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A cross-over study on the effect of various therapeutic approaches to morning breath odour

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Abstract

Objective: The aim of this study was to investigate the effect of tongue scraping and inter-dental flossing on morning bad breath in periodontally healthy subjects.

Methods: A four-step blind, cross-over study was conducted in 19 volunteers, divided into four groups: Group I: tooth brushing; Group II: tooth brushing and inter-dental flossing; Group III: tooth brushing and tongue scraping; and Group IV: tooth brushing, inter-dental flossing and tongue scraping. The volunteers performed these oral hygiene procedures three times a day for 7 days. Seven-day wash-out intervals were observed. Morning mouth breath was assessed organoleptically and by volatile sulphur compound concentrations.

Results: The highest mean organoleptic and volatile sulphur compound measurement values were found in the treatment groups in which tongue scraping was not performed and there were statistical differences between the two groups ($p<0.05$). In the organoleptic evaluation ($p>0.05$), inter-dental flossing did not show any statistical improvement in the effect of tongue hygiene on morning bad breath, but it significantly reduced the concentration of volatile sulphur compounds ($p<0.05$).

Conclusion: The findings suggest that tongue scraping appears to be the most important hygienic procedure to reduce morning bad breath in periodontally healthy subjects.

Key words: bad breath; dental plaque; halitosis; morning breath odour; tongue scraping; volatile sulphur compounds

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Halitosis, often called oral malodour or bad breath, is influenced by a combination of several factors (Tonzetich & Ng 1976). Although numerous non-oral sites and systemic causes have been suggested (nasal inflammation, chronic sinusitis, diabetes mellitus, ureamia, etc.), an estimated 80–90% of all bad breath odours originate in the mouth itself (Petri et al. 1992, Miyazaki et al. 1995, Rosenberg 1996, Delanghe et al. 1997, Loesche & Kazor 2002, Tangerman et al. 2002, Lee et al. 2003).

It is well accepted that the pathogenesis of oral malodour is associated with the bacterial degradation of sulphur-containing amino acids (methionine, cysteine and cystine) into volatile sulphur compounds (VSCs), of which hydrogen sulphide (H_2S), methyl mercaptan (CH_3SH) and, to a lesser extent, dimethyl sulphide ($(CH_3)_2S$) are the principal components (Tonzetich & Carpenter 1971, Persson et al. 1990 Rosenberg et al. 1991a, Kleinberg & Westbay 1992, Miyazaki et al. 1995, Waler 1997, Morita & Wang 2001, Roldan et al. 2003a). In addition, other compounds in mouth air may also be offensive. There are non-sulphur-containing products, such as volatile aromatic compounds (indole, skatole), organic acids (acetic, propionic) and amines, i.e. cadaverine (Goldberg et al. 1994) and putrescine (Reingewirtz 1999).

Halitosis exists in several different clinical situations. Malodorous breath upon awakening after a night's sleep is a common condition known as "morning bad breath". This problem tends to be, in contrast to the persistent oral halitosis, transient in nature. Both malodour conditions appear to result, in great part, from the above-mentioned excessive quantities of sulphur-containing gases of bacterial origin (Rosenberg et al. 1991a, De Boever & Loesche 1995). It has been postulated that decreased salivation during sleep promotes bacteria proliferation that releases offending gases (Rosenberg et al. 1991a, 1996, Rosenberg & McCullock 1992, Suarez et al. 2000). Therapies that reduce morning bad breath do not imply efficacy in the treatment of oral halitosis; however, morning breath has often been used as a model to test the clinical efficacy of different therapies on oral halitosis (Tonzetich & Ng 1976, Suarez et al. 2000, van Steenberghe et al. 2001, Carvalho et al. 2004), instead of working with real halitosis patients. This model has been accepted, as the recruitment of real halitosis subjects is difficult (privacy, embarrassed feeling, unpleasant examinations) and difficult to standardize (origin, behaviour) (Suarez et al. 2000, van Steenberghe et al. 2001, Quirynen et al. 2002, Carvalho et al. 2004), although there is no evidence that treatment protocols that are efficacious for morning malodour would be equally efficacious in the treatment of halitosis.

Any oral site where microbial accumulation and putrefaction can occur is suspected of producing VSCs. The sites that most commonly produce intra-oral malodour are the tongue and inter-dental and subgingival areas (Rosenberg 1996), and the tongue is considered to be largely responsible for it. Moreover, different oral hygiene procedures for preventing oral malodour, such as tooth brushing, dental prophylaxis, flossing and tongue scraping, contribute to the problem when they are inefficiently performed (Tonzetich & Ng 1976, Seemann et al. 2001, Quirynen 2003). Thus, the aim of this study was to investigate the effect of tongue hygiene and flossing on the concentration of volatile sulphur compounds and morning bad breath in periodontal healthy subjects.

Material and Methods

Patient population

Nineteen dental student volunteers, six men and 13 women, (aged 19–22 years) from the Faculty of Dentistry of Maringá-UEM, volunteered to participate in this blind, randomized, cross-over clinical trial. All the volunteers were submitted to an anamneses and oral clinical exam. The exclusion criteria were as follows: subjects with medical disorders; visible tongue coating; anyone undergoing antibiotic or other antimicrobial therapy in the previous 6 months; smokers; pregnant women; anyone presenting a probing depth $\geqslant 3$ mm with bleeding on probing and attachment loss $\geqslant 2$ mm; caries; tongue coating; alterations in the oral and nasopharyngeal mucosa; and anyone with fewer than 20 natural teeth. The Institutional Committee of Ethics in Clinical Research of the University of Maringá approved the study protocol.

Randomization and allocation concealment

Each subject was given a code number at the enrolement visit and they were randomly assigned by a computer-generated table in one of the four treatment groups. The coordinator of the study (R. M. H.) assigned participants to their groups and was responsible to cross-over the subjects in different treatments during the experimental phase.

A researcher (G. C.) prepared a hygiene kit in plastic bags containing toothbrushes (Dental Prev, SP, Brazil), inter-dental flossing (Anakol Ind. Com. Ltda-Kolynos do Brasil, – Colgate Palmolive Co., São Bernardo do Campo, SP, Brazil) and tongue scraper (Dental Prev), and was also responsible for giving the kits to the subjects. All study personnel, including the examiner researcher (M. F.) and biostatistician, were blinded to treatment assignment for the duration of the study.

Pre-treatment

One week before the beginning of the study, all subjects went through motivation sessions in which standard oral hygiene instructions were given. These instructions included conventional dental plaque control (toothbrushes; Dental Prev), inter-dental plaque control (inter-dental flossing – Anakol Ind. Com. Ltda- Kolynos do Brasil – Colgate Palmolive Co.) and tongue cleaning (tongue scraper; Dental Prev). All the volunteers received the same toothpaste (Sorriso®, Anakol Ind. Com. Ltda-Kolynos do Brasil – Colgate Palmolive Co.). This instruction was given only at this moment.

In order to standardize baseline measurements and avoid interference from the presence of dental plaque, the volunteers' dental plaque was kept under strict controls. One day before each experimental phase, all volunteers had professional supragingival plaque removal performed. Supragingival plaque was revealed using a disclosing solution. Visible supragingival plaque was removed using scalers, following by tooth polishing using a rubber cup and dentifrice. In addition, the volunteers necessarily had to present a mean plaque index $\leqslant 1$ (Silness & Löe 1964) at the beginning of each treatment phase.

Study design

This study was a randomized, blind comparison of 19 volunteers divided into four cross-over groups, performed in four experimental periods of 7 days. In each period, every volunteer performed the following oral hygiene procedures three times a day:

Group I (Control): tooth brushing (B);
Group II: tooth brushing and inter-dental flossing (BF);
Group III: tooth brushing and tongue scraping (BT); and
Group IV: tooth brushing, inter-dental flossing and tongue scraping (BFT).

Each experimental period was followed by a 7-day wash-out interval. In between the experimental periods, the subjects maintained the standard oral hygiene that was given during pre-treatment period. Compliance was assessed by calling the subjects every day during the experimental phase by a researcher (G. C.).

Breath evaluations

At the beginning and at the end of all experimental phases, the quality of the subject's mouth air was evaluated. For the measurements (08:00 hours), the volunteers refrained from tooth brushing, drinking, eating, gargling and using scented cosmetic products (Rosenberg 1996, Neiders & Ramos 1999). All measurements were performed by the same examiner (M. F.), who was blind to the treatment used.

Organoleptic assessmenter

The organoleptic quality of the subject's mouth air was scored by a trained and calibrated judge. Subjects were asked to close their mouths for 60 s and not to swallow during this period. The subjects and the judge were separated by a screen, in which a disposable tube was inserted, leading the mouth air straight to the judge's nose. The judge immediately recorded the odour rating on a four-point scale (Schmidt et al. 1978): 0, no odour; 1, slight malodour; 2, moderate malodour; and 3, high malodour.

Volatile sulphur concentration

The volatile sulphur concentration was scored immediately after organoleptic assessment, using a portable industrial sulphide monitor (Halimeter®, Interscan Corp., Chatsworth, CA, USA), zeroed to ambient air before each measurement, using the technique established by Rosenberg et al. (1991a, b).

Three measurements were taken, and the mean of these values was determined in parts per billion (p.p.b.) of sulphide equivalents.

Statistical analysis

According to the cross-over design, the organoleptic and VSCs concentration data were compared among treatments, applying the ANOVA and the Tukey tests. The difference in changes was calculated by ΔVSC (ΔVSC = VSC concentration after − VSC concentration before). The relationship between the VSC concentration and organoleptic assessment was obtained by Spearman's correlation. For all the analyses, a 5% significance level was adopted and the data were analysed using the software SAS/LAB 8.02 (1999, Cary, NC, USA).

Table 1. Mean values of organoleptic ratings before and after the treatments and the difference in changes (Δscores) before and after for each treatment (mean ± SD; $n = 19$)

Treatment/groups	Organoleptic score		Δscore
	before	after	
Brushing	$1.05 \pm 0.52^{A, a}$	$2.00 \pm 0.33^{B, a}$	0.94 ± 0.52^{a}
Brushing and flossing	$1.05 \pm 0.40^{A, a}$	$1.94 \pm 0.22^{A, b}$	0.89 ± 0.56^{a}
Brushing and tongue scraping	$1.05 \pm 0.52^{A, a}$	$1.26 \pm 0.45^{A, b}$	0.21 ± 0.41^{b}
Brushing, flossing and tongue scraping	$0.89 \pm 0.65^{A, a}$	$1.10 \pm 0.45^{A, b}$	0.21 ± 0.63^{b}

ANOVA and Tukey's test; $p < 0.05$.
Different lower letters in columns indicate statistically significant differences among groups.
Different capital letters in lines indicate statistically significant differences between time points.

Table 2. Mean values of volatile sulphur compound (VSCs) concentrations before and after the treatments and the difference in changes (ΔVSC) before and after for each treatment (mean ± SD; $n = 19$)

Treatment/groups	VSCs concentration		Difference
	before	after	
Brushing	$44.0 \pm 10.0^{A, a}$	$133.7 \pm 32.2^{B, a}$	89.7 ± 23.5^{a}
Brushing and flossing	$44.2 \pm 19.4^{A, a}$	$120.8 \pm 34.5^{B, a}$	76.5 ± 6.4^{a}
Brushing and tongue scraping	$40.0 \pm 16.8^{A, a}$	$58.4 \pm 21.6^{A, b}$	18.3 ± 9.1^{b}
Brushing, flossing and tongue scraping	$40.9 \pm 18.2^{A, a}$	$46.5 \pm 16.1^{A, b}$	5.5 ± 8.0^{c}

ANOVA and Tukey's test; $p < 0.05$.
Different lower letters in columns indicate statistically significant differences among groups.
Different capital letters in lines indicate statistically significant differences between time points.

Results

All the selected subjects ($n = 19$) completed the study. Before the treatments, there were no statistically significant differences ($p > 0.05$) for VSCs and organoleptic ratings among the 19 volunteers at the beginning of each experimental period. Furthermore, considering the wash-out periods, it was observed that no carry-over effect occurred between the treatments.

Organoleptic measurements

Mean values and standard deviations for organoleptic measurements are presented in Table 1. The highest mean organoleptic measurement values were found in the groups in which tongue scraping was excluded and the two groups differed from those that performed tongue hygiene. Similarly, the Δscore showed statistically higher values for B and BF, compared with BT and BFT ($p < 0.05$).

Volatile sulphide measurements

Table 2 shows the sulphide monitor measurements. The highest mean VSC concentration values were found in the groups B and BF, in which tongue scraping was excluded, and differed from BT and BFT. The B presented the highest ΔVSC mean (89.7 ± 23.5), followed by BF, BT and BFT. There were no statistical differences for ΔVSC between BF and B ($p > 0.05$) and the two showed higher mean values compared with the other groups. The comparison between BFT and BT revealed a statistically significant difference ($p < 0.05$).

In general, tongue scraping demonstrated a statistical reduction in the VSC concentration and organoleptic scores. Although the absence of inter-dental flossing showed a tendency to contribute to an increased VSC concentration in the mouth, no statistical difference was found in comparison with the organoleptic scores.

In addition, there was a significant positive correlation between organoleptic scores and volatile sulphide monitor measurements ($r = 0.60$; $p < 0.0001$).

Discussion

Over the last few years, halitosis has been of interest both to the scientific community and to people who suffer from it. Epidemiological studies have demonstrated a high prevalence in the population (Loesche & Kazor 2002). Thus, further studies are necessary to enable the mechanism of malodour formation to be understood, in order to provide more precise and safer treatment.

In this clinical trial, the contribution made by tongue and inter-dental hygiene to reducing morning oral malodour in periodontally and systemically healthy individuals was investigated. The results demonstrated that lack of tongue hygiene had an impact in spite of teeth being brushed. The individuals who cleaned their tongues showed significantly lower values compared with the subjects who did not. These data were observed in the organoleptic and volatile sulphur measurements (Tables 1 and 2). This can be explained because the tongue has a large surface and its papillary structure represents an anaerobic niche in the oral cavity, favouring the accumulation of oral debris and microorganisms responsible for the VSC formation. Tongue coating is composed of blood components and other nutrients, large amounts of desquamated epithelial cells and bacteria (Yaegaki & Sanada 1992). Moreover, in individuals who have tongue coating, the bacterial load is about 25 times greater than in those who do not have it (De Boever & Loesche 1995).

The results of the present study are in agreement with other studies that have demonstrated that the tongue is the major site for the production of VSCs (Tonzetich & Ng 1976, Yaegaki & Sanada 1992, Bosy et al. 1994, De Boever & Loesche 1995, Waler 1997, Suarez et al. 2000, Çiçek et al. 2003, Kazor et al. 2003, Lee et al. 2003, Pedrazzi et al. 2004, Liu et al. 2006). However, most of these studies selected volunteers with features that would interfere with halitosis at some level, for example: individuals with periodontal disease, individuals with tongue coating, or halitosis induced artificially by the use of mouthrinses containing sulphur amino acids. It is known that periodontal pockets are colonized by anaerobic bacteria (Ximenez-Fyvie et al. 2000), microbial flora capable of producing VSCs (Figueiredo et al. 2002). In addition, Yaegaki & Sanada (1992) found six times more tongue coating in patients with periodontitis than in those who were periodontally healthy. Therefore, individuals with periodontal disease will probably present with microbial flora more favourable to exacerbating VSC formation in comparison with healthy individuals. In this study, all volunteers were periodontally healthy subjects and their plaque control was strictly maintained. The results were not affected by any oral factors that could have contributed to the increase of VSC concentration. Therefore, the period of 7 days without tongue hygiene was the major factor responsible for the increase in VSCs.

The absence of inter-dental flossing increased the VSC concentration in the morning bad breath. The VSC measurement values ($p<0.05$) (Table 2) of the individuals who performed all the oral hygiene procedures differed statistically from those of the groups that did not perform inter-dental flossing. However, this trend was not observed in the organoleptic measurements (Table 1). These results are in disagreement with Ceravolo et al. (1973), who suggested a possible influence of inter-dental plaque on the pathogenesis of malodour when using organoleptic measurement. The instruments currently used to assess oral malodour are the gas chromatograph, sulphide monitors and sensory odour judges. The gas chromatograph and sulphide monitor quantify, and in some cases, identify volatile sulphur compounds in concentrations as low as a few p.p.b. (Tonzetich 1977, Schmidt et al. 1978, Rosenberg et al. 1991a, b, Yeagaki & Sanada 1992). However, these sensors are only useful for identifying VSCs and sensor odour judges are not only able to detect and recognize compounds (VSCs) but can also discriminate complex mixtures (non-sulphur compounds). Thus, sensor odour judges are considered the gold standard for oral malodour assessment. According to the results of the present study, inter-dental flossing has no added value with regard to reducing morning bad breath.

The manufacturer of the sulphide monitor (Halimeter®, Interscan Corp.) had not stated a definitive value of ppb for normal reading for many years. Yaegaki & Sanada (1992) recommended 75 p.p.b. as the perceived level of malodour in mouth air. Miyazaki et al. (1995) also utilized the same standard in their studies for halitosis in Japan. Recently, the manufacturer suggested 110 p.p.b. or below as a normal reading in their instructions (http://www.halimeter.com/halcal.htm). In this study, VSC measurements demonstrated in all treatment groups lower values at baseline (mean <45 p.p.b.) that would mean a normal level of mouth odour according to the literature. However, organoleptic scores were higher than the corresponding values of VSC measurements. The lack of consensus between these values can be attributed to the fact that the measurement device does not allow a calibration procedure with known standard gases in order to obtain a more exact result. Thus, the readings' values are totally dependable on the sulphide sensor and they are more useful in comparative results than absolute results. In addition, other important odourants, such as volatile aromatic compounds (indole, skatole) and organic acids (acetic, propionic), could contribute to the morning bad breath and not be detected by the sulphide monitor. The finding values at baseline readings were a consequence of our experimental population, which consisted of subjects without periodontal disease, had excellent oral hygiene and were dentistry students.

There are no accepted standards of care for treating halitosis, and clinical protocols for the diagnosis and treatment of this problem vary widely (Rosenberg 2002, Çiçek et al. 2003). Antiseptic rinses have also been used to control morning bad breath (van Steenberghe et al. 2001, Roldan et al. 2003b, Winkel et al. 2003, Carvalho et al. 2004, Roldan et al. 2004, Fine et al. 2005). These mouthrinses contain several masking and antimicrobial agents. However, further studies are necessary to analyse the effects of these agents on the oral cavity over a long period. This study demonstrated that when a person in general and periodontal good health performs adequate oral hygiene, but has a persistent oral malodour problem, tongue cleaning is an effective way to treat morning bad breath. This result was in agreement with Tonzetich & Ng (1976), who reported that tongue cleaning is twice as effective as tooth brushing for reducing oral malodour. Thus, tongue cleaning should be a part of daily home oral hygiene procedures.

In the present study, a significant Spearman correlation among organoleptic and monitor sulphur measurements ($r=0.60$; $p<0.0001$) was found. These data are in agreement with other studies that demonstrated a correlation coefficient ranging from $r=0.42$ to 0.64, $p\leqslant0.001$ (Rosenberg et al. 1991a, b, Rosenberg 1996). Thus, the sulphur monitor has been considered a valid instrument for use in clinical studies of malodour, within its limitations (Rosenberg & McCulloch 1992).

To sum up, the results suggest that the absence of tongue cleaning was able to increase VSC concentration and promote morning bad breath. The presence of inter-dental biofilm did not contribute significantly to the increase in VSC concentration in the healthy population. Thus, it was concluded that tongue scraping appears to be the most important hygiene procedure to reduce morning mouth breath in periodontally healthy subjects and should be a part of daily home oral hygiene procedures.

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Clinical Relevance

Scientific rationale for study: A review of the scientific literature showed that morning bad breath is a very common condition, even in periodontally healthy individuals. However, very little is known about the oral hygiene procedures, including tongue and inter-dental hygiene, that these patients should implement to obtain relief.

Principal findings: Among the procedures evaluated, tongue scraping appeared to be the most effective, as the lack of it was shown to be capable of inducing halitosis.

Practical implication: In order to prevent morning bad breath, periodontally healthy subjects should not neglect tongue hygiene.