Relationship Between Sulcular Sulfide Level and Oral Malodor in Subjects With Periodontal Disease

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Background: The relationship between oral malodor and sulfide levels in periodontal pockets (pS) has not yet been determined. The aims of this study were: 1) to identify the correlation among oral malodor, pS levels, and the BANA (benzoyl-DL-arginine-naphthylamide) test and 2) to recognize the interaction between oral malodor, demographic factors, tongue coating, and periodontal condition.

Methods: Eighty-one peridontal patients participated in this study. A portable sulfide monitor and organoleptic method were used to evaluate oral malodor. Demographic data included age, gender, race, and smoking habits. The volume of tongue coating and periodontal condition for all teeth were assessed. The pS levels of 3 different radiographic bone loss (RBL) sites: RBL <2 mm, healthy; RBL ≥2 to <4 mm; low to moderate; RBL ≥4 mm, severe, were measured using an industrial sulcular sulfide-monitoring device. Subgingival plaque samples from the above 3 sites and tongue scraping were examined by the BANA test.

Results: The volume of tongue coating ($P<0.001$), extent of periodontal disease ($P<0.05$), pS levels of the sites with low to moderate bone loss ($P<0.05$), and BANA score of tongue scrapings ($P<0.05$) were significantly associated with oral malodor. Stepwise multiple regression analysis examined the degree of association between oral malodor and potential explanatory variables. The volume of tongue coating and percent of sites BOP (bleeding on probing) were significantly associated with oral malodor. Females and smoking habit were negatively correlated with organoleptic measurements.

Conclusions: The pS level of the representative sites with low to moderate bone loss demonstrated a modest association with oral malodor. Oral malodor in periodontal patients was primarily associated with tongue coating and gingival inflammation. *J Periodontol 2001;72:79-84.*

KEY WORDS

Periodontal diseases; BANA test; halitosis; gingivitis; sulfur compounds; demography; tongue diseases; gingival crevicular fluid/analysis.

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Patients with periodontal disease often suffer from oral malodor. Primary substances associated with oral malodor include volatile sulfur compounds (VSC) in mouth air such as hydrogen sulfide, methyl mercaptan, and dimethyl sulfide.[1,2] These compounds are produced primarily by anaerobic, Gram-negative periodontal pathogens.[3,4] Several clinical studies reported the positive correlation between oral malodor and the severity of periodontal disease.[5,6] However, the relationship between oral malodor and VSC in periodontal pockets has not yet been clarified.

Three oral bacterial species highly associated with periodontal disease—*Porphyromonas gingivalis, Treponema denticola,* and *Bacteroides forsythus*—are among the most active VSC producers in vitro.[4] The presence of these organisms in dental plaque can be detected based on their ability to hydrolyze the synthetic trypsin substrate N-benzoyl-DL-arginine-2-napthylamide (BANA).[7] When subjects reporting oral malodor were studied, the BANA scores obtained from various loci (saliva, periodontal pockets, and tongue) were positively associated with oral malodor.[8,9]

Limited data are available in terms of the relationship between oral malodor and sulcular sulfide levels in periodontal subjects. Therefore, the purposes of this study were to examine the association between: 1) whole-mouth malodor and sulfide levels and demographic factors; and 2) whole-mouth malodor and sulfide levels and whole-mouth clinical param-

eters, and 3) whole-mouth malodor and sulfide levels and site-specific sulcular sulfide levels and site type BANA results.

MATERIALS AND METHODS

Study Population

After the Institutional Review Board of the University of Michigan approved the study protocol, 81 patients being treated in Graduate Periodontics at the University of Michigan School of Dentistry were selected. Inclusion criteria included: 1) adult males or females older than 20 years of age; and 2) presence of 3 representative periodontal sites with radiographic evidence of bone loss (RBL): RBL <2.0 mm, healthy; RBL ≥2.0 to <4 mm, low to moderate; and RBL ≥4.0 mm, severely diseased sites.[10] RBL was expressed as the distance in mm from the cemento-enamel junction (CEJ) to the alveolar crest. Exclusion criteria included: 1) subjects taking any antibiotics within the last 3 months; 2) subjects with the evidence of any systemic diseases (e.g., diabetes mellitus, chronic renal failure, cirrhosis of the liver, gastrointestinal disorder, respiratory dysfunction, and neoplasia, etc.) that may influence oral malodor;[11] and 3) subjects who were pregnant or lactating.

All subjects completed a demographic–medical–smoking interview after which they received an intraoral examination. Smoking habit was determined as the number of pack years (number of packs of cigarettes smoked per day × number of years smoked).[10]

Oral Malodor Assessment

Subjects were asked to refrain from oral activities (e.g., eating, drinking, chewing, brushing, and mouth rinsing) for 2 hours prior to data collection. They were also requested to refrain from using commercial mouth rinses for a period of 24 hours prior to this visit.

The principal investigator (MM) utilized both VSC level measurements in mouth air and organoleptic evaluation to assess oral malodor.[12] The VSC level in mouth air (VSC) was measured with a portable sulfide monitor‡ zeroed on ambient air prior to each measurement. Each subject sat quietly without talking for 2 minutes prior to VSC measurement. A disposable plastic straw was attached to the air inlet of the monitor. The subject was instructed to bring his mouth slightly opened over the straw so that it extended into the oral cavity approximately 4 cm. Subjects were then asked to breathe through the nose during the measurement. The sulfide monitor contains a pump that pulls air through the plastic straw. As the sample of mouth air passes through an electrolytic sensor, the concentration of VSC is detected. Peak VSC level was determined in parts per billion (ppb) sulfur equivalents by direct reading from the digital scale of the monitor.

In the organoleptic measurement, each subject remained quiet and kept their lips closed for a period of 2 minutes. They were asked to exhale through the mouth briefly with moderate force at a distance of appropriately 10 cm from the principal investigator. Organoleptic malodor rating (OR) was estimated on a scale of 0 to 5 as follows: 0, no odor; 1, barely noticeable; 2, slight, but clearly noticeable; 3, moderate; 4, strong; and 5, extremely strong.

The principal investigator also estimated the tongue coating status by visual examination based on the criteria as follows: score 0, non visible; 1, less than one-third of tongue dorsum surface covered; 2, less than two-thirds; and 3, more than two-thirds.[13]

Periodontal Examination

The clinical examination included assessment of soft tissue, dental caries, and the periodontal parameters probing depth (PD) and bleeding on probing (BOP). PD and BOP were scored for all teeth present in the dentition by calibrated examiners. The PD measurement was made to the nearest 1 mm utilizing a Michigan "O" style dental probe. Six sites per each tooth were scored: mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, and disto-lingual. BOP was expressed as presence or absence at 30 seconds after probing. The mean PD, percentage of the pockets ≤4 mm and ≤6 mm, and percentage of the sites with BOP positive (the bleeding index) were calculated for each subject.

Sulcular Sulfide Level and the BANA Test

The principal investigator performed the measurement of sulcular sulfide (pS) level and BANA score. The pS levels of the 3 representative sites with different severity of bone loss were obtained for each subject using an industrial sulcular sulfide-monitoring device.§ This device consists of an electric control unit and a disposable sensor tip that combines a standard Michigan "O" style dental probe with a sulfide sensor.[14] The probe tip with a sulfide sensor responds to the various forms of sulfides expected in periodontally-involved pockets. The electronic control unit reports the sulfide level at each site in a digital score ranging from 0.0 (undetectable pS; less than 10^{-7} M of sulfide) to 5.0 (more than or equal to 10^{-2} M of sulfide) in increments of 0.5. This digital score, pS, is defined by pS = (7 + Log S) in the molar concentration of sulfide in an "equivalent model sulcus fluid."

The presence of putative pathogens *T. denticola, P. gingivalis,* and *B. forsythus* in subgingival plaque samples and on the surface of tongue dorsum were determined using a commercially available test kit.‖ Subgingival plaque was sampled from 3 representative sites with a sterile curet. The posterior surface of the tongue dorsum was scraped with a wooden stick, and

‡ Hallimeter, Interscan Corp., Chatsworth, CA.
§ Diamond Probe/Perio 2000, Diamond General Development Corp., Ann Arbor, MI.
‖ Perioscan, Oral B, Redwood City, CA.

Table 1.

Mean ± Standard Deviation (SD) and Range of Oral Malodor Measurements, Clinical Parameters, Sulcular Sulfide Level, and BANA Results (n = 81)

	Mean ± SD	Range
Oral malodor measurements		
Organoleptic rating	1.5 ± 1.1	0-4
VSC concentration	145.6 ± 89.1	49-452
Volume of tongue coating	1.6 ± 0.9	0-3
Periodontal measurements		
Number of teeth	24.8 ± 4.1	1.0-32.0
Mean probing depth	3.4 ± 0.7	1.9-5.4
% of pockets (≥4 mm)	32.8 ± 18.4	1.3 ± 84.1
% of pockets (≥6 mm)	9.5 ± 10.0	0.0-46.2
% of sites with BOP positive	27.4 ± 21.7	2.0-78.4
Sulcular sulfide level		
Healthy sites	0.10 ± 0.23	0-1.0
Low to moderate sites	0.43 ± 0.58	0-2.5
Severe sites	1.17 ± 0.87	0-2.5
BANA test (pocket)		
Healthy sites	0.32 ± 0.61	0-2
Low to moderate sites	0.73 ± 0.74	0-2
Severe Sites	1.15 ± 0.74	0-2
BANA test (tongue)	0.26 ± 0.52	0-2

the scrapings were collected. The plaque and tongue samples were placed on a BANA impregnated strip along the lowered border of a test card. An upper reagent strip containing Evan's black dye was then activated through dampening with distilled water, and the 2 strips were folded over so they contacted one another. After folding, the card was incubated at 35°C for 5 minutes.[15] Results were recorded as: strong, dark blue spots (score = 2); weak, light blue spots (score = 1); or no color change (score = 0).

Examiner Calibration

The inter-examiner agreements of PD and CAL were 0.96 and 0.95, respectively, using the Pearson correlation test. This correlation test was done in 30 randomly-selected sites and compared at two different time points a week apart. The intra-examiner pS, VSC, and organoleptic agreement by the principal investigator MM was 0.95, 0.93, and 0.90, respectively, when expressed as the Pearson correlation coefficient of the scores examined on two separate occasions.

Data Analysis

The mean value of OR and VSC were compared between the subgroups with different demographic factors using analysis of variance (ANOVA). Pearson correlation coefficients were calculated to determine the association of each clinical variable to the OR and VSC. The ordinal stepwise multiple regression analysis was carried out to detect the degree of association between oral malodor level and potential explanatory variables using age, gender, race, smoking habit, tongue coating, clinical periodontal indices, sulcular sulfide level, and BANA test results as independent variables. Calculations were undertaken using a statistical software package.¶

Table 2.

Relationship Between Oral Malodor and Subject's Demographic Factors (mean ± SD)

	N	Organoleptic Rating (Mean ± SD)	VSC in Mouth Air (Mean ± SD)
Gender			
Male	43	1.89 ± 1.17*	165.6 ± 106.0†
Female	38	1.05 ± 0.87	122.9 ± 59.2
Age			
≤39	17	1.65 ± 1.17	155.3 ± 107.3
40-49	22	1.64 ± 1.05	161.5 ± 92.5
50-59	19	1.37 ± 1.21	142.2 ± 82.0
≥60	23	1.35 ± 1.12	137.8 ± 78.3
Race			
Caucasian	58	1.36 ± 1.04	137.8 ± 78.3
African-American	11	1.45 ± 1.29	155.9 ± 103.2
Asian	12	2.17 ± 1.19	174.1 ± 122.3

* Significantly higher than females by 1-way ANOVA ($P<0.001$).
† Significantly higher than females by 1-way ANOVA ($P<0.05$).

RESULTS

Subjects consisted of 38 females and 43 males with the mean age of 52.1 ± 14.4 years old. There were 58 Caucasian, 11 African-American, and 12 Asian subjects. Twenty-nine of the subjects were current smokers. The mean OR and VSC in mouth air for all subjects was 1.5 ± 1.1 and 145.6 ± 89.1, respectively. Significant correlation (r = 0.729, $P<0.001$) was observed between OR and VSC. Mean ± standard deviation and range of the clinical parameters are listed in Table 1.

Table 2 shows the relationship between oral malodor and the subject's demographic factors. The mean OR and VSC of male subjects were significantly higher than the corresponding values of female subjects (OR: 1.89 versus 1.05, $P<0.01$; VSC: 165.6 versus 122.9, $P<0.05$). No significant difference was observed between the 4 age subgroups. The Asian subjects tended to have higher OR and VSC than African-American and Caucasian subjects. However, there was no significant difference among the ethnic subgroups.

¶ SPSS Inc., Chicago, IL.

Table 3.

Pearson Correlation Coefficients (r) Between Oral Malodor Measurements, Pack Year, Clinical Parameters, Sulcular Sulfide Level, and BANA Results (n = 81)

	Organoleptic Rating		VSC in Mouth Air	
	r	P	r	P
Smoking pack/year	–0.249	0.025	–0.070	0.537
Tongue coating	0.428	<.001	0.552	<.001
Probing depth	0.289	0.009	0.353	0.001
% of pockets (≥4 mm)	0.241	0.030	0.303	0.006
% of pockets (≥6 mm)	0.371	0.001	0.411	<.001
Bleeding index*	0.489	<.001	0.472	<.001
Sulcular sulfide (pS) level				
Healthy sites	0.123	0.233	0.115	0.308
Low to moderate sites	0.264	0.017	0.335	0.002
Severe sites	0.165	0.140	0.169	0.130
BANA test				
Healthy sites	0.021	0.850	0.038	0.739
Low to moderate sites	0.269	0.015	0.217	0.052
Severe sites	0.226	0.042	0.173	0.123
Tongue	0.272	0.014	0.397	<.001

* Percentage of sites with positive BOP.

As shown in Table 3, the volume of tongue coating was significantly correlated with OR (r = 0.428, P <0.001) and VSC (r = 0.552, P<0.001). Smoking habit (pack year) showed a significant negative correlation with OR (r = –0.249, P<0.05). There was a significant relationship between oral malodor and periodontal parameters. The bleeding index had the highest correlation coefficients with OR (r = 0.489, P<0.001) and VSC (r = 0.472, P <0.001) among the periodontal parameters examined. The pS levels in the sites with low to moderate bone loss showed a significant correlation with OR (r = 0.264, P <0.05) and VSC (r = 0.335, P <0.01). However, there was no significant correlation between pS levels of healthy and severe sites and oral malodor measurements. The BANA score of low to moderate (r = 0.269, P <0.05) and severe (r = 0.226, P <0.05) diseased sites showed a significant correlation with OR, however, not with VSC. The BANA test in the tongue scraping was significantly correlated with VSC (r = 0.253, P <0.05).

The degree of association between oral malodor and potential explanatory variables was examined using stepwise multiple regression analysis with OR and VSC as the dependent variables (Table 4). The volume of tongue coating (P <0.001) and the bleeding index (P <0.01) were the strongest factors for both OR and VSC. For example, if the tongue volume score increased by one, then the VSC in mouth air increased by 42.8 ppb (parts per billion) according to the non-standardized beta. Gender (female) and smoking habit (pack year) were negatively associated with OR (P <0.01). The BANA score in the tongue scraping was significantly associated with VSC (P <0.05).

DISCUSSION

Results from this study indicate that oral malodor was associated with pS levels of low to moderate disease sites (mean PD = 3.5 mm). This finding suggests the possible contribution of sulcular sulfide to oral malodor. Interestingly, pS levels in sites with severe bone loss (mean PD = 5.4 mm) did not correlate with oral malodor. This may be because the volatile sulfide within deep pockets is not released into the oral cavity. In addition, stepwise multiple regression analysis did not recognize pS levels as a significant factor for oral malodor. We measured pS levels from 3 representative periodontal sites. Hence, it is suggested that pS levels from these representative sites may not be good indicators for oral malodor.

The BANA results of low to moderate and severe disease sites significantly correlated with OR. However, no significant correlation was observed between VSC and BANA results. This is in agreement with the

Table 4.

Stepwise Multiple Regression Analysis of Oral Malodor Measurements

		Non-Standardized		Standardized	
Dependent Variables	Independent Variable	ß	SE	ß	P
Organoleptic rating	Tongue coating	0.427	0.112	0.340	<.001
	Bleeding index*	0.015	0.005	0.306	0.001
	Smoking pack/year	–0.018	0.006	–0.283	0.002
	Gender†	–0.580	0.199	–0.259	0.005
				Multiple R = 0.462	
VSC in mouth air	Tongue coating	42.8	8.83	0.429	<.001
	Bleeding index*	1.21	0.37	0.292	0.002
	BANA (tongue)	36.7	15.3	0.214	0.019
				Multiple R = 0.461	

* Percentage of sites with positive BOP.
† Male (1), female (2).

report by Kozlovsky et al.[8] It is possible that bacteria associated with the pocket BANA test contribute to non-sulfide odorants, such as cadaverine,[16] indole, and pyridine.[17,18] Stepwise multiple regression analysis did not identify pocket BANA scores as significant factors for oral malodor. Therefore, like pS levels, BANA scores from representative sites may not be a good indicator for oral malodor.

The tongue BANA score was significantly correlated with VSC. This result conflicts with the report by Kozlovsky et al.[8] They found no significant association between tongue BANA score and VSC. The difference in this result may be due to the varying characteristics of the subject population. Kozlovsky et al.[8] examined individuals reporting oral malodor regardless of their periodontal condition (mean probing depth = 2.9 mm), whereas we recruited only periodontitis subjects (mean pocket depth = 3.4 mm). Yaegaki and Sanada[19] indicated a greater contribution of tongue coating from periodontitis subjects to VSC formation than that from healthy individuals. When stepwise multiple regression analysis was performed, both tongue coating volume and tongue BANA score were identified as significant factors for VSC. This suggests that tongue coating in periodontitis subjects may harbor many kinds of VSC-producing bacteria other than the three BANA-hydrolyzing bacterial species.

This study showed that both OR and VSC significantly correlated with volume of tongue coating and periodontal disease condition. Moreover, the bleeding index (% of the sites with BOP positive) rather than % of deep pockets (≥6 mm) was highly associated with oral malodor. These findings are consistent with past clinical studies.[13,19] Miyazaki et al.[13] surveyed VSC levels in mouth air, tongue coating status, periodontal conditions, and dental plaque, as well as oral health habits in the general population (2,672 individuals) of Japan. A significant correlation was observed among VSC level, periodontal conditions, and volume of tongue coating. They further suggested that the disease activity is more responsible for oral malodor production than the presence of deep periodontal pockets.[13] This is supported by Bosy et al.[12] who reported that periodontally healthy individuals could also exhibit significant levels of oral malodor.

The smokers had significantly lower levels of OR than the non-smokers. In fact, it was hard to detect oral malodor behind tobacco smell. Although tobacco smoke itself contains VSC,[20] our study could not detect positive correlation between VSC level in mouth air and amount of cigarette smoking. Conversely, Miyazaki et al.[13] found a significant negative relationship between VSC and smoking habit. Therefore, smoking may have a role in reducing VSC as well as masking oral malodor.[21,22]

Males had significantly higher OR and VSC than females. This result is in agreement with the findings reported by Sulser et al.[23] and Rosenberg and Leib.[24] Oral malodor is not caused by a single factor, but by a combination of variable factors including, but not limited to, salivary components[16-18] and salivary flow rate.[13] These factors may explain the difference noted between males and females.

In summary: 1) oral malodor had a weak association with sulcular sulfide level of low to moderate bone loss sites, but not with severe bone loss sites; 2) organoleptic rating was negatively associated with females and smoking; and 3) oral malodor was primarily associated with tongue coating volume and gingival inflammation.

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