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Original

Detection of *Streptococcus anginosus* and 8-hydroxy-deoxyguanosine in saliva

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Abstract: Several studies have demonstrated a close association between *Streptococcus (S.) anginosus* infection and head and neck cancer. Accumulation of 8-hydroxy-deoxyguanosine (8-OHdG), which may result from the continuous generation of reactive oxygen species associated with chronic inflammation, has been reported in human preneoplastic lesions and in cancerous tissues. The purpose of the present investigation was to assess the salivary levels of *S. anginosus* and 8-OHdG in patients with periodontitis. Salivary levels of *S. anginosus* were measured by real-time PCR. *S. anginosus* was detected in 28 out of 38 (73.7%) of subjects. The 8-OHdG level was significantly higher in patients positive for *S. anginosus* than in patients negative for the bacterium. A significant decrease in *S. anginosus* and 8-OHdG levels was observed after initial periodontal treatment. Our findings indicate that, although the levels of *S. anginosus* are relatively low, there is a correlation between the salivary level of *S. anginosus* and 8-OHdG, and that periodontal treatment can decrease the levels of these hazard factors. (J. Oral Sci. 45, 181-184, 2003)

Key words: *Streptococcus anginosus*; 8-hydroxy-deoxyguanosine; saliva.

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Introduction

Streptococcus anginosus, one of the oral streptococci, is considered a common commensal organism found in the human oral cavity. Several studies have demonstrated a close association between this organism and head and neck cancer (1-3). Despite its recently increased clinical significance, little information is available on how *S. anginosus* could cause carcinogenesis. Several mechanisms have been proposed by which bacterial infection might lead to predisposition to cancer. Among these mechanisms, DNA damage by oxygen radicals induced by persistent inflammatory cell infiltration may lead to gene alteration and result in the development of cancer (4-9). 8-Hydroxy-deoxyguanosine (8-OHdG) is a product of oxidative DNA damage, arising from specific enzymatic cleavage after 8-hydroxylation of the guanine base. Singlet oxygen, photodynamic action or hydroxyl radicals are responsible for the formation of 8-OHdG. At present, 8-OHdG is one of the most commonly used markers for evaluating oxidative damage. Increases in 8-OHdG levels have been implicated in a number of disorders, including cancer and infections (10-14).

The purpose of the present investigation was to assess the salivary levels of *S. anginosus* and 8-OHdG in patients with periodontitis.

Materials and Methods

Patients and saliva samples

The study group comprised 38 systemically healthy subjects with periodontitis, mean age 48.7 years (range 25-

68 years) (15). The clinical criteria for periodontitis involved standard measurements of clinical pocket depth. Periodontitis patients had at least two sites showing probing depths greater than 4 mm. Saliva samples were taken at the first appointment in all patients and after initial periodontal treatment in 13 periodontitis patients (7 women and 6 men, age range 36-68 years). The 2 to 4 months of initial periodontal treatment consisted mainly of oral hygiene instruction, and scaling and root planing. Paraffin wax-stimulated whole saliva was collected, and the samples were stored at - 80°C until analyzed.

Determination of salivary 8-hydroxy-deoxyguanosine by ELISA

Saliva samples were centrifuged at 10,000 × *g* for 10 min and the supernatant was used to determine 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).

Real-time PCR

The samples were boiled for 10 min and then centrifuged at 10,000 × *g* for 5 min, and 5 μl of the supernatant was used as a template for PCR. Real-time PCR was performed essentially as reported previously (15). Briefly, each reaction tube contained 50 μl of reaction mixture, including 5 μl of sample, 1 × Universal PCR Master Mix (Applied Biosystems; ABI, Foster City, USA), 900 nM each primer and 250 nM probe.

Amplification of total bacterial rDNA was carried out in a separate reaction at the same time under the same conditions as those used for the *S. anginosus*-specific amplification. Data were analyzed using ABI Sequence Detection System software. The number of bacterial cells was determined by using DNA from known amounts of bacteria. *S. anginosus* levels were expressed as a percentage of total bacteria. Only C_T (threshold cycle) values smaller than 36 were considered positive to avoid errors resulting from inaccurate and unreliable quantification values. The detection limit of the real-time PCR assay (C_T = 36) was about 2,000 bacterial cells per ml of saliva (100 copies/reaction, data not shown).

Statistical analyses

Differences in salivary 8-OHdG levels between *S. anginosus*-positive patients and negative patients were analyzed by the Mann-Whitney *U* test. Correlations between salivary levels of *S. anginosus* and 8-OHdG were analyzed by the Spearman rank correlation test. Differences in the salivary levels of *S. anginosus* and 8-OHdG before and after the initial periodontal treatment were analyzed by Student's *t* test. Statistical analyses were performed using SPSS® software (SPSS, Chicago, IL, USA).

Results

PCR analysis detected *S. anginosus* in 28 (73.7%) of the 38 patients with periodontitis. The 8-OHdG level was significantly higher in patients positive for *S. anginosus* than in those negative for the bacterium (Table 1). We searched for correlations between salivary levels of *S. anginosus* and 8-OHdG, but there was no statistically significant relationship (R^2 = 0.00). *S. anginosus* and 8-OHdG levels in whole saliva were compared before and after periodontal treatment in 13 periodontitis patients. A significant decrease in *S. anginosus* and 8-OHdG levels was observed after the treatment (Tables 2 and 3).

Discussion

Accumulation of 8-OHdG, which may result from the continuous generation of reactive oxygen species associated

Table 1 Detection of salivary *S. anginosus* and 8-OHdG in periodontitis patients (N = 38)
All values are expressed as means ± SE. Statistically significant differences by the Mann-Whitney *U* test between positive and negative are indicated by $*P < 0.01$, $**P < 0.05$.

Variable	Positive (N = 28)	Negative (N = 10)
S. anginosus Levels (%)	$0.69 \pm 0.44 \times 10^{-2}$	0*
Number of Total Bacteria / ml	$1.20 \pm 0.22 \times 10^{9}$	$1.12 \pm 0.27 \times 10^{9}$
Age	49.2 ± 2.6	47.5 ± 4.7
8-OHdG (ng/ml)	5.11 ± 1.20	2.42 ± 0.28**

Table 2 Changes in salivary *S. anginosus* levels and 8-OHdG in periodontitis patients (N = 13) before and after treatment
All values are expressed as means ± SE. Statistically significant differences by Student's *t* test between before and after periodontal treatment are indicated by $*P < 0.01$.

Variable	Before	After
S. anginosus Levels (%)	$1.76 \pm 0.46 \times 10^{-2}$	$0.67 \pm 0.24 \times 10^{-2}$*
Number of Total Bacteria/ml	$1.66 \pm 0.58 \times 10^{9}$	$1.78 \pm 0.30 \times 10^{9}$
8-OHdG (ng/ml)	5.06 ± 1.01	2.05 ± 0.51*

Table 3 Salivary *S. anginosus* levels and 8-OHdG in periodontitis patients (N = 13) before and after treatment

	Before		After	
Patient No.	8-OHdG(ng/ml)	*S. anginosus* Levels (10^{-2}%)	8-OHdG(ng/ml)	*S. anginosus* Levels (10^{-2}%)
1	9.65	0.61	3.12	0.18
2	2.59	1.98	2.46	0.75
3	13.40	3.97	6.15	2.13
4	6.65	3.45	1.89	0
5	3.60	0.27	3.35	0.27
6	2.21	3.45	1.71	1.31
7	2.11	1.85	1.57	0.40
8	6.05	0.86	3.07	0.50
9	3.65	0.33	0.45	0.27
10	2.63	0.33	0.55	0
11	5.12	3.70	0.62	2.13
12	1.84	0.12	0.88	0.05
13	6.38	1.74	0.80	0.66

with chronic inflammation, has been reported in human preneoplastic lesions and in cancerous tissues (12,16-19). Research in gastric cancer indicates that DNA damage due to oxygen radicals induced by *Helicobacter pylori*-associated inflammation in the gastric mucosa may lead to genetic alterations and result in the development of carcinoma (10). A recent study has shown that *S. anginosus* can interact with host cells to induce synthesis of nitric oxide and accumulation of inducible nitric oxide synthase (20).

In our study, the mean 8-OHdG level in *S. anginosus*-positive patients was significantly higher than in negative patients. Salivary *S. anginosus* and 8-OHdG levels decreased in response to initial periodontal treatment. Elimination of dental plaque and inflammation may contribute to the decrease in salivary *S. anginosus* and 8-OHdG. Our findings indicate that, although the levels of *S. anginosus* are relatively low, there is a correlation between salivary levels of *S. anginosus* and 8-OHdG, and that periodontal treatment can decrease the levels of these hazard factors. These results also support the hypothesis that oxidative damage due to *S. anginosus* infection could be a driving force that leads from chronic inflammation to head and neck cancer. Further investigation is needed to provide clear evidence for this relationship.

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