# ORIGINAL ARTICLE

C Calil FL Liberato AC Pereira M de Castro Meneghim JM Goodson FC Groppo

# The relationship between volatile sulphur compounds, tongue coating and periodontal disease

#### Authors' affiliations:

Caroline Calil, Discipline of Periodontics, Department of Stomatology, School of Dentistry, University of São Paulo, São Paulo, Brazil and Department of Physiologic Sciences, Piracicaba Dental School, State University of Campinas, Piracicaba, SP, Brazil Fabiola Laurino Liberato, Antonio Carlos Pereira, Marcelo de Castro Meneghim, Department of Social and Preventive Dentistry, Piracicaba Dental School, State University of Campinas, Piracicaba, SP, Brazil Jo Max Goodson, Department of Periodontology, The Forsyth Institute, Boston, MA, USA Francisco Carlos Groppo, Department of Physiologic Sciences, Piracicaba Dental School, State University of Campinas, Piracicaba, SP, Brazil

#### Correspondence to:

*Francisco Carlos Groppo* CP 52, State University of Campinas 13414-903, Piracicaba SP Brazil Tel.: + 55 19 2106 5310 Fax: + 55 19 2106 5310 E-mail: fcgroppo@fop.unicamp.br

#### Dates:

Accepted 12 January 2009

#### To cite this article:

Int J Dent Hygiene 7, 2009; 251–255 DOI: 10.1111/j.1601-5037.2009.00366.x Calil C, Liberato FL, Pereira AC, de Castro Meneghim M, Goodson JM, Groppo FC. The relationship between volatile sulphur compounds, tongue coating and periodontal disease.

© 2009 John Wiley & Sons A/S

Abstract: The purpose of the present study was to observe the casual levels of volatile sulphur compounds (VSC) in volunteers with different clinical scores of tongue coating, periodontal pockets depth and Gingival Bleeding Index. Seventy-two subjects who attended for the first time at the dental clinic of the University were randomly selected for intra-oral and periodontal examinations. Systemic and dental histories were also obtained. The subjects were unaware of all procedures. The level of VSC was assessed by using a portable sulphide monitor (Halimeter<sup>®</sup>; Interscan Co., Chatsworth, CA, USA). High tongue coating levels were related with more VSC counts (multivariate anova, P = 0.01). No statistically significant relation (multiple linear regression, P > 0.05) was observed among the VSC levels considering age, bleeding and periodontal pockets sites (depth >4 mm). We concluded that the tongue coating was one of the main factors influencing the VSC levels.

Key words: halitosis; periodontal disease; tongue coating

## Introduction

Halitosis is the general term used to describe any disagreeable odour in expired air, regardless of whether the odorous substances originate from oral or non-oral sources (1, 2). The malodour is usually caused by poor oral conditions, periodontal disease and gingival bleeding (3, 4). Halitosis can be also caused by non-oral aetiological factors such as diabetes, gastrointestinal disorders, psychopathological symptoms and stress (5–7).

Hydrogen sulfide (H<sub>2</sub>S), methyl mercaptan (CH<sub>3</sub>SH) and dimethyl sulfide [(CH<sub>3</sub>)<sub>2</sub>S] comprise about 90% of the volatile

sulphur compounds (VSCs) found in exhaled air (8) and they are probably the major contributors to the objectionable odours presenting in bad breath. These compounds result from the proteolytic degradation of various sulphur-containing substrates from mouth (food debris, saliva, blood and epithelial cells), which are produced mainly by anaerobic gram-negative oral microorganisms (9).

As oral malodour is a perceived olfactory stimulus, direct sampling and assessment by human judges (organoleptic measurements) has been pointed as the most logical measurement approach. However, this method raises several disadvantages. There is a considerable variation among judges on the ranking of the unpleasantness of different odours. Moreover, it may be equivocal to judge the rank of an odour using ordinal scales as there are no standards (10). The development of the Halimeter<sup>®</sup> (Interscan Co., Chatsworth, CA, USA) has led to increasing halitosis researches due to its convenience, portability and reproducibility (7, 10–12).

The two main anatomical sources of VSC identified in the oral cavity are gingival sulcus and the tongue (1). Tongue coating has components, such as blood debris, nutrients, large amounts of desquamated cells and bacteria. These substances are responsible for putrefaction (13) and strongly correlate with malodour (14, 15).

Although some studies have assumed a positive correlation between VSC levels in the air from mouth and the number of periodontal pockets, the relationship between the periodontal disease and malodour remains contradictory (15). The actual evidence for periodontal disease as being the major source of oral malodour has been considered unequivocal. The aim of the present study was to correlate the VSC levels with tongue coating, periodontal disease and gingival bleeding in dentate Brazilian adults.

## Patients and methods

This study was approved (protocol 112/2004) by the Ethical Committee at the Piracicaba Dental School/Unicamp (State University of Campinas). Subjects of both gender (31 males), aging from 18 to 60 years old (39.8  $\pm$  10.9 years) were selected. These subjects were attending for the first time at the dental clinic of the University and they were unaware of all procedures of the study. This condition was important to assure that the subjects did not perform any other oral hygiene measure than the usual.

They were informed of the nature, potential risks and benefits of study. Brief systemic and dental histories were obtained after signing the informed consent form. Intra-oral and periodontal examinations were also performed in all subjects by two examiners. All subjects were examined between 10:00 am and 12:00 pm hours. All the data were analysed anonymously.

Exclusion criteria were pregnancy, smoking, previous periodontal therapy in the past 3 months, absence of all teeth and total prosthesis wearing. In addition, patients taking medication for the past 3 months (except over-the-counter medication), which could interfere in the VSC production such as antibiotics and antidepressants (salivary flow reduction) were excluded. In addition, subjects with localized juvenile periodontitis, rapidly progressive periodontitis, or acute necrotizing ulcerative gingivitis were also excluded. Only the subjects that refrained from eating, drinking, smoking, brushing and rinsing their mouth for 2 h before the evaluation of VSC were accepted.

Measures of bleeding on probing (BOP, 0/1) and probing pocket depth (PPD) were taken at four sites per tooth (mesial, buccal, distal and lingual) of all teeth. Probing pocket depth was recorded to the nearest millimeter using a North Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA). Four or more teeth showing at least one PPD greater than 4 mm was considered as indicative of periodontal disease.

The VSC measurements were made using a portable sulphide monitor (Halimeter) as previously described (7, 11). Subjects were instructed to refrain from talking for 1 min prior to measurements. The monitor was zeroed on ambient air and the measurement performed by inserting a short length of teflon tube (connected to the monitor inlet), fitted with a disposable suction tube approximately 4 cm into the oral cavity. The volunteer was oriented to avoid breathing during measurement in order to prevent contamination. Results of peak of sulphide concentration (ppb) were recorded. Measurements were made in duplicate and the mean value was calculated (16).

Tongue coating was scored using a clinical index (10, 17). This score ranged from 0 to 3 (0 – no tongue coating apparent; 1 – tongue coating covering less than 1/3 of tongue dorsum; 2 – tongue coating covering less than 2/3 of tongue dorsum; and 3 – tongue coating covering greater than 2/3 of tongue dorsum). Two examiners performed all measurements. The percentage agreement (Kappa Index) of tongue coating scores between the two examiners was 92% (k = 0.92).

## Statistical analysis

A power calculation was performed by using NQuery 4.0 software (Statistical Solutions, Boston, MA, USA), considering data from a previous study (7). A sample size of 30 subjects will have 90% power to detect a difference in means of at least 11.0 ppb in the VSC levels, when using a two group *t*-test calculation with a 0.05 significance level.

Data were analysed using of the Systat for Windows (Systat Software Inc., Chicago, IL, USA). Multivariate ANOVA, multiplelinear regression and Kruskal–Wallis (Mann–Whitney) tests were used to compare the data, considering 5% of significance level.

## Results

Eighteen (25%) subjects met the periodontitis disease criteria and 49 (68%) subjects presented more than 50% of the teeth



*Fig. 1.* Volatile sulphur compounds (VSC; mean  $\pm$  SEM) according to the tongue coating levels. The dashed line (80 ppb) represents the social acceptable VSC level.

### Table 1. VSC and tongue coating levels according to age, gender, periodontal disease and number of BOP and PPD sites

sites bleeding. All volunteers presented tongue coating level higher than zero.

Figure 1 shows the relationship between VSC (mean  $\pm$  SEM) and the tongue coating levels. Table 1 shows the relationship among VSC levels and age, gender, the percentage of bleeding sites and the percentage of pocket depth sites (>4 mm).

Multivariate ANOVA considering gender, tongue coating, periodontal status (disease or not) and race showed that only tongue coating (P = 0.01) influenced the VSC levels (Table 1). There were no statistically significant correlation (multiple linear regression, P > 0.05) among age, number of sites with BOP and number of sites with PPD >4 mm with VSC levels.

## Discussion

We found a significant correlation between VSC and tongue coating scores for all age groups, which was the same reported by Hinode *et al.* (18) and Liu *et al.* (19). It has become increasingly clear that tongue coating is the major factor inducing halitosis (13).

The fissures and crypts of the tongue can harbour large amounts of bacterial species (20). These innumerable depressions in the tongue surface are ideal niches for bacterial adhesion and growth, sheltered from cleaning action. These bacteria can be an important source for VSC, which are major components in oral malodour. Unfortunately, many clinicians

	VSC (mean ± SEM) [95% CI]	P	Tongue coating (median) [lower and upper quartiles]	P
Age (vears)				
18-29 (n = 16)  30-44 (n = 33)  45-60 (n = 23)	100.8 (10.9) [77.5–124.0] 126.6 (±17.7) [90.6–162.7] 120 (±15.9) [87.1–153.0]	0.667*	2 [1–2] 2 [1.5–3] 2 [2–2]	0.169 <sup>†</sup>
Gender Male ( <i>n</i> = 31) Female ( <i>n</i> = 41)	132.9 (±17.0) [98.1–167.6] 108.1 (±11.4) [85.0–131.2]	0.220 <sup>‡</sup>	2 [2–3] 2 [1–2]	0.477 <sup>§</sup>
Periodontal disease				
Yes $(n = 18)$ No $(n = 54)$	126.1 (±23.3) [76.8–175.3] 116.4 (±10.7) [94.9–137.8]	0.672 <sup>‡</sup>	2.5 [2–3] 2 [1–2]	0.002 <sup>§</sup>
BOP sites Up to 25% ( <i>n</i> = 16) Up to 50% ( <i>n</i> = 7) Up to 75% ( <i>n</i> = 16)	123.6 (±27.35) [65.3–181.9] 132.9 (±28.76) [62.5–203.2] 130.4 (±22.81) [81.8–179.0]	0.472*	2 [1.25–2.75] 3 [2–3] 2 [2–3]	0.097†
More than 75% ( $n = 33$ ) PPD sites	107.8 (±11.82) [83.7–131.9]		2 [1–2]	
None ( <i>n</i> = 48) Up to 25% ( <i>n</i> = 19) More than 25% ( <i>n</i> = 5)	114 (±11.37) [91.2–136.9] 113.5 (±14.01) [84.1–142.9] 184.2 (±75.09) [24.3–392.7]	0.142*	2 [1–2] 2 [2–3] 3 [1.5–3]	0.008†

BOP, bleeding on probing; PPD, probing pocket depth; VSC, volatile sulphur compounds. \*Multiple linear regression; <sup>†</sup>Kruskal–Wallis test; <sup>‡</sup>Multivariate anova; <sup>§</sup>Mann–Whitney *U*-test.

still believe that periodontal disease is the only cause for bad breath and they ignore that the tongue should be the first structure verified when facing a patient complaining about halitosis.

Gordon and Gibbons (21) were the first to analyse the tongue microbiota and they identified several anaerobic species (*Bacteroides, Fusobacteria spp, Peptococcus and Peptostreptococcus*) among the prominent cultivable microorganisms (22). Since then, most of the studies that evaluated the tongue microorganisms have concluded that it was characterized by a wide variable and diverse microbiota, with high proportion of anaerobic bacteria (23).

Although most patients perceive the condition of bad breath primarily as a cosmetic problem, reports of tissue toxicity at even extremely low concentrations of VSC have appeared (24), possibly causing damage in periodontal tissues (25). For these reasons, instructions for the prevention of halitosis should be included in oral health promotion activities.

There has been some disagreement regarding the effect of the aging on halitosis levels. Rosenberg *et al.* (11) suggested that sulphide measurements with the monitor were positively related to the age of the subject. However, Miyazaki *et al.* (10) demonstrated that age was a risk factor for deterioration of gum disease but not related to VSC increase. Our results suggest that oral malodour problems may not occur in older people having healthy periodontal conditions and little tongue coating.

Previous studies have shown that periodontal disease and tongue coating are a major source of VSC, and therefore, the cause of offensive odours (3, 13, 26). As most of the oral bacteria that produce malodorous compounds (e.g. *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsynthensis*, etc) are periodontal pathogens, it was logical to assume a positive correlation between VSC levels in the mouth air and the extent of periodontal pocket depths and the gingival bleeding tendency (14). The present study, however, showed that there was a much weaker association between VSC and periodontal conditions, which was also reported by Bosy et al. (15). These observations tend to suggest that oral malodour is not caused by periodontal disease alone but by a combination of factors, including periodontal conditions and tongue coating.

Another interesting data of our results was that 9 out of 16 periodontal patients with tongue coating level 3 had periodontal disease and 1 out of 16 patients with tongue coating level 1 had the same problem, which has lead us to speculate that tongue coating is a contributing factor for periodontal diseases. Moreover, Faveri *et al.* (27) reported that the tongue surface could be an important reservoir for periodontal pathogens and may play a role in the colonization of tooth surfaces and in the aetiology of halitosis.

Bad breath becomes an important factor in social relations and can cause concern, not only related to health aspects but also to psychological changes that lead to social and personal isolation (28). In addition, clinical studies revealed that brushing the teeth exclusively was not very effective in reducing oral malodour scores (13). Brushing the dorsum of the tongue with toothpaste was more effective than brushing the teeth. Therefore, it is clear that tongue cleaning is clinically highest priority to reduce oral malodour. Thus, health education should be realized with a view to a dynamic balance (29), involving the physical and psychological aspects of human beings as well as their social interactions, so that individuals do not turn into puzzles of sick parts.

## References

- Rosenberg M. Clinical assessment of bad breath: current concepts. J Am Dental Assoc 1996; 127: 475–482. Review. Erratum in: J Am Dent Assoc 1996 May; 127(5): 570.
- 2 Tangerman N. Halitosis in medicine: a review. *Int Dental J* 2002; **52**: 201–206.
- 3 Tonzetich J, Preti G, Huggins GR. Changes in concentration of volatile sulphur compounds of mouth air during the menstrual cycle. J Int Med Res 1978; 6: 245–254.
- 4 Kleinberg I, Westbay G. Salivary and metabolic factors involved in oral malodor formation. J Periodontol 1992; 63: 768–775.
- 5 Queiroz CS, Hayacibara MF, Tabchoury CPM, Marcondes FK, Cury JA. Relationship among stressful situations, salivary flow rate and oral volatile sulfur-containing compounds. *Eur J Oral Sci* 2002; **110:** 337–340.
- 6 Kurihara E, Marcondes FK. Oral concentration of sulfur compounds in stressed rats. *Stress* 2002; 5: 295–298.
- 7 Calil CM, Marcondes FK. Influence of anxiety on the production of oral volatile compounds. *Life Sci* 2006; **79:** 660–664.
- 8 Tonzetich J. Direct gas chromatographic analysis of sulphur compounds in mouth air in man. Arch Oral Biol 1971; 16: 587-597.
- 9 Tonzetich J, Yaegaki K, Coil JM. Collagen metabolism by fibroblasts cultures in presence of methyl mercaptan. *J Dental Res* 1986; 64: 786.
- 10 Miyasaki H, Sakao S, Katoh Y, Takehara T. Correlation between volatile sulphur compounds and certain oral health measurements in the general population. *J Periodontol* 1995; 66: 679–684.
- 11 Rosenberg M, Knaan T, Cohen D. Association among bad breath, body mass index, and alcohol intake. J Dent Res 2007; 86: 997– 1000.
- 12 Rosenberg M, Kulkarni GV, Bosy A, McCulloch CAG. Reproductibility and sensitivity of oral malodor measurements with a portable sulfide monitor. *J Dent Res* 1991; **70**: 1436–1440.
- 13 Yaegaki K, Sanada K. Volatile sulfur compounds in mouth air from clinically healthy subjects with and without periodontal disease. *J Periodont Res* 1992; 21: 434–439.
- 14 Coli JM, Tonzetich J. Characterization of volatile sulphur compounds production at individual gingival crevicular sites in humans. *J Clin Dent* 1992; 3: 97–103.

- 15 Bosy A, Kulkarni GV, Rosenberg M, McCulloch CA. Relantionship of oral malodor to periodontitis: evidence of independence in discrete subpopulations. *J Periodontol* 1994; 6: 37–46.
- 16 Monteiro AMF, Chinellato LEM, Tárzia O, Rezende MLR. Evaluation of oral and nasal odor in patients with and without cleft lip and palate: preliminary report. *Cleft Palate Craniofac J* 2004; 41: 661–663.
- 17 Oho T, Yoshida Y, Shimazaki Y, Yamashita Y, Koga T. Characteristics of patients complaining of halitosis and the usefulness of gas chromatography for diagnosing halitosis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001; **91:** 531–534.
- 18 Hinode D, Fukui M, Yokoyama N, Yokoyama M, Yoshioka M, Nakamura R. Relationship between tongue coating and secretoryimmunoglobulin A level in saliva obtained from patients complaining of oral malodor. J Clin Periodontol 2003; 30: 1017–1023.
- 19 Liu XN, Shinada K, Chen XC, Zhang BX, Yaegaki K, Kawaguchi Y. Oral malodor-related parameters in the Chinese general population. *J Clin Periodontol* 2006; **33**: 31–36.
- 20 De Boever EH, Loesche WJ. Assessing the contribution of anaerobic microflora of the tongue to oral malodor. J Am Dent Microbiol 1996; 34: 537–542.
- 21 Gordon DF, Gibbons RJ. Studies of the predominant cultivable micro-organisms from the human tongue. Arch Oral Biol 1966; 11: 627–632.

- 22 Goldberg S, Cardash H, Browning H, Sahlyh H, Rosenberg M. Isolation of enterobacteriadae from the mouth and potential association with malodor. *J Dental Res* 1997; **76**: 1770–1775.
- 23 Loesche WJ, Kazor CE. Microbiology and Treatment of halitosis. *Periodontol 2000* 2002; 28: 256–279.
- 24 Ratcliff PA, Johnson PW. The relationship between oral malodor, gingivitis, and periodontitis. A review. *J Periodontol* 1999; 5: 485– 489.
- 25 Johnson PW, Yaegaki K, Tonzetich J. Effect of volatile thiol compounds on protein metabolism by human gingival fibroblasts. *J Periodontal Res* 1992; 27: 553–561.
- 26 Schmidt NF, Tarbet WS. The effect of oral rinse on organoleptic mouth odor ratings and levels of VSC. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1978; 45: 876–883.
- 27 Faveri M, Feres M, Shibli JA, Hayacibara RF, Hayacibara MM, de Figueiredo LC. Microbiota of the dorsum of the tongue after plaque accumulation: an experimental study in humans. *J Periodontol* 2006; **77:** 1539–1546.
- 28 Sanz M, Roldán S, Herrera D. Fundamentals of breath malodour. J Contemp Dent Pract 2001; 2: 1–17.
- 29 Elias MS, Ferriani MGC. Historical and social aspects of halitosis. *Rev Latino-Am Enfermagem* 2006; 14: 821–823.

Copyright of International Journal of Dental Hygiene is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.