Malodor reductions and improved oral hygiene by toothbrushing and mouthrinsing

Nandlal B, Shahikumar P¹, Avinash BS¹, Sreenivasan PK², Subramanyam R²

ABSTRACT

Objective: This clinical study compared the effects of an antibacterial regimen, comprising a triclosan toothpaste and a 0.075% cetylpyridinium chloride (CPC) mouthrinse, on malodor, self-reported malodor, and oral hygiene measures such as dental plaque, gingivitis, and bleeding relative to brushing with a fluoride toothpaste.

Materials and Methods: At baseline, 36 subjects were evaluated for malodor (9-point organoleptic scale [OLT]), dental plaque (Turesky modification of Quigley-Hein; PI), gingivitis (Löe-Silness; GI) and bleeding (Ainamo and Bay; BI) and randomized to (1) tooth brushing with fluoride toothpaste, or (2) a regimen comprising tooth brushing with a triclosan toothpaste and mouth rinsing with CPC mouthrinse. After the first use of assigned treatments, subjects were evaluated for malodor 2 h after breakfast (OLT-2 h) and used provided treatments for the next 14 days. On the 7th and 14th days, subjects refrained from oral hygiene for 12 h before evaluations (OLT, PI, GI, and BI) and then performed oral hygiene at the dental clinic. Subjects were evaluated for malodor 2 h after breakfast (OLT-2 h) and self-assessed their malodor on a 100 mm visual analog scale (VAS).

Results: Treatment groups demonstrated no significant differences in OLT, PI, GI, BI at baseline ($P > 0.05$). OLT-2 h scores after the first use of regimen and after tooth brushing alone were 5.94 and 6.21, respectively, and were statistically significantly different ($P < 0.05$). Correspondingly, the regimen demonstrated progressive reductions in OLT and OLT-2 h on the 7th and 14th day evaluations (5.81, 4.88, and 5.09, 4.20, respectively) and were significantly lower than after tooth brushing alone (6.49, 6.18, and 6.35, 5.99, respectively) ($P < 0.05$). From the 7th to 14th days, the regimen also demonstrated progressively lower PI, GI, BI, and self-reported malodor (VAS scores) which were significantly lower than tooth brushing alone ($P < 0.05$).

Conclusions: Results from this study demonstrated that a regimen comprising a triclosan toothpaste and CPC mouthrinse demonstrated significant malodor reductions 2 h after the first use and progressively increasing reductions in malodor, dental plaque, gingivitis, bleeding and self-reported malodor from the 7th to 14th days than tooth brushing alone.

Key words: Oral hygiene, malodor, mouthrinse, toothpaste

Halitosis commonly referred to as bad breath is defined as an offensive odor from the mouth.$¹^{1,2}$ Surveys suggest the widespread prevalence of halitosis$³$ with estimates suggesting 25–50% of individuals afflicted with the condition$¹$ and a higher proportion of the population who perceive bad breath.$²$ An extensive literature describes the significant impacts of halitosis on social interactions with this condition representing an important reason for dental visits.$⁴,⁵$
The origin of bad breath is associated with the mouth and the ability of Gram-negative bacteria to putrify sulfur-containing proteinaceous substrates. Biochemical studies have characterized the ability of organisms found on the tongue surface as a predominant source of malodorous compounds. Based on these laboratory analyses, volatile sulfur compounds such as hydrogen sulfide, methyl mercaptan, and dimethyl sulfate and others are identified with malodor.

The practical consequences of oral malodor and its social implications represent important reasons to identify effective treatments. New ingredients with potential application to reduce malodor and clinical studies with formulations are reported in the literature. An important focus of these efforts is an assessment of the antimicrobial efficacy of ingredients on oral bacteria including those associated with malodor. In addition, clinical studies have examined the effects of dentifrices and mouthwashes with antimicrobial agents such as chlorhexidine, triclosan, cetylpyridinium chloride (CPC), and herbal ingredients to control malodor. Oral hygiene regimens that include a dentifrice and mouthwash represent an additional approach to control malodor.

Brushing with a toothpaste formulated with antimicrobial ingredients followed by rinsing with mouthwash may provide greater control of oral organisms within the residual plaque that remains inaccessible to toothbrushing. Accordingly, this clinical study compared the effects of an antibacterial regimen, comprising a triclosan toothpaste and a 0.075% CPC mouthrinse, on malodor, self-reported malodor, and oral hygiene measures such as dental plaque, gingivitis, and bleeding relative to brushing with a fluoride toothpaste. In addition, the study evaluated malodor effects evaluated after initial use with evaluations conducted after breakfast representing an outcome of practical relevance along with longer-term effects conducted after 14 days use.

MATERIALS AND METHODS

The study was a randomized, double-blind, parallel design clinical study comparing the effects of two treatment regimens. The protocol for the study was reviewed and approved by the Ethical Review Board of JSS Dental College and Hospital, Mysore, India.

Males and female subjects 18–70 years of age in good general health who completed voluntary informed consent and a health questionnaire were evaluated by a dentist to assess oral soft and hard tissues. Adults with a minimum of 20 natural teeth without crowns and other restorations and maintained adequate oral hygiene along with malodor scores of 6 or more on a hedonic scale ranging from 1 to 9 were selected. In addition to organoleptic evaluations, subjects who registered a score of 60 mm or more on a 100 mm visual analog scale (VAS) for a malodor self-assessment were enrolled.

Subjects with a history of allergic reactions to oral hygiene products were ineligible for this study. Those presenting oral symptoms such as a severe periodontal disease, mobile teeth, gingival enlargement, and grossly decayed teeth were excluded. Other exclusion criteria included self-reported pregnancy, lactating mothers, smokers, and systemic diseases. In addition, subjects prescribed antibiotics in the past month or reporting participation in clinical studies were also excluded.

Following study enrollment, subjects discontinued the use of all other oral hygiene aids and completed a 10 days washout phase with a commercially available fluoride toothpaste and a soft-bristled toothbrush. Baseline examinations were conducted after the washout phase with subjects refraining from oral hygiene for 12 h and from any food or drink for 4 h before their evaluation. Baseline assessments included whole mouth examinations for dental plaque, gingival inflammation, and gingival bleeding by a calibrated dental examiner with calibrations conducted before study commencement. Three calibrated examiners conducted an organoleptic malodor examination (OLT) and the subject completed a VAS based self-assessment of malodor.

Subjects were randomly assigned to one of the test groups using a computer-generated assignment sequence. The control group was assigned to brush with a commercially available fluoride toothpaste (CIBACA Toothpaste, Colgate-Palmolive Company, India; Control Group) whereas the test group was assigned to brush with a commercially available triclosan toothpaste (Colgate Strong Teeth, Colgate-Palmolive Company, India) and a 0.075% CPC mouthwash (Colgate Plax Mouthwash, Colgate-Palmolive Company, India; Test Group) with all test articles overwrapped and provided a unique code. Test products were issued by other study personnel in an area separate from those used for clinical evaluations. At the dental clinic, subjects performed oral hygiene using their assigned treatments and completed their breakfast. Subjects returned 2 h later for an organoleptic evaluation (OLT-2 h) and recorded their malodor status using the VAS scale. Volunteers were instructed to use their assigned treatments twice daily for the next 14 days.

Posttreatment examinations were conducted after 7 days and 14 days use of assigned treatments. Subjects refrained from eating and drinking for 4 h and all oral hygiene procedures for 12 h before their posttreatment visits and were evaluated for gingival inflammation, gingival bleeding, dental plaque, and organoleptic scores (OLT). After the evaluation, volunteers performed oral hygiene with their assigned treatments and completed their breakfast. Subjects returned 2 h later for an organoleptic evaluation (OLT-2 h) and completed a malodor self-assessment using the VAS scale.
Statistical analysis
Analyses were performed separately for each evaluation, i.e., dental plaque, gingival inflammation, gingival bleeding, organoleptic scores (OLT and OLT-2 h), and VAS. Sample size calculations were based on demonstrating a statistically significant difference of 1 unit for malodor with a standard deviation of 0.5 at 80% power. Treatment groups were compared for gender and age using a Chi-square and an independent t-test, respectively. Comparisons within treatment groups from baseline were conducted using paired t-test. An analysis of covariance (ANCOVA) utilized the baselines to compare the effects of treatments at each posttreatment evaluation. All statistical tests were conducted using MINITAB (State College, PA, USA) and employed a level of significance of $\alpha = 0.05$.

RESULTS
Thirty-six adults (average age 35.36 ± 7.74; comprising 19 males and 17 females) were enrolled in the study. All subjects complied with the study protocol and completed the clinical study with no adverse events. Study subjects were stratified into two treatment groups based on age, gender, and baseline malodor scores. There were no significant differences between treatment groups for age, gender, and evaluated parameters at baseline ($P < 0.05$).

Malodor scores
Shown in Table 1 is a summary of the malodor scores at each evaluation. Malodor scores were 6.70 in both treatment groups at baseline with no significant differences ($P > 0.05$). Both treatment groups demonstrated lower scores from their baselines to the evaluation conducted 2 h after the first use of either treatment with the control and test groups registering average scores of 6.21 and 5.94, respectively. The test group demonstrated a significant reduction in malodor scores of 4.34% in comparison to the control at the 2 h evaluation ($P < 0.05$). Average malodor scores for the control and test groups were 6.49 and 6.18, respectively at the evaluation conducted in the morning of the 7th day, 12 h after use of the treatments. The test group demonstrated a significant reduction of 4.77% in comparison to the control at this evaluation ($P < 0.05$). Average malodor scores (OLT-2 h) on the 7th day for the control and test groups were 5.81 and 4.88, respectively at the evaluation conducted 2 h after use of these treatments and were significantly different ($P < 0.05$). The test group demonstrated 17.2% lower malodor scores than the control group at this evaluation conducted 2 h after product use.

Malodor scores for the control and test groups were 6.35 and 5.99 respectively during the evaluation conducted on the 14th day, 12 h after product use and were significantly different ($P < 0.05$). The test demonstrated 5.6% lower malodor scores than the control at this evaluation. Average OLT-2 h malodor scores on the 14th day for the control and test groups were 5.09 and 4.20, respectively with the test registering significantly lower scores ($P < 0.05$). The test demonstrated 17.4% lower malodor scores than the control group at this evaluation conducted 2 h after product use.

Plaque index scores
Table 2 presents a summary of the plaque index scores over the study duration. Average plaque index scores in the control and test groups were 2.89 and 2.77, respectively at the baseline evaluation and were not significantly different ($P > 0.05$). Mean plaque index scores in the control and test groups at the 7 days evaluations were 2.78 and 2.42, respectively and were significantly different ($P < 0.05$). Plaque levels were 12.94% lower for the test group than the control group at the evaluation conducted after 7 days use of treatments. Average plaque index scores in the control and test groups were 2.66 and 1.97, respectively and demonstrated a statistically significant difference of 25.93% on the evaluations conducted on day 14 ($P < 0.05$).

Gingival index scores
Average scores for the gingival index in the treatment groups is shown in Table 3. The scores for the control and the test groups were 1.44 and 1.46, respectively at the baseline evaluation and were not statistically significant ($P > 0.05$). Average gingival index scores in the control and test groups were 1.36 and 1.20, respectively after 7 days use of assigned treatments and were significantly different ($P < 0.05$). Gingival index scores were 11.76% lower for the test group than the control at the evaluation conducted after 7 days use of assigned treatments. The average gingival index scores in the control and test groups were 1.23 and 0.94, respectively at the 14 days evaluation and demonstrated a statistically significant difference of 23.57% ($P < 0.05$).

Bleeding index scores
The average bleeding index scores in the treatment groups over the study duration is shown in Table 4. Average

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Table 1: Summary of malodor (organoleptic) scores from subjects who completed the clinical study

| Treatment | Day 1 | Day 7 | Day 14 |
|-----------|------|------|-------|
|           | Prior to treatment | 2 h after treatment | Prior to treatment | 2 h after treatment | Prior to treatment | 2 h after treatment |
| Control   | 6.70±0.53 | 6.21±0.71 | 6.49±0.45 | 5.81±0.57 | 6.35±0.40 | 5.09±0.37 |
| Test (%)  | 6.70±0.52 | 5.94±0.44\(^a\) (4.34)\(^b\) | 6.18±0.50\(^c\) (4.77)\(^c\) | 4.88±0.49\(^d\) (17.2)\(^d\) | 5.99±0.45\(^e\) (5.6)\(^e\) | 4.20±0.25\(^f\) (17.4)\(^f\) |

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\(^a\)Brushing with a fluoride toothpaste, \(^b\)Brushing with a triclosan toothpaste and rinsing with a CPC mouthwash, \(^c\)Significantly different from control at corresponding evaluation ($P<0.05$), \(^d\)Percentage differences between treatments at each posttreatment evaluation. Results indicate average±SD at each evaluation. CPC=Cetylpyridinium chloride, SD=Standard deviation
bleeding index scores in the control and test groups were 44.08 and 45.43 at the baseline evaluation with no statistically significant differences (\(P > 0.05\)). At the evaluation conducted after 7 days of assigned treatments, bleeding index scores in the control and test groups were 42.15 and 40.86, respectively and were significantly different (\(P < 0.05\)). Bleeding index scores were 3.06% lower for the test group than the control group at the evaluation conducted after 7 days of treatments. The average bleeding index scores in the control and test groups were 40.69 and 34.33, respectively at the 14 days evaluation and demonstrated a statistically significant difference of 15.6% (\(P < 0.05\)).

**Visual analog scale scores**

Table 5 presents a summary of the VAS scores from subjects over the study duration. Mean VAS scores were 64.17 for subjects in the control group and 63.44 in the test group at the baseline examination and were not significantly different (\(P > 0.05\)). At the posttreatment evaluations on the 7th day, VAS scores were 61.39 in the control and 55.94 in the test and were significantly different (\(P < 0.05\)). The test group exhibited a 8.8% difference from the control at the 7 days evaluation. Average VAS score in the control and test groups were 58.89 and 46.39, respectively at the 14 days evaluation and demonstrated a statistically significant difference of 21.22% (\(P < 0.05\)).

**DISCUSSION**

This randomized clinical study was designed to examine the relative effects of a regimen on oral malodor among adult subjects. This study determined the effect of an oral hygiene regimen of a triclosan toothpaste and a CPC mouth rinse in comparison to a toothbrushing with a fluoride dentrifice over a 14 days period. The evaluated treatments were commercially available and incorporated triclosan and CPC as active agents.\(^{[32,33]}\) Previous studies have determined the effects of formulations with these agents on malodor and oral bacteria associated with halitosis.\(^{[7,20,26,32,34]}\) Notably, this study sought to evaluate the effects of oral hygiene using these formulations as a regimen.

Several features incorporated in the study design remain novel and require highlight. The short- and long-term effects of treatments on malodor in conjunction with a malodor self-assessment by the subjects represented the evaluated outcomes. Enrolled subjects were community-dwelling adults who had not participated in clinical studies based on interviews and did not include health-care professionals who are associated with better oral hygiene.\(^{[35,36]}\) Subjects underwent a washout phase prior to the start of the study to reduce the influences of oral hygiene products they had used before study enrollment. Washout phases are used in some short-term studies\(^{[38]}\) to discern treatment differences due to the short-term nature of this study. Malodor evaluations were by a group of examiners and in accordance with accepted procedures used to evaluate bad breath.\(^{[29]}\) Furthermore, there were no changes in diet\(^{[37]}\) and individuals did not report dietary restrictions\(^{[15,38]}\) that may contribute to differences between treatment groups. Over the study duration, subjects were informed of their schedules and reminded of their appointments using text messages to maintain study compliance.\(^{[39]}\) Together, the inclusion of these steps resulted in statistically similar baseline scores between the treatment groups at the baseline evaluation.

Another component of this study was a malodor assessment 2 h after breakfast representing short-term effects of the assigned treatment. While both treatments reduced malodor scores, these effects were significantly greater in the group assigned the toothpaste and the mouthwash. From a practical standpoint, a malodor evaluation 2 h after breakfast offers an additional feature of practical significance. Whereas daily activities\(^{[40]}\) and everyday stresses are potential contributors to malodor\(^{[41]}\) with additional influences on

**Table 2: Summary of plaque index scores for subjects who completed the study (average±standard deviation)**

| Evaluation | Control\(^{†}\) | Test\(^{*}\) |
|------------|----------------|-------------|
| Baseline   | 2.89±0.26      | 2.77±0.32   |
| Day 7      | 2.78±0.25      | 2.42±0.37*  |
| Day 14     | 2.66±0.26      | 1.97±0.32*  |

\(^{†}\)Brushing with a fluoride toothpaste, \(^{*}\)Brushing with a triclosan toothpaste and rinsing with a CPC mouthwash, \(^{*}\)Significantly different from control at corresponding evaluation (\(P<0.05\)). CPC=Cetylpyridinium chloride

**Table 3: Summary of gingival index scores for subjects who completed the study (average±standard deviation)**

| Evaluation | Control\(^{†}\) | Test\(^{*}\) |
|------------|----------------|-------------|
| Baseline   | 1.44±0.20      | 1.46±0.32   |
| Day 7      | 1.36±0.20      | 1.20±0.30*  |
| Day 14     | 1.23±0.19      | 0.94±0.27*  |

\(^{†}\)Brushing with a fluoride toothpaste, \(^{*}\)Brushing with a triclosan toothpaste and rinsing with a CPC mouthwash, \(^{*}\)Significantly different from control at corresponding evaluation (\(P<0.05\)). CPC=Cetylpyridinium chloride

**Table 4: Summary of bleeding index scores for subjects who completed the study (average±standard deviation)**

| Evaluation | Control\(^{†}\) | Test\(^{*}\) |
|------------|----------------|-------------|
| Baseline   | 44.0±10.58     | 45.4±11.49  |
| Day 7      | 42.1±10.42     | 40.8±10.35* |
| Day 14     | 40.6±10.19     | 34.3±9.19*  |

\(^{†}\)Brushing with a fluoride toothpaste, \(^{*}\)Brushing with a triclosan toothpaste and rinsing with a CPC mouthwash, \(^{*}\)Significantly different from control at corresponding evaluation (\(P<0.05\)). CPC=Cetylpyridinium chloride

**Table 5: Summary of visual analog scale scores for subjects who completed the study (average±standard deviation)**

| Evaluation | Control\(^{†}\) | Test\(^{*}\) |
|------------|----------------|-------------|
| Baseline   | 64.1±4.57      | 63.4±5.49   |
| Day 7      | 61.39±4.84     | 55.9±5.14*  |
| Day 14     | 58.89±4.70     | 46.3±4.98*  |

\(^{†}\)Brushing with a fluoride toothpaste, \(^{*}\)Brushing with a triclosan toothpaste and rinsing with a CPC mouthwash, \(^{*}\)Significantly different from control at corresponding evaluation (\(P<0.05\)). CPC=Cetylpyridinium chloride
oral hygiene, the ability of a regimen on malodor during the day incorporates daily lifestyle activities and represent useful observations. There are few studies on the immediate effects of mouthrinses. To the best of our knowledge, there are few evaluations on the immediate effects of oral hygiene on malodor. A study reported reductions in organoleptic scores and \( H_2S \) evaluated 15 min after use of mouthrinses and attributed to the dilution of odoriferous compounds in the mouth and temporary malodor relief offered by oral hygiene and flavoring agents of formulations. Dadamo et al. also report significantly lower malodor after 7 days use of antimicrobial rinses which was attributed to the therapeutic effects offered by the rinses evaluated.

All parameters evaluated 12 h after use of assigned treatments in both treatment groups registered progressively lower scores with the regimen demonstrating significantly higher effects than the control. Correspondingly, all evaluated clinical parameters also demonstrated significantly greater reductions in the group assigned both the toothpaste and mouthwash with the greatest improvement registered during the final visit. Self-reported malodor by subjects were similar to the observed results. These results support an overnight control of malodor by the assigned treatments, representing outcomes evaluated in previous studies. Malodor evaluations conducted 2 h after product use on day 7 and day 14 were lower than those recorded before treatment and support the results observed after the first use of treatment.

Results from this study are consistent with reductions in dental plaque and gingivitis reported previously. Rinsing with a 0.05% CPC mouthrinse for 7 days demonstrated greater reductions in whole mouth plaque, interproximal plaque, and plaque severity indices with these evaluations conducted 12 h after final use of formulation. These clinical observations are supported by the antimicrobial effects reported for a 0.05% CPC mouthrinse on a range of oral bacteria and polymicrobial samples collected from subjects. The longer term clinical effects of CPC mouthrinses have been reported recently over a 6-month period in which subjects were instructed to brush 3 times daily followed by rinsing with a 0.07% CPC or control mouthwash. These investigators report reductions in dental plaque and bleeding on probing in the CPC mouthwash group but not in other clinical parameters. Additional studies have reported the significant reductions in plaque and bleeding indices over an 8-week period among subjects assigned a 0.1% CPC mouthwash. Several studies report the effects of a triclosan toothpaste on malodor, the organisms associated with bad breath and clinical parameters of oral hygiene with these evaluations conducted 12 h after brushing with the toothpaste.

Concurrent assessments of malodor and other measures of oral hygiene, i.e., dental plaque, gingival inflammation, gingival bleeding highlights a methodological difference of this study. Most clinical studies commonly assess the effects of regimens on a few clinical parameters. Results from this study demonstrating reductions in malodor and clinical parameters may offer an additional means to motivate subjects to adopt oral hygiene regimens. In summary, results presented in this report consistently demonstrate significantly greater control of malodor and lower clinical scores amongst subjects assigned a regimen comprising toothbrushing with a triclosan toothpaste and rinsing with a CPC mouthwash. Effects on malodor were noted at short and long-term assessments including those conducted 12 h after use of assigned treatments. The results are noteworthy since participants assigned the regimen also registered lower self-rated malodor scores.

CONCLUSIONS

A regimen comprising a triclosan toothpaste and CPC mouthrinse demonstrated significant malodor reductions 2 h after the first use and progressively increasing reductions in malodor, dental plaque, gingivitis, bleeding and self-reported malodor from the 7th to 14th days than tooth brushing alone.

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Conflicts of interest

P. K. Sreenivasan and R. Subramanyam are employees of Colgate-Palmolive Company.

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