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Low salivary flow and volatile sulfur compounds in mouth air

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Objective. The purpose of this study was to determine whether a reduction of salivary flow would influence the production of methylmercaptan (CH₃SH) and hydrogen sulfide (H₂S), which are volatile sulfur compounds (VSCs) known to cause oral malodor.

Study design. The VSCs in mouth air were measured by means of gas chromatography. Spitting and masticatory (stimulated) methods were used to determine the salivary flow rates of 174 patients.

Results. There was no significant correlation between the level of VSCs and salivary flow rate. However, subjects with extremely low resting salivary flow rates had significantly higher CH₃SH and H₂S concentrations and tongue-coating scores than those with higher resting salivary flow rates. Moreover, logistic analyses revealed that extremely low resting salivary flow, the increase in tongue coating, and a probing pocket depth greater than 4 mm were strong explanatory factors for the generation of VSCs, which could have caused oral malodor.

Conclusions. These findings suggested that an extreme reduction in resting saliva influenced the generation of CH₃SH and H₂S in mouth air.

(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96:38-41)

Volatile sulfur compounds (VSCs) such as hydrogen sulfide (H₂S) and methylmercaptan (CH₃SH) are the main causes of oral malodor,¹⁻³ which is a common complaint in different populations. VSCs originate from the bacterial metabolism of amino acids in materials such as food debris, desquamated cells from oral mucosa, and leukocytes that accumulate in the oral cavity.³⁻⁵ The intensity of clinical bad breath is significantly correlated with the level of the intraoral VSCs.^{6,7} The generation of oral VSCs is influenced by various factors in the oral cavity. The tongue coating and the periodontal pocket are the main sources of VSC production with respect to the bacterial profile. It is evident that an increase in the amount of tongue coating and the number of periodontal pockets significantly correlates with an increase in the concentration of VSCs in mouth air.⁸⁻¹²

Dry mouth is generally regarded as another of the major contributory factors in the production of oral

malodor because decreased salivary flow weakens the normal cleansing mechanism of the mouth and predisposes the oral flora toward the gram-negative organisms responsible for the malodor.^{13,14} The oral malodor called morning breath is one of the phenomena caused by reduced salivary flow during sleep.^{3,14} However, previous studies have shown no evidence that a reduction in the salivary flow rate significantly correlates with increases in oral malodor or concentration of VSCs in mouth air.^{6,12} Thus, the general view that a reduction of salivary flow is linked to the production of oral malodor is merely a hypothesis.

The purpose of this study was to determine whether a reduction in salivary flow would influence the production of oral malodor, so we evaluated the relationship between the salivary flow rate and the level of VSCs in mouth air and between salivary flow rate and other parameters that are related to oral malodor, such as tongue coating and periodontal health.

SUBJECTS AND METHODS

The subjects were adult individuals who were selected at random from patients visiting for care at the Preventive Dentistry and Breath Odor Clinic of Kyushu Dental College (Kitakyushu, Japan). The procedures were explained to the subjects, and their informed consent was obtained before the investigation. Because systemic diseases and a decrease in the number of remaining teeth might influence the correlation between the generation of VSCs and the salivary flow rate, the subjects who were undergoing treatment for

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Received for publication May 8, 2002; returned for revision Jul 11, 2002; accepted for publication Feb 3, 2003.

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1079-2104/2003/\$30.00 + 0

doi:10.1016/S1079-2104(03)00162-8

Table I. Profiles of the subjects in this study (means \pm SDs)

Parameters	Total (N = 174)	Men (n = 52)	Women (n = 122)
Age (y)	48.6 \pm 13.7	50.1 \pm 13.3	48.0 \pm 13.9
No. of teeth	26.5 \pm 2.9	26.7 \pm 3.1	26.4 \pm 2.8
Resting salivary flow rate (mL/min)	0.3 \pm 0.2	0.4 \pm 0.3	0.3 \pm 0.2
Stimulated salivary flow rate (mL/min)	1.4 \pm 0.8	1.6 \pm 0.8	1.4 \pm 0.8
Concentration of CH ₃ SH (ng/10 mL)	5.6 \pm 18.1	9.0 \pm 26.7	4.2 \pm 12.7
Concentration of H ₂ S (ng/10 mL)	9.4 \pm 31.3	18.8 \pm 53.1	5.5 \pm 13.2
No. of PD4 (sites)	10.2 \pm 16.9	16.3 \pm 22.7*	7.7 \pm 13.1
No. of BOP (sites)	15.5 \pm 17.7	17.1 \pm 19.3	14.8 \pm 16.9
Tongue-coating score	1.2 \pm 0.6	1.4 \pm 0.7*	1.1 \pm 0.6
PCR (%)	46.2 \pm 20.5	49.7 \pm 19.2	44.7 \pm 20.9

CH₃SH, Methylmercaptan; H₂S, hydrogen sulfide; PD4, probing pocket depth \geq 4 mm; BOP, bleeding on probing; PCR, plaque control record.
*Mann-Whitney U test, P < .05.

systemic diseases or who had fewer than 20 teeth¹⁵ were excluded from this study.

All subjects underwent a general dental examination. Probing pocket depth (PD) and bleeding on probing (BOP) were evaluated by using a Williams probe. Probing was performed at 3 points on the buccal and lingual sides of each tooth. Sites with probing pocket depths greater than 4 mm (PD4) and sites with BOP were counted. The tongue coating was rated in terms of the extent of the distribution (by using a previously described method¹²) as follows: 0, none (or invisible); 1, light (ie, less than one third of the tongue dorsum surface covered); 2, medium (ie, less than two thirds of the dorsum covered); 3, heavy (ie, more than two thirds covered). The amount of dental plaque was estimated by using the plaque control record (PCR; in percent) developed by O'Leary et al.¹⁶

Gas chromatography was used in the oral malodor evaluation. In this study, H₂S and CH₃SH concentrations in mouth air were determined by applying a previously described method⁸ to the use of a gas chromatograph (G2800 gas chromatograph; Yanaco, Kyoto, Japan) equipped with a flame photometric detector and a 3.4-mm \times 3-m glass column packed with 1,2,3-Tris propane 25% (2-cyanoethoxy) in chromosorb W AW-DMCS 60/80 mesh (GL Sciences, Tokyo, Japan). The column conditions were as follows: column temperature, 60°C; injection port temperature, 120°C; flame photometric detector temperature, 120°C; nitrogen gas flow pressure, 1.2 kg/cm²; hydrogen gas flow pressure, 1.0 kg/cm²; and air flow pressure, 1.0 kg/cm². Each CH₃SH and H₂S result was dichotomized into a high-concentration group and a low-concentration group in terms of each standard level (ie, CH₃SH > 0.5 ng/10 mL and H₂S of 1.5 ng/10 mL) that was found objectionable; the standard level was demonstrated in a previous study.¹⁷

The flow rate of whole saliva was determined by

Table II. Spearman correlation coefficients between VSC levels and salivary flow rates and between VSC and parameters of considerable oral malodor

Parameters	CH ₃ SH concentration (ng/10 mL)		H ₂ S concentration (ng/10 mL)	
	r	P	r	P
Resting salivary flow rate (mL/min)	-0.103	.190	-0.044	.581
Stimulated salivary flow rate (mL/min)	0.055	.487	0.098	.219
No. of PD4 (sites)	0.262	.001	0.311	<.001
No. of BOP (sites)	0.310	<.001	0.276	<.001
Tongue-coating score	0.386	<.001	0.340	<.001
PCR (%)	0.052	.541	-0.086	.315

VSC, Volatile sulfur compound.

using the draining (resting) and the paraffin-chewing (stimulated) methods as described elsewhere.¹⁸

The subjects were classified into 2 groups in terms of their resting and stimulated salivary flow rates. The following classifications were based on the methods of Ericsson and Hardwick¹⁹: resting saliva (RS) flow rate < 0.1 mL/min, or group RS1; RS \geq 0.1 mL/min, or group RS2; stimulated saliva (SS) flow rate < 0.7 mL/min, or group SS1; SS \geq 0.7 mL/min, or group SS2. The Mann-Whitney U test was used to evaluate the differences between men and women and the differences between groups classified in terms of the salivary flow rate. Spearman rank correlation analyses were used to evaluate the relationship among the concentration of the VSCs (CH₃SH and H₂S), the salivary flow rate, and the parameters related to oral malodor. Stepwise logistic regression analyses were performed to isolate the strongest explanatory factor among the extremely low resting salivary flow rates and the stimulated salivary flow rates and the increase in the tongue

Table III. Comparison of parameters between groups on the basis of salivary flow rate

Variables	Salivary flow rates			
	RS rate		SS rate	
	RS1 (n = 24 [8/16])	RS2 (n = 150 [44/106])	SS1 (n = 25 [7/18])	SS2 (n = 149 [45/104])
Age (y)	52.9 ± 14.5	47.9 ± 13.5	45.4 ± 17.3	49.1 ± 13.0
No. of teeth	25.4 ± 3.3	26.7 ± 2.8	26.8 ± 3.0	26.4 ± 2.9
Concentration of CH ₃ SH (ng/10 mL)	19.6 ± 31.0*	3.2 ± 13.5	8.5 ± 25.1	5.1 ± 16.5
Concentration of H ₂ S (ng/10 mL)	18.4 ± 29.1*	8.0 ± 31.6	8.0 ± 16.3	9.7 ± 33.3
No. of PD4 (sites)	16.3 ± 22.8	9.3 ± 15.7	8.2 ± 15.6	10.6 ± 17.1
No. of BOP (sites)	23.8 ± 25.7	14.2 ± 15.7	17.8 ± 18.1	15.1 ± 17.6
Tongue-coating score	1.5 ± 0.5*	1.1 ± 0.6	1.2 ± 0.7	1.1 ± 0.6
PCR (%)	50.5 ± 19.6	45.4 ± 20.6	52.5 ± 22.7*	44.9 ± 19.8

RS, Resting saliva; RS1, resting saliva less than 0.1 mL/min; RS2, resting saliva greater than or equal to 0.1 mL/min; SS, stimulated saliva; SS1, stimulated saliva less than 0.7 mL/min; SS2, stimulated saliva greater than or equal to 0.7 mL/min; n, number of subjects (men/women).

*Mann-Whitney U test, $P < .05$.

Table IV. Odds ratios of parameters for producing high concentrations of CH₃SH and H₂S

Parameters	Odds ratio (95% CI)	
	CH ₃ SH ≥ 0.5 ng/10 mL	H ₂ S ≥ 1.5 ng/10 mL
RS flow rate <0.1 mL/min	4.0 (1.1-13.6)*	4.2 (1.2-14.8)*
Increase of 1 score in tongue coating	2.8 (1.4-5.8) [†]	2.0 (1.0-4.2)*
Increase of 1 site in no. of PD4	1.1 (1.0-1.1) [†]	1.1 (1.0-1.2) [†]

* $P < .05$.

[†] $P < .01$.

coating score, PD4, BOP, and PCR for the high concentration of CH₃SH and H₂S in mouth air. Statistical analyses were performed with SPSS software (SPSS Inc, Chicago, Ill).

RESULTS

The profiles of the subjects in this study are shown in Table I. The study population consisted of 52 men and 122 women with a mean age and SD of 48.6 ± 13.7 years. There was no significant difference between men and women in terms of the parameters, except for the number of PD4 and the tongue-coating score. That is, men had significantly greater numbers of PD4 and higher tongue-coating scores than did women.

Table II shows the correlations between the VSC levels and the salivary flow rate, in addition to the correlations between VSC levels and the parameters of considerable oral malodor. Significant correlations were found between the VSC (CH₃SH and H₂S) concentrations and the numbers of PD4 and BOP and between VSC concentrations and the tongue-coating score. The resting and stimulated salivary flow rates had no significant correlation with the VSCs.

The mean values of parameters among groups classified in terms of the difference in the resting salivary flow rate or the stimulated salivary flow rate are summarized in Table III. The VSC concentrations and tongue-coating scores were significantly higher in the RS1 group than in the RS2 group, and the PCR score was significantly higher in the SS1 group than in the SS2 group. There was no significant difference between the groups with respect to the other parameters. On the other hand, the 7 subjects with RS1 also had SS1.

Table IV shows the odds ratios calculated for stepwise logistic regression analyses to evaluate the associations between the high concentrations of CH₃SH and H₂S (CH₃SH ≥ 0.5 ng/10 mL; H₂S ≥ 1.5 ng/10 mL) and the resting salivary flow rate, the tongue coating, and the number of PD4. The odds ratios calculated from subjects with high concentrations of CH₃SH and H₂S in mouth air were significantly greater, by 4.0-fold and 4.2-fold ($P < .05$), respectively, if the resting salivary flow rate was extremely low (ie, <0.1 mL/min). The odds ratios calculated from subjects with high concentrations of CH₃SH and H₂S were significantly greater, by 2.8-fold and 2.0-fold ($P < .01$), respectively, in terms of a 1-point increase in the tongue-coating score. In addition, the odds ratios were greater, by 1.1-fold ($P < .01$), with an increase in the number of PD4 at 1 site. Other parameters (the stimulated salivary flow rate and the number of BOP sites and PCRs) were not extracted as strong factors related to the objectionable VSC concentrations.

DISCUSSION

Saliva plays an important role in the maintenance of oral health. Thus, reduced salivary flow predisposes individuals to oral disease and discomfort. Our results reveal that the proportions of subjects with a resting salivary flow rate of less than 0.1 mL/min and a stim-

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CH₃SH and H₂S are the main substances that cause oral malodor.¹⁻³ This study has shown no significant correlation between the CH₃SH and H₂S levels in mouth air and the resting and stimulated salivary flow rate; however, the results did reveal that subjects with a resting salivary flow rate of less than 0.1 mL/min had higher CH₃SH and H₂S levels in mouth air than those with a flow rate greater than 0.1 mL/min. Therefore, it was suggested that an extreme reduction in the RS flow rate might be one of the risk factors for the generation of oral malodor but that RS flow may not significantly influence the intensity of the oral malodor if the rate is within normal limits. In contrast, the VSC levels of subjects with an extremely low stimulated salivary flow rate of less than 0.7 mL/min did not significantly differ from those of the other subjects; therefore, the reduction in the SS was not believed to be associated with the generation of VSCs. This result is similar to the findings of Miyazaki et al,²⁰ who showed no correlation between the VSC level and the SS. Thus, we suggest that a reduction in the stimulated saliva might not be a risk factor for oral malodor.

It was evident from a review of previous studies that tongue coating was strongly related to VSC production⁸⁻¹² and that the VSC concentration was higher in individuals with periodontitis than in those without periodontitis.⁸⁻¹⁰ This study showed that the extreme reduction in RS flow, in addition to the increase in the amount of tongue coating and periodontal pockets, was one of the main explanatory factors for the high-level generation of VSCs (CH₃SH and H₂S) in mouth air. On the other hand, subjects with an extremely low RS flow rate had a significantly higher tongue-coating score than the other subjects, and they tended to have poor periodontal health compared with the others. Therefore, it was suggested that a reduction of RS flow might influence the production of tongue coating and the periodontal health and that oral malodor was caused by the interaction of multiple risk factors.

In conclusion, in addition to tongue coating and periodontal health, a reduction in the RS flow rate influenced the generation of the VSCs (CH₃SH and H₂S). Thus, an extreme reduction in the RS flow rate is associated with the generation of oral malodor.

REFERENCES

1. Tonzetich J. Direct gas chromatographic analysis of sulphur compounds in mouth air in man. *Arch Oral Biol* 1964;16:587-97.
2. Tonzetich J, Richer VJ. Evaluation of volatile odoriferous components of saliva. *Arch Oral Biol* 1971;9:39-45.
3. Kleinberg I, Westbay G. Oral malodor. *Crit Rev Oral Biol Med* 1990;1:247-59.
4. McNamara TF, Alexander JF, Lee M. The role of microorganisms in the production of oral malodor. *Oral Surg Oral Med Oral Pathol* 1972;34:41-8.
5. Morita M, Wang HL. Association between oral malodor and adult periodontitis: a review. *J Clin Periodontol* 2001;28:813-9.
6. 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;91:531-4.
7. Rosenberg M, Septon I, Eli I, Bar-Ness R, Gelernter J, Brenner S, et al. Halitosis measurement by an industrial sulphide monitor. *J Periodontol* 1991;62:487-9.
8. Yaegaki K, Sanada K. Volatile sulfur compounds in mouth air from clinically healthy subjects and patients with periodontal disease. *J Periodontol Res* 1992;27:233-8.
9. Yaegaki K, Sanada K. Biochemical and clinical factors influencing oral malodor in periodontal patients. *J Periodontol* 1992;63:783-9.
10. Morita M, Wang HL. Relationship between sulcular sulfide level and oral malodor in subjects with periodontal disease. *J Periodontol* 2001;72:79-84.
11. De Buever EH, Loesche WJ. Assessing the contribution of anaerobic microflora of the tongue to oral malodor. *J Am Dent Assoc* 1995;126:1384-93.
12. Miyazaki H, Sakao S, Katoh Y, Takehara T. Correlation between volatile sulphur compounds and certain oral health measurements in the general population. *J Periodontol* 1995;66:679-84.
13. Messadi DV. Oral and nonoral sources of halitosis. *J Calif Dent Assoc* 1997;25:127-31.
14. McDowell JD, Kassebaum DK. Diagnosing and treating halitosis. *J Am Dent Assoc* 1993;124:55-64.
15. Goto M, Ishii T, Sakakihara Y. Studies of mastication as an indicator of adult dental health: II. Comparative study of age and number of missing teeth. *J Dent Hlth* 1987;37:444-445.
16. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. *J Periodontol* 1972;43:38.
17. Tonzetich J. Production and origin of oral malodor: a review of mechanisms and methods of analysis. *J Periodontol* 1977;48:13-20.
18. Heintze U, Birkhed D, Björn H. Secretion rate and buffer effect of resting and stimulated whole saliva as a function of age and sex. *Swed Dent J* 1983;7:227-38.
19. Ericsson Y, Hardwick L. Individual diagnosis, prognosis and counselling for caries prevention. *Caries Res* 1978;12:94-102.
20. Miyazaki H, Fujita C, Soh I, Takehara T. Relationship between volatile sulfur compounds and oral conditions in the general Japanese population. In: van Steenberghe D, Rosenberg M, editors. *Bad breath: a multidisciplinary approach*. Leuven, Belgium: Leuven University Press; 1996. p. 165-79.

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n = 149 [45/104]

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