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Relationship between oral status and prevalence of periodontopathic bacteria on the tongues of elderly individuals

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Colonization of periodontopathic bacteria is associated with increased risk of systemic diseases. However, few studies have investigated the relationships between oral status factors and health-related quality of life (HR-QOL) and the prevalence of such bacteria in elderly individuals. This study investigated the prevalence of *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola* and *Tannerella forsythia* in 165 community-dwelling functionally independent 85-year-old Japanese individuals (93 dentate, 72 edentulous) and the relationship to oral status, including oral malodour and HR-QOL. All four of the studied periodontopathic bacteria were found more frequently in tongue coating samples from dentate than edentulous subjects, and the prevalence of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Treponema denticola* was significantly related to the number of teeth with a periodontal pocket depth $\geqslant 4$ mm. These results suggest the existence of a stable circulation of periodontopathic bacteria between the gingival sulcus and tongue coating over time with teeth. In addition, the presence of teeth with a deep pocket and colonization of *Treponema denticola* were positively related to the level of CH_3SH, whilst the number of present teeth contributed positively to HR-QOL, especially with regard to mental health. In conclusion, as the dentate state can retain colonization of periodontopathic pathogens in the oral cavity, both periodontal treatment and tongue care are important for maintaining a healthy oral status in the elderly, and possibly result in avoidance of risk for tooth loss and decline in HR-QOL, as well as protecting from systemic diseases.

INTRODUCTION

Recent cohort analyses of late functioning and mortality risk have indicated that oral health is significantly associated with survival in elderly individuals (Thorstensson & Johansson, 2009) and that tooth loss has an effect on healthy eating and, consequently, seems to be correlated to reduced longevity (Shimazaki *et al.*, 2001; Hämäläinen *et al.*, 2003; Kitamura *et al.*, 2009). A leading cause of the loss of permanent teeth is periodontal disease initiated by a select group of Gram-negative anaerobes, i.e. periodontopathic bacteria including *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia* and *Treponema denticola*, which colonize and form biofilms in the oral cavity (Anonymous, 1996). In addition to oral infectious diseases, dentate status in elderly individuals is possibly related to the risk of systemic diseases caused by oral bacteria, such as aspiration pneumonia (Terpenning *et al.*, 2001; Awano *et al.*, 2008), infective endocarditis (Ohara-Nemoto *et al.*, 2005; Lockhart *et al.*, 2009), atherosclerotic coronary disease (Herzberg & Meyer, 1996) and decreased kidney function (Kshirsagar *et al.*, 2007). Furthermore, the involvement of periodontopathic pathogens has recently been emphasized as a causative factor in the development of cardiovascular diseases (Iwai *et al.*, 2005). Therefore, a more detailed investigation of the prevalence of these bacteria in the oral cavities of elderly individuals has become important.

The preferred habitat of periodontopathic bacteria is periodontal pockets; thus, the presence of teeth is a permissive factor for colonization by these bacterial organisms. Following the loss of all natural teeth, the prevalence of *Porphyromonas gingivalis* and *Tannerella*

Abbreviations: HR-QOL, health-related quality of life; SF-36, Outcome Study Short Form 36; VSC, volatile sulfur compound.

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forsythia on oral mucous membranes or in saliva is significantly decreased (Danser *et al.*, 1994; Cortelli *et al.*, 2008; Sachdeo *et al.*, 2008), suggesting that dentate elderly individuals have a higher risk of harbouring periodontopathic pathogens than those who are edentulous.

In addition to periodontal pockets, the tongue dorsum is another permissive habitat for periodontopathic bacteria. We demonstrated previously that the prevalence of one or more of the species *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia* and *Treponema denticola* was relatively high (~70 %) in tongue coating, even in periodontally healthy young adults (Kishi *et al.*, 2002). In addition, the amount of tongue coating and levels of volatile sulfur compounds (VSCs), which are metabolites of periodontopathic bacteria, were closely related to the prevalence of *Porphyromonas gingivalis*. Based on these observations and the fact that dentate status and salivation vary widely among elderly individuals, tongue coating is thought to be a suitable representative specimen for evaluation of the colonization of periodontopathic bacteria in the oral cavities of elderly people. In the present study, we examined the prevalence of periodontopathic bacteria in tongue coating samples obtained from 165 subjects aged 85 years and investigated the relationship with their oral conditions, related variables, levels of VSCs and health-related quality of life (HR-QOL).

METHODS

Subjects and oral examinations. We examined 165 subjects, each of whom was 85 years old (71 males, 94 females; 93 dentate, 72 edentulous; Table 1), after obtaining informed consent for participation in the study. They were functionally independent and community-dwelling residents of Iwate Prefecture, Japan. This study protocol received ethical approval from the Ethics Committees of Iwate Medical University School of Dentistry (approval no. D-01053).

Clinical measurements, including numbers of present teeth, decayed teeth and decayed and filled teeth, as well as periodontal status, were assessed. Remaining teeth without a crown but with a root were counted as present teeth. Teeth with treated and untreated root caries were considered to be filled and decayed teeth, respectively. Periodontal status was assessed by a community periodontal index (CPI), and the subjects were divided into those with periodontal pocket depths <4 mm and those with depths $\geqslant 4$ mm. Dental caries status as well as periodontal status assessed by CPI was determined according to methods presented by the WHO (1997). No gender differences were found among the oral health variables. In addition, the amount of tongue coating was assessed as described below.

Sampling of tongue coating. Tongue coating samples were collected as described previously (Kishi *et al.*, 2002). Briefly, after expectorating saliva, the tongue coating was removed from the circumvallate papilla to the apex of the tongue dorsum using three strokes with a sterile toothbrush. Collected samples were immediately immersed in sterile PBS (pH 7.4) and dispersed by sonication on ice. After centrifugation at 12 000 ***g*** for 15 min, the precipitate was resuspended in 1 ml ice-cold PBS and centrifuged. This washing step was repeated three times. The amount of tongue coating was calculated by measuring the optical density of the dispersed suspension sample at 550 nm (OD_{550}) with a calibration curve (correlation between OD_{550} and wet weight of tongue coating, $r^2=0.950$), which was obtained in a preliminary examination. The tongue coating samples were stored at −80 °C until use.

Bacterial species-specific PCR. Genomic DNA was purified from the tongue coating samples using a Wizard Genomic DNA Purification kit (Promega). 16S rRNA gene-based species-specific PCR assays were performed to detect *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola* and *Tannerella forsythia*, as described previously (Kimura *et al.*, 2002). To detect *Streptococcus mutans*, a primer set for the *gtfB* gene (GenBank accession no. M17361; 5′-ATGGACAAGAAAGTGCGTTATA-3′ and 5′-GAAGTTTTGTCAACTGTAGTTG-3′) was designed and synthesized. PCR amplifications were performed in 20 μl reaction mixture containing 0.5 μg DNA and 0.2 μM each primer. Amplification of the 16S rRNA gene with universal primers was confirmed for every DNA sample. PCR was performed for 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, and the products were separated by electrophoresis on agarose gels. We examined the sensitivity of the PCR method using serial dilutions of genomic DNA purified from appropriate type or laboratory strains, and confirmed that 0.5–5 pg DNA corresponding to approximately 10^2–10^3 c.f.u. was easily detected under the test conditions, as reported previously (Kimura *et al.*, 2002; Ikeda *et al.*, 2004).

Measurements of H_2S and CH_3SH concentrations. Concentrations of H_2S and CH_3SH, predominant sulfur compounds of oral malodour, in mouth air were measured in 115 of the 165 subjects who did not have any oral activities within 2 h before the sampling. Using a previously reported method (Senpuku *et al.*, 2004) with slight modifications, the subjects held a 1 ml disposable syringe firmly in their mouth for 30 s. Thereafter, 0.5 ml mouth air was collected with the syringe and subjected to a portable gas chromatography device (Oral Chroma CHM-1; Abilit). To examine the relationship with QOL, oral malodour-positive subjects were classified as those with a

Table 1. Measurement groups to determine prevalence of bacteria and concentrations of VSC in subjects aged 85 years

The prevalence of the four tested periodontopathic bacteria and *S. mutans* was determined using a bacterial-species specific PCR. Concentrations of H_2S and CH_3SH in mouth air were measured using gas chromatography. Numbers of teeth are given as means ± SD (range).

Measurement groups	Oral status	Male/female	Present teeth	Decayed teeth	Decayed and filled teeth
Prevalence of bacteria (n=165)	Dentate (n=93)	49/44	9.9 ± 7.2 (1–26)	1.2 ± 1.7 (0–8)	7.3 ± 5.4 (0–23)
	Edentulous (n=72)	22/50	–	–	–
Concentrations of VSCs (n=115)	Dentate (n=49)	28/21	10.1 ± 7.7 (1–26)	1.2 ± 1.8 (0–8)	7.1 ± 5.3 (1–19)
	Edentulous (n=66)	20/46	–	–	–

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higher concentration of either H_2S or CH_3SH compared with the human odour threshold [115 parts per billion (p.p.b.) for H_2S and 26 p.p.b. for CH_3SH] in their mouth air sample (Tonzetich & Ng, 1976).

Assessment of HR-QOL. To assess the HR-QOL of our subjects, we used the Outcome Study Short Form 36 (SF-36) Health Survey (Japanese edition, version 1.2; Fukuhara *et al.*, 1998a, b) and interviewed the subjects according to the manual provided with the SF-36 (Fukuhara *et al.*, 2001). The SF-36 contains the following eight subscales: physical functioning, limitations in role functioning for physical reasons (role – physical), bodily pain, general health, vitality, social functioning, limitations in role functioning for mental reasons (role – emotional) and mental health. Possible scores ranged from 0 to 100 for each subscale and were standardized to norm-based scores that showed a deviation value (50 ± 10) in comparison with the general population in Japan. Two summary norm-based scorings (physical component score and mental component score) were also calculated according to the manual. Briefly, the eight subscales were summarized after being adjusted by factor coefficients derived from principal components analyses of the scores in a general Japanese population sample. For all scales, higher scores indicated a better HR-QOL.

Statistical analysis. Statistical analyses were conducted using the SPSS 15.0J software package. Differences in the rates of incidence of the examined bacteria between edentulous and dentate subjects were assessed using a chi-squared test. For dentate subjects, multiple logistic regression analyses were performed to determine the most significant factor related to the prevalence of bacteria. Comparisons of H_2S and CH_3SH concentrations were made using a Kruskal–Wallis test, and pairwise comparisons of the combination in each group were made using a Mann–Whitney U test with Bonferroni correction. For multifactorial regression analysis, the presence of periodontopathic bacteria was treated as a binary variable, and oral status including H_2S and CH_3SH concentrations was transferred to a rank, due to the considerable distribution.

RESULTS AND DISCUSSION

Prevalence of periodontopathic bacteria in tongue coatings of 85-year-old dentate and edentulous individuals

PCR analyses showed that 114 (69.1 %) of our 165 subjects harboured one or more species of periodontopathic bacteria in their tongue coating samples. The prevalence of periodontopathic bacteria was significantly higher in dentate subjects (85/93, 91.4 %) compared with edentulous subjects (29/72, 40.3 %) ($P<0.001$; Fig. 1). In contrast, the rate of incidence of the cariogenic bacterium *S. mutans* was not significantly correlated with either of the groups ($P=0.087$). Among the four species, *Tannerella forsythia* was detected most frequently (104/165, 63.0 %), followed by *Porphyromonas gingivalis* (83/165, 50.3 %), *Treponema denticola* (62/165, 37.6 %) and *Prevotella intermedia* (41/165, 24.8 %), and each of these prevalence rates was significantly higher in the dentate than in the edentulous subjects ($P<0.001$). The prevalence tendency in the dentate elderly subjects closely resembled that reported previously for periodontally healthy young adults (24.8 ± 3.2 years old; Kishi *et al.*, 2002). In addition, the relatively high prevalence of *Tannerella forsythia* was in accordance with

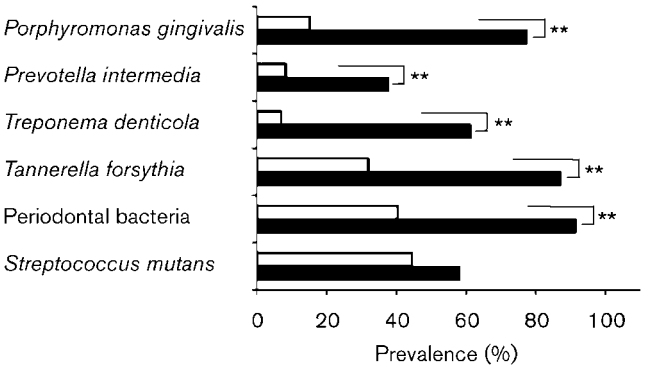

Fig. 1. Prevalence of periodontopathic bacteria in 85-year-old subjects with and without teeth. Detection of the four tested periodontopathic bacteria and *S. mutans* was carried out using species-specific PCR with genomic DNA purified from tongue coating samples. The prevalence rates for each individual bacterium and one or more periodontopathic bacteria are indicated as open (edentulous subjects, $n=72$) and closed (dentate subjects, $n=93$) bars. **, $P<0.001$, chi-squared test.

previous reports that used dental plaque and tongue coating specimens (Kishi *et al.*, 2002; Saito *et al.*, 2009). These observations suggested strongly that colonization by periodontopathic bacteria in the tongue coating reaches an equilibrium in individuals in their 20s and is maintained throughout the dentate period until reaching old age.

There is limited information regarding the succession of residential oral microflora according to age. With regard to periodontopathic pathogens, *Porphyromonas gingivalis* and *Treponema denticola* were scarcely detected in dental plaque specimens obtained from young children under 13 years (Kimura *et al.*, 2002). A prominent emergence of *Porphyromonas gingivalis* and *Prevotella intermedia* was observed in subjects older than 19 years, and the prevalence rate remained nearly constant from 19 to 60 years (Cortelli *et al.*, 2008). Therefore, after considering the bacterial prevalence shown in dental plaque and tongue coating specimens, we speculate that an adequately stable circulation of periodontopathic bacteria between the gingival sulcus and tongue coating occurs over time in dentate individuals. In addition, tooth loss, which is synonymous with loss of the gingival sulcus, may affect the oral microflora population, resulting in a significant decrease in periodontopathic bacteria.

Colonization of mutans streptococci is closely related to tooth eruption (Caufield *et al.*, 1993). However, our finding that the rate of incidence of *S. mutans* colonization did not differ significantly between the dentate and edentulous subjects (Fig. 1) indicated that this bacterium, which initially and preferably colonizes tooth surfaces, probably colonizes both the tongue coating and the epithelial mucosa of elderly individuals. These results are in accordance with a previous report, which found that the

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prevalence of mutans streptococci in saliva (60–88 %) was not significantly different among groups ranging from 20 to 80 years of age (Percival *et al.*, 1991).

Relationship between prevalence of periodontopathic bacteria in tongue coating and periodontal status

To evaluate the permissive role of the gingival sulcus, the relationship between bacterial colonization and number of teeth with a periodontal pocket was analysed in the 93 dentate subjects (Fig. 2). The prevalences of *Treponema denticola* (P=0.012) and *Porphyromonas gingivalis* (P=0.024) were significantly higher in subjects with periodontal pockets ($\geqslant$4 mm) compared with those without pockets (<4 mm). A higher prevalence of *Prevotella intermedia* was also found in subjects with pockets, although it was not significant (P=0.086). On the other hand, the prevalence of *Tannerella forsythia* and *S. mutans* was not correlated with periodontal pocket depth (P=0.137 and 0.535, respectively). These results suggested that the four periodontopathic bacteria examined do not relate equally to periodontal disease, and that *Porphyromonas gingivalis* and *Treponema denticola* are the most likely to be related to the disease. Furthermore, it was of interest that dentate status, as shown in Fig. 1, was more significantly correlated with the prevalence of the periodontopathic bacteria than the presence of teeth with periodontal pockets, indicating that the existence of teeth, and thus the existence of gingival sulcus, is important to allow colonization by periodontopathic bacteria in the oral cavity.

We also analysed the relationship of the presence of each micro-organism in the dentate subjects with variables including gender, frequency of tooth-brushing each day, smoking habits and dental data [number of present teeth, teeth with periodontal pockets $\geqslant$4 mm, teeth with active caries (decayed teeth), decayed and filled teeth and amount of tongue coating] using multiple logistic regression analysis (Table 2). In the dentate subjects, the presence of *Porphyromonas gingivalis* and *Treponema denticola* was significantly associated with the number of teeth with pockets $\geqslant$4 mm, whilst the presence of *Prevotella intermedia* was closely related to the number of present teeth, but not to pocket depth. No variable was associated with the prevalence of *Tannerella forsythia* and *S. mutans*. These results clearly demonstrated that *Porphyromonas gingivalis* and *Treponema denticola* have a stronger relationship with the development of periodontal diseases than *Prevotella intermedia* and *Tannerella forsythia.*

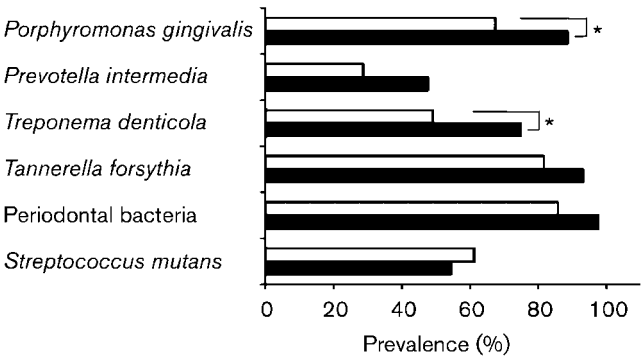

Fig. 2. Prevalence of periodontopathic bacteria in dentate elderly individuals with and without periodontal pockets. Detection of the four tested periodontopathic bacteria and *S. mutans* was carried out using species-specific PCR with genomic DNA purified from tongue coating samples. The prevalence rates for each bacterium and one or more periodontopathic bacteria are indicated by open [dentate subjects without pockets (<4 mm), n=49] and closed [dentate subjects with pockets ($\geqslant$4 mm), n=44] bars. *, P<0.05, chi-squared test.

Relationships among oral status, colonization of periodontopathic bacteria and concentrations of H_2S and CH_3SH in mouth air

We demonstrated previously that oral malodour is related to the colonization of *Porphyromonas gingivalis* in tongue coating samples obtained from periodontally healthy young adults (Kishi *et al.*, 2002). To investigate this relationship in elderly individuals, the concentrations of H_2S and CH_3SH in mouth air were measured in 115 of the 165 subjects (48 males, 67 females; 49 dentate, 66 edentulous; Table 1), who did not have oral activities within the 2 h before the examination. There were no significant differences with regard to the numbers of present teeth, decayed teeth or decayed and filled teeth between this group of subjects and the others (n=50; P=0.985, P=0.541 and P=0.740, respectively). Among this group, CH_3SH concentrations were significantly higher in the dentate subjects with periodontal pockets $\geqslant$4 mm compared with dentate subjects without pockets and edentulous subjects

Table 2. Variables related to prevalence of the four tested periodontopathic bacteria in tongue coating from dentate subjects (n=93) shown by multiple logistic regression analysis

The P values given are from the final model of stepwise analysis. The first step of the regression model included the following independent variables: number of present teeth, teeth with periodontal pockets ($\geqslant$4 mm), teeth with active caries (decayed teeth), decayed and filled teeth and amount of tongue coating.

Species	Final model of multiple logistic regression after stepwise procedure	
	Related variable	P value
Porphyromonas gingivalis	Number of teeth with pockets	0.010
Prevotella intermedia	Number of present teeth	0.001
Treponema denticola	Number of teeth with pockets	0.016
Tannerella forsythia	No related variable found	–
Streptococcus mutans	No related variable found	–

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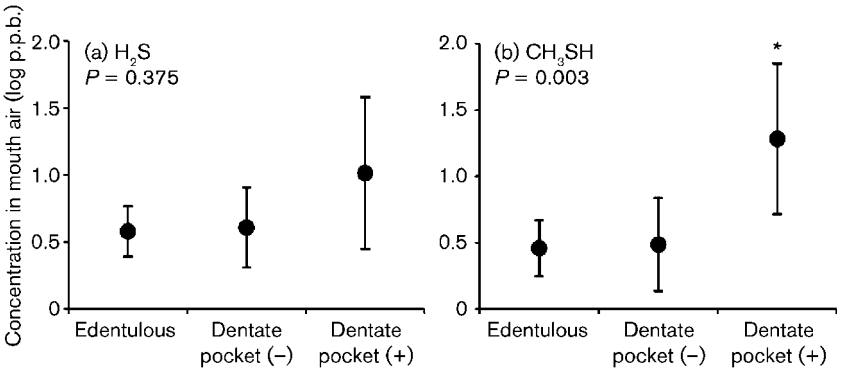

Fig. 3. Concentrations of H_2S and CH_3SH in mouth air samples collected from 85-year-old subjects. The concentrations of H_2S (a) and CH_3SH (b) were measured in edentulous subjects (n=66), dentate subjects without periodontal pockets (<4 mm, n=30) (–) and with periodontal pockets (⩾4 mm, n=19) (+). Values are shown as means with 95 % confidence intervals. P values were calculated using a Kruskal–Wallis test for comparisons among the three groups. *, P<0.05 by Mann–Whitney U test with Bonferroni correction.

(P=0.003) (Fig. 3). The same tendency was true for H_2S concentration, although the difference was not significant.

The relationships among H_2S and CH_3SH concentrations in mouth air, colonization by the periodontopathic bacteria in tongue coating and oral status were analysed using multiple linear regression analysis (Table 3). The results showed that both colonization by *Treponema denticola* and the number of teeth with pockets ⩾4 mm were significantly related to CH_3SH concentration in the dentate subjects (n=49), whilst none of the variables was related to H_2S concentrations. The correlation of number of teeth with periodontal pockets indicated that there was a large subgingival niche available for species colonization in those subjects. For the edentulous subjects (n=66), there were no correlations between bacterial colonization and concentrations of H_2S and CH_3SH found, whilst the amount of tongue coating was closely related to H_2S concentration.

Relationships between HR-QOL and oral status factors

Previous studies have demonstrated that having a larger number of present teeth is positively related to HR-QOL (Akifusa *et al.*, 2005; Hugo *et al.*, 2009). In contrast, oral malodour is considered to have a negative influence on HR-QOL, as individuals who complain of halitosis have been shown to have a reduced QOL (Kishi *et al.*, 2005; Ng & Leung, 2006). In the present study, we analysed the relationship of HR-QOL with oral status factors. An SF-36 questionnaire was completed by 111 (47 dentate, 64 edentulous) of the 115 subjects whose VSC levels were measured. A comparison of SF-36 scores between dentate and edentulous subjects showed that the mental component score was significantly higher in dentate (59.0±6.86) than edentulous (55.6±7.33) subjects (P=0.015 assessed by Student's t-test), indicating that remaining teeth contributed positively to HR-QOL. When the number of present teeth, having oral malodour and gender were used as independent variables, multiple linear regression analyses revealed the strongest correlation between the number of present teeth and mental component score, although it was not significant. In contrast, the presence of oral malodour did not have a negative effect on HR-QOL, possibly because none of our subjects complained of having halitosis.

In conclusion, the present results indicate that dentate status in elderly individuals allows periodontopathic

Table 3. Variables related to concentrations of H_2S and CH_3SH in dentate and edentulous subjects shown by multiple linear regression analysis

For dentate subjects, the first step of the regression model included the following independent variables: colonization by *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola* and *Tannerella forsythia*, number of present teeth, teeth with periodontal pockets ⩾4 mm, teeth with active caries (decayed teeth), decayed and filled teeth and amount of tongue coating. For edentulous subjects, the first step of the regression model included the following independent variables: colonization by *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola* and *Tannerella forsythia* and amount of tongue coating. P values are from the final model of stepwise analysis.

Subjects	VSC	Related variable	P value
Dentate (n=49)	H_2S	No related variable found	–
	CH_3SH	Colonization of *Treponema denticola*	0.024
		Number of teeth with pockets	0.011
Edentulous (n=66)	H_2S	Amount of tongue coating	0.002
	CH_3SH	No related variable found	–

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pathogens to colonize the oral cavity, possibly increasing the risk of both periodontal and systemic diseases. Such problematic particulars were most strongly correlated with the number of teeth with periodontal pockets. Therefore, treatment of periodontitis as well as tongue care is still required for elderly individuals to maintain oral and systemic health, as well as HR-QOL.

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