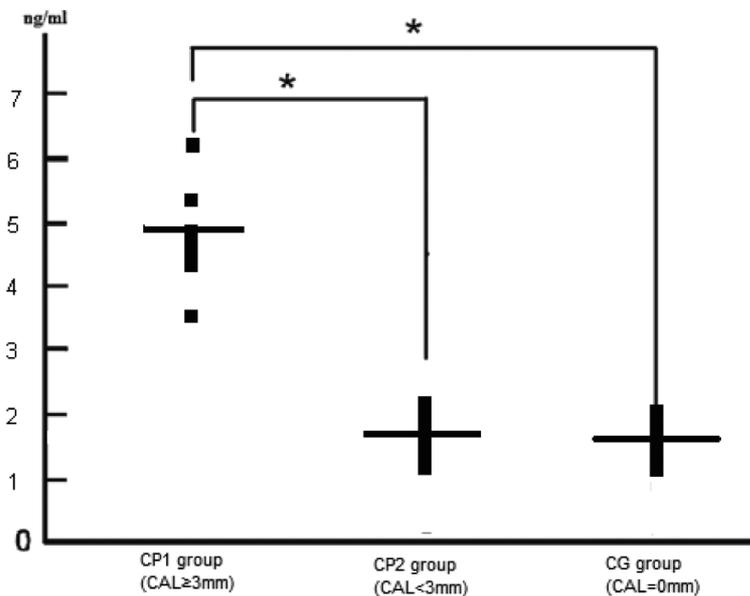


Fig. 1. Distribution of the salivary 8-OHdG levels from Chronic periodontitis patients with $CAL \geq 3$ (CP1) group Chronic periodontitis patients with $CAL < 3$ (CP2) group and chronic gingivitis patients. Difference is significant between CP1 and CP2, CG groups. * $p < 0.001$ Difference is significant.

3.3. Correlation of the salivary 8-OHdG levels with clinical parameters in the CP1 group

Relationships among the occurrence of salivary 8-OHdG levels with the clinical parameters PI, GI, PD, CAL and age) were evaluated in the CP1 group. Statistically significant positive correlation was only observed between the salivary levels of 8-OHdG and $CAL \geq 3$ mm patients ($p < 0.001$). (Table 4).

4. Discussion

The oxidative DNA damages are reported to be involved in the pathogenesis of many chronic conditions, including neurodegenerative disease [28], dia-

Table 3
Correlation between the salivary 8-OHdG level and age, clinical parameters of periodontitis in Chronic periodontitis (CP) group

| | CP | GI | PI | PD | CAL | Age |
|--------|-------|-------|--------------------|--------------------|--------|-----|
| 8-OHdG | 0.119 | 0.122 | 0.814 [†] | 0.843 [†] | 0.495* | |

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

[†]Correlation is significant ($p < 0.001$).

betes [29], cancer [30] and chronic inflammatory conditions [31,32]. However, information on markers of ROS reaction with DNA in periodontitis is limited. Majority of published data on oxidative damage to DNA reported higher 8-OHdG levels in saliva of periodontitis patients [20–25]. Although elevated 8-OHdG levels were detected in Gingival Crevicular Fluid (GCF) [21], the analyses of GCF can offer only site specific infor-

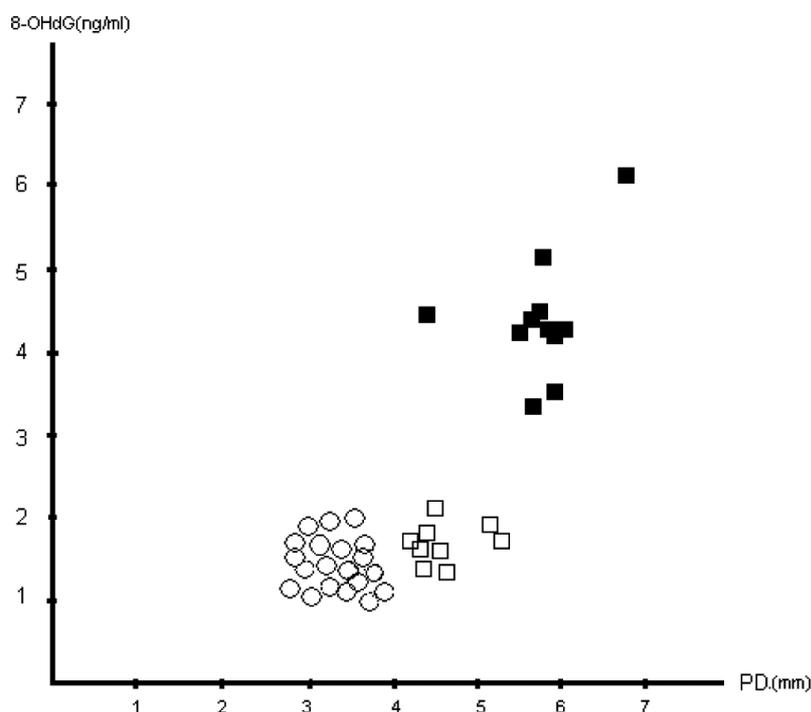


Fig. 2. Distribution of the salivary 8-OHdG levels and probing depth levels of periodontitis and gingivitis patients. The gingivitis group was demonstrated with circles where CAL ≥ 3 mm periodontitis patients were shown with black squares and CAL < 3 were shown with white squares.

mation and its use in routine diagnosis seems not to be in the near future because of the difficulties in collection and analysis of the samples. 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 [33]. Elevated levels of salivary 8-OHdG [20–25] seems to be derived from GCF of the deep pockets of the periodontitis patients.

These studies evaluated the salivary levels of 8-OHdG in samples from only subjects with chronic periodontitis and periodontally healthy controls. The salivary levels of 8-OHdG in gingivitis patients were first evaluated within this study. Although there were slightly elevated levels of salivary levels of 8-OHdG in gingivitis patients compared with healthy individuals, this difference was statistically not significant. This means that inflammation itself is not enough for causing a significant elevation for salivary levels of 8-OHdG.

In an early study, Takane et al. [22] reported that salivary 8-OHdG levels may be a useful marker for pe-

Table 4
Correlation between the salivary 8-OHdG level and age, clinical parameters of periodontitis in Chronic periodontitis patients with CAL ≥ 3 (CPI) group

| | CP1 | GI | PI | PD | CAL | Age |
|--------|-----|-------|-------|-------|--------|-------|
| 8-OHdG | | 0.216 | 0.073 | 0.147 | 0.603* | 0.099 |

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

†Correlation is significant ($p < 0.001$).

riodontitis. On the other hand, we previously found that the increased salivary 8-OHdG level was not detected in all periodontitis patients, but it might signify premature oxidative mtDNA damage of diseased periodontium in patients with periodontitis [23]. In another study carried out by Takane et al. [21], authors found significantly higher salivary 8-OHdG levels in subject with periodontally hopeless teeth than those in subject without periodontally hopeless teeth and those in clinically healthy controls. If so, the increased salivary 8-OHdG level has a strong relation with clinical condition of periodontitis patients.

In this study, we measured salivary 8-OHdG level, clinical periodontal parameters such as clinical attachment level (CAL), probing depth (PD), gingival index (GI) and plaque index (PI), and age in periodontally healthy and diseased individuals. Our study showed that the salivary 8-OHdG levels were significantly high-

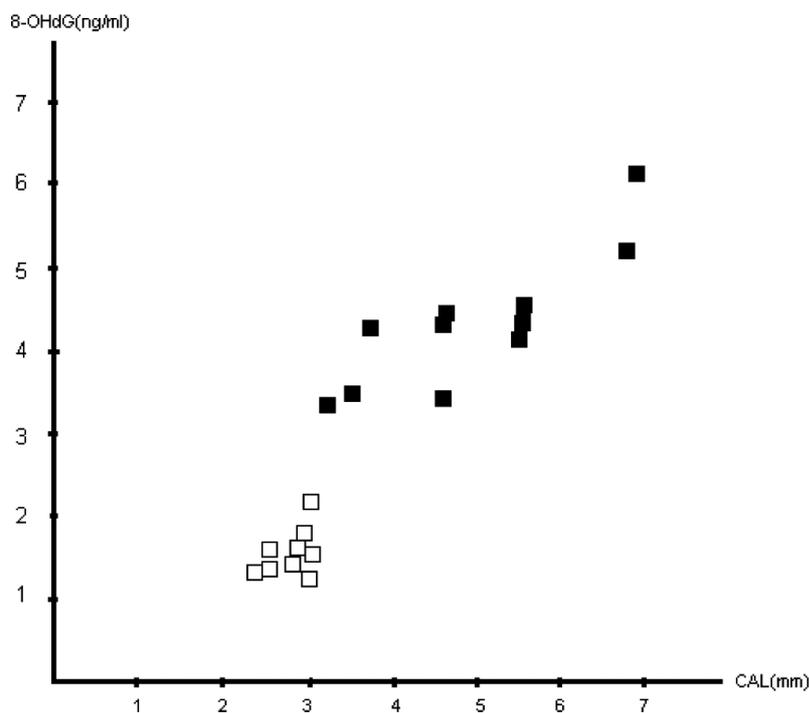


Fig. 3. Distribution of the salivary 8-OHdG levels and CAL of periodontitis patients. $CAL \geq 3$ mm periodontitis patients were shown with black squares and $CAL < 3$ were shown with white squares.

er in periodontitis patients than patients with gingivitis and healthy controls. In addition, we found that both salivary 8-OHdG levels had significant positive correlations with age, PD and CAL, and no significant correlations with PI and GI in periodontitis group. In chronic periodontitis patients these positive correlations between oxidative DNA damage marker (8-OHdG level), age and clinical parameters (PD and CAL) may be related to the duration of periodontitis and indirectly disease severity. This result confirms findings of limited previous studies [20–25]. For the first time in this study, the salivary 8-OHdG levels of periodontitis patients were measured by considering CAL. We found that 8-OHdG levels in patients with $CAL \geq 3$ mm were significantly higher than those of periodontitis patients with $CAL < 3$ mm. Interestingly 8-OHdG levels in periodontitis patients with $CAL < 3$ mm were not statistically different from those of patients with gingivitis and healthy individuals. Also the 8-OHdG levels of patients with $CAL \geq 3$ mm (CP1 group) had statistically significant positive correlation with only CAL, any correlation did not detected in patients with $CAL < 3$ mm (CP2 group) (data not shown).

In periodontitis patients, clinical periodontal parameters (CAL, PD, PI and GI) do not reflect disease activity but show the disease severity. CAL is the most

important and useful parameter for detecting the severity of periodontitis. On the other hand, we previously reported that oxidative DNA damage markers such as 8-OHdG level and DNA deletions may signify disease activity [23]. In a previous study undertaken by Takane et al. [21], 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 which was detected by measured CAL. Some results of this study confirm limited previous studies [20–25], and the findings of oth-

ers may give a different approach about meaning of salivary 8-OHdG in periodontitis patients.

5. Conclusion

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

Conflict of interest

The authors declare that they have no conflict of interest.

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