Original

A marker of oxidative stress in saliva: association with periodontally-involved teeth of a hopeless prognosis

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Abstract: The aim of this study was to determine the association between levels of a marker of oxidative stress, 8-hydroxydeoxyguanosine (8-OHdG), in saliva and the presence of teeth with a hopeless prognosis as a result of advanced periodontitis. Thirty-four periodontitis patients were divided into two groups based on the presence or absence of periodontally-involved teeth of hopeless prognosis. Salivary levels of 8-OHdG in those with were significantly higher than in subjects without periodontally-involved teeth of hopeless prognosis (4.78 ± 0.14 ng/ml and 2.35 ± 0.18 ng/ml, respectively). We also evaluated 8-OHdG levels in gingival crevicular fluid (GCF) of teeth with advanced periodontal destruction (mean probing depth = 7.2). In this case, 8-OHdG was detected only from those periodontally-involved teeth of hopeless prognosis, and only in some cases (8 out of 18 samples). These data suggest that periodontally-involved teeth of hopeless prognosis are a major source of salivary 8-OHdG. Measurement of salivary 8-OHdG levels may prove to be useful in identifying patients with teeth of hopeless prognosis. (J. Oral Sci. 47, 53-57, 2005)

Keywords; 8-hydroxydeoxyguanosine; saliva; periodontally-involved teeth of hopeless prognosis.

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Introduction

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 (1,2). Diagnosis of periodontal disease has been primarily based upon clinical and radiographic measures of periodontal tissue destruction. While these parameters provide a measure of past destruction, they are of limited use in diagnosis (3-5).

In periodontitis, neutrophils play a central role in the initial host inflammatory response to the periodontal pathogens (2). As a result, oxidative stress is enhanced during periodontitis (2,6-11). 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 fluids with DNA repair. Several studies have demonstrated that the 8-OHdG in bodily fluids can act as a biomarker of oxidative stress (12-24) and 8-OHdG is commonly used as a marker to evaluate oxidative damage in disorders including chronic inflammatory diseases. Previously, we have demonstrated that the mean 8-OHdG level in saliva is a useful marker to screen for periodontal disease (6). Compared with healthy subjects, salivary 8-OHdG levels were higher in the presence of periodontitis, and they decreased significantly following periodontal treatment. There were no significant correlations between salivary 8-OHdG levels and either age, probing depth, or bleeding on probing at baseline.

The aim of the current study was to determine the relationship between 8-OHdG levels in saliva and the

presence of periodontally-involved teeth of hopeless prognosis.

Materials and Methods

Study population and saliva samples

The study group (n = 34) comprised systemically healthy subjects with chronic periodontitis (i.e. at least 2 sites of probing depth > 4 mm). The mean age of the study group was 51.1 years (range 21-68 years) and 17 were male. Individuals with a clinically healthy periodontium served as controls (n = 17). The mean age was 34.2 years (range 24-64 years) and 11 were males.

The clinical examination involved measurement of both probing depths (PD) and bleeding on probing (BOP), performed by three experienced examiners who measured the same clinical parameters throughout the study. Clinical measurements were performed at the first appointment and after initial periodontal treatment, which consisted of 2-6 months of oral hygiene instruction, scaling and root planing and extraction of teeth with a hopeless prognosis.

Before initial periodontal treatment, those individuals with periodontitis were divided into two subgroups based on the presence/absence of periodontally involved teeth of hopeless prognosis. Those individuals with periodontally-involved teeth of hopeless prognosis (n = 16) had a mean age 48.2 (range 26-68 years), and 7 were male. The mean number of teeth of hopeless prognosis per subject was 2.38 ± 0.20. In those individuals without teeth of hopeless prognosis (n = 18), the mean age was 53.2 years (range 21-67), and 10 were male.

Teeth were defined as being of hopeless prognosis based on the criteria of Becker et al. (25), whereby 2 of the following requirements needed to be fulfilled; 1) loss of over 75% bone support, 2) PD greater than 8 mm, 3) class β furcation involvement, 4) class β mobility with movement in the mesial, distal, and vertical directions, 5) a poor crown-root ratio, 6) root proximity with minimal interproximal bone, 7) evidence of horizontal bone loss and 8) a history of repeated periodontal abscess formation.

During the clinical examination, paraffin wax-stimulated whole saliva was collected, and samples were stored at -80°C until analyzed. A single freeze and thaw process showed no effect on 8-OHdG levels. Samples of gingival crevicular fluid (GCF) were obtained from periodontal pockets after drying the gingival margin and gently inserting a filter paper strip until resistance was felt. (26,27) The strip was left for 30 seconds and then immediately immersed in 100 µl of distilled water.

The protocol was approved by the Nihon University School of Dentistry Institutional Review Board, and informed consent was obtained from the subjects prior to all saliva collections.

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 a competitive ELISA kit. The determination range was 0.125 to 200 ng/ml.

Statistical analyses

Differences in 8-OHdG levels between groups at baseline were analyzed by the Mann-Whitney's U-test. Differences in 8-OHdG levels in saliva samples, PD, BOP before and after initial periodontal treatment were analyzed using a Student's t-test. All statistical analyses were performed using statistical software. Statistical significance was defined as $P < 0.05$.

Results

The mean salivary 8-OHdG level in subjects with a clinically healthy periodontium was 1.56 ± 0.1 ng/ml. In periodontitis subjects possessing periodontally-involved teeth of hopeless prognosis, the mean 8-OHdG level was 4.78 ± 0.14 ng/ml. This was significantly higher than that in periodontitis subjects without teeth of hopeless prognosis (2.35 ± 0.18 ng/ml) and periodontally healthy controls (Fig. 1).

The mean PD and BOP was significantly reduced following the initial periodontal treatment (Tables 1, 2). A statistically significant decrease in 8-OHdG levels in whole saliva was observed after treatment of subjects with but not those without periodontally involved teeth of hopeless prognosis (Fig. 2).

We also examined 8-OHdG levels in GCF of teeth with advanced periodontal destruction (mean PD = 7.2). Eighteen out of 36 samples were from periodontally-involved teeth of hopeless prognosis. 8-OHdG was detected in 8 out of these 18 samples but not in any sample from subjects with periodontally-involved teeth of better prognosis. The range of 8-OHdG levels in samples from periodontally-involved teeth of hopeless prognosis was 0 - 1.79 ng/ml (Table 3).

Discussion

Numerous studies have evaluated the use of various host-derived factors in saliva for diagnosis of periodontal disease (28-36). The correlation between 8-OHdG levels in saliva, and periodontal health has been previously investigated in adult periodontitis patients (6). Patients with periodontitis had an increased 8-OHdG concentration compared with controls, and, following periodontal
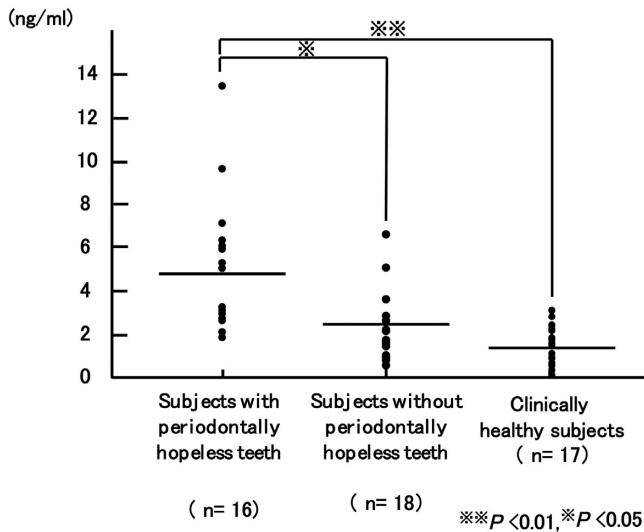
Fig. 1 Distribution of 8-OHdG levels in whole saliva from periodontitis and healthy subjects. The periodontitis subjects were divided into two subgroups, based on the presence/absence of periodontally-involved teeth of hopeless prognosis. Significant difference between groups (*$P < 0.05$, **$P < 0.01$, Mann-Whiteny's U-test).
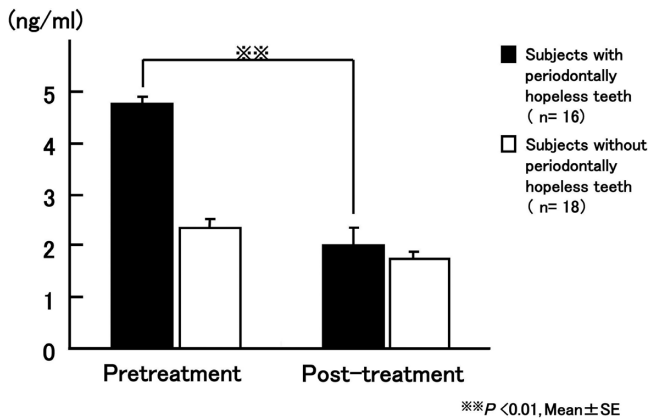

Fig. 2 The effect of initial periodontal treatment on salivary 8-OHdG levels. The Student's t-test was used to identify statistically significant differences between before and after treatment. *$P < 0.05$, **$P < 0.01$.

Table 1 Changes in clinical measurements and salivary 8-OHdG levels of subjects with periodontally-involved teeth of hopeless prognosis

	Pre	Post
8-OHdG (ng/ml)	4.78 ± 0.41	2.02 ± 0.31 **
PD (mm)	3.80 ± 0.12	2.96 ± 0.07 *
BOP (%)	54.0 ± 0.6	21.2 ± 1.6 **

**$P < 0.01$, *$P < 0.05$, n= 16, Mean ± SE

Table 2 Changes in clinical measurements and salivary 8-OHdG levels of subjects without periodontally-involved teeth of hopeless prognosis

	Pre	Post
8-OHdG (ng/ml)	2.35 ± 0.18	1.73 ± 0.16
PD (mm)	3.13 ± 0.08	2.61 ± 0.08 *
BOP (%)	41.4 ± 2.8	18.2 ± 1.1 *

*$P < 0.05$, n = 16, Mean ± SE

Table 3 Levels of 8-OHdG in gingival crevicular fluid

	Periodontally hopeless teeth (n=16)	Better teeth (n=16)
Detection of GCF 8-OHdG(%)	8/18(44%)	0/18(0%)
Average of the deepest PD (mm)	7.22±0.10	7.22±1.11
Average(ng/ml)	0.48±0.04	0

treatment, these levels declined and approached those observed in controls. However, no significant correlations were observed between clinical parameters and salivary 8-OHdG levels. The current results demonstrate a correlation between salivary 8-OHdG levels and periodontally-involved teeth of hopeless prognosis. In addition, 8-OHdG could not be detected in GCF of those periodontally-involved teeth of better prognosis. These data suggest that periodontally-involved teeth of hopeless prognosis are a major source of salivary 8-OHdG. In those

teeth of hopeless prognosis, the frequency and duration of destructive episodes induced by oxidative stress may be increased (37-39).

8-OHdG was not detected in 10 out of 18 samples from periodontally-involved teeth of hopeless prognosis. This suggests that saliva sampling may be preferable to GCF sampling for the detection of 8-OHdG.

Traditional methods for evaluating tooth prognosis involve assessment of clinical parameters (5,40,41). A complete periodontal examination is time consuming and

the tooth prognosis is determined based primarily on the skill and judgment of the dentist. The ability of a method to permit accurate prediction of survival is the ultimate test for any system used in the assessment of prognosis. 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. Further studies are required to determine the relationship between salivary 8-OHdG levels and progression of periodontal disease.

Acknowledgments

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