The Association of Periodontopathic Bacteria Levels in Saliva and Tongue Coating with Oral Malodor in Periodontitis Patients

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Purpose: To elucidate the association oral malodor with the levels of periodontopathic bacteria in saliva and tongue coating of periodontitis patients with oral malodor.

Materials and Methods: In 25 periodontitis patients, the organoleptic test (OLT) was performed, the levels of volatile sulfur compounds (VSCs) were measured, tongue coating (TC) score was determined, and periodontal parameters and the proportions of *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Treponema denticola, Tannerella forsythia* and *Prevotella intermedia* in the saliva and the tongue coating were evaluated.

Results: The proportions of *T. denticola* and *T. forsythia* in the saliva of patients with an OLT score ≥ 2 were significantly higher than those with OLT scores < 2. The proportion of *P. gingivalis* in the saliva significantly correlated with periodontal parameters, whereas *T. denticola* and *T. forsythia* levels in the tongue coating correlated with VSC levels. However, the five periodontopathic bacteria were found in the tongue coating at levels approximately 12 times lower than in the saliva.

Conclusions: The findings suggest that the levels of *T. denticola* and *T. forsythia* in the saliva of periodontitis patients correlate with oral malodor, and that the prevalence of *P. gingivalis* in the saliva is related to periodontitis. Periodontopathic bacteria in the tongue coating contribute minimally to oral malodor in periodontitis patients.

Key words: oral malodor, periodontopathic bacteria, saliva, tongue coating

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Volatile sulfur compounds (VSCs), such as hydrogen sulphide (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulphide [$(CH_3)_2SH$], are considered to be the principal compounds responsible for oral malodor.[12-14] Periodontal pathogens, including *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia* and *Treponema denticola,* have been implicated as the principal culprits in the production of significant amounts of H_2S and CH_3SH.[7] Previous studies have indicated that VSC levels in breath correlate with the periodontal health status,[13,17,18] and our own group has reported that periodontal status correlates with oral malodor parameters, including the organoleptic test score and VSC levels.[9] These findings strongly suggest that periodontopathic bacteria are associated with oral malodor. Awano et al[1] reported that the proportions of *T. forsythia, P. gingivalis* and *P. intermedia* in saliva influence the concentrations of VSCs in breath, and Tanaka et al[11] showed that periodontopathic bacteria on the dorsum of the tongue may strongly relate to VSC production. Conversely, other studies have refuted the association between periodontopathic bacteria in the tongue coating and either periodontal status or VSC levels in breath.[4] Thus, whether the presence of periodontopathic bacteria in the saliva or tongue coating influences oral malodor and periodontal status remains controversial. The aim of the present study was to determine whether any correlation exists between oral malodor and the levels of periodontopathic bacteria in the saliva and tongue coating in chronic periodontitis patients.

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Table 1 Oligonucleotide primers and probes

Primer or probe	Sequence
Primers	
Porphyromonas gingivalis-F	5'-TACCCATCGTCGCCTTGGT-3'
Porphyromonas gingivalis-R	5'-CGGACTAAAACCGCATACACTTG-3'
Aggregatibacter actinomycetemcomitans-F	5'-CAGCATCTGCGATCCCTGTA-3'
Aggregatibacter actinomycetemcomitans-R	5'-TCAGCCCTTTGTCTTTCCTAGGT-3'
Tannerella forsythia-F	5'-ATCCTGGCTCAGGATGAACG-3'
Tannerella forsythia-R	5'-TACGCATACCCATCCGCAA-3'
Treponema denticola-F	5'-AGAGCAAGCTCTCCCTTACCGT-3'
Treponema denticola-R	5'-TAAGGGCGGCTTGAAATAATGA-3'
Prevotella intermedia-F	5'-TGTCGGTTTACTGGCTATGTTCTC-3'
Prevotella intermedia-R	5'-CTTGTCTGTTGGCCATCTTGAAG-3-'
Fluorescent probes	
Porphyromonas gingivalis	5'-FAM-GCTAATGGGACGCATGCCTATCTTACAGCT-TAMRA-3'
Aggregatibacter actinomycetemcomitans	5'-FAM-TCGAGTATTCCTCAAGCATTCTCGCACG-TAMRA-3'
Tannerella forsythia	5'-FAM-ATGTAACCTGCCCGCAACAGAGGGATAAC-TAMRA-3'
Treponema denticola	5'-FAM-CAGCGTTCGTTCTGAGCCAGGATCA-TAMURA-3'
Prevotella intermedia	5'-FAM-TCAAAGACGCACGTACCAATCCAGACC-TAMRA-3'

MATERIALS AND METHODS

Subjects

Between September 2008 and November 2010, 25 periodontitis patients (16 women and 9 men) who visited Kagoshima University Medical and Dental Hospital for dental treatment were enrolled in the study. Informed consent was obtained from all patients, a condition of permission for this study granted by the Ethics Committee of Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan. The patients had at least one tooth with a probing pocket depth ≥4 mm and alveolar bone loss based on full-mouth radiographs. Patients who had received antibiotic treatments within the previous 3 months or had any systemic disease that might influence oral malodor were excluded.

Oral Malodor Measurement

Each subject was requested to abstain from eating and drinking, performing oral hygiene, smoking and using scented cosmetics for 3 h before oral malodor measurement. Oral malodor measurement was carried out as described previously.[9] The organoleptic test (OLT) was performed by three dentists who directly smelled expelled breath from a distance of approximately 10 cm. OLT scoring was recorded on a scale of 0–5 as follows; 0: absence of odor; 1: questionable odor, 2: slight odor; 3: moderate odor; 4: strong odor, 5: severe odor. VSC levels were measured with a gas chromatograph (GC-14B, Shimadzu; Kyoto, Japan) equipped with a flame photometric detector. Five ml of breath was collected with a gas-tight syringe. The samples were injected into the gas chromatography column at 70°C. A Teflon column was packed with 5% five-ring polyphenyl ether on 80–100 mesh Uniport HP (GL Sciences; Tokyo, Japan).

Clinical Examination

Tongue coating (TC) was measured as a score of 0–4 as follows; 0: absence of tongue coating; 1: thin tongue coating covering 1/3 of tongue dorsum; 2: thin tongue coating covering 2/3 of the tongue dorsum or thick tongue coating covering 1/3 of tongue dorsum; 3: thin tongue coating covering more than 2/3 of the tongue dorsum or thick tongue coating covering 2/3 of tongue dorsum; 4: thick tongue coating covering more than 2/3 of tongue dorsum. TC score measuring was carried out by three dentists.

Probing pocket depth (PPD) and bleeding on probing (BoP) were evaluated using a Williams probe at six points around all teeth. Periodontal status was classified as the percentage of teeth characterised by PPD ≥ 4 mm (PPD%). The gingival index (GI)[5] was used to quantify gingival inflammation, and the Turesky-Gilmore-Glickman modified plaque index (PII)[15] was used to quantify adherent dental plaque.

Periodontal Treatment and Tongue Cleaning

Thirteen of the 25 patients underwent periodontal treatment, including of oral hygiene instruction, scaling and root planing, and received tongue cleaning. Tongue cleaning with a tongue brush was performed to clean the posterior tongue following the protocol described by Yaegaki et al.[16] Briefly, patients were instructed in a tongue brushing technique which ensured that the brush was moved from the terminal sulcus to the front of the tongue, that the tonsils were avoided, and that infection of the respiratory system was not precipitated. Oral malodor parameters, periodontal parameters, and detection of the proportion of periodontopathic bacteria in the saliva and tongue coating were performed both before (baseline) and after treatment.

Sampling and Quantitative Analysis of Tongue Coating and Saliva

Tongue coating samples were collected from the circumvallate papilla area using sterile swabs (BD BBL CultureSwab EZ, Becton Dickinson; Franklin Lakes, NJ, USA). Subjects were asked to chew gum for 3 min and stimulated saliva samples were collected in sterile plastic tubes. Periodontopathic bacteria (including *P. gingivalis, Aggregatibacter actinomycetemcomitans, T. denticola, T. forsythia* and *P. intermedia*) in these samples were quantified by real-time PCR (Oral Check Centre, GC; Tokyo, Japan) using the species-specific primers and TaqMan probes listed in Table 1.

Statistical Analysis

Data are expressed as mean ± standard deviation. Features of the proportions of periodontopathic bacteria at baseline were analysed using the Mann-Whitney U-test, and any correlation between clinical parameters and the proportions of periodontopathic bacteria at baseline was determined using Pearson's correlation. Changes in the proportions or populations of periodontopathic bacteria as a consequence of treatment were evaluated using the Wilcoxon signed-rank test.

RESULTS

The amount of the five periodontopathic bacterial species (*P. gingivalis, A. actinomycetemcomitans, T. denticola, T. forsythia* and *P. intermedia*) measured in this study was approximately 12 times lower in the tongue coating than in the saliva (Table 2). Subjects were subclassified according to OLT score (< 2 or ≥ 2), VSC levels (< 200 or ≥ 200 ppb) and TC score (< 2 or ≥ 2) at baseline. Table 3 shows the proportions of each bacterial species in these subclasses. In the saliva, the proportions of *T. denticola* and *T. forsythia* were significantly higher in patients with an OLT score ≥ 2 than those with an OLT score < 2. The total number of bacteria was significantly greater in the group with a TC score ≥ 2 than in the group with a TC score < 2. Conversely, in the tongue coating, there was no significant difference in bacteria levels between the respective subclasses of OLT, VSC levels, or TC scores.

Table 2 Characteristics of the subjects at baseline, n = 25

Characteristic	
Gender	n
Women (n)	16
Men (n)	9
Total (n)	25
Age (years)	56.52 (12.70)
H_2S (ppb)	344.76 (458.01)
CH_3SH (ppb)	150.01 (278.12)
$(CH_3)_2SH$ (ppb)	20.01 (23.12)
VSCs (ppb)	514.78 (712.25)
OLT score	2.29 (1.19)
TC score	2.26 (0.83)
PPD (mm)	2.86 (0.63)
PPD%	17.50 (18.89)
BoP (%)	29.08 (26.24)
GI	1.09 (0.59)
PII	1.43 (0.75)
Bacteria in saliva	
Pg %	3.33E-02 (5.15E-02)
Aa %	5.36E-04 (1.52E-03)
Td %	4.24E-02 (1.48E-01)
Tf %	8.98E-03 (3.23E-02)
Pi %	1.24E-02 (3.54E-02)
5 bacteria %	9.76E-02 (2.14E-01)
Total bacteria (cells/ml)	6.86E+10 (5.45E+10)
Bacteria in tongue coating	
Pg %	6.50E-03 (1.91E-02)
Aa %	3.88E-04 (1.05E-04)
Td %	6.46E-04 (9.56E-04)
Tf %	2.30E-04 (4.30E-04)
Pi %	4.82E-04 (1.76E-03)
5 bacteria %	8.24E-03 (2.11E-02)
Total bacteria (cells/mg)	7.24E+08 (8.39E+08)

Values represent mean ± standard deviation. Significant difference between OLT scores < 2 or ≥ 2, VSCs levels < 200 ppb or ≥ 200 ppb, TC scores < 2 or ≥ 2 (*p < 0.05).

Table 3 Characteristics of the proportions of periodontopathic bacteria: organoleptic test (OLT) score, volatile sulfur compounds (VSCs) levels and tongue coating (TC) score at baseline (N = 25)

		Saliva						
		Pg %	Aa %	Td %	Tf %	Pi %	5 bacteria %	Total bacteria (cell)
OLT score	< 2 (n = 9)	3.96E-02 (6.61E-02)	ND	1.48E-03 (2.49E-03)	1.35E-03 (2.52E-03)	2.76E-03 (8.26E-03)	4.52E-02 (6.95E-02)	4.61E+10 (2.70E+10)
	≥ 2 (n = 16)	2.98E-02 (4.34E-02)	8.37E-04 (1.85E-03)	6.55E-02 1.83E-01)*	1.33E-02 (4.02E-02)*	1.78E-02 (4.33E-02)	1.27E-01 (2.61E-01)	8.12E+10 (6.24E+10)
VSCs	< 200 ppb (n = 14)	2.90E-02 (5.48E-02)	7.08E-04 (1.85E-03)	6.80E-02 (1.97E-01)	1.39E-02 (4.31E-02)	1.80E-02 (4.59E-02)	1.30E-01 (2.80E-01)	6.17E+10 (5.14E+10)
	≥ 200 ppb (n = 11)	3.87E-02 (4.91E-02)	3.16E-04 1.00E-03)	9.85E-03 (1.48E-02)	2.71E-03 (3.47E-03)	5.23E-03 (1.26E-02)	5.68E-02 (6.78E-02)	7.72E+10 (5.95E+10)
TC score	< 2 (n = 7)	1.07E-02 (1.40E-02)	ND	2.90E-02 (7.57E-02)	1.86E-03 (3.77E-03)	3.99E-03 (1.05E-02)	4.56E-02 (8.91E-02)	3.09E+10 (1.45E+10)
	≥ 2 (n = 18)	4.21E-02 (5.82E-02)	7.44E-04 (1.76E-03)	4.76E-02 (1.70E-01	1.17E-02 (3.79E-02)	1.56E-02 (4.11E-02)	1.18E-01 (2.45E-01)	8.32E+10 (5.75E+10)*

		Tongue coating						
		Pg %	Aa %	Td %	Tf %	Pi %	5 bacteria %	Total bacteria
OLT score	< 2 (n = 9)	1.17E-02 (2.96E-02)	4.50E-04 (1.35E-03)	2.45E-04 (4.07E-04)	2.31E-04 (3.70E-04)	4.58E-04 (1.37E-03)	1.31E-03 (3.09E-02)	8.23E+08 (9.99E+08)
	≥ 2 (n = 16)	3.56E-03 (9.63E-03)	3.53E-04 (8.80E-04)	8.71E-04 (1.11E-03)	2.30E-04 (4.73E-04)	4.96E-04 (1.98E-03)	5.51E-03 (1.34E-02)	6.69E+08 (7.64E+08)
VSCs	< 200 ppb (n = 14)	7.70E-03 (2.39E-02)	4.47E-04 (1.13E-03)	4.63E-04 (8.03E-04)	1.13E-04 (2.16E-04)	2.94E-04 (1.10E-03)	9.02E-03 (2.49E-02)	9.49E+08 (9.56E+08)
	≥ 200 ppb (n = 11)	4.97E-03 (1.15E-02)	3.12E-04 (9.84E-04)	8.79E-04 (1.12E-03)	3.79E-04 (5.83E-04)	7.21E-04 (2.39E-03)	7.26E-03 (1.60E-02)	4.39E+08 (5.83E+08)
TC score	< 2 (n = 7)	1.30E-02 (3.39E-02)	5.79E-04 (1.53E-03)	5.53E-04 (1.04E-03)	2.17E-04 (3.37E-04)	ND	1.44E-02 (3.51E-02)	4.25E+08 (4.29E+08)
	≥ 2 (n = 18)	3.97E-03 (9.35E-03)	3.14E-04 (8.34E-04)	6.82E-04 (9.50E-04)	2.35E-04 (4.70E-04)	6.69E-04 (2.05E-03)	5.87E-03 (1.30E-02)	8.41E+08 (9.36E+08)

Correlation analysis between clinical parameters and bacteria levels at baseline is shown in Table 4. The proportion of *P. gingivalis* in the saliva significantly correlated with CH_3SH levels, TC score, PPD, PPD%, BoP and PII, whereas the proportion of *P. gingivalis* in the tongue coating correlated only with BoP. The proportion of *T. forsythia* in the tongue coating significantly correlated with the levels of H_2S, CH_3SH, $(CH_3)_2SH$ and total VSCs, and the proportion of *T. denticola* in the tongue coating significantly correlated with CH_3SH levels. Moreover, the proportions of *T. denticola, T. forsythia,* and *P. intermedia* in the tongue coating were each significantly correlated with some or all of the periodontal parameters.

Thirteen patients received tongue cleaning education and professional periodontal treatment. After the treatment, mean VSC levels, OLT score, TC score and PPD% all de-

Table 4 Correlation analysis between clinical parameters and the proportion of periodontopathic bacteria at baseline (n = 25)

	Saliva						
	Pg %	Aa %	Td %	Tf %	Pi %	5 bacteria %	Total bacteria
H_2S	0.25	-0.11	-0.10	-0.08	-0.07	-0.03	-0.03
CH_3SH	0.43*	0.19	-0.09	-0.07	0.05	0.04	-0.19
$(CH_3)_2SH$	0.07	0.06	-0.18	-0.14	-0.16	-0.15	-0.05
VSCs	0.33	0.01	-0.10	-0.08	-0.03	-0.01	-0.09
OLT score	0.13	0.12	0.08	0.09	0.17	0.13	0.24
TC score	0.41*	0.22	-0.04	0.02	0.06	0.09	0.31
PPD	0.62**	0.22	-0.08	-0.08	0.10	0.10	-0.13
PPD %	0.63**	0.17	-0.06	-0.06	0.14	0.13	-0.15
BoP	0.49*	0.18	-0.06	-0.07	0.08	0.08	-0.11
GI	0.33	0.27	-0.08	-0.03	0.05	0.03	0.05
PII	0.44*	-0.02	-0.04	0.01	0.03	0.08	0.10

	Tongue coating						
	Pg %	Aa %	Td %	Tf %	Pi %	5 bacteria %	Total bacteria
H_2S	-0.02	-0.04	0.20	0.50*	0.10	0.01	-0.24
CH_3SH	0.16	0.29	0.56**	0.74**	0.54**	0.25	-0.22
$(CH_3)_2SH$	-0.12	-0.11	0.11	0.53**	0.07	-0.09	-0.16
VSCs	0.05	0.09	0.35	0.63**	0.28	0.10	-0.25
OLT score	-0.05	0.09	0.37	0.31	0.30	0.01	-0.18
TC score	-0.13	-0.03	0.10	0.15	0.30	-0.09	0.29
PPD	0.30	0.39	0.49*	0.63**	0.69**	0.38	-0.03
PPD %	0.28	0.32	0.54**	0.76**	0.79**	0.37	-0.03
BoP	0.43*	0.33	0.36	0.60**	0.62**	0.48*	-0.14
GI	0.31	0.29	0.12	0.24	0.37	0.34	-0.17
PII	-0.05	-0.12	0.03	0.25	0.25	-0.02	0.23

*$p < 0.05$, **$p < 0.01$.

creased (from 785.55 ppb to 209.98 ppb; 2.68 to 1.59; 2.65 to 1.74; and 26.85% to 8.48%, respectively), as did the proportions of periodontopathic bacteria (Table 5), although this latter change was not significant. The numbers of *P. gingivalis, T. denticola, T. forsythia* and total bacteria in the saliva, and of *T. denticola, T. forsythia* and total bacteria in the tongue coating, significantly decreased after treatment.

DISCUSSION

It was demonstrated that subjects with an OLT score of ≥ 2 had significantly greater proportions of *T. denticola* and *T. forsythia* in their saliva than those with an OLT score ≥ 2 (Table 2). Kurata et al[4] observed no significant difference in the proportions of *P. gingivalis, T. denticola, T. forsythia, P. intermedia* and *Prevotella nigrescens* in saliva in peri-

Table 5 Changes of the proportions (A) and populations of periodontopathic bacteria (B) from baseline to after treatment (n = 13)

(A) Proportions of periodontopathic bacteria							
		Pg %	Aa %	Td %	Tf %	Pi %	5 bacteria %
Saliva	Baseline	5.39E-02 (6.44E-02)	5.56E-04 (1.36E-03)	6.28E-02 (2.00E-01)	1.57E-02 (4.45E-02)	1.96E-02 (4.78E-02)	1.53E-01 (2.83E-01)
	Post-treatment	4.21E-02 (7.01E-02)	1.37E-03 (4.42E-03)	3.72E-03 (6.11E-03)	2.19E-03 (2.74E-03)	8.39E-03 (2.01E-02)	5.77E-02 (8.64E-02)
Tongue coating	Baseline	5.47E-03 (1.07E-02)	3.09E-04 (9.09E-04)	8.50E-04 (1.06E-03)	3.24E-04 (5.32E-04)	9.27E-04 (2.39E-03)	7.88E-03 (1.50E-02)
	Post-treatment	3.28E-03 (4.14E-03)	4.33E-05 (1.56E-04)	7.11E-04 (2.28E-03)	7.73E-05 (1.09E-04)	2.05E-04 (7.40E-04)	4.31E-03 (6.12E-03)

(B) Populations of periodontopathic bacteria								
		Pg	Aa	Td	Tf	Pi	5 bacteria	Total bacteria
Saliva (cells/ml)	Baseline	2.63E+07 (3.29E+07)	8.09E+04 (2.69E+05)	4.15E+06 (5.72E+06)	1.74E+06 (1.55E+06)	2.52E+06 (5.96E+06)	3.48E+07 (3.48E+07)	6.04E+10 (4.71E+10)
	Post-treatment	1.29E+07 (2.84E+07)*	4.85E+05 (1.73E+06)	1.02E+06 (2.10E+06)*	7.06E+05 (1.0w5E+06)**	1.41E+06 (5.02E+06)	1.65E+07 (3.35E+07)*	1.94E+10 (1.85E+10)**
Tongue coating (cells/mg)	Baseline	2.12E+04 (3.34E+04)	8.78E+02 (2.64E+03)	3.49E+03 (4.54E+03)	1.61E+03 (2.47E+03)	3.38E+03 (8.27E+03)	3.06E+04 (4.61E+04)	7.91E+08 (9.18E+08)
	Post-treatment	2.68E+03 (2.82E+03)	1.38E+02 (4.99E+02)	6.75E+02 (1.74E+03)*	7.85E+01 (1.11E+02)**	1.54E+02 (5.55E+02)	3.72E+03 (4.60E+03)*	1.24E+08 (9.92E+07)**

Values represent mean ± standard deviation. Significant difference between baseline and post-treatment values: *p < 0.05, **p < 0.01.

odontitis patients with or without oral malodor, and subclassified these patients based on their VSC levels. On the other hand, the present study showed that there was no significant difference between the proportions of *T. denticola* and *T. forsythia* in the saliva of patients with VSC levels ≥ 200 ppb and those with VSC levels < 200 ppb. Therefore, it is suggested that *T. denticola* and *T. forsythia* in saliva are related to OLT score.

The proportion of *P. gingivalis* in the saliva significantly correlated with CH_3SH levels, TC score, and periodontal parameters. *P. gingivalis* is a gram-negative anaerobic rod that has been recognised as a major pathogen in periodontitis[2] and produces significant amounts of CH_3SH.[7] CH_3SH in breath is positively correlated with the degree of periodontal disease,[18] so the proportion of *P. gingivalis* in the saliva may be an indicator of periodontal status.

There was no significant difference in the proportions of periodontopathic bacteria in the tongue coating between the OLT subgroups, VSC subgroups, or TC score subgroups. This absence of difference may be due to the low proportions of the five periodontopathic bacteria in the tongue coating – there were 12 times fewer bacteria in the tongue coating than in the saliva. Kazor et al[3] showed that among the bacteria in the tongue coating of subjects with oral malodor, the species most related to oral malodor are *Atopobium parvulum*, a phylotype of *Dialister*, *Eubacterium sulci, Solobacterium moorei,* and a phylotype of *Streptococcus*. However, in the present study, the proportions of *T. denticola* and *T. forsythia* in the tongue coating were significantly correlated with VSC levels and PPD, although not with OLT score. Tanaka et al[10] found that the proportion of periodontopathic bacteria in the tongue coating is strongly correlated with VSC levels, but only weakly correlated with OLT scores, suggesting that periodontopathic bacteria in the tongue coating contribute more significantly to VSC production. This agrees with our conclusion that the proportions of

T. denticola and *T. forsythia* in tongue coating may be related to VSC levels rather than OLT score.

Kurata et al[4] reported that the proportions of *P. gingivalis, T. denticola,* and *T. forsythia* in the saliva were significantly reduced after periodontal treatment in periodontitis patients with oral malodor, and suggested that the prevalence of *P. gingivalis, T. denticola* and *T. forsythia* in saliva could be associated with oral malodor and periodontal health status. In the present study, tongue cleaning and periodontal treatment induced no significant decrease in the proportions of five periodontopathic bacteria in the saliva, although clinical parameters were improved. However, the absolute numbers of *P. gingivalis, T. denticola,* and *T. forsythia* in the saliva were significantly decreased, and the proportions of *T. denticola* and *T. forsythia* tended to decrease after tongue cleaning and periodontal treatment ($p = 0.074$ and 0.087, respectively). Thus, the results suggest that the levels of *T. denticola* and *T. forsythia* in the saliva may be associated with oral malodor in periodontitis patients.

Pham et al[8] used scores from a N-benzoyl-DL-arginine-2-napthilamide (BANA) test (which reflects the presence of *P. gingivalis, T. denticola* or T. forsythia[6]) to show that these bacteria in the tongue coating of periodontitis patients were significantly reduced by periodontal treatment. The current data also show decreased numbers of *T. denticola* and *T. forsythia* in the tongue coating after tongue cleaning and periodontal treatment. However, the levels of the five periodontopathic bacteria in the tongue coating were comparatively low and, although correlated to VSC levels, the proportions of *T. denticola* and *T. forsythia* in the tongue coating did not correlate with the OLT score. Thus, periodontopathic bacteria in the tongue coating may not contribute directly or significantly to oral malodor in periodontitis patients. Further studies with larger sample sizes may be necessary to quantify the contribution of these bacteria to oral malodor.

CONCLUSION

The present findings suggest that the levels of *T. denticola* and *T. forsythia* in the saliva of periodontitis patients correlate with the existence of oral malodor, and that the prevalence of *P. gingivalis* in the saliva of these patients is related to the severity of their periodontitis. However, periodontopathic bacteria in the tongue coating only contribute minimally to oral malodor in periodontitis patients.

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REFERENCES

1. Awano S, Gohara K, Kurihara E, Ansai T, Takehara T. The relationship between the presence of periodontopathogenic bacteria in saliva and halitosis. Int Dent J 2002;52(suppl 3):212–216.
2. Darveau RP, Tanner A, Page RC. The microbial challenge in periodontitis. Periodontol 2000 1997;14:12–32.
3. Kazor CE, Mitchell PM, Lee AM, Stokes LN, Loesche WJ, Dewhirst FE, et al. Diversity of bacterial populations on the tongue dorsa of patients with halitosis and healthy patients. J Clin Microbiol 2003;41:558–563.
4. Kurata H, Awano S, Yoshida A, Ansai T, Takehara T. The prevalence of periodontopathogenic bacteria in saliva is linked to periodontal health status and oral malodour. J Med Microbiol 2008;57:636–642.
5. Loe H. The Gingival Index, the Plaque Index and the Retention Index Systems. J Periodontol 1967;38(suppl):610–616.
6. Loesche WJ, Kazor CE, Taylor GW. The optimization of the BANA test as a screening instrument for gingivitis among subjects seeking dental treatment. J Clin Periodontol 1997;24:718–726.
7. Persson S, Edlund MB, Claesson R, Carlsson J. The formation of hydrogen sulfide and methyl mercaptan by oral bacteria. Oral Microbiol Immunol 1990;5:195–201.
8. Pham TA, Ueno M, Zaitsu T, Takehara S, Shinada K, Lam PH, et al. Clinical trial of oral malodor treatment in patients with periodontal diseases. J Periodontal Res 2011;46:722–729.
9. Takeuchi H, Machigashira M, Yamashita D, Kozono S, Nakajima Y, Miyamoto M, et al. The association of periodontal disease with oral malodour in a Japanese population. Oral Dis 2010;16:702–706.
10. Tanaka M, Anguri H, Nishida N, Ojima M, Nagata H, Shizukuishi S. Reliability of clinical parameters for predicting the outcome of oral malodor treatment. J Dent Res 2003;82:518–522.
11. Tanaka M, Yamamoto Y, Kuboniwa M, Nonaka A, Nishida N, Maeda K, et al. Contribution of periodontal pathogens on tongue dorsa analyzed with real-time PCR to oral malodor. Microbes Infect 2004;6:1078–1083.
12. Tonzetich J. Direct gas chromatographic analysis of sulphur compounds in mouth air in man. Arch Oral Biol 1971;16:587–597.
13. Tonzetich J. Oral malodour: an indicator of health status and oral cleanliness. Int Dent J 1978;28:309–319.
14. Tonzetich J. Production and origin of oral malodor: a review of mechanisms and methods of analysis. J Periodontol 1977;48:13–20.
15. Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of victamine C. J Periodontol 1970;41:41–43.
16. Yaegaki K, Coil JM, Kamemizu T, Miyazaki H. Tongue brushing and mouth rinsing as basic treatment measures for halitosis. Int Dent J 2002;52(suppl 3):192–196.
17. Yaegaki K, Sanada K. Biochemical and clinical factors influencing oral malodor in periodontal patients. J Periodontol 1992;63:783–789.
18. Yaegaki K, Sanada K. Volatile sulfur compounds in mouth air from clinically healthy subjects and patients with periodontal disease. J Periodontal Res 1992;27:233–238.