J Periodont Res 2008; 43: 186–193

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2007.01011.x

The levels of volatile sulfur compounds in mouth air from patients with chronic periodontitis

C-C. Tsai, H-H. Chou, T-L. Wu, Y-H. Yang, K-Y. Ho, Y-M. Wu, Y-P. Ho

Graduate Institute of Dental Sciences College of Dental Medicine and Chung-Ho Memorial Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan

Tsai C-C, Chou H-H, Wu T-L, Yang Y-H, Ho K-Y, Wu Y-M, Ho Y-P. The levels of volatile sulfur compounds in mouth air from patients with chronic periodontitis. J Periodont Res 2008; 43: 186–193. © 2007 The Authors. Journal compilation © 2007 Blackwell Munksgaard

Background and Objective: Volatile sulfur compounds may be the main source of oral malodor. The aim of this study was to clarify the relationship between periodontal parameters and volatile sulfur compounds and to evaluate the improvement of several halitosis-related outcomes by tongue scraping, nonsurgical periodontal treatment (including oral hygiene instruction) and oral hygiene instruction/chlorhexidine + cetyl pyridinium gargling.

Material and Methods: Seventy-two chronic periodontitis patients with heavy tongue coating were assessed for oral malodor and periodontal status. Oral malodor was evaluated by measuring the levels of volatile sulfur compounds using OralChroma^{TM} and the organoleptic test score. Thirty participants were selected for the subsequent experiments: tongue scraping; nonsurgical periodontal treatment; and oral hygiene instruction/chlorhexidine + cetyl pyridinium gargling. Twenty-five participants completed all experimental stages.

Results: Significant correlations were observed between the organoleptic test score and hydrogen sulfide (H_2S), methyl mercapton (CH_3SH), tongue coating score and volatile sulfur compounds, which was also significantly correlated with bleeding on probing percentage and tongue coating score. Tongue scraping significantly reduced the levels of volatile sulfur compounds. Further reduction of volatile sulfur compounds after nonsurgical periodontal treatment and oral hygiene instruction/chlorhexidine + cetyl pyridinium gargling were noted compared with baseline.

Conclusion: Volatile sulfur compounds, with H_2S and CH_3SH as the main components, in mouth air are the prominent elements of malodor. Volatile sulfur compounds were decreased by more than 50% after tongue scraping. Nonsurgical periodontal treatment and oral hygiene instruction/chlorhexidine + cetyl pyridinium gargling maintained a significantly lower level of malodor compared with baseline.

Chi-Cheng Tsai, DDS, PhD, College of Dental Medicine, Kaohsiung Medical University, Kaohsiung City, Taiwan
Tel: +886 07 3121101, ext. 2112
Fax: +886 07 3210637
e-mail: chchts@kmu.edu.tw

Key words: chlorhexidine; nonsurgical periodontal treatment; oral malodor; periodontitis; tongue coating; volatile sulfur compounds

Accepted for publication March 30, 2007

Tonzetich (1) indicated that the principal components of bad breath are volatile sulfur compounds. Hydrogen sulfide (H_2S) and methyl mercapton (CH_3SH) comprise 90% of the total volatile sulfur compounds and are the most important factors for determining the degree of bad breath. Miyazaki *et al.* (2) measured volatile sulfur compound levels by a portable sulfide monitor, and when examining the periodontal status (community periodontal treatment need and attachment loss), dental plaque and tongue coating, they found that the concentration

of volatile sulfur compounds had a high correlation with the periodontal status and the amount of tongue coating. These results indicated that periodontal disease and tongue coating are the major causes of foul odor of breath.

In addition, Yaegaki *et al.* (3) detected, by gas chromatography, the presence of volatile sulfur compounds in 31 patients with periodontal disease and they found that the CH_3SH concentration and the CH_3SH/H_2S ratio in the group with probing pocket depth ≥ 4 mm were higher than in the group with probing pocket depth < 4 mm. After removal of the tongue coating, the average amount of volatile sulfur compounds decreased, further suggesting that tongue coating and periodontal pockets are the main factors determining the presence of volatile sulfur compounds, and that the periodontal condition had a greater effect on CH_3SH than on H_2S. Yaegaki *et al.* (4) also indicated that the saliva of patients with periodontal disease contained high levels of 2-ketobutyrate. As 2-ketobutyrate is an intermediate metabolite in the conversion of methionine to CH_3SH, faster metabolism of methionine to CH_3SH could occur in patients with periodontal disease. The analysis of gingival crevicular fluid samples found that methionine is the major amino acid. This result indicated that saliva and gingival crevicular fluid might be the sources of volatile sulfur compounds.

The main direct approach to assess breath odor is organoleptic assessment (rating); however, this rating has low interexaminer agreement, low reproducibility and is too subjective (5). Tonzetich (1) measured volatile sulfur compounds by gas chromatography. At the present time, gas chromatography is the gold standard for measuring breath malodor. Schmidt *et al.* (6) assessed 102 oral breath samples by gas chromatography and also evaluated them by organoleptic rating. These investigators found a statistically significant correlation between the two methods. However, the drawbacks of gas chromatography equipment are that it is too large in size, expensive and technically sensitive (7). Rosenberg *et al.* (5) surveyed malodor in 77 individuals using both a sulfide monitor and organoleptic measurements. These researchers found that the concentration of volatile sulfur compounds measured by a sulfide monitor is highly correlated with the organoleptic rating, suggesting that the sulfide monitor could be a convenient instrument for diagnosing mouth malodor and evaluating the treatment effects.

Quirynen *et al.* (8) found that one-stage full-mouth periodontal treatment has a better result than multiple-stage treatment in the prevention of mouth malodor. Bosy *et al.* (9) assessed volatile sulfur compounds by a sulfide monitor in 127 subjects and found significant decreases of volatile sulfur compounds after oral rinsing with 0.2% chlorhexidine for 7 d. This result suggested that besides periodontal treatment, oral rinsing with antiseptics could improve breath odor.

The present study aimed to examine Taiwanese subjects with moderate periodontitis suffering from bad breath to assess the impact of nonsurgical periodontal treatment and the adjunctive effect of chlorhexidine + cetyl pyridinium on breath-related outcome variables.

Material and methods

Subjects

Patients diagnosed with moderate periodontitis (as described in the acceptance criteria) and having breath malodor were recruited from July 2004 to December 2005 at the Department of Periodontics, Kaohsiung Medical University Hospital, Taiwan. Patients gave their informed consent to participate in this study and the protocol was reviewed by the Clinical Trials Committee of the Kaohsiung Medical University Hospital. The acceptance criteria were that patient must have: (a) at least 15 functional teeth with a minimum of six teeth having a probing pocket depth of ≥ 5 mm on one or more site(s); (b) a minimum of six teeth with bone loss over one-third of the root length; (c) a tongue coating score of ≥ 4 (2); and (d) an organoleptic testing score of ≥ 2 (5). The exclusion criteria were that patient: (a) had received periodontal treatment, including prophylactic scaling, subgingival curettage and periodontal surgery, within the 6 mo prior to the study; (b) had received radiotherapy of the head and neck region and had salivary gland atrophy with xerostomia; (c) had systemic disease(s), including of the gastrointestinal tract, liver and kidneys, or chronic respiratory diseases; (d) had received antibiotics within 6 mo prior to the study; (e) had more than 4 units of fixed crown-bridge or removable partial denture; or (f) was a current smoker or betel quid chewer.

Pilot study

Five patients were selected to evaluate the reproducibility of volatile sulfur compound measurements assessed by the OralChroma^{TM} (Abilit Corp., Osaka City, Japan). Subjects were asked to have two volatile sulfur compound tests 10-min apart. The levels of volatile sulfur compounds of the two periods were highly reproducible ($r = 0.98$, $p = 0.0002$).

Experimental design

The experiment on each patient was carried out sequentially, as outlined in Table 1. Seventy-two subjects (42 men, 30 women, 19–64 years, mean age 46.83 ± 10.18 years) were selected, according to the aforementioned acceptance and exclusion criteria, to participate in the baseline data collection. Thirty subjects (17 men, 13 women, mean age 46.13 ± 10.71 years) were sampled at random from these 72 patients to participate in the 'tongue scraping' experiment. Lastly, 25 patients (15 men, 10 women, mean age 46.56 ± 11.09 years) completed the nonsurgical periodontal treatment, oral hygiene instruction/chlorhexidine-2 and oral hygiene instruction/chlorhexidine-4 experiments. At the completion of each stage of the experiment, patients received a volatile sulfur compound assessment, organoleptic testing and periodontal status evaluation. The concentration of volatile sulfur compounds was measured using

Table 1. Flow chart of investigation

I. Baseline	
1	Volatile sulfur compounds measurement.
2	Organoleptic test score.
3	Tongue coating score (0–12).
4	Periodontal examination: plaque index, gingival index, probing pocket depth, clinical attachment loss, percentage of bleeding on probing.
5	Full-mouth radiographs (parallel technique).
II. Tongue scraping	
1	Thorough removal of tongue coating (tongue coating score = 0) with a plastic tongue scraper (President OrganicShops Ltd. Taiwan) immediately after baseline data collection.
2	Volatile sulfur compounds measurement immediately after tongue scraping.
III. Nonsurgical periodontal therapy	
1	Nonsurgical periodontal therapy including scaling and root planing, removal of ill-fitting prostheses, oral hygiene instruction [Bass method tooth brushing, interdental brushing and flossing, tongue brushing (10 times once, twice a day with a toothbrush)] beginning within 2 wk after 'II. Tongue scraping' and completed within 2 wk.
2	Volatile sulfur compounds measurement, organoleptic test score, tongue coating score and periodontal examination 1 mo after the last date of 'III. Nonsurgical periodontal therapy'.
IV. Oral hygiene instruction/chlorhexidine-2, oral hygiene instruction/chlorhexidine-4	
1	Oral hygiene instruction as 'III. Oral hygiene instruction.
2	15–20 mL of '0.12% chlorhexidine + 0.05% cetyl pyridinium' (Skuber Gargle™; Washington Drug Co., Taiwan) mouth gargling, for 30 s every morning and night. Two weeks and 4 wk later, data collections were made (volatile sulfur compounds measurement, organoleptic test score, tongue coating score, periodontal parameters).

the OralChroma™. A new plastic 1-mL syringe was inserted deep into the patient's oral cavity and held between the lips for 3 min. Then, the plunger was pulled slowly, pushed in again and pulled for a second time before removal from the mouth. A dedicated needle was attached and the sample of oral gas was ejected to 0.5-mL (one-half calibration) by the plunger. The remaining oral gas was injected into the inlet on the main unit of the OralChroma™. The measurement was then started automatically. Further operations were handled by the 'OralChroma™ Data Mananger'. Then, the organoleptic test score was determined to evaluate malodor. The subject was asked to close his/her lips tightly for 3 min and then to exhale briefly through the mouth, at a distance $\approx$10 cm from the nose of the examiner (the second author). A privacy screen with a hole was placed between the subject and the examiner. The organoleptic test score was recorded on a scale of 0–5 (0, no odor; 1, really noticeable odor; 2, light but clearly noticeable odor; 3, moderate odor; 4, strong odor; 5, extremely foul odor) (5,10). To avoid smell fatigue, the second patient was evaluated at least 30 min after the previous one. Following organoleptic testing, tongue coating was assessed on a scale of 0–3 by inspecting the areas of the tongue (the tongue was divided into four areas by the sulcus terminalis and the linea mediana: anterior right, anterior left, posterior right and posterior left) and scoring the coating (0, no coating apparent; 1, less than one-third of the area of the tongue dorsum coated; 2, between one-third and two-thirds of the surface covered; and 3, more than two-thirds of the surface coated) (2). The tongue coating score of a tongue could therefore range from 0 to 12. Patients then received periodontal examinations, including the determination of plaque index (11), gingival index (12), probing pocket depth using the Williams Periodontal Probe (Hu-Fridy™), clinical attachment loss and bleeding on probing (13). Full-mouth radiographs were taken by a parallel technique for the evaluation of bone levels (at baseline only).

After data collection at baseline, 30 patients were randomly chosen to receive tongue scraping immediately followed by measurements for volatile sulfur compounds and organoleptic testing. Twenty-five patients received nonsurgical periodontal treatment, consisting of scaling and root planing, removal of unsatisfactory restorations and oral hygiene instructions (Bass method tooth brushing, flossing and interdental brushing, tongue brushing 10 times once, twice a day) within 2 wk after the first tongue scraping. Four weeks after the completion of nonsurgical periodontal treatment, similar data were collected as at baseline. Then, patients were asked to gargle with chlorhexidine/cetyl pyridinium (15–20 mL, twice a day: in the morning and at night for 30 s). Data were collected at 2- and 4-wk time-periods.

Statistical analysis

Statistical analysis was carried out using JMP5.0 and SAS (SAS Institute, Cary, NC, USA). Pearson correlation was used to determine the association between the concentration of volatile sulfur compounds and the organoleptic test score, tongue coating score and clinical parameters. The Boferroni strategy was used to define Pearson correlation for p-value analysis [$p = 0.05/(^{12}_{2}) = 0.000758$]. The Wilcoxon Sign-Ranks test was used to determine the differences in the values collected from the five-stage experiments (baseline, tongue scraping, nonsurgical periodontal treatment, oral hygiene instruction/chlorhexidine-2 and oral hygiene instruction/chlorhexidine-4) including volatile sulfur compounds, organoleptic test score, tongue coating score and clinical periodontal parameters.

Results

Organoleptic test score, volatile sulfur compounds, tongue coating score and periodontal parameters at baseline

The Pearson correlation coefficients between organoleptic test score and concentrations of volatile sulfur

Table 2. Pearson correlation of organoleptic score, sulfide measurements and periodontal measurements

TCS	OLTS	H_2S	CH_3SH	$(CH_3)_2S$	VSCs	PPD	CAL	GI	PLI	BOP%	PPD%
TCS	0.68*	0.58*	0.40*	0.14	0.49*	− 0.01	0.04	0.09	0.33	0.39	0.19
OLTS		0.61*	0.45*	0.16	0.54*	0	− 0.02	0.2	0.44*	0.54*	0.22
H_2S			0.77*	0.39	0.91*	− 0.14	− 0.10	− 0.06	0.35	0.42*	0.15
CH_3SH				0.71*	0.96*	− 0.13	− 0.1	− 0.09	0.25	0.36	0.21
$(CH_3)_2S$					0.65*	− 0.13	− 0.11	− 0.08	0.05	0.21	0.15
VSCs					− 0.14	− 0.11	− 0.09	0.3	0.41*	0.20	
PPD							0.92*	0.27	− 0.16	0.16	0.40*
CAL							0.25	− 0.17	0.17	0.32	
GI									0.49*	0.06	0.19
PLI										0.33	0.03
BOP%											0.35
PPD%											

*p-value: 0.000758 [apply for Boferroni strategy, $p = 0.05/(^{12}_{2}) = 0.05/66 = 0.000758$]. There were 72 subjects.
BOP%, percentage of sites with bleeding on probing; CAL, clinical attachment level (mm), mean of the six most severely affected teeth; GI, gingival index, mean of the six most severely affected teeth; OLTS, organoleptic test score; PLI, plaque index, mean of the six most severely affected teeth; PPD, probing pocket depth (mm), mean of the six most severely affected teeth; PPD%, percentage of pockets ≥ 5 mm; TCS, tongue coating score; VSCs, volatile sulfur compunds: $H_2S + CH_3SH + (CH_3)_2S$ (ng/10 mL).

compounds and periodontal parameters are depicted in Table 2. Volatile sulfur compounds were correlated with organoleptic test score. Among the variables, the correlations of organoleptic test score and H_2S, CH_3SH, tongue coating score, plaque index mean and percentage of bleeding on probing were statistically significant, but this was not the case between organoleptic test score and $(CH_3)_2S$, probing pocket depth mean, clinical attachment loss mean and percentage of probing pocket depth ≥ 5 mm.

Volatile sulfur compounds, individual sulfur components and periodontal parameters

The Pearson correlation coefficients between the concentrations of volatile sulfur compounds and individual sulfur components are presented in Table 2. The concentration of volatile sulfur compounds was significantly correlated with H_2S, CH_3SH, $(CH3)_2S$ and percentage of bleeding on probing, but not with probing pocket depth mean, gingival index mean, plaque index mean or percentage of probing pocket depth ≥ 5 mm.

Correlation between tongue coating score and volatile sulfur compounds and periodontal parameters

As shown in Table 2, tongue coating score was significantly correlated with volatile sulfur compounds, H_2S and CH_3SH, but not with $(CH_3)_2S$, probing pocket depth mean, clinical attachment loss mean, gingival index mean, plaque index mean, bleeding on probing percentage and percentage of probing pocket depth ≥ 5 mm.

Table 3. The differences of sulfide measurements and organoleptic score between baseline and after tongue scraping

	Baseline	TS	*p*-value (TS vs. baseline)
OLTS	3.83 ± 1.20	3.00 ± 1.31	0.0004
H_2S (ng/10 mL)	5.25 ± 5.51	2.23 ± 1.91	0.0018
CH_3SH (ng/10 mL)	3.32 ± 3.61	1.55 ± 1.42	0.0030
$(CH_3)_2S$ (ng/10 mL)	0.18 ± 0.47	0.08 ± 0.21	0.3501
VSCs (ng/10 mL)	8.75 ± 8.68	3.86 ± 3.08	0.0011

Data are expressed as the mean ± standard deviation. There were 30 subjects.
OLTS, organoleptic test score; TS, tongue scraping; VSCs, volatile sulfur compunds: $H_2S + CH_3SH + (CH_3)_2S$ (ng/10 mL).

Differences of sulfur measurements and organoleptic test score between baseline and after tongue scraping

As shown in Table 3, after tongue scraping, the organoleptic test score, the levels of H_2S, CH_3SH and volatile sulfur compounds, but not the $(CH_3)_2S$, were significantly lower than at baseline.

Sulfur measurements and periodontal parameters at baseline and experimental stages

Data [organoleptic test scores, H_2S, CH_3SH, $(CH_3)_2S$, volatile sulfur compounds, tongue coating score, probing pocket depth, clinical attachment loss, gingival index, plaque index, bleeding on probing percentage and probing pocket depth percentage] collected at different experimental stages are presented in Table 4.

Statistical analyses of sulfur measurements and periodontal parameters among experimental stages

Statistically significant differences of the organoleptic test score were observed between baseline and after tongue scraping, nonsurgical periodontal treatment, and oral hygiene instruction/chlorhexidine + cetyl pyridinium mouth rinsing (Table 5). Statistically significant differences in the organoleptic test score were also observed between after tongue scraping and nonsurgical periodontal

Table 4. Measurements of sulfur compounds and periodontal parameters at baseline and during the experiment

Variable	Baseline	TS	NSPT	OHI/CHX-2	OHI/CHX-4
OLTS	3.80 ± 1.26	3.00 ± 1.22	2.44 ± 1.19	1.44 ± 0.96	1.24 ± 0.78
H_2S	6.06 ± 7.03	2.18 ± 1.86	1.49 ± 1.35	0.87 ± 0.92	0.85 ± 0.58
CH_3SH	3.14 ± 3.44	1.30 ± 0.96	0.84 ± 0.92	0.28 ± 0.31	0.21 ± 0.30
$(CH_3)_2S$	0.19 ± 0.51	0.08 ± 0.22	0.11 ± 0.35	0.17 ± 0.40	0.06 ± 0.18
VSCs	9.39 ± 9.87	3.55 ± 2.36	2.43 ± 1.84	1.33 ± 1.08	1.18 ± 0.76
TCS	8.24 ± 3.11	–	3.96 ± 2.35	2.88 ± 1.30	2.16 ± 1.21
PPD	6.83 ± 1.26	–	5.03 ± 1.29	4.4 ± 1.16	4.07 ± 1.21
CAL	7.08 ± 1.40	–	5.94 ± 1.64	5.54 ± 1.62	5.09 ± 1.38
GI	2.21 ± 0.22	–	1.47 ± 0.51	1.10 ± 0.44	0.77 ± 0.41
PLI	2.22 ± 0.43	–	1.09 ± 0.58	0.64 ± 0.41	0.35 ± 0.20
BOP%	40.88 ± 14.41	–	19.2 ± 9.99	12.88 ± 6.82	9.57 ± 5.30
PPD%	33.67 ± 14.96	–	16.02 ± 8.54	12.24 ± 7.05	10.08 ± 6.82

Data are expressed as the mean ± standard deviation. There were 25 subjects.
CAL, mean clinical attachment level (mm); CHX, chlorhexidine; GI, mean gingival index; OHI, oral hygiene instruction; OLTS, organoleptic test score; NSPT, nonsurgical periodontal treatment; PLI, mean plaque index; PPD, mean probing pocket depth (mm); TCS, tongue coating score; VSCs, volatile sulfur compounds = H_2S + CH_3SH + $(CH_3)_2S$ (ng/10 mL).

treatment, and between nonsurgical periodontal treatment and oral hygiene instruction/chlorhexidine + cetyl pyridinium mouth rinsing for the first 2 wk.

Compared with baseline measurements, volatile sulfur compounds after nonsurgical periodontal treatment, and after oral hygiene instruction/chlorhexidine + cetyl pyridinium mouth rinsing for the first 2 wk, and organoleptic test scores after oral hygiene instruction/chlorhexidine + cetyl pyridinium mouth gargling for 4 wk, were significantly different. Among the experimental stages, the volatile sulfur compounds were significantly different between after tongue scraping and after nonsurgical periodontal treatment.

Regarding the changes of H_2S, significant reductions of H_2S were observed between baseline and after tongue scraping, after nonsurgical periodontal treatment and after oral hygiene instruction/chlorhexidine + cetyl pyridinium mouth rinsing. Among different experimental stages, H_2S reductions were significant between after tongue scraping and post-nonsurgical periodontal treatment, and between post-nonsurgical periodontal treatment and oral hygiene instruction/chlorhexidine + cetyl pyridinium mouth gargling for the first 2 wk.

CH_3SH after tongue scraping and after oral hygiene instruction/chlorhexidine + cetyl pyridinium mouth rinsing were significantly different from baseline measurements. CH_3SH concentrations were also significantly different between after tongue scraping and after nonsurgical periodontal treatment, and between after nonsurgical periodontal treatment and after oral hygiene instruction/chlorhexidine + cetyl pyridinium mouth gargling for the first 2 wk.

As shown in Fig. 1, the changes in malodor measurements were decreased over time-treatment courses. Furthermore, as shown in Fig. 2, the tongue coating score, decreased to 0 by tongue scraping, remained significantly lower over all the experimental stages compared with baseline values. A similar trend of changes in organoleptic test score, as in tongue coating score, was observed. Regarding the differences of tongue coating score and organoleptic test score between after oral hygiene instruction/chlorhexidine +

Table 5. Statistical analyses of sulfur compound measurements and periodontal parameters

	Wilcoxon Sign-Ranks *p*-value							
Variable	TS vs. baseline	NSPT vs. vs. baseline	OHI/CHX-2 vs. TS	OHI/CHX-4 vs. OHI/CHX-2	Baseline vs. TS	OHI/CHX-2 vs. baseline	OHI/CHX-4 vs. baseline	OHI/CHX-4 vs. OHI/CHX-2
OTLS	< 0.001	< 0.001	< 0.001	< 0.001	0.016	< 0.001	< 0.001	0.408
H_2S	< 0.001	< 0.001	< 0.001	< 0.001	0.008	< 0.001	0.005	0.350
CH_3SH	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001	< 0.001	0.135
$(CH_3)_2S$	0.502	0.438	0.563	0.453	0.828	0.578	0.781	0.188
VSCs	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.188
TCS	–	< 0.001	< 0.001	< 0.001	–	0.011	< 0.001	< 0.001
PPD	–	< 0.001	< 0.001	< 0.001	–	< 0.001	< 0.001	< 0.001
CAL	–	< 0.001	< 0.001	< 0.001	–	0.010	< 0.001	0.003
GI	–	< 0.001	< 0.001	< 0.001	–	< 0.001	< 0.001	< 0.001
PLI	–	< 0.001	< 0.001	< 0.001	–	< 0.001	< 0.001	< 0.001
BOP%	–	< 0.001	< 0.001	< 0.001	–	< 0.001	< 0.001	< 0.001
PPD%	–	< 0.001	< 0.001	< 0.001	–	< 0.001	< 0.001	< 0.001

Data are expressed as the mean ± standard deviation. There were 25 subjects.
BOP%, percentage of sites with bleeding on probing; CAL, mean clinical attachment level (mm); CHX, chlorhexidine; GI, mean gingival index; NSPT, nonsurgical periodontal treatment; OHI, oral hygiene instruction; OLTS, organoleptic test score; PLI, mean plaque index; PPD, mean probing pocket depth (mm); TCS, tongue coating score; TS, after tongue scraping; VSCs, volatile sulfur compounds = H_2S + CH_3SH + $(CH_3)_2S$ (ng/10 mL).

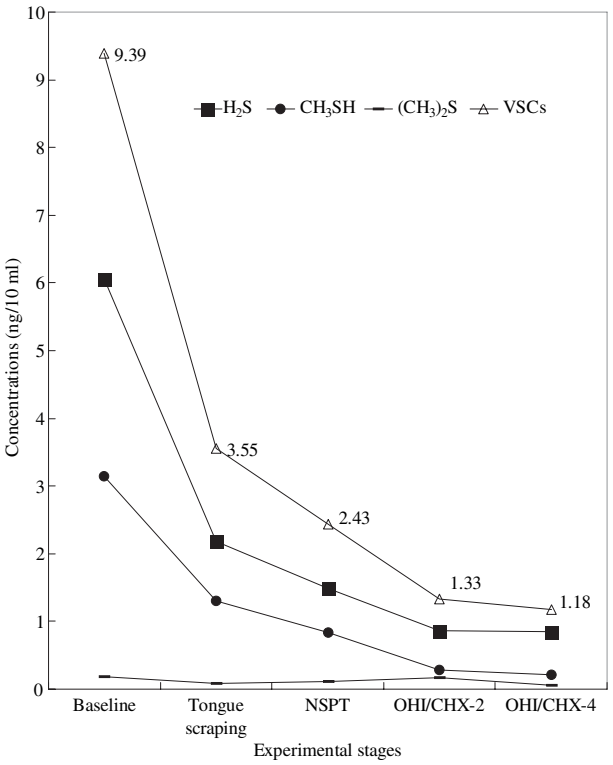

Fig. 1. The concentrations of sulfide compounds at different experimental stages. Measurements of sulfide compounds and treatments were as described in the Material and methods. Data are expressed as the mean concentrations ± standard deviation from 25 subjects. The concentrations of sulfur compounds were changed by different treatments and were dependent on the treatment stages. Significant differences in the levels of H_2S, CH_3SH and volatile sulfur compounds were observed among the experimental stages (but not oral hygiene instruction/chlorhexidine-4 vs. oral hygiene instruction/chlorhexidine-2). CHX, chlorhexidine; NSPT, nonsurgical periodontal treatment; OHI, oral hygiene instruction; VSCs, volatile sulfur compounds = H_2S + CH_3SH + $(CH_3)_2S$ (ng/10 mL).

cetyl pyridinium mouth rinsing for the first 2 wk and after nonsurgical periodontal treatment, however, the organoleptic test score was not significantly different between after oral hygiene instruction/chlorhexidine + cetyl pyridinium mouth rinsing for the first 2 wk and for 4 wk.

Discussion

The present study, which evaluated breath parameters of 72 patients with moderate periodontitis and breath malodor, found that the concentration of volatile sulfur compounds, assessed by the OralChroma™, was significantly correlated with organoleptic test score. Our results are in agreement with the results of Tanaka *et al.* (14), Oho *et al.* (15) and Schmidt *et al.* (6). In addition, our conclusion was also in agreement with the results of Rosenberg *et al.* (5), who analyzed sulfides using a sulfide monitor. Therefore, the concentrations of volatile sulfur compounds assessed by gas chromatography or a sulfide monitor were significantly correlated with organoleptic test score, suggesting that volatile sulfur compounds assessed by gas chromatography can represent oral breath malodor to a certain extent.

Our data indicated that the concentrations of H_2S and CH_3SH, but not of $(CH_3)_2S$, were significantly correlated with the organoleptic test score. This suggests that H_2S and CH_3SH are the important components of volatile sulfur compounds affecting the organoleptic test score. In fact, the present study, and that of Tonzetich (1), found that 90–98% of the total volatile sulfur compounds were H_2S and CH_3SH. We found no significant correlation between organoleptic test score and probing pocket depth mean, clinical attachment loss mean, gingival index mean or percentage of probing pocket depth ≥5 mm. This is in agreement with the report of Bosy *et al.* (9). However, the organoleptic test score was significantly correlated with plaque index mean and percentage of bleeding on probing. The difference was that Bosy *et al.* (9) did not set any exclusion criteria, but in our study we included patients with moderate periodontitis and breath malodor, and without periodontal treatment within 6 mo prior to the study.

The concentrations of volatile sulfur compounds were not significantly correlated with probing pocket depth mean, clinical attachment loss mean, gingival index mean, plaque index mean or percentage of probing pocket depth ≥5 mm. Only percentage of bleeding on probing was significantly correlated with the concentration of volatile sulfur compounds. Our current data suggest that an inflammatory condition is the major factor for oral malodor. Bosy *et al.* (9) also indicated that deep periodontal pocket depth (≥ 5 mm) is not correlated with the concentration of volatile sulfur compounds. This is in agreement with our current data. However, Yaegaki *et al.* (4) found that the concentration of intrapocket volatile sulfur compounds is correlated with the percentage of probing pocket depth ≥5 mm. The difference between the result of Yaegaki *et al.* (4) and that of Bosy *et al.* (9) and our results might be because we measured the volatile sulfur compounds directly, not intrapocket volatile sulfur compounds, which might not spread into the oral cavity and therefore would not be detected by gas chromatography. Also, Miyazaki *et al.* (2) and Liu *et al.* (16) reported that the concentration of volatile sulfur compounds is related to periodontal status and plaque index. Coli & Tonzetich (17), Bosy *et al.* (9) and Rosenberg (18) all reported strong correlations between the amount of tongue coating and organoleptic test score. Tonzetich & Ng (19) not only found a relationship between tongue coating and oral

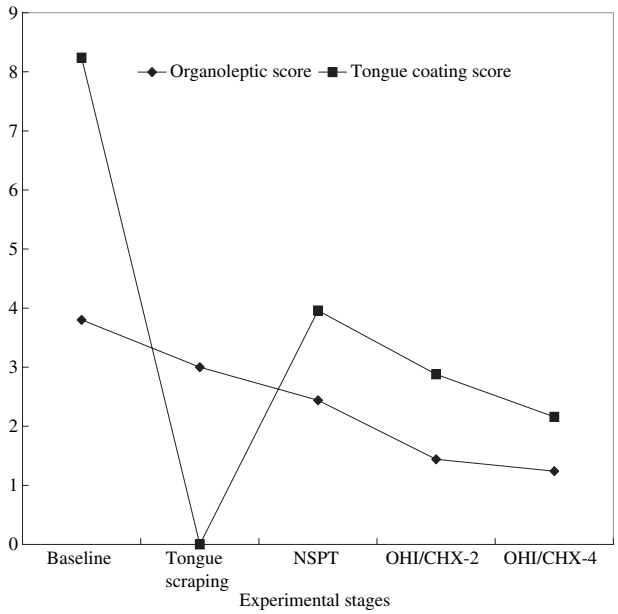

Fig. 2. Changes on the organoleptic and tongue coating scores. Organoleptic and tongue coating scores were obtained as described in the Material and methods. Data are expressed as the mean ± standard deviation from 25 subjects. Both scores were decreased by different treatments and were stage-dependent. Significant differences in organoleptic test score were observed in nonsurgical periodontal treatment vs. tongue scraping, oral hygiene instruction/chlorhexidine-2 vs. nonsurgical periodontal treatment and oral hygiene instruction/chlorhexidine-4 vs. nonsurgical periodontal treatment, but not in oral hygiene instruction/chlorhexidine-4 vs. oral hygiene instruction/chlorhexidine-2. Significant differences in tongue coating scores were noted among all experimental stages. CHX, chlorhexidine; NSPT, nonsurgical periodontal treatment; OHI, oral hygiene instruction.

malodor, but also suggested that tongue coating is strongly related to oral malodor. Our present study also indicated that tongue coating is highly correlated with the organoleptic test score and with the concentration of volatile sulfur compounds. In addition, the tongue coating score is not related to periodontal parameters (probing pocket depth mean, clinical attachment loss mean, gingival index mean, plaque index mean, percentage of probing pocket depth ≥ 5 mm (probing pocket depth percentage) and percentage of bleeding on probing, suggesting that tongue coating formation is poorly related to periodontal status or to the cleanness of teeth.

The organoleptic test score and the concentrations of volatile sulfur compounds and CH_3SH were significantly reduced only by the removal of tongue coating. Besides our present study, Yaegaki & Sanada (3) also reported that the concentration of volatile sulfur compounds in the periodestructive group (mean probing pocket depth > 0.4 mm) could be reduced, suggesting that the removal of tongue coating could reduce the H_2S, CH_3SH and volatile sulfur compounds by up to 50% (i.e. proper management of tongue coating could eliminate at least 50% of breath malodor caused by sulfide compounds). However, the organoleptic test score was reduced by only up to 20%, suggesting that oral malodor not only came from sulfide compounds but also from other foul odor substances that were not related to the tongue coating.

After completely removing the tongue coating and undergoing nonsurgical periodontal treatment, the organoleptic test score and the concentrations of volatile sulfur compounds and CH_3SH were significantly decreased, but there was no significant change in the concentration of $(CH_3)_2S$. The concentration of volatile sulfur compounds and the tongue coating score were also significantly reduced after tongue scraping and nonsurgical periodontal treatment. From these results, we concluded that nonsurgical periodontal treatment could markedly improve the breath malodor, both in the organoleptic test score and in the objective measurement of volatile sulfur compounds. Although tongue coating could re-accumulate, through oral hygiene education (including tooth brushing and tongue brushing) the amount of tongue coating could be maintained at a very low level. Yaegaki & Sanada (3) reported more CH_3SH in patients with active periodontal disease than in periodontally healthy individuals, but we did not find a similar result in our untreated periodontal disease patients.

After the completion of nonsurgical periodontal treatment, patients were given oral hygiene instruction/chlorhexidine + cetyl pyridinium mouth rinsing for 2 wk and 4 wk. A significant adjunctive reduction in organoleptic test score and in the concentrations of volatile sulfur compounds, H_2S and CH_3SH were obtained by oral hygiene instruction/chlorhexidine + cetyl pyridinium mouth rinsing. The mouth rinsing solution of chlorhexidine + cetyl pyridinium was reported by Quirynen & Avontroodt (20) to have antiplaque activity. Quirynen & Zhao (21) indicated that patients with moderate periodontitis receiving the combination of nonsurgical periodontal treatment and chlorhexidine + cetyl pyridinium mouth rising for 6 mo achieved a significant reduction in the concentration of volatile sulfur compounds and of tongue coating score. Quirynen *et al.* (8) concluded that periodontitis patients who received a one-stage, full-mouth nonsurgical periodontal treatment and chlorhexidine + cetyl pyridinium mouth rinsing for 2 mo showed a significant reduction of organoleptic test score, but no reduction of volatile sulfur compounds. This could be a result of the high correlation of CH_3SH with periodontal status, and the metabolic relationship between H_2S and tongue-coating microbes. Quirynen only performed nonsurgical periodontal treatment without tongue scraping; therefore, there was a greater

reduction in CH_3SH than in H_2S concentrations by nonsurgical periodontal treatment. Also, Quirynen *et al*. (8) used a portable sulfide monitor, which has low sensitivity for CH_3SH. This could lead to a greater reduction in CH_3SH, which might not be detected. Therefore, the organoleptic test score could be markedly reduced, but the concentrations of volatile sulfur compounds were not significantly reduced.

The results of the present study indicated that in patients with moderate periodontitis and a high tongue coating score, tongue scraping alone could improve oral malodor significantly. Nonsurgical periodontal treatment combined with chlorhexidine + cetyl pyridinium gargling did have a significant effect on the tongue coating score as well as on periodontal status, further reducing malodor.

References

1. Tonzetich J. Production and origin of oral malodor: a review of mechanisms and methods of analysis. *J Periodontol* 1977;**48:**13–20.
2. 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:**679–684.
3. Yaegaki K, Sanada K. Volatile sulfur compounds in mouth air from clinically healthy subjects and patients with periodontal disease. *J Periodont Res* 1992; **27:**233–238.
4. Yaegaki K, Sanada K. Biochemical and clinical factors influencing oral malodor in periodontal patients. *J Periodontol* 1992; **63:**783–789.
5. Rosenberg M, Kulkarni GV, Bosy A, McCulloch CA. Reproducibility and sensitivity of oral malodor measurements with a portable sulphide monitor. *J Dent Res* 1991;**70:**1436–1440.
6. Schmidt NF, Missan SR, Tarbet WJ. The correlation between organoleptic mouth-odor ratings and levels of volatile sulfur compounds. *Oral Surg Oral Med Oral Pathol* 1978;**45:**560–567.
7. Claus D, Geypens B, Rutgeerts P, Ghyselen J, Hoshi K, van Steenberghe D, Ghoos Y. Where gastroenterology and periodontology meet; determination of oral volatile organic compounds using closed loop trapping and high resolution gas chromatograph ion trap detection. In: van Steenberghe D, Rosenberg M, eds. *Bad Breath: A Multidisciplinary Approach*. Leuven: Leuven University Press, 1996; 15–28.
8. Quirynen M, Mongardini C, van Steenberghe D. The effect of a 1-stage full-mouth disinfection on oral malodor and microbial colonization of the tongue in periodontitis. A pilot study. *J Periodontol* 1998;**69:**374–382.
9. Bosy A, Kulkarni GV, Rosenberg M, McCulloch CA. Relationship of oral malodor to periodontitis: evidence of independence in discrete subpopulations. *J Periodontol* 1994;**65:**37–46.
10. Murata T, Yamaga T, Lida T, Miyazaki H, Yaegaki K. Classification and examination of halitosis. *Int Dent J* 2002;**52:** 181–186.
11. Silness J, Löe H. Periodontal disease in pregnancy. I. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;**22:**121–135.
12. Loe H. The gingival index, the plaque index and the retention index systems. *J Periodontol* 1987;**38:**610–616.
13. Lang NP, Nyman S, Senn C, Joss A. Bleeding on probing as it relates to probing pressure and gingival health. *J Clin Periodontol* 1991;**18:**257–261.
14. Tanaka M, Anguri H, Tarbet WJ. Reliability of clinical parameters for predicting the outcome of oral malodor treatment. *J Dent Res* 2003;**82:**518–522.
15. 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.
16. Liu XNK, 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.
17. Coli JM, Tonzetich J. Characterization of volatile sulphur compounds production at individual gingival crevicular sites in humans. *J Clin Dent* 1992;**3:**97–103.
18. Rosenberg M. Clinical assessment of bad breath: current concepts. *J Am Dent Assoc* 1996;**127:**475–482.
19. Tonzetich J, Ng SK. Reduction of malodor by oral cleansing procedures. *Oral Surg Oral Med Oral Pathol* 1976;**42:** 172–181.
20. Quirynen M, Avontroodt P. Effect of different chlorhexidine formulations in mouthrinses on de novo plaque formation. *J Clin Periodontol* 2001;**28:**1127–1136.
21. Quirynen M, Zhao H, Soers C,*et al*. The impact of periodontal therapy and the adjunctive effect of antiseptics on breath odor-related outcome variables: a double-blind randomized study. *J Periodontol* 2005;**76:**705–712.