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Oral prophylaxis and its effects on halitosis-associated and inflammatory parameters in patients with chronic periodontitis

Abstract: *Objective:* A controlled clinical trial was conducted to evaluate the effects of oral prophylaxis on halitosis-associated, immunological and microbiological parameters. *Methods:* Thirty subjects were included in this controlled clinical trial (patients with generalized chronic periodontitis and controls without clinical attachment loss; each $n = 15$). Before oral prophylaxis and 14 days after (including tongue cleaning) volatile sulphur compounds (VSC), organoleptic scores and a tongue coating index were evaluated. The levels of IL-1β, IL-8, IL-10 and MMP-8 were measured in GCF, and also major periodontal pathogens were detected. Data were statistically analysed using ANOVA and paired *t*-test. *Results:* Supragingival plaque and calculus removal with combined tongue cleaning was able to reduce significantly ($P < 0.05$) the VSC values in both groups (no significant differences between both groups). Two weeks after periodontal debridement, the VSC values were observed in the periodontitis group, but not in the control group, similar to the baseline values. The difference between the groups was statistically significant ($P < 0.05$). Only a repeated prophylaxis session in the periodontitis group was able to reduce VSC values significantly in comparison with baseline ($P < 0.05$). Organoleptic scores (10 and 30 cm) were significantly different ($P < 0.05$) between both groups before and after the treatment. Periodontal pathogens and host-derived markers were not significantly affected by a single prophylaxis session. *Conclusions:* Oral prophylaxis may result in a significant decrease in VSC values. However, in periodontal diseases, a more complex treatment seems to be necessary.

Key words: interleukins; oral prophylaxis; periodontal pathogens; periodontitis; volatile sulphur compounds

Introduction

Patients with periodontal diseases frequently suffer from pathological malodour caused mainly by volatile sulphur compounds (VSC), which are primarily hydrogen sulphide (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulphide ($(CH_3)_2S$). These compounds are caused by the proteolytic degradation of sulphur-containing substrates, for example food debris, blood or epithelial cells (1, 2). Epidemiological studies in Asia demonstrated that pathological VSC values may be caused by tongue coating or periodontal diseases (3, 4). In Europe, a representative study from Switzerland indicated that one-third of the subjects had oral

malodour that was associated with high VSC values and tongue coating (5). Tongue coating was also identified as influencing factor regarding the VSC values in a Brazilian population (6). A survey among the US dentists showed that 41% of them saw six or more patients a week with malodour breath (7). A recent study from Turkey reported that halitosis occurred at higher rates among males than among females in a study population of 268 dental students (8).

Causes of oral malodour usually originated from the oral cavity (>85%) (9). In these cases, it originates from the posterior dorsum of tongue and/or periodontal diseases, situations where food and bacterial biofilms can accumulate and anaerobic ecosystems can develop (10). Microbiological analyses showed that periodontal pathogenic bacteria, for example anaerobic gram-negatives, can contribute to increased VSC production (CH_3SH) in the oral cavity (11). *Porphyromonas gingivalis*, *Prevotella intermedia* and *Treponema denticola* are able to produce high VSC levels (12). For this reason, it was supposed that VSC levels in the breathing air may be correlated with the extent of periodontal destruction (13, 14). However, not all patients suffering from gingivitis or periodontitis have oral malodour (2).

The tongue is one of the most important anatomical structures in the mouth harbouring one of the most complex microbiological niches. With its papillary structure and the fissures on the dorsum, the tongue offers a large surface for the accumulation of oral debris and microorganisms (15). Several periodontal pathogens were detected on the dorsum of the tongue, for example *P. gingivalis* (16) and *P. intermedia* (17).

The purpose of this study was to evaluate the effects of oral prophylaxis, as one of the corner stones of anti-infective periodontal treatment (18), (i) on halitosis-associated parameters and (ii) on immunological and microbiological parameters.

Materials and methods

Subject recruitment

Thirty subjects (subjects with generalized severe chronic periodontitis or periodontally healthy controls, respectively, each $n = 15$) were cumulatively recruited from patients of the Center of Dental Medicine, Jena University Hospital, Germany, from April until September 2011. Treatment and follow-up visits were performed between October 2011 and April 2012.

The definition of chronic periodontitis was based on the classification system of the 'International Workshop for a Classification System of Periodontal diseases and Conditions' from 1999 (19).

Individuals with generalized severe chronic periodontitis were included when they demonstrated probing pocket depths ≥5 mm at more than 30% of sites and when they were at an age of ≥35 years. Periodontally healthy subjects with no evidence of periodontal disease (all probing depths ≤3 mm, clinical attachment loss = 0 mm) were recruited as controls.

The participants of this study had no obvious complaints of oral malodour.

Subjects with significant systemic diseases (e.g. diabetes mellitus, cancer or coronary heart disease), subjects who took antibiotic therapy within the last 6 months and pregnant or lactating females were excluded. Further diseases in the nasopharyngeal zone and the daily use of a tongue scraper were exclusion criteria. Only non-smokers with no history of smoking were included in the study.

Ethical approval was obtained from local ethics committee of the University of Jena (3076-03/11). The procedures were in accordance with the ethical standards with the Helsinki Declaration of 1975, as revised in 1983. Written informed consent was obtained from each subject prior to participation.

Study design

The study was designed as controlled clinical trial. The change in VSC levels was defined as primary outcome parameter, while immunological and microbiological parameters were secondary outcome measures. An a-priori sample size calculation was made (G*Power, free statistical software; Heinrich-Heine-University, Dusseldorf, Germany) based on the results obtained from a pilot study (10 subjects who were not included in the main study). A sample size of $n = 14$ in each group should reach a statistical power of 90%.

Clinical parameters, volatile sulphur compounds, organoleptic parameters, tongue coating, microbiological and immunological parameters were determined at baseline (t_0). After a full-mouth professional tooth cleaning (t_1), the volatile sulphur compounds were detected again. Both groups, the subjects suffering from periodontitis ($n = 15$) and the controls ($n = 15$), were treated in the same manner. Supragingival calculus was removed using a piezoelectric ultrasonic scaler (P4 Scaler tip, Vector®; Duerr Dental AG, Bietigheim-Bissingen, Germany), followed by interdental and proximal manual instrumentation (Scaler #2; Hu-Friedy Co., Chicago, IL, USA). Tooth floss was applied to clean interproximally beneath contact areas (GABA GmbH, Lörrach, Germany), and scaled tooth surfaces were polished (Prophy Cup; Hager Werken GmbH & Co KG, Duisburg, Germany).

Afterwards, all subjects received oral hygiene instructions and were instructed how to use a tongue scraper (one drop only, Berlin, Germany) for personal usage at home (2 times per day). They were asked to clean their tongue with 15 pullings from the posterior part to the front by demonstrating the procedure in front of a mirror.

Following the professional full-mouth tooth cleaning (t_1), the tongue was cleaned. Immediately after tongue cleaning (t_2), the volatile sulphur compounds were measured again. Two weeks later (t_3), VSC, organoleptic parameters, tongue coating, inflammatory and microbiological data were repeatedly determined. In the periodontitis group, the treatment was repeated in the same manner as described above, and after 14 days (in sum 28 days after baseline evaluation), the VSC values were measured again (t_4).

The flow of participants through each stage of the study and a timeline of all measurement time points (t_0–t_4) are shown in Fig. 1. There were no deviations from the study protocol.

Clinical data

Probing depths (PD) were measured using a periodontal probe (PCP UNC-15; Hu-Friedy Co.) at six sites per tooth. Plaque index (PI) (20) and bleeding on probing (BoP) (21) were recorded as the percentage of surfaces demonstrating plaque or bleedings.

One examiner (AG) recorded the clinical data and collected the GCF samples at baseline (t_0) and 2 weeks after intervention (t_3).

Collection of GCF samples

Gingival crevicular fluid (GCF) samples from each subject were collected in the periodontitis group from the deepest periodontal pocket in each quadrant and from the mesiobuccal site of each first molar in the control group (22). Periodontal pathogenic bacteria and inflammatory parameters were determined in these pooled samples.

The sample sites were isolated with cotton rolls and gently air-dried. Afterwards, supragingival plaque was carefully removed. Paper strips (Dentognostics GmbH, Jena, Germany) were then inserted into the periodontal pocket (periodontitis group) or into the gingival sulcus (controls) for 20 s. Subsequently, the paper strips were put in a screw top plastic vial for freezing at −20°C (22).

Inflammatory parameters

For the host-derived biomarkers interleukin-1 beta (IL-1β), IL-8, IL-10 and matrix metalloproteinase-8 (MMP-8), the GCF samples were stored at −80°C until assayed. A day before analysis, samples were eluted at 4°C overnight into 700 µl phosphate-buffered saline containing proteinase inhibitors (Sigma-Aldrich, St Louis, MO, USA). After being centrifuged at 400 *g* for 4 min, the paper strips were removed and 100 µl aliquots of the supernatant were added. The concentrations of total MMP-8 and IL-1β, IL-8, IL-10 were determined using commercially available enzyme-linked immunosorbent assay kits (R&D Systems Europe Ltd, Abingdon, UK) according to the manufacturer's instructions. The detection levels of the kits ranged from 1 pg/site for

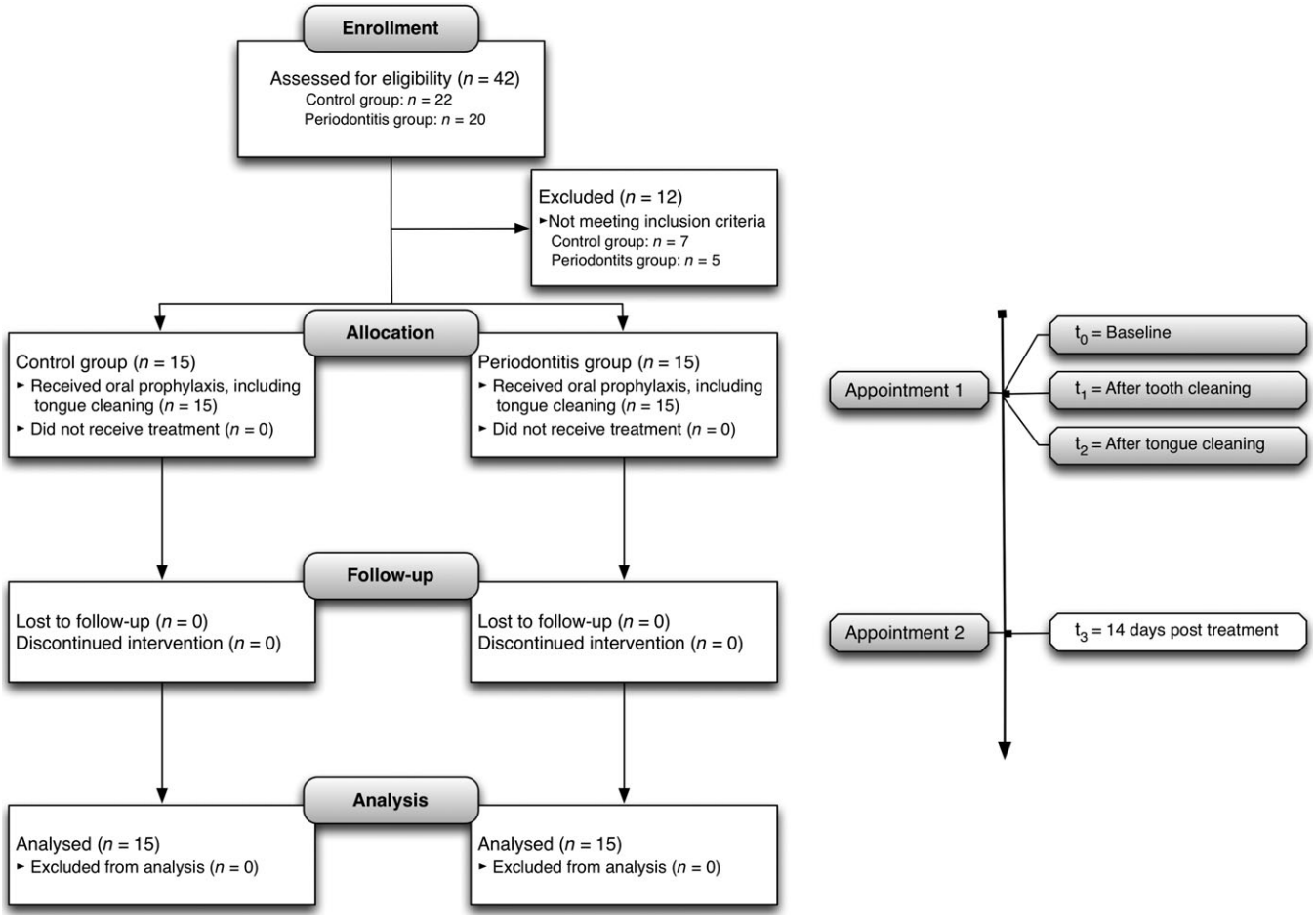

Fig. 1. Flow of participants through each stage of the study and timeline of measurement time points (t_0–t_4). Only patients with chronic periodontitis were examined at t_4.

IL-1β to 50 pg/site for MMP-8. Each 100 μl of the eluate was used.

Detection of selected bacterial species associated with periodontitis

The semiquantitative loads of selected periodontal pathogens (*Aggregatibacter actinomycetemcomitans*, *P. gingivalis*, *Tannerella forsythia*, *T. denticola*) were determined by using the micro-Ident® (Hain Lifescience, Nehren, Germany) as described recently (23). In brief, DNA was extracted by using a DNA extraction system (High Pure PCR Template Preparation Kit; Roche, Mannheim, Germany) according to the recommendations of the manufacturer. PCR amplification was carried out in a reaction volume of 25 μl consisting of 2.5 μl of template DNA and 22.5 μl of reaction mixture containing 17.5 μl of primer–nucleotide mix (microIDent® and microIDent®plus, respectively), 2.5 μl of 10 × PCR buffer, 2.5 μl of 25 mM $MgCl_2$ and 1 U Taq polymerase (MBI Fermentas, Vilnius, Lithuania). PCR cycling was carried out in a Mastercycler (Eppendorf, Hamburg, Germany). The cycling conditions comprised an initial denaturation step at 95°C for 5 min., 10 cycles at 95°C for 30 s and at 60°C for 2 min, 20 cycles at 95°C for 10 s, at 55°C for 30 s and at 72°C for 30 s and a final extension step at 72°C for 10 min. For the subsequent reverse hybridization, the biotinylated amplicons were denatured and incubated at 45°C with hybridization buffer and strips coated with two control lines and five species-specific probes. After the PCR products had bound to their respective complementary probe, a highly specific washing step removed any unspecifically bound DNA. Streptavidin-conjugated alkaline phosphatase was added, the samples were washed, and hybridization products were visualized by adding a substrate for alkaline phosphatase. Finally, the counts were determined semiquantitatively: no band, $\leq 10^4$, 10^5, $\geq 10^6$.

VSC measurement

In this study, a portable Halimeter® (Interscan Corp., Chatsworth, CA, USA) was used to detect the volatile sulphur compounds. Prior to each measurement, the apparatus had to be calibrated to zero on ambient air.

Patients received instruction to refrain from smoking, chewing gum, performing oral hygiene (tooth brushing, interdental and tongue cleaning) and eating 2 h before the evaluation of VSC.

The patient was asked to close the mouth for 3 min. After this period, the end of a straw was inserted into patient's mouth placed near the dorsal region of the tongue. The lips should be almost closed, and breathing through the nose should be preferred. After 30 s, the Halimeter® showed the maximum peak value of VSC in parts per billion. This procedure was recapitulated three times, and the results were averaged out. It was taken care that the measurement was taken approximately at the same time of day to avoid variability during the day.

Organoleptic assessment

For this measurement, the patients were asked to close their mouth for 1 min. Afterwards, they were asked to exhale slowly through the mouth by keeping approximately 10 cm, 30 cm and 1 m distances to the nose (24).

Tongue coating (Winkel tongue coating index, WTCI)

The dorsum of the tongue was divided into six areas (three in the anterior and three in the posterior part). In these areas, the coating was assessed using a scale of 0–2: 0 = no coating, 1 = slight coating and 2 = heavy coating.

Data analysis

Statistical data analysis was performed using a statistical software program (SPSS, version 19.0; IBM Corporation, New York, NY, USA). Individual mean values and standard deviations (SD) were calculated. The subject was the statistical unit.

Normal distribution of the clinical data was verified by Kolmogorov–Smirnov and Shapiro–Wilk tests. Differences in VSC between the times of measurements were analysed using analysis of variance (ANOVA) with univariate repeated measurements. A *P*-value of (<0.05) was considered to be statistically significant. Differences within the groups were analysed using a paired *t*-test.

The changes in microbiological, inflammatory, organoleptic parameters and tongue coating between baseline and 2 weeks after treatment were analysed within the groups using Wilcoxon test because of no normal distribution. Moreover, Mann–Whitney *U*-test was used for the determination of significant differences between the groups at the baseline measurement and at the measurement after 2 weeks. Correlations were investigated using Spearman's test.

Results

Demographic and clinical parameters are presented in Table 1. Oral prophylaxis was able to reduce the plaque index significantly in both groups ($P < 0.05$), but shows only limited effect within 2 weeks on probing depth and bleeding.

Volatile sulphur compounds

Statistically significant differences in the volatile sulphur compounds of both groups were observed between baseline (t_0), after professional tooth cleaning (t_1), after tongue cleaning (t_2) and after 2 weeks tongue cleaning at home (t_3) ($P < 0.05$). Oral prophylaxis decreased the VSC levels significantly (Table 2).

VSC values were correlated with organoleptic test scores at baseline (t_0) and 2 weeks after treatment (t_3), as well as with the tongue coating index (WTCI) at (t_3). At baseline (t_0): VSC versus 10 cm distance ($R = 0.47$, $P = 0.\ 01$). After 2 weeks (t_3): VSC versus 10 cm ($R = 0.45$, $P = 0.01$) and VS versus

Table 1. **Demographic and clinical data**

	Control $n = 15$		Periodontitis $n = 15$	
Age (mean; range) (years)	26 (23–39)		52 (25–70)	
Gender (f:m)	11: 4		7: 8	
	Baseline (t_0)	After 2 weeks (t_3)	Baseline (t_0)	After 2 weeks (t_3)
PD (mean ± SD) (mm)	1.50 ± 0.51	1.38 ± 0.47	3.91 ± 0.66**	3.40 ± 0.86**
PI (mean ± SD) (%)	60.31 ± 19.92	35.34 ± 12.46*	87.86 ± 10.45**	45.24 ± 12.68*,**
BoP (mean ± SD) (%)	21.50 ± 24.15	15.80 ± 12.38	75.07 ± 29.85**	62.80 ± 15.48**

*Significantly different from baseline ($P < 0.05$; ANOVA).
**Significantly different between control and periodontitis group ($P < 0.05$; *t*-test).

Table 2. **VSC values (in ppb)**

VSC in ppb	Control	Periodontitis
Baseline (t_0)	76.5 ± 29.7	77.7 ± 23.0
After tooth cleaning (t_1)	51.8 ± 28.3*	47.6 ± 24.3*
After tongue cleaning (t_2)	48.4 ± 27.2*	45.7 ± 24.3*
14 days post-prophylaxis (t_3)	45.8 ± 16.4*	76.9 ± 34.4**
28 days post-prophylaxis (t_4)	–	47.0 ± 23.0*

*Significantly different from baseline ($P < 0.05$; ANOVA).
**Significantly different between control and periodontitis group ($P < 0.05$; *t*-test).

30 cm ($R = 0.43$, $P = 0.01$) and VSC versus tongue coating score (WTCI) ($R = 0.38$; $P = 0.04$). Probing depths were also positively correlated with the VSC values ($R = 0.39$, $P = 0.04$).

Tongue coating

In both groups, it was observed that the posterior part of the tongue was more coated than the anterior one. As shown in Fig. 2, there was a significant improvement in consequence of an oral prophylaxis session, which includes tooth and tongue cleaning procedures (control group – sextants A, B, C; periodontitis group – sextants B, C, E) ($P < 0.05$). Differences between both groups were not significant ($P > 0.05$).

Organoleptic measurement

Regarding the organoleptic levels (Fig. 3), a significant improvement ($P < 0.05$) was detected in both groups (control group – distance of 10 cm, and periodontitis group – distances of 10 and 30 cm). At baseline, organoleptic values were correlated with the tongue coating score (10 cm – $R = 0.37$; $P = 0.04$).

Inflammatory parameters and periodontal pathogens

The levels of the anti-inflammatory cytokine IL-10 were lower in the periodontitis group compared to periodontally healthy subjects at baseline and 2 weeks after treatment (Table 3). Oral prophylaxis reduced the levels of MMP-8 and IL-8 in the control group ($P < 0.05$). After oral prophylaxis, the levels of MMP-8 and IL 1-β were lower in the GCF of periodontally healthy subjects compared to patients with periodontitis ($P < 0.05$).

In the periodontitis group, significantly higher counts of *P. gingivalis*, *T. denticola* and *T. forsythia* were found at baseline ($P < 0.05$). Oral prophylaxis decreased only the counts of *T. forsythia* in the periodontitis group ($P < 0.05$). Nevertheless, the counts of *T. forsythia* were still higher in the periodontitis group than in periodontally healthy controls 2 weeks after starting intensive oral prophylaxis (Table 3).

Discussion

The removal of plaque, calculus and stains from the exposed and unexposed surfaces of the tooth by scaling and polishing is a preventive procedure for the control of local irrational factors (25). It provides the basis for the prevention of periodontal diseases (26) and is part of the disease control phase in the treatment for chronic and aggressive periodontitis, as well as an element of the supportive treatment phase (18).

The findings of the present study demonstrate that oral prophylaxis was able to alter halitosis-associated parameters. Supra- and subgingival plaque and calculus removal with combined tongue cleaning reduced the volatile sulphur compounds in patients with periodontitis and periodontally healthy controls. However, patients suffering from periodontal diseases demonstrated 2 weeks after oral prophylaxis comparable VSC levels to those measured before treatment. Only when the oral prophylaxis session was repeated in the periodontitis group, the VSC values were significantly reduced and reached the values comparable to the controls. However, it seems to be likely that this may be a short-term effect, and if a more complex treatment (scaling and root planing) is focused on arresting the inflammation, the VSC values will follow.

Previous results indicate that patients with periodontal diseases were at a higher risk of halitosis detection than individuals with healthy periodontium (27). In patients with periodontitis, the intensity of malodour is greater than in healthy subjects (28). These findings were weak in the present study as it also was reported by other authors (2,6). However, VSC levels were positively correlated with the pocket depth.

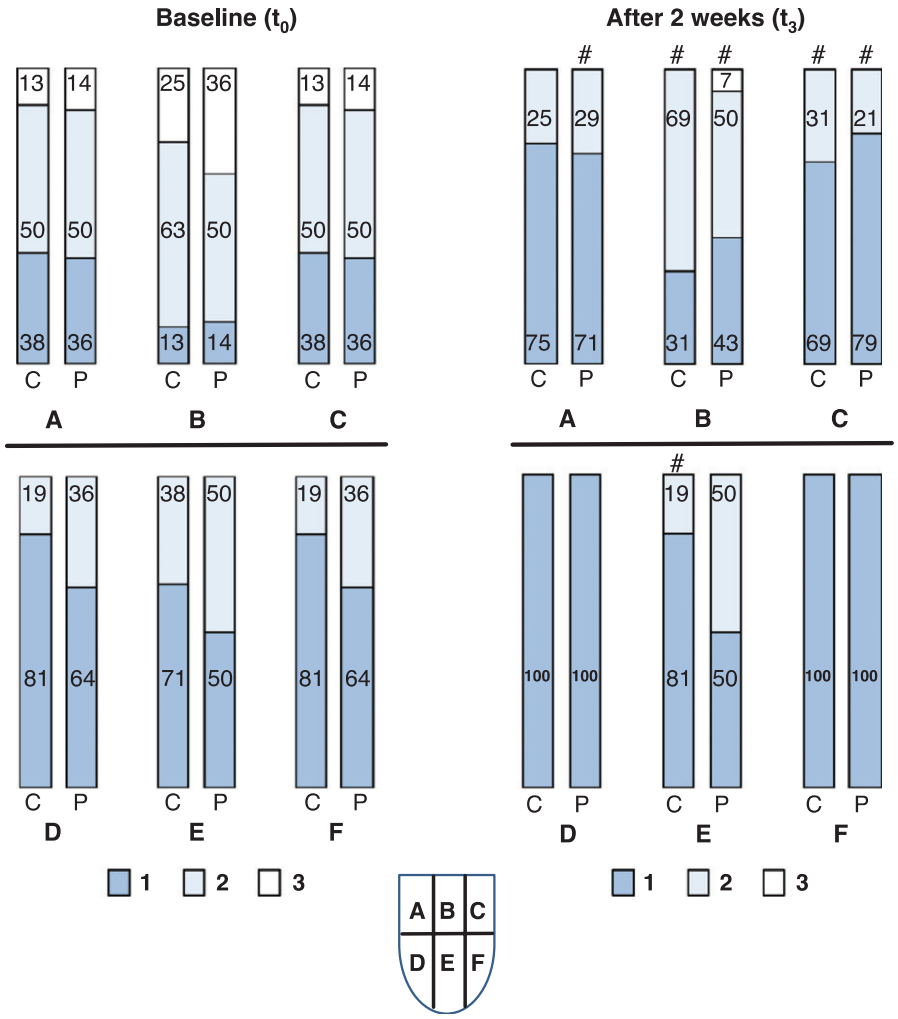

Fig. 2. Tongue coating index before and 2 weeks after oral prophylaxis. Most coating was detected in both groups (C – controls, P – periodontitis) in the posterior tongue region (a, b and c). After oral prophylaxis including tongue cleaning, the coating was significantly reduced (#$P < 0.05$ in comparison with baseline; ANOVA).

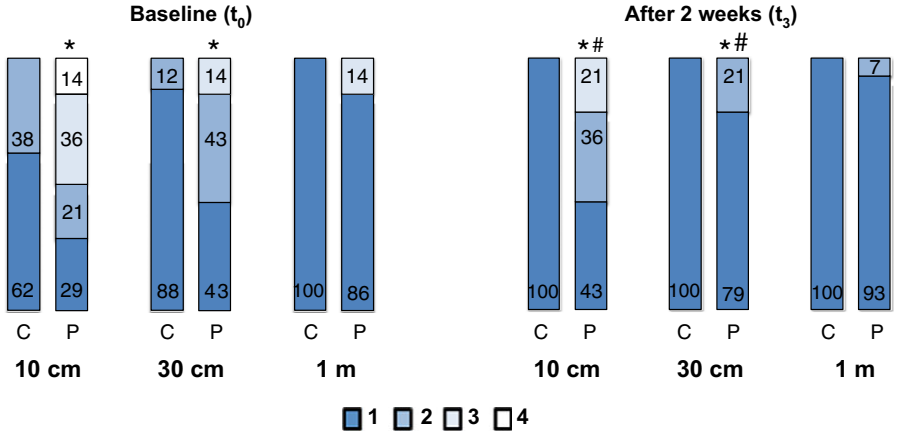

Fig. 3. The organoleptic scores were significantly higher in the periodontics group (P) in comparison with healthy controls (*$P < 0.05$; *t*-test), but were reduced after treatment (#$P < 0.05$ in comparison with baseline; ANOVA).

The treatment of malodour is primary directed towards treating oral/dental diseases, reducing the accumulation of food debris and the development of biofilm, and malodour-producing oral bacteria by improving oral hygiene and reducing the tongue coating (10). Apatzidou *et al.* reported that the posterior tongue odour might be an important source of odorous compounds, regardless of periodontal conditions. This can be confirmed by the results of the present study. The reduction in posterior tongue coating was associated with decreased VSC values, especially in the control group. Several studies identified a significant correlation between VSC and tongue coating scores (6, 29, 30). The anatomical situation of this area with its

Table 3. **Inflammatory and microbiological parameters**

	Baseline (t_0)		2 weeks post-(t_3)	
	Control	Periodontitis	Control	Periodontitis
MMP-8 (mean ± SD) (pg/site)	2328.0 ± 339.8	2741.0 ± 351.8	1621.5 ± 324.7*	3527.1 ± 336.1**
IL-1β (mean ± SD) (pg/site)	8.2 ± 5.8	24.2 ± 6.2	6.9 ± 5.3	33.3 ± 5.7**
IL-8 (mean ± SD) (pg/site)	24.0 ± 8.7	41.1 ± 9.3	14.81 ± 6.2*	33.09 ± 6.7
IL-10 (mean ± SD) (pg/site)	29.5 ± 3.2	8.1 ± 3.4**	25.7 ± 3.3	4.9 ± 3.5**
A. actinom. (positive/$\geq 10^6$ (%/%)	0/0 (0/0)	1/1 (7;7)	0/0 (0/0)	1/1 (7/7)
Porphyromonas gingivalis (positive/$\geq 10^6$ (%/%))	0/0 (0/0)	7/4 (47/27)**	0/0 (0/0)	7/6 (47/40)**
T. forsythia (positive/$\geq 10^6$ (%/%))	0/0 (0/0)	9/7 (60/47)**	0/0 (0/0)	8/3 (53/20)**
Treponema denticola (positive/$\geq 10^6$ (%/%))	0/0 (0/0)	9/6 (60/40)**	0/0 (0/0)	6/2 (40/13)*, **
Prevotella intermedia (positive/$\geq 10^6$ (%/%)	0/0 (0/0)	2/1 (13/7)	0/0 (0/0)	1/1 (7/7)

A.a., *A. actinomycetemcomitans;* P.g., *Porphyromonas gingivalis*; T.f., *T. forsythia;* T.d., *Treponema denticola;* P.i., *Prevotella intermedia)*; IL-1ß (detection level of 5 pg/site); IL-10 (detection level of 10 pg/site); IL-8 (detection level of 10 pg/site).
*Significantly different from baseline ($P < 0.05$; Wilcoxon test).
**Significantly different between control and periodontitis group ($P < 0.05$; Mann–Whitney *U*-test).

fissures and crypts establishes an ideal basis for bacterial species that are able to produce VSC and other toxins (31). Among the cosmetic problem of bad breath, VSC are tissue toxic even in extremely low concentrations and may cause periodontal tissue damage (32, 33). Mechanical approaches such as tongue brushing or scraping to clean the dorsum of the tongue can reduce breath odour and tongue coating (34).

A further focus of this study was to investigate the effect of oral prophylaxis and how or if microbiological and immunological parameters were affected. Often GCF of patients suffering from periodontitis was collected after hygiene phase before scaling and root planing were performed to avoid the contamination of the sample with blood (35). The results of the present study showed that the differentiation between healthy and diseased periodontal situations using immunological and microbiological parameters obtained from GCF samples was possible before (t_0) and after oral prophylaxis (t_3).

The host response to infections caused by periodontal pathogenic bacteria is characterized by infiltration of the tissue by neutrophils, macrophages and lymphocytes and by the generation of high concentrations locally of cytokines, eicosanoids and other destructive mediators such as the matrix metalloproteinases (MMPs) (36). MMP-8 (collagenase-2) is increased due to the inflammatory process in periodontitis (37, 38). Increased levels of MMP-8 in case of periodontitis were described in several studies (39–41) and were also observed in the present survey. Additionally, the MMP-8 value increased in periodontitis group after the prophylaxis session that may be the result of a stronger inflammatory response.

Cytokines function in complex networks involving both pro- and anti-inflammatory effects (36). IL-1β is mainly a pro-inflammatory cytokine that stimulates the expression of many genes associated with inflammation (42) and is an important mediator of connective tissue destruction of the gingiva and the periodontal ligament, as well as of resorption of the alveolar bone (43). Besides its function as chemokine, IL-8 has a pro-inflammatory function, activates neutrophils (44) and induces the release of granule enzymes from PMNs (45). IL-10 has an anti-inflammatory role and inhibits the activity of Th1, Th2 and NK cells as well as macrophages (36).

Increased levels of expression and synthesis of IL-1, TNF-α, IL-6 and IL-8 have been detected in periodontal tissues (46). The results of this study showed that IL-10 level was significantly higher in periodontally healthy controls. On the other hand, IL-1β and IL-8 levels were higher in periodontitis. This is in line with recent findings that IL-1β, IL-8 and MMP-8 are detectable in higher (47) and IL-10 can be found in lower levels (48) in patients with periodontitis.

There is evidence that even the subgingival ecosystem may be influenced by repeated professional supragingival plaque removal (49, 50). However, a single prophylaxis treatment is not able to alter the subgingival biofilm for longer time period in patients who suffer from periodontitis. The re-colonization of the subgingival environment occurs via the down-growth of supragingival plaque and the re-growth of the subgingivally remaining bacteria (51).

Scaling and polishing lead in patients with gingivitis or periodontitis to a reduction in plaque level and gingival inflammation (52). Oral hygiene instructions, in addition to a professional oral prophylaxis (53) and self-performed mechanical plaque removal (54), are able to increase this effect. In chronic periodontitis, a single prophylaxis session did not influence significantly the bacterial composition of the subgingival biofilm and was not able to suppress VSC values, even over a short-term period. In patients with periodontitis, a more complex, multistage treatment is necessary (18).

Conclusions

Within the limitations of this study, the results demonstrated that oral prophylaxis is able to affect halitosis-associated parameters, such as the amount of volatile sulphur compounds or organoleptic scores, and showed limited effects on microbial and immunological parameters.

However, a simple prophylaxis session is only effective to reach a short-term decrease in VSC in periodontitis, and a

repeated treatment and further anti-infective procedures seem to be necessary.

A differentiation between diseased and healthy periodontal situations regarding inflammatory parameters in gingival crevicular fluid is possible before and after oral prophylaxis.

Clinical relevance

Scientific rationale for the study

Oral malodour is mainly caused by tongue coating and/or periodontal diseases. Periodontopathogens are able to produce volatile sulphur compounds (VSC).

Principal findings

In chronic periodontitis, a single prophylaxis session did not influence significantly the bacterial composition of the subgingival biofilm and was not able to suppress VSC values, even over a short-term period.

Practical implication

Full-mouth tooth cleaning, flossing, polishing and tongue cleaning not only prevent caries or periodontal diseases, but can also reduce the symptoms of halitosis in healthy subjects. In chronic periodontitis, a more complex treatment is necessary.

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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 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.
3 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.
4 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.
5 Bornstein MM, Kislig K, Hoti BB, Seemann R, Lussi A. Prevalence of halitosis in the population of the city of Bern, Switzerland: a study comparing self-reported and clinical data. *Eur J Oral Sci* 2009; **117**: 261–267.
6 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. *Int J Dent Hyg* 2009; **7**: 251–255.
7 Anonoymous. Survey conducted at ADA reveals interesting trends. *Dent Econ* 1995; **85**: 6.
8 Evigren S, Kamburoglu K. Effects of tongue coating and oral health on halitosis among dental students. *Oral Health Prev Dent* 2013; **11**: 169–173.
9 Eldarrat AH. Influence of oral health and lifestyle on oral malodour. *Int Dent J* 2011; **61**: 47–51.
10 Scully C, Greenman J. Halitology (breath odour: aetiopathogenesis and management). *Oral Dis* 2012; **18**: 333–345.
11 Tonzetich J, McBride BC. Characterization of volatile sulphur production by pathogenic and non-pathogenic strains of oral Bacteroides. *Arch Oral Biol* 1981; **26**: 963–969.
12 Persson S, Edlund MB, Claesson R, Carlsson J. The formation of hydrogen sulfide and methyl mercaptan by oral bacteria. *Oral Microbiol Immunol* 1990; **5**: 195–201.
13 Coli JM, Tonzetich J. Characterization of volatile sulphur compounds production at individual gingival crevicular sites in humans. *J Clin Dent* 1992; **3**: 97–103.
14 Yaegaki K, Sanada K. Effects of a two-phase oil-water mouthwash on halitosis. *Clin Prev Dent* 1992; **14**: 5–9.
15 Roldán S, Herrera D, Sanz M. Biofilms and the tongue: therapeutical approaches for the control of halitosis. *Clin Oral Investig* 2003; **7**: 189–197.
16 Lee KH, Tanner AC, Maiden MF, Weber HP. Pre- and post-implantation microbiota of the tongue, teeth, and newly placed implants. *J Clin Periodontol* 1999; **26**: 822–832.
17 Van Winkelhoff AJ, Van der Velden U, Winkel EG, de Graaff J. Black-pigmented Bacteroides and motile organisms on oral mucosal surfaces in individuals with and without periodontal breakdown. *J Periodontal Res* 1986; **21**: 434–439.
18 Dentino A, Lee S, Mailhot J, Hefti A. Principles of periodontology. *Peridontol* 2000; **2013**: 16–53.
19 Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999; **4**: 1–6.
20 Silness J, Loe H. Periodontal disease in pregnancy. Ii. Correlation between oral hygiene and periodontal condition. *Acta Odont Scand* 1964; **22**: 121–135.
21 Lang NP, Joss A, Orsanic T, Gusberti FA, Siegrist BE. Bleeding on probing. A predictor for the progression of periodontal disease? *J Clin Periodontol* 1986; **13**: 590–596.
22 Guentsch A, Kramesberger M, Sroka A, Pfister W, Potempa J, Eick S. Comparison of gingival crevicular fluid sampling methods in patients with severe chronic periodontitis. *J Periodontol* 2011; **82**: 1051–1060.
23 Eick S, Straube A, Guentsch A, Pfister W, Jentsch H. Comparison of real-time polymerase chain reaction and DNA-strip technology in microbiological evaluation of periodontitis treatment. *Diagn Microbiol Infect Dis* 2011; **69**: 12–20.
24 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.
25 AAP. *Glossary of Periodontal Terms*. Chicago, IL: AAP, 2001.
26 Needleman I, Suvan J, Moles DR, Pimlott J. A systematic review of professional mechanical plaque removal for prevention of periodontal diseases. *J Clin Periodontol* 2005; **32**: 229–282.
27 Apatzidou AD, Bakirtzoglou E, Vouros I, Karagiannis V, Papa A, Konstantinidis A. Association between oral malodour and periodontal disease-related parameters in the general population. *Acta Odont Scand* 2012; **71**: 189–195.

28 Yaegaki K, Sanada K. Volatile sulfur compounds in mouth air from clinically healthy subjects and patients with periodontal disease. *J Periodontal Res* 1992; **27** (4 Pt 1): 233–238.
29 Hinode D, Fukui M, Yokoyama N, Yokoyama M, Yoshioka M, Nakamura R. Relationship between tongue coating and secretory-immunoglobulin A level in saliva obtained from patients complaining of oral malodor. *J Clin Periodontol* 2003; **30**: 1017–1023.
30 Chang YC, Yang SF, Lai CC, Liu JY, Hsieh YS. Regulation of matrix metalloproteinase production by cytokines, pharmacological agents and periodontal pathogens in human periodontal ligament fibroblast cultures*J Periodontal Res* 2002; **37**: 196–203.
31 De Boever EH, Loesche WJ. Assessing the contribution of anaerobic microflora of the tongue to oral malodor. *JADA* 1995; **126**: 1384–1393.
32 Johnson P, Yaegaki K, Tonzetich J. Effect of volatile thiol compounds on protein metabolism by human gingival fibroblasts. *J Periodontal Res* 1992; **27**: 553–561.
33 Johnson P, Yaegaki K, Tonzetich J. Effect of methyl mercaptan on synthesis and degradation of collagen. *J Periodontal Res* 1996; **31**: 323–329.
34 Van der Sleen M, Slot D, Van Trijffel E, Winkel E, Van der Weijden GA. Effectiveness of mechanical tongue cleaning on breath odour and tongue coating: a systematic review. *Int J Dent Hyg* 2010; **8**: 258–268.
35 Puklo M, Guentsch A, Hiemstra P, Eick S, Potempa J. Analysis of neutrophil-derived antimicrobial peptides in gingival crevicular fluid suggests importance of cathelicidin LL-37 in the innate immune response against periodontogenic bacteria. *Oral Microbiol Immunol* 2008; **23**: 328–335.
36 Preshaw PM, Taylor JJ. How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? *J Clin Periodontol* 2011; **38**(Suppl 11): 60–84.
37 Nomura T, Ishii A, Oishi Y, Kohma H, Hara K. Tissue inhibitors of metalloproteinases level and collagenase activity in gingival crevicular fluid: the relevance to periodontal diseases. *Oral Dis* 1998; **4**: 231–240.
38 Yakob M, Kari K, Tervahartiala T *et al*. Associations of periodontal microorganisms with salivary proteins and MMP-8 in gingival crevicular fluid. *J Clin Periodontol* 2012; **39**: 256–263.
39 Gursoy UK, Kononen E, Pradhan-Palikhe P *et al*. Salivary MMP-8, TIMP-1, and ICTP as markers of advanced periodontitis. *J Clin Periodontol* 2010; **37**: 487–493.
40 Munjal SK, Prescher N, Struck F, Sorsa T, Maier K, Netuschil L. Evaluation of immunoassay-based MMP-8 detection in gingival crevicular fluid on a point-of-care platform. *Ann NY Acad Sci* 2007; **1098**: 490–492.
41 Sorsa T, Hernandez M, Leppilahti J, Munjal S, Netuschil L, Mantyla P. Detection of gingival crevicular fluid MMP-8 levels with different laboratory and chair-side methods. *Oral Dis* 2010; **16**: 39–45.
42 Dinarello C. The IL-1 family and inflammatory diseases. *Clin Exp Rheumatol* 2002; **20**: S1–S13.
43 Liu Y, Lerner U, Teng Y. Cytokine response against periodontal infection: protective and destructive roles. *Peridontol* 2000; **2010**: 163–206.
44 Bickel M. The role of interleukin-8 in inflammation and mechanisms of regulation. *J Periodontol* 1993; **64**: 456–460.
45 Schröder J. The monocyte-derived neutrophil activating peptide (NAP/interleukin 8) stimulates human neutrophil arachidonate-5-lipoxygenase, but not the release of cellular arachidonate. *J Exp Med* 1989; **170**: 847–863.
46 Lamont R, Jenkinson H. Life below the gum line: pathogenic mechanisms of *Porphyromonas gingivalis*. *Microbiol Mol Biol Rev* 1998; **62**: 1244–1263.
47 Konopka L, Pietrzak A, Brzezińska-Błaszczyk E. Effect of scaling and root planing on interleukin-1β, interleukin-8 and MMP-8 levels in gingival crevicular fluid from chronic periodontitis patients. *J Periodontal Res* 2012; **47**: 681–688.
48 de Lima Oliveira A, de Faveri M, Gursky L *et al*. Effects of periodontal therapy on GCF cytokines in generalized aggressive periodontitis subjects. *J Clin Periodontol* 2012; **39**: 295–302.
49 Katsanoulas T, Renee I, Attstrom R. The effect of supragingival plaque control on the composition of the subgingival flora in periodontal pockets. *J Clin Periodontol* 1992; **19**: 760–765.
50 Hellstrom MK, Ramberg P, Krok L, Lindhe J. The effect of supragingival plaque control on the subgingival microflora in human periodontitis. *J Clin Periodontol* 1996; **23**: 934–940.
51 Quirynen M, De Soete M, Dierickx K, van Steenberghe D. The intra-oral translocation of periodontopathogens jeopardises the outcome of periodontal therapy. A review of the literature. *J Clin Periodontol* 2001; **28**: 499–507.
52 Needleman I, Suvan J, Moles DR, Pimlott J. A systematic review of professional mechanical plaque removal for prevention of periodontal diseases. *J Clin Periodontol* 2005; **32**(Suppl 6): 229–282.
53 van der Weijden F, Slot DE. Oral hygiene in the prevention of periodontal diseases: the evidence. *Periodontol 2000* 2011; **55**: 104–123.
54 van der Weijden GA, Hioe KP. A systematic review of the effectiveness of self-performed mechanical plaque removal in adults with gingivitis using a manual toothbrush. *J Clin Periodontol* 2005; **32**(Suppl 6): 214–228.