

# Relationship of Oral Malodor to Periodontitis: Evidence of Independence in Discrete Subpopulations

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ASSOCIATIONS BETWEEN ORAL MALODOR, measures of periodontal disease, and trypsin-like activity of periodontal pathogens on tongue and teeth were examined in 127 subjects. Volatile sulphur compound (VSC) measurements were made with a portable sulphide monitor; oral malodor was also estimated by organoleptic methods. Measurements repeated one week apart indicated that steady-state VSC levels ( $r = 0.72$ ;  $P = 0.0001$ ) and peak VSC levels ( $r = 0.63$ ;  $P = 0.0001$ ) were reproducible but these  $r$  values were not significantly different ( $P > 0.1$ ). There was a significant correlation between tongue odor and peak VSC levels ( $r = 0.40$ ;  $P = 0.0001$ ) and between tongue odor and whole mouth organoleptic measures ( $r = 0.55$ ;  $P = 0.0001$ ). To study the effect of reducing microbial colonization on oral malodor, chlorhexidine gluconate (0.2%) rinsing was prescribed for 7 days. Reductions of VSC levels were significant for both peak (37%) and steady-state (41%) data ( $P = 0.0001$ ). Anaerobic periodontal pathogens on the tongue estimated by the proportions of positive BANA tests were reduced 19% ( $P = 0.001$ ) and this was concomitant with a 40% ( $P = 0.0001$ ) decrease in organoleptic measurement of the tongue dorsum. Mean pH measurements of the tongue dorsum showed large reductions from 6.9 initially to 6.3 post-treatment ( $P = 0.0001$ ). Subjects were divided into periodontitis/no periodontitis based on periodontal inflammation and probing depth ( $\geq 5$  mm). Of the 37 subjects with periodontitis, 23 had oral malodor whereas 52 out of 90 periodontally healthy subjects exhibited malodor. Chi square analysis comparing halitosis in subjects with and without periodontitis showed no statistically significant association ( $X^2 = 0.208$ ;  $P 0.65$ ) between these two factors although the intensity of malodor as based on VSC concentration in periodontally healthy subjects was 19% less (mean = 111 ppb) than in subjects with periodontitis (mean = 136 ppb). The odds ratio was 1.2, indicating that oral malodor was not associated with periodontitis. These data indicate that a large proportion of individuals with oral malodor are periodontally healthy and that the mucosal surface of the tongue is a major site of oral malodor production. *J Periodontol* 1994;65:37-46.

**Key Words:** Chlorhexidine/therapeutic use; halitosis/etiology; tongue diseases/pathogenesis; sulfur compounds; periodontal diseases/pathogenesis.

Halitosis affects a large proportion of the population<sup>1</sup> and may be the cause of a significant social and psychological handicap<sup>2</sup> to those who suffer from it. Although little is known about the specific causes of oral malodor there are strong associations with periodontal diseases. For example, saliva from individuals with periodontitis putrefies more rapidly and the odor is more disagreeable in comparison to saliva from healthy individuals.<sup>1,3,4</sup> This is attributable in part to the degradation of blood and host cellular products

that provide substrates for generation of volatile sulphur compounds (VSC) and that may in turn accelerate bacterial growth and proteolysis.<sup>5</sup> Conversely, uptake of volatile sulphurs by epithelial cells may play an important role in the pathogenesis of periodontal disease:<sup>5</sup> it has been proposed that volatile sulphurs may alter the permeability of affected cells and facilitate the access of toxic metabolites into the underlying connective tissue<sup>5,6</sup> thereby contributing to collagen degradation. There is also general agreement that the VSC content of exhaled mouth air<sup>5,7,8</sup> and the concentration of VSC precursors<sup>9-11</sup> increases with the severity of periodontal disease. Conversely VSC are reduced in subjects with good oral hygiene compared to those with gingivitis<sup>12</sup>

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and odor detected from dental floss is more intense after removal of mature plaque in comparison to nascent plaque.<sup>13</sup> Thus the presence of periodontal diseases and tooth-bound plaque appear to be strongly related to the detection of VSC in mouth air.

However, there also appears to be a distinct and separate population with detectable oral malodor that does not exhibit any periodontal disease.<sup>11,14</sup> Although the VSC content of mouth air in these individuals is not as high as those with periodontal diseases,<sup>1,11,15</sup> the breath of these individuals may be very offensive. Therefore, multiple etiologies of oral malodor independent of periodontal diseases and plaque are not only possible<sup>16</sup> but may represent a substantial proportion of the affected population. The objectives of this study were to examine quantitatively the association between oral malodor and periodontitis and to assess the relationship between measurements of VSC and bacteria on the tongue and dental sites.

## MATERIALS AND METHODS

### Subjects

Subjects were selected from two pools. First, all individuals who attended the Halitosis Assessment Clinic at the University of Toronto (n = 107) over the period from December 1, 1991 to June 21, 1992 were included. These subjects all reported halitosis as their chief complaint and had responded to a newspaper article on bad breath, to local and national television programs on this subject, or by referral from their dentist or physician. No exclusion criteria were used. Second, to ensure that subjects with a wide range of oral conditions were included, subjects without a chief complaint of halitosis were chosen at random from a large (n ~ 2000) pool of staff, students, and patients at the Faculty of Dentistry (n = 26). This group was seen for one appointment only. The choice of sample size was based on convenience as there were inadequate previous data to provide accurate *a priori* calculations.

### Subject Preparation

Subjects were asked to fill out a consent form and a medical history questionnaire. Conduct of this study was approved in advance by the Human Ethics Experimentation Committee at the University of Toronto. Subjects were asked to refrain from oral activities including eating, drinking, chewing, brushing, and mouth rinsing for 2 hours prior to each appointment. Subjects were also asked not to use commercial mouthrinse for one day prior to the appointment to reduce artifactual errors of volatile sulphur measurements associated with alcohol or zinc-containing mouthwashes.

### Study Design

All subjects were measured at the first appointment to establish their baseline measurement. Only those subjects presenting to the Halitosis Assessment Clinic were measured

at the second and third appointments which were held at intervals of approximately 1 week and at approximately the same time of day. For these subjects measurements from the second appointment were compared with those from the first appointment and assessed for reproducibility of the baseline data. Subjects were assessed by one of two evaluators who had been calibrated previously for organoleptic and periodontal measurements over a period of several clinical sessions prior to the study. To evaluate any differences in assessment each evaluator examined the same patients independently over several clinical sessions and the results were compared and the examiners calibrated.

Following the second appointment subjects were instructed to rinse with a 0.2% chlorhexidine gluconate (CHX) mouthrinse 2 times per day for a period of 1 week to assess any decrease in odor intensity due to microbial activity. Rinsing was done in the morning after breakfast and toothbrushing and in the evening just before bedtime. Each treatment consisted of brushing the tongue with a toothbrush soaked with CHX followed by a 60-second rinse and was performed from the evening of the second appointment to the evening of the third appointment, 1 week later. Rinsing with water following the CHX rinse was not encouraged. If subjects found the rinse too bitter, a period of 30 minutes waiting time was required prior to rinsing with water. Subjects were advised of possible side-effects due to CHX and asked to inform the principal investigator of these sequelae. All measurements were repeated on the third appointment and compared with the baseline data for any post-rinse differences.

### Volatile Sulphurs

VSC measurements were made with a portable industrial sulphide monitor<sup>‡</sup> zeroed on ambient air prior to each measurement. Two monitors, both with new sensors, were used and were calibrated for inter-monitor agreement (difference < 5 parts per billion; ppb). This method of VSC measurement has been reported previously.<sup>17,18</sup> Briefly, each subject sat quietly without talking for 3 minutes prior to measurement of VSC levels. A disposable plastic straw was attached to the air inlet of the monitor. The subject was then instructed to bring his slightly open mouth 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 sulphide monitor contained a pump which sucks air from the plastic straw placed inside the subject's mouth at approximately 1500 mL/min. As the sample of mouth air passed through an electrolytic sensor, the concentration of volatile sulphurs was estimated. Peak (maximum; VSCPK) and steady-state levels (VSCSS) were determined in ppb sulphur equivalents by direct reading from the analog scale of the monitor.

<sup>‡</sup>Model 1170, Interscan Corp., Chatsworth, CA.

### Organoleptic Assessment

Subjects remained quiet and kept their lips closed for a period of 2 minutes. They were then asked to exhale through the mouth briefly with moderate force at a distance of approximately 10 cm from the nose of the evaluator. Following this, subjects exhaled through the nose for a nasal odor evaluation. Organoleptic and nasal malodor scores were estimated on a scale of 0 to 5 as follows: 0 = no odor; 1 = barely noticeable odor; 2 = slight but clearly noticeable odor; 3 = moderate odor; 4 = strong odor; and 5 = extremely foul odor. The reproducibility and validity of this method of oral malodor measurement have been previously reported.<sup>18</sup>

Tongue malodor was assessed by scraping the posterior tongue dorsum with gauze and immediately smelling its odor. The malodor was estimated using the same organoleptic scale. The amount of coating on the tongue dorsal surface was estimated by visual examination as heavy, medium, light, or none.

Malodor in dental sites was assessed by passing waxed dental floss interproximally between all teeth, smelling the floss immediately and recording the odor based on the organoleptic scale. Different segments of floss were used for each passage. The maximum organoleptic floss score and the mean value of sites with odor were used to assess the contribution of interproximal sites to oral malodor.

### Periodontal Measurements

The probing depth of periodontal pockets was measured with a Michigan O probe to the nearest mm marking and recorded as > 5 mm or > 7 mm. Measurements of plaque index (PI)<sup>19</sup> and gingival index (GI)<sup>20</sup> were recorded, using the 6 Ramfjord sample teeth<sup>21</sup> at each appointment following malodor measurements.

A commercially available diagnostic test kit<sup>§</sup> for bacterial trypsin-like activity, based on the hydrolysis of benzoyl DL arginine-naphthylamide (BANA), was used to assess the presence of one or more of *T. denticola*, *P. gingivalis*, and *B. forsythus*. These species and some *Capnocytophaga* species possess an enzyme capable of hydrolysing the synthetic trypsin substrate, BANA, an activity not identified in other microbial species found in plaque.<sup>22</sup> The species identified by the BANA test have been tentatively implicated as putative periodontal pathogens and can also degrade proteins to volatile sulphur compounds.

In this study subgingival plaque samples from the mesial of first molars were obtained with an explorer and tested. The posterior surface of the tongue dorsum was scraped with a wooden tongue depressor and the scrapings were collected. Thick debris was discarded, the tongue was scraped again and the collected material was placed on strips that were incubated for 15 minutes.<sup>22</sup> A result which produced

a definite blue to a pale blue color was recorded as positive. No reaction was recorded as a negative.

### Salivary Measurement

Oral mucosal wetness was measured using paper strips and an instrument developed specifically for measurement of salivary flow rates.<sup>¶</sup> The accuracy of the unit in measuring small volumes of crevicular fluid has been determined previously.<sup>23,24</sup> The model we used has been adapted to measure larger volumes such as oral mucosal wetness and the flow of saliva. Measurements were made by placing small paper strips over the given area of the oral mucosa. For the purpose of this study paper strips were placed on the palate to measure flow from the palatal glands as well as the right and left posterior vestibular regions to measure parotid flow. These areas were dried with gauze prior to placement. Following a 5-second application, the strips were placed immediately into the sensor of the measuring unit. The displayed reading was converted to microliters using a standard calibration curve supplied by the manufacturer relating the readings to the volume of fluid (in microliters) absorbed by the paper strips.<sup>#</sup>

Litmus indicator paper was used to estimate the pH levels of the tongue dorsum, sublingual sites, and in the right and left posterior vestibules. One inch strips of pH indicator paper were placed on each of the surfaces and held until wet. The color was compared against standards and the pH was recorded.

### Dental Measurements

The number of teeth, broken fillings, and caries were recorded. Fixed prostheses were examined for leaks and plaque and food impaction. Removable prostheses were taken out and measured organoleptically for fit and cleanliness. Lateral borders of the tongue, palate, and buccal mucosa were assessed for pathology and lesions. Desquamation, keratinization, and cheek biting were recorded. Pharyngeal tissues were checked for tonsillar enlargement and redness. Lip seal, mouthbreathing, and enlargement of lymph nodes were noted.

### Statistical Analysis

The reproducibility of the VSC data were assessed by plots of differences against the means, by a paired *t*-test and by Pearson correlation. An analysis of covariance was used to determine systemic reduction in VSC values over time. Intra-examiner agreement was assessed by unweighted kappa statistic. The sensitivity of periodontal and malodor tests in detecting a reduction in values after CHX was assessed by paired *t*-tests. The Pearson correlation was used to assess

<sup>¶</sup>Periotron 6000, Model II, Interstate Drug, Amityville, NY.

<sup>§</sup>Sialopaper, Pro Flow, Inc., Amityville, NY.

<sup>#</sup>pHydrion Vivid 6-8, Micro Essential Laboratory, Brooklyn, NY.

<sup>§</sup>Perioscan, Oral B, Redwood City, CA.

the strength of association between various malodor measurements and periodontal parameters. Backward stepwise regression analysis with the GLM (general linear model) procedure\*\* was used to determine the malodor and periodontal parameters most strongly related to VSC. Positive BANA tests were counted separately for subjects with and without periodontitis and calculated as a percentage of the total number of tongue or tooth sites with a positive test.

## RESULTS

One hundred and twenty seven subjects completed this study of whom 83 were female ( $43.2 \pm 11.5$  years) and 44 were male ( $43.1 \pm 16.5$  years). The duration of the study was 7 months. Of the 107 subjects from the Halitosis Clinic, 101 completed all three visits. Reasons for not attending all three sessions included unsuitability due to dental caries, long distances to travel, and failure to return for the last appointment. Data from these subjects were not included in the analyses.

Of the 127 subjects who completed the study, only 2 were edentulous with complete upper and lower dentures. The dentate subjects had an average of 26.5 teeth and 13 of these subjects wore partial dentures. When the dentures were examined, only 1 full denture and 3 partial dentures had organoleptically measurable odor. All but 5 subjects reported regular dental examinations every 6 months or less. One subject had 2 carious lesions, 3 subjects had one caries each, and 1 subject had a broken filling. When the soft tissues were examined, 8 subjects exhibited minor pharyngeal inflammation, 2 had enlarged tonsils, and 4 subjects had some palatal inflammation. Seven subjects had deep fissures on the tongue dorsum and 2 had a geographic tongue. Seven subjects exhibited cheek biting and moderate desquamation on cheeks or edentulous areas. When checked for habits that may impact on their oral malodor, only 2 subjects were mouthbreathers and 9 were smokers.

Of the 101 subjects that were given CHX on the second appointment, 90 responded to a questionnaire on adverse reactions to the rinse. The most common reaction experienced by the subjects was the change in the taste of food (58.9%). A substantial number of subjects (25.6%) experienced discomfort in the form of a burning sensation on the tip of the tongue. Subjects also reported tongue dorsum staining (16.7%) and tooth staining (12.2%). Four subjects (4.4%) reported sloughing of tissues or gingival pain as a result of possible allergic reaction while only 11 subjects (12.2%) reported having no adverse reactions to rinsing with CHX.

### Reproducibility

In contrast to our earlier study<sup>18</sup> we did not find a significant decrease in the sensitivity of measurements obtained from

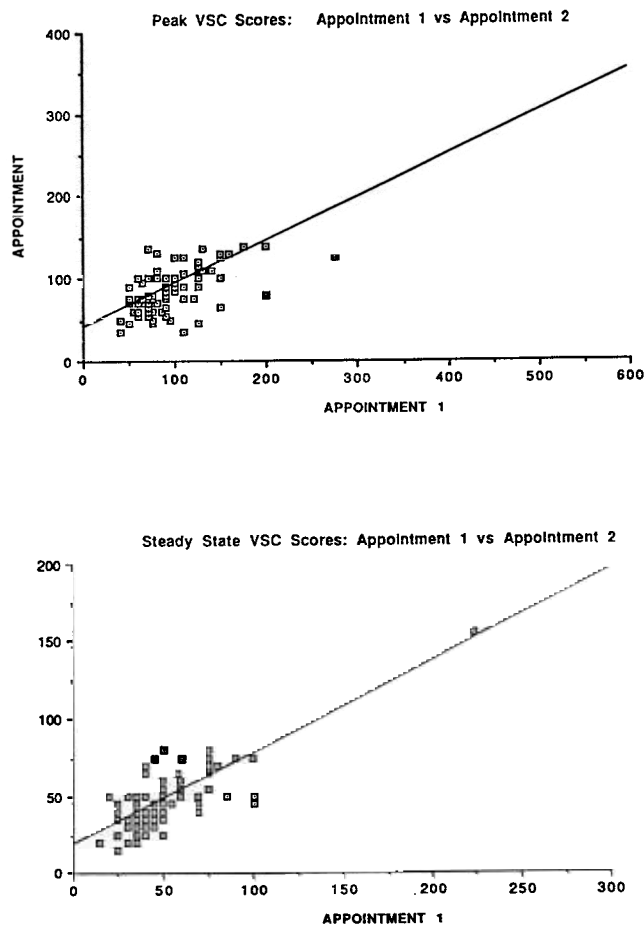


Figure 1. Scatter plots of peak and steady state VSC scores from first and second appointments showing the relationship between replicate measurements of VSC level. For steady state VSC,  $r = 0.72$ ; for peak VSC,  $r = 0.63$ .

the monitor during the course of the study (6 months), indicating that the variations of VSCPK and VSCSS were due to biological responses and not due to instrument drift. Reproducibility measurements repeated one week apart (Fig. 1) indicated that steady-state VSC levels were more reproducible ( $r = 0.72$ ;  $P = 0.0001$ ) than peak VSC levels ( $r = 0.63$ ;  $P = 0.0001$ ) but this difference was not statistically significant. Organoleptic measurements ( $r = 0.72$ ;  $P = 0.0001$ ) between appointments 1 and 2 were as reproducible as steady-state VSC. A paired *t*-test showed no significant difference ( $P > 0.1$ ) between appointment 1 and appointment 2 for peak VSC levels and for organoleptic scores. A significant difference ( $P < 0.05$ ) between the two appointments for steady state VSC measurements was reduced to no difference ( $P > 0.1$ ) when two extreme measures (difference of 70 and 75) were removed.

Intra-examiner agreement of whole mouth organoleptic measurements assessed with the unweighted kappa statistic indicated moderate consistency for whole mouth organoleptic measurement (examiner 1 = 0.48; examiner 2 = 0.54). Agreement of tongue dorsum scores was similarly

\*\*SAS 6.03, SAS Institute, Cary, NC.

Table 1. Pearson Correlation Analysis of Periodontal and Malodor Measurements

VSCPK	VSCSS	Oral Odor	Nasal Odor	Tongue	GI	PI	Average Floss	Maximum Floss	Pockets*	
									>5mm	>7mm
Oral		1.000	0.148		0.148	0.118		0.202	0.108	
Nasal			1.000		0.028	0.152		0.101	-0.105	
Tongue				1.000	0.233†	0.037		0.168	0.054	
Gingival index					1.000	0.259†		0.292†	0.192†	
Plaque index						1.000		0.177	0.050	
Average floss								0.590†	0.191	
Maximum floss								1.000	0.287†	
Pockets > 5 mm									1.000	
Pockets > 7 mm										1.000

Pearson correlation coefficients/prob>|R| under Ho:Rho=0. Data from first appointment (all subjects) were analyzed. VSCPK=peak VSC scores; VSCSS=steady-state VSC scores; oral odor=whole mouth organoleptic scores; tongue odor=tongue organoleptic scores; GI=gingival index; PI=plaque index. Average floss=overall organoleptic scores for floss odor; maximum floss=maximum organoleptic scores for floss odors.

\*Mean number of pockets/subject.

†Significant at  $P > 0.01$ .

analyzed and yielded Kappa values of 0.22 and 0.21 respectively.

### VSC-Organoleptic Relationship

The relationship between whole mouth odor (measured organoleptically) and volatile sulphurs (measured by monitor) was assessed using data from the first appointment for all subjects. Peak VSC levels were more strongly associated with whole mouth organoleptic scores ( $r = 0.53$ ;  $P = 0.0001$ ) than with steady state VSC levels ( $r = 0.44$ ;  $P = 0.0001$ ; Table 1) but this difference was not statistically significant ( $P = 0.18$ ). Nasal odors were weakly associated with peak VSC levels ( $r = 0.37$ ;  $P = 0.003$ ), and steady state VSC levels ( $r = 0.37$ ;  $P = 0.0001$ ) but not with whole mouth organoleptic scores ( $r = 0.15$ ;  $P = 0.14$ ).

### Contribution of Oral Sites of Malodor Formation

Pearson correlation analysis (Table 1) of data from the first appointment was used to assess, specifically, the contribution of two separate intraoral loci to malodor formation. Tongue malodor assessed organoleptically was significantly correlated with peak VSC scores ( $r = 0.40$ ;  $P = 0.0001$ ), steady state scores ( $r = 0.37$ ;  $P = 0.0001$ ), and whole mouth organoleptic scores ( $r = 0.55$ ;  $P = 0.0001$ ) and increases in tongue malodor were directly proportional to the amount of coating present on the dorsal surface of the tongue ( $r = 0.45$ ;  $P = 0.0001$ ). Floss odors were assessed as an average score of at least 6 sites and secondly as the maximum, organoleptically-rated interproximal odor. The contribution of both these assessments to measures of oral malodor was small and of marginal significance. A backward step wise regression analysis of log transformed steady state VSC values from all subjects on the first appointment (Table 2) showed that the strongest associations of VSC were (in descending order), organoleptic scores ( $P = 0.0001$ ), tongue odor ( $P = 0.0001$ ), and maximum floss odor ( $P = 0.02$ ).

Table 2. Backward Step Wise Regression Analysis of Log-Transformed Steady State VSC

Variable	Slope $\pm$ S.E.	F	Prob > F
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Overall regression equation - R-Square = 0.362; Intercept = 3.009  $\pm$  0.126 F = 570.00 ( $P = 0.0001$ ). Backward step-wise regression analysis with log-transformed steady state VSC values shown in descending order of the relative strength of associations of VSC.

### Contribution of Bacteria to Oral Malodor

To examine the association of trypsin-like activity detected by the BANA test with oral malodor, BANA scores were compared to odors from teeth and tongue. The four tooth sites that were sampled showed a moderately strong relationship ( $X^2 = 16.84$ ;  $P = 0.001$ ) with floss odors. When related with tongue odor, the association of tongue dorsum BANA scores was comparatively weak ( $X^2 = 6.52$ ;  $P = 0.01$ ). There was no relationship between the presence of bacteria on the tongue dorsum and the presence of bacteria on teeth ( $r = 0.08$ ;  $P = 0.4$ ) using Pearson correlation. A calculation of positive test sites for all subjects showed that when periodontitis was present, 87.5% of tooth sites and 92.5% of tongue sites were BANA positive, while in healthy individuals, 74.4% of tooth sites and 94.3% of tongue sites were BANA positive.

Although oral dryness has been positively associated with oral malodor,<sup>1</sup> Pearson correlation assessment demonstrated no relationship between measures of oral wetness and oral measurement. This result was consistent with the results from a previous study.<sup>18</sup> There was also no relationship ( $r = 0.02$ ;  $P = 0.7$ ) between the pH of the tongue dorsum and the BANA test scores. Further, when the tongue pH values of healthy individuals were compared to those with periodontitis there was no detectable difference.

To study the effect of reducing the overall oral microbial

Table 3. Comparison of Malodor and Periodontal Variables for All 3 Appointments (n = 101)

Variable	Appointment			% Change*
	1	2	3	
VSC PK	103.8±5.8	95.5±4.9	65.8±1.5	36.6
VSC SS	54.3±2.8	50.5±2.3	32.0±1.9	41.1
Oral odor	2.9±0.1	2.8±0.01	1.0±0.1	65.5
Nasal odor	0.7±0.1	0.8±0.1	0.3±0.1	57.1
Tongue odor	2.5±0.1	2.6±0.05	2.5±0.1	40.2
Tongue coat	2.7±0.1	2.6±0.1	2.0±0.1	25.9
Gingival index	1.2±0.1	1.2±0.1	1.0±0.6	16.7
Plaque index	1.7±0.1	1.6±0.1	1.0±0.1	41.2
Floss odor*	3.3±0.3	3.0±0.2	2.1±0.2	36.3
Pockets ≥5 mm	0.8±0.1	0.8±0.1	0.6±0.2	25.0
Pockets ≥7 mm	0.2±0.1	0.2±0.1	0.2±0.2	0.0
pH Dorsum	6.9±0.1	7.0±0.1	6.3±0.1	400

Measurements are mean ± SE. Comparison of malodor and periodontal measurements for all 3 appointments and the percent change between the first and third appointment.

\* Significant at  $P = 0.0001$  except for gingival index, where  $P = 0.05$  and for periodontal pockets where there was no change.

load on levels of oral malodor, CHX (0.2%) rinsing was prescribed for 7 days. When post-rinse measurements were compared to those obtained at the first appointment, large and significant ( $P = 0.0001$ ) reductions of peak VSC levels (36.6%), steady state VSC levels (41.1%), and organoleptic scores (65.5%) were found (Table 3). Plaque indices were reduced by 41.2% ( $P = 0.0001$ ) and there was a 25% reduction in the number of 5 mm and 6 mm pockets ( $P = 0.0001$ ). The number of deep periodontal pockets ( $\geq 7$  mm) did not change, however, the mean pH measurements of the tongue dorsum were significantly reduced ( $P = 0.0001$ ; pH 6.9 to 6.3 before and after CHX treatment). Gingival indices were reduced by 16.7% ( $P = 0.05$ ). Although this reduction was statistically significant, it was considerably less than the value ( $P = 0.0001$ ) for all other reductions. There was a dramatic reduction in the proportion of BANA positive reactions in samples taken from tooth sites of healthy individuals (to 35.5%) and a smaller reduction in positive reactions of tongue sites (to 75% of total sites). In individuals with periodontitis, post-CHX BANA positive tooth sites were decreased to 54.7% and positive tongue sites to 71%.

#### Relationship of Measures of Periodontal Disease to Oral Malodor

For this study, plaque indices, gingival indices, and the mean number of pockets  $\geq 5$  mm and  $\geq 7$  mm per subject were used as measures of periodontitis. The association of these measures with VSC levels and organoleptic scores were assessed at the first appointment by Pearson correlation analysis. Plaque indices and gingival indices were not associated with VSC levels and only weakly with organoleptic scores. The presence of pockets was also not associated with VSC levels or organoleptic scores.

To examine the relationship between oral malodor and periodontal diseases from another perspective, all subjects were classified into 4 groups based on the presence of periodontitis and halitosis (Table 4). The presence of periodontitis was defined as the clinical detection of 1 or more

Table 4. Dichotomous Analysis of Subjects Based on Halitosis and Periodontitis

	No Periodontitis		Total
	Periodontitis	No Periodontitis	
Halitosis	N = 23 (18.1%)	N = 52 (40.9%)	N = 75 (59.0)
No Halitosis	N = 14 (11.1%)	N = 38 (29.9%)	N = 52 (41.0)
Total	N = 37 (29.1%)	N = 90 (70.9%)	N = 127

Presentation of numbers of subjects in groups with and without halitosis and periodontitis based on criteria described in Materials and Methods.

pockets of  $\geq 5$  mm. Although malodor has been measured by several ordinal scales,<sup>15,18</sup> no standardized recognition value has been established for any measurement method. Based on data from a previous study<sup>18</sup> and by inspection of preliminary data, the presence of halitosis was defined in this study as an organoleptic measurement of  $\geq 3$ . This threshold value was computed by subtracting the 95% confidence limit of the mean from the mean organoleptic score for the whole population.<sup>18</sup> The rationale for the application of this threshold was to include all subjects with easily detected oral malodor and to eliminate any subject with only mildly objectional levels. When the groups were compared with this dichotomous approach, only 23 subjects exhibited both periodontitis and organoleptic scores above the threshold (Table 5). Fifty-two subjects exhibited organoleptic scores above the threshold but no periodontitis; these individuals comprised the largest subpopulation. The second largest group had no periodontitis and organoleptic scores which were well below the threshold limit. A small group of 14 subjects with periodontitis had very low organoleptic scores. Chi square analysis comparing halitosis in subjects with and without periodontitis showed no statistically significant association ( $X^2 = 0.208$ ;  $P = 0.65$ ) between malodor and periodontitis. The odds ratio was 1.2 indicating that oral malodor was not associated with periodontitis.

Fisher's exact test was conducted to determine if associations between oral malodor and periodontitis were present in subjects (n = 26) recruited through sources other

**Table 5. Malodor and Periodontal Parameters for Groups With and Without Periodontitis**

Variable	Periodontitis/ Halitosis	No Periodontitis/ Halitosis	Periodontitis/ No Halitosis	No Periodontitis/ No Halitosis
	50.5 ± 2.9	40.8 ± 1.9	49.6 ± 3.7	39.6 ± 1.6
	135.7 ± 18.8	110.4 ± 6.3	73.9 ± 8.1	69.1 ± 3.3
	67.6 ± 8.3	56.2 ± 3.2	36.4 ± 3.5	39.1 ± 2.6
	3.5 ± 0.1	3.4 ± 0.1	1.3 ± 0.2	1.7 ± 0.1
	2.7 ± 0.2	2.7 ± 0.1	1.7 ± 0.2	1.7 ± 0.1
	6.9 ± 0.8	6.9 ± 0.1	6.9 ± 0.2	6.9 ± 0.1
	3.1 ± 0.6	0.0	2.0 ± 0.4	0.0
	0.4 ± 0.2	0.0	0.7 ± 0.3	0.0
	1.5 ± 0.1	1.2 ± 0.08	1.4 ± 0.1	1.0 ± 0.1
	1.9 ± 0.1	1.8 ± 0.07	1.6 ± 0.1	1.5 ± 0.1

Measurements of malodor and periodontitis (mean and standard error) for each of the 4 groups identified in Table 4.

**Table 6. Dichotomous Analysis of the Subset Based on Halitosis and Periodontitis**

	Periodontitis	No Periodontitis	Total
Halitosis	N = 3 (11.5%)	N = 3 (11.5%)	N = 6 (23.0%)
No halitosis	N = 7 (26.9%)	N = 13 (50.0%)	N = 20 (76.9%)
Total	N = 10 (38.5%)	N = 16 (61.5%)	N = 26

Presentation of numbers of subjects other than those seen reporting oral malodor in groups with and without halitosis and periodontitis based on criteria described in Materials and Methods.

than the oral malodor clinic (Table 6). Three subjects had both oral malodor and periodontitis; 3 subjects had oral malodor without periodontitis; 7 subjects had periodontitis without malodor; and 13 subjects had no periodontitis and no malodor (Table 7). These data indicated that no statistically significant ( $P = 0.29$ ) associations were found between oral malodor and periodontitis in either the whole study sample ( $n = 127$ ) or the subjects recruited ( $n = 26$ ) outside of the oral malodor clinic.

## DISCUSSION

### Relationship of Periodontal Disease to Oral Malodor

Previous studies have indicated that oral malodor often accompanies periodontal diseases.<sup>9-12</sup> Higher VSC levels have been found in subjects with probing depths >4 mm than in

subjects with healthy periodontium<sup>11</sup> and the intensity of the odor increases with the severity of the disease.<sup>10-12</sup> Substantial evidence demonstrates that oral malodor may also accelerate the rate of progression of periodontal diseases.<sup>4,6</sup> We have assessed this relationship quantitatively. The results of the study indicated that moderate to severe malodor was detected organoleptically in individuals with both periodontal health and disease. Further, VSC measurements were in good agreement with whole mouth organoleptic measurements which supported this finding. Although the mean VSC level of healthy subjects was not as high as the group with periodontitis, the difference was less than 10% and was not statistically significant ( $P > 0.05$ ). This small difference in recorded VSC levels between the two groups could be attributed to the reduced sensitivity of the sulphide monitor in detecting the elevated amounts of methyl mercaptan produced in periodontitis.<sup>6,11</sup> Mercaptans, which are organoleptically more objectionable than hydrogen sulphide, are detected at approximately 50% of corresponding levels of hydrogen sulphide yet are important contributors to oral malodor. Hydrogen sulphide, a compound formed in both periodontally healthy and diseased mouths,<sup>6,17</sup> is measured much more efficiently by the sulphide monitor (unpublished data). Since significant positive correlations have been established between organoleptic measures and instrumental measures of total oral VSC,<sup>15</sup>

**Table 7. Malodor and Periodontal Parameters for Groups With and Without Periodontitis**

Variable	Periodontitis/ Halitosis	No Periodontitis/ Halitosis	Periodontitis/ No Halitosis	No Periodontitis/ No Halitosis
	35.0 ± 2.9	51.0 ± 1.9	49.3 ± 3.7	38.3 ± 1.6
	126.7 ± 18.8	120.4 ± 6.3	55.7 ± 8.1	71.9 ± 3.3
	46.7 ± 8.3	83.3 ± 3.2	29.3 ± 3.5	39.2 ± 2.6
	3.7 ± 0.2	3.7 ± 0.1	1.0 ± 0.2	1.2 ± 0.1
	2.3 ± 0.2	3.3 ± 0.1	1.3 ± 0.2	1.4 ± 0.1
	7.7	6.9	6.9	6.9
	2.7 ± 0.6	0.0	2.3 ± 0.4	0.0
	0.3 ± 0.2	0.0	0.3 ± 0.3	0.0
	1.3 ± 0.1	1.5 ± 0.08	1.3 ± 0.1	1.2 ± 0.1
	2.1 ± 0.1	1.7 ± 0.07	1.5 ± 0.1	1.6 ± 0.1

Measurements of malodor and periodontitis (mean and standard error) for each of the 4 groups identified in Table 6.

the insignificant (4%) difference in mean organoleptic measurements between the periodontally healthy and diseased groups indicates that an accurate estimate was obtained from the sulphide monitor. Consequently, in contrast to other reports,<sup>7,9,11</sup> we have good reason to believe that both periodontally healthy and diseased individuals can exhibit significant malodor levels and that malodor and VSC are unlikely to be diagnostic of the presence of periodontitis.

Although periodontal pockets are believed to be important sites for VSC production,<sup>6,11</sup> an association between the presence of deep pockets and VSC level was not established in this study. The positive associations reported by other studies may be attributed to the direct, within pocket sampling methods that were used to measure VSC composition and levels<sup>6,11</sup> and that may not be detectable in whole mouth air sampling. Further, due to the inclusion of large numbers of individuals in this study who were self-reported for halitosis consultation, we cannot rule out the existence of confounding bias, the origins of which are currently unknown.

The amount of supragingival plaque estimated by the plaque index was not associated with VSC levels. Eight to 24 hours of maturation are generally required before plaque deposits produce VCS;<sup>29</sup> as the subjects in this study brushed several times per day, smooth surface deposits were repeatedly disrupted before maturation. Thus, in contrast with our previous study,<sup>18</sup> we were unable to demonstrate the importance of readily accessible supragingival plaque in oral malodor production. Although it is possible that each of the studies assessed very different populations, the inclusion of data from all three appointments for the Pearson correlation analysis in our original study may account, in part, for this difference. Since the possibility existed that subjects might alter their oral hygiene habits and subsequently the levels of oral malodor, only first appointment data were analyzed for associations in the present study. When data from all three appointments in our present study were analyzed together, the associations were similar to those found in our previous study.<sup>18</sup> Further, in agreement with an earlier review indicating that protected plaque in approximal sites produces substantial odors and is associated with overall VSC levels,<sup>4</sup> our observations indicated that oral malodor production in healthy individuals may originate, at least in part, from protected interdental sites.

#### Relationship of Tongue Dorsum to Oral Malodor

Toothbrushing accompanied by tongue cleaning results in a substantial reduction in VSC levels.<sup>30</sup> However, the removal of plaque from tooth surfaces by brushing has been found to be less than half as effective in reducing oral malodor as tongue brushing alone. This suggests that the tongue appears to be an important source of oral malodor<sup>11,25</sup> in periodontally-healthy subjects. Indeed the results of this study indicated that the tongue dorsum contributed to oral malodor and to the VSC content of whole mouth air. These findings are consistent with other

studies<sup>11,25,31</sup> indicating that the tongue dorsum is a principal source of oral malodor. However, in contrast with other studies,<sup>11</sup> no difference was found between mean values of tongue odor in subjects with and without periodontitis. This finding supports the observation described above in which oral malodor is not significantly different between periodontally healthy and diseased individuals and further implicates the tongue as an important site of odor production.<sup>31</sup>

Tonzetich<sup>5</sup> showed that brushing the tongue decreased VSC by approximately 75% and reduced oral malodor to an undetectable level in most cases. In contrast, toothbrushing resulted in less than 25% reduction of hydrogen sulphide and mercaptan. Recent work by Yaegaki and Sanada<sup>11</sup> has shown that the tongue is the major contributor in healthy mouths to oral malodor production; but in subjects with periodontal involvement, both tooth-bound plaque and tongue coating contribute to oral malodor. In this context, the coating of the tongue may be an important factor that accelerates VSC production in subjects with and without periodontitis.<sup>9,32</sup> It has been demonstrated that high VSC levels, rich in methyl mercaptans, are produced on the tongue dorsum in subjects with periodontal diseases and that this differs considerably from the amount and composition of VSC (predominantly hydrogen sulphide) produced in healthy subjects.<sup>11</sup> The lower sample size and different patient population used in the Yaegaki and Sanada<sup>11</sup> study may have produced data that are not comparable to our results.

#### Relationship of Bacteria to Oral Malodor

Oral Gram-negative microorganisms such as spirochetes, fusiforms, vibrios, veillonella, and some bacteroides theoretically have the capacity to produce oral malodor, presumably through putrefaction<sup>33,34</sup> of sulphur-containing protein substrates such as cysteine, cystine, and methionine.<sup>4,6,9,35</sup> The presence of one or more putative periodontal pathogen such as *T. denticola*, *P. gingivalis*, and *B. forsythus* was identified by the BANA test on 74.4% of tooth and 92.5% of the tongue dorsum sites in healthy individuals. Since the test has a reported sensitivity of 85%, a specificity of 53%, and an accuracy of 79%,<sup>22</sup> there is strong indication that at least one of these periodontal pathogens was present on the tongue of healthy individuals. Indeed, earlier studies have indicated that bacteria are essential for the production of oral malodor<sup>33,34</sup> and, in particular, *F. nucleatum*<sup>36,37</sup> and strains of *P. gingivalis* are capable of metabolizing cysteine and methionine to form VSC.<sup>31,39</sup> The large surface area and papillary structure of the tongue can retain considerable quantities of food and debris and can support and protect a large bacterial population.<sup>40,41</sup> *P. gingivalis*,<sup>42</sup> *T. denticola*,<sup>43</sup> *A. actinomycetemcomitans*,<sup>43</sup> various motile organisms,<sup>44</sup> and *P. intermedia* are found predominantly in deep pockets,<sup>42</sup> but have also been found in healthy gingival sites and on the tongue dorsum of individuals with and without disease.<sup>42</sup> A significant correlation has been found between the pres-

ence of motile organisms ( $P < 0.05$ ) and *P. intermedia*<sup>43</sup> on the tongue in subjects with periodontitis as opposed to periodontally-healthy subjects. This indicates that the tongue may act as a reservoir for some periodontopathogens that contribute to oral malodor. Thus the diverse microorganisms found on the tongue surface<sup>45</sup> could influence the microbiota of the entire oral cavity.

Optimum activity for the production of oral malodor occurs in anaerobic conditions with low carbohydrate<sup>4</sup> availability and above neutral pH.<sup>34,35</sup> No direct association was found between pH and the presence of oral bacteria in this study but there was a decrease in pH after CHX treatment and this corresponded to a statistically significant decrease in tongue and teeth BANA test scores. Regardless of periodontal status, the most objectionable odor is produced when large numbers of disintegrated epithelial cells covered with bacteria are trapped in plaque and crevices of the tongue dorsum.<sup>4</sup> Although we found no significant relationship between decreased salivary activity and oral malodor, oral dryness and/or abstinence from food and liquid provide optimal conditions for putrefaction<sup>1</sup> on dental and tongue sites.

#### Antimicrobial Effects on Oral Malodor

We used a one week period of CHX rinsing to probe experimentally the importance of bacteria in oral malodor production. Previous clinical studies<sup>46,47</sup> have shown that CHX rinsing significantly reduces the number of plaque microorganisms. Large and significant reductions in VSC levels, organoleptic scores, and positive BANA test scores were measured following a one week rinse with CHX but only small reductions in gingival inflammation and no change in the numbers of deep pockets were noted. These data indicate that the reduction of the microbial load in the oral cavity is important for the control of oral malodor and that, consequently, the growth of bacteria appears to be critical for oral malodor production. However, we were unable to determine quantitatively which oral sites exhibited the largest reduction in bacteria although it appears that dental sites are probably reduced as much as other non-dental sites. For example, the large and contemporaneous reductions of floss odor, VSC, organoleptic, and approximal BANA test scores suggest that oral malodor production from these sites is an important contributor to whole mouth odor.

Our data suggest that dental and tongue sites may be colonized by putative pathogens without evidence of previous periodontal disease. The existence of a carrier state<sup>48</sup> and a prolonged latency period before pathogens initiate overt periodontitis suggests the possibility that the presence of periodontal pathogens in moderate numbers may create oral malodor prior to the initiation of active periodontitis.

#### CONCLUSIONS

1. Some subjects with periodontitis exhibited high levels of oral malodor but overall these levels were not significantly different from subjects with healthy periodontium. Therefore, oral malodor is not directly associated with the

presence of periodontitis; substantial oral malodor may be present in individuals without periodontitis.

2. Organoleptic assessments of the tongue dorsum and approximal plaque were associated with VSC content of whole mouth air and consequently appear to be important sources of oral malodor.

3. Conditions favoring a positive BANA test on the tongue dorsum and at tooth sites were associated with oral malodor production.

4. Rinsing with chlorhexidine resulted in significant reductions of VSC and oral malodor.

#### Acknowledgments

This work was supported by grants from Oral-B and from the Faculty of Dentistry, University of Toronto. Dr. McCulloch is supported by a Career Science Award from the Ontario Ministry of Health.

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Accepted for publication June 28, 1993.