A Crossover Clinical Trial to Assess the Effectiveness of Different Oral Hygiene Regimens on the Reduction of Morning Bad Breath in Healthy Young Adults

Abstract

Context: Bad breath causes embarrassment and affects interpersonal social communication. Morning breath odor is a commonly encountered oral problem which should be rectified with effective oral hygiene measure. Aim: The aim of this study was to assess and compare the effectiveness of different oral hygiene regimens on the reduction of morning bad breath, plaque formation, and gingivitis in healthy young adults. Settings and Design: A four-step, crossover clinical trial was conducted among 40 young adults aged 18–22 years residing in one of the nongovernmental organizations in New Delhi. Materials and Methods: Study participants were divided into four groups, which underwent intervention for 7 days each. Group 1: tooth brushing; Group 2: tooth brushing and mouthwashing; Group 3: tooth brushing and tongue scraping; and Group 4: combination of all. A washout interval of 14 days was employed in between the groups. Breath scores were measured at three time intervals whereas oral health status was recorded at the beginning and the end of each interventional period. Statistical Analysis Used: Appropriate tests such as paired t-test, ANOVA test, and Pearson correlation tests were used in the study. Results: The highest reduction in mean value of breath scores (2.03 ± 0.69) was found in Group 4 followed by Group 3. Similarly, Group 4 showed the highest reduction in mean value of plaque score (0.79 ± 0.19) and gingival score (0.54 ± 0.23) followed by Group 2. Conclusion: Combination of mechanical and chemical oral hygiene measures is an effective regimen for the reduction of morning bad breath.

Keywords: Crossover studies, dental plaque, gingivitis, halitosis

Introduction

Oral malodor, also called halitosis or bad breath, is a frequently experienced oral health problem.[1] Humans emit a variety of volatile and nonvolatile molecules that are influenced by genetics, diet, stress, and disease.[2]

Halitosis is defined as an oral health condition characterized by offensive odors emanating consistently from the oral cavity attributed to extrinsic and intrinsic causes. Extrinsic causes include tobacco, alcohol, and certain foods such as onions, garlic, and certain spices.[3] Intrinsic causes of bad breath include both oral (90%) and systemic in origin (10%).[4] Oral causes may include the faulty restorations, sites of food impaction, deep carious lesions, periodontal disease, oral infections, and mucosal ulcerations.[5] Systemic diseases also contribute toward nonoral sources of breath odor.[6]

The principle components of oral malodor are volatile sulfur compounds (VSCs), especially hydrogen sulfide (H$_2$S), methyl mercaptan (CH$_3$SH), and dimethyl sulfide.[7] The basic pathophysiology involves microbial degradation of an organic substrate by predominantly anaerobic gram-negative oral microorganisms giving off such volatile gases. Any area in the oral cavity where microbial accumulation and putrefaction occur can produce VSCs.[8] The primary source of VSCs production is a coating on the dorsum surface of the tongue due to its large structure and high retention capacity with rough and papillary structure.[9,10]

Halitosis exists in several different clinical situations. Malodorous breath upon awakening after a night sleep is a common condition known as “morning bad breath.” One experiences oral malodor after any prolonged period of decreased salivary flow as in fasting or sleeping condition due to continuous bacterial proliferation that releases offending gases.[11]
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 instead of working with real halitosis patients.\cite{12}

Different treatment strategies, including mechanical interventions such as tooth brushing and tongue cleaning, and chemical methods comprised various antimicrobial compounds such as chlorhexidine (CHX), cetylpyridinium chloride are available for controlling bad breath.\cite{13-15} Still, there is plenty of disagreement among the results and their real effectiveness in the treatment of halitosis.\cite{16}

There are few studies which have compared the effects of the combination of mechanical and chemical oral hygiene procedures on the reduction of bad breath in young adults.\cite{16,17} Therefore, this study was undertaken with an aim to assess and compare the effects of different oral hygiene procedures, that is, tooth brushing, tongue cleaning and mouthwashing alone, and in combination, on the reduction of morning bad breath and oral health status in young adults.

Materials and Methods

Study population and study setting

The study was conducted among 40 young adults, 20 males and 20 females (aged 18–22 years) residing in one of the nongovernmental organizations (NGOs) in New Delhi from April 2016 to June 2016. The sample size was estimated using an expected mean breath level difference of 1, a within-participant variance around the mean breath level difference of 0.5, a 95% confidence interval with a power of 80%. The results indicated a required sample size of 32 participants for a crossover design. Therefore, after consideration of dropout rate, a sample size of 40 participants was chosen in the study.

All participants underwent an oral examination before the start of the study. Participants with good oral hygiene and willing to participate were included in the study. The exclusion criteria were as follows: participants with dental caries, medical disorders, undergoing any antibiotic therapy in the previous 6 months, acute sinusitis, using tobacco in any form and those who, on presudy clinical screening, presented a probing pocket depth ≥3 mm with bleeding on probing and attachment loss ≥2 mm were excluded from the study.

Ethical considerations

Ethical clearance was obtained from the Ethical Review Board of the institute. An official permission to conduct the study was obtained from the NGO authority, and informed consent from the participants was taken.

Study design and procedures

The present study was designed as a prospective crossover, double-blind study with an examination period of 3 months. Consolidated Statement of Reporting Trials guidelines were used in reporting of the present study. Study participants were divided into four crossover groups, performed in four experimental periods of 7 days. In each period, every volunteer performed the following oral hygiene measures two times a day:

- **Group 1**: tooth brushing only using a toothbrush (Colgate) with fluoridated toothpaste (Colgate Total – sodium fluoride –1000 ppm) Colgate-Palmolive Co.
- **Group 2**: tooth brushing and use of mouthwash (CHX mouth rinse [Rexidine 0.2% w/v CHX gluconate solution] for 30 S (Colgate-Palmolive Co.)
- **Group 3**: tooth brushing and tongue scraping (Ajanta Tongue Cleaner-Bombay Brush Co., India); and
- **Group 4**: tooth brushing, tongue scraping, and use of mouthwash.

All the participants underwent pre-experimental motivation sessions in which standard oral hygiene instructions were given. Participants were instructed to abstain from foods such as garlic, onion, and certain spices producing a strong odor. They were asked to not to use any oral hygiene aid for 20 h before the commencement of the experiments. On the day of baseline and postinterventional assessment of every experimental trial, participants were instructed to be in complete fasting situation without performing any type of oral hygiene, and they should not use any type of cosmetics that liberates odors/perfumes.\cite{19} According to crossover design, the participants received one of an assigned experimental group that they were allowed to use during the following 7-day period.

Assessment plan (7-day trial for each experimental group)

The Baseline or Preintervention Assessment (1st day) was carried out in 2 parts

Part 1

A structured proforma was developed consisting of general information and oral hygiene practices of study participants. It also included the format for recording breath odor.

The levels of VSCs were measured by asking the volunteers to expel air through their mouths into a hand-held sulfide monitor, Breath AlertTM (Tanita Corporation, Japan), according to the manufacturer’s instructions as performed previously in a study done by Godha et al.\cite{19} and Oliveira-Neto et al.\cite{19}

Breath Alert is an innovative palm-size monitor, quick, easy to operate, and relatively cheap device that detects and measures the presence of annoying or embarrassing breath odors. It detects and measures the presence of VSCs responsible for bad breath and displays the degree of odor in just 9 s in one of the six levels.

- Level 0-No odor
- Level 1-Slight odor
• Level 2-Moderate odor
• Level 3-Heavy odor
• Level 4-Strong odor
• Level 5-Intense odor.
The breath of each participant was examined at the following three time intervals:
• \( T_{00} \) = Before using the assigned oral hygiene measure
• \( T_{0} \) = Immediately after using the assigned oral hygiene measure
• \( T_{1} \) = After 2 h using the assigned oral hygiene measure.

Part 2
A clinical examination was undertaken for all the participants by one examiner. Plaque index (PI) (Silness P and Loe H) and gingival index (GI) (Loe and Silness) were recorded to assess their oral health status.[29]

Interventional period (2nd day to 6th day)
The interventional or experimental period was for 1 week duration. A coordinator organized a hygiene kit containing toothbrushes, tongue scrapers, and mouthwashes and was liable for giving the kits to the participants. The study participants used only the assigned experimental oral hygiene measure during experimental.

Postinterventional assessment (7th day)
Breath scores were recorded again for all the participants at the same three time intervals as used in baseline assessment. Clinical assessment of oral hygiene and gingival health status was also reassessed on the 7th day.

Compliance was assessed by calling the participants every day during each experimental period by an investigator.

Washout period
Each experimental group underwent 1 week assessment plan comprised baseline assessment, interventional period and postinterventional assessment followed by 14 days washout interval. In this period, the study participants maintained the standard oral hygiene that was practiced by them throughout pre-experimental motivation sessions.

Training and calibration
Before the commencement of the study, the examiner was trained and calibrated to ensure the consistent clinical judgment. All the measurements were performed by the same examiner, who was blinded to the treatment need. The intraexaminer reliability for recording the breath odor, plaque and gingival scores using Cohen’s Kappa statistics (chance-corrected proportional agreement) was found to be 0.9 showing total agreement.

Statistical analysis
The collected data were entered into Microsoft Excel 2007 and subjected to statistical analysis using SPSS version 20.0 (IBM Statistics Inc., Chicago, IL, USA). The statistical tests used were paired t-test, ANOVA with post hoc Tukey’s test, and Pearson’s correlation coefficient test. The difference was considered to be of statistical significance if the \( P \leq 0.05 \).

Results
A total of 40 participants, equally distributed males and females, participated in the study. The mean age of participants was found to be 20.42 ± 1.32. The proper method of brushing (modified bass technique) and use of dentifrice was reported by 45% and 100% of participants, respectively, whereas the frequency of changing toothbrush (≥3 months) and use of any other oral hygiene aids was reported by only 15% of participants.

Table 1 shows intergroup comparisons of mean values of breath scores before intervention (day 1). Before the intervention no significant results (\( P > 0.05 \)) for breath scores were observed among the all four groups at each time interval from \( T_{00} \) to \( T_{1} \), whereas highly significant results (\( P < 0.001 \)) for breath scores were observed on intergroup comparison after intervention (day 7) from baseline (\( T_{00} \)) to each time interval (\( T_{0} \) and \( T_{1} \)) in which post hoc analysis results showed significant difference of mean values of breath scores of Group 4 with all other groups (1, 2, and 3) at each time interval, that is, \( T_{00} \), \( T_{0} \), and \( T_{1} \) [Table 2].

Table 3 shows intragroup comparison of mean values of breath scores and mean reduction in scores before and after intervention at a different time interval. The highest reduction in mean values of breath scores was found in Group 4 followed by Group 3 and then in Group 2 whereas the highest mean values of breath scores before and after the intervention were observed in Group 1.

Tables 4 and 5 present the reduction of mean PI and GI scores within the individual group before and after the intervention. Both index mean values show highly significant reduction among all the four groups. The highest reduction in mean plaque value (0.79 ± 0.19) and mean gingival value (0.54 ± 0.23) was found for Group 4 which shows that Group 4 was significantly (\( P < 0.001 \)) more effective than Groups 1, 2, and 3 in improving oral hygiene and gingival health status.

| Table 1: Intergroup comparison of mean value of breath scores at different time intervals before intervention (day 1) (mean±standard deviation; \( n=40 \)) |
|---|---|---|
| Groups | \( T_{00} \) | \( T_{0} \) | \( T_{1} \) |
| Group 1 | 3.15±0.73 | 2.60±0.44 | 2.90±0.37 |
| Group 2 | 3.08±0.57 | 2.55±0.48 | 2.85±0.36 |
| Group 3 | 2.98±0.27 | 2.43±0.59 | 2.78±0.42 |
| Group 4 | 2.88±0.33 | 2.28±0.64 | 2.68±0.52 |
| \( P \) | 0.09 | 0.18 | 0.10 |

ANOVA test, \( T_{00} \)=Before using oral hygiene measure, \( T_{0} \)=Immediately after using oral hygiene measure, \( T_{1} \)=After 2 h using oral hygiene measure.
Table 6 shows an intergroup comparison of mean plaque score and mean gingival score before and after the intervention. The results demonstrated that no significant difference was recorded in the plaque scores ($P = 0.07$) and gingival scores ($P = 0.29$) on day 1, but highly significant reduction ($P < 0.001$) was observed in their scores on day 7 on comparing all groups.

Furthermore, a significant positive correlation was observed between plaque scores and gingival scores before intervention (day 1) ($r = 0.433; P < 0.001$) and after intervention (day 7) ($r = 0.747; P < 0.001$) [Tables 7 and 8].

**Discussion**

Halitosis frequently causes embarrassment and may affect interpersonal social communication irrespective of any age group. For an oral malodor study, selection of participants is crucial because many different factors affect oral malodor. To exclude the influence of oral and systemic diseases on oral malodor, healthy young adults were recruited for this study. Further, all participants, as full-time NGO residents, lived in the same place and had a similar lifestyle, including the content, time, and frequency of meals. Thus, the problem of different food and eating habits, which could affect oral malodor, was avoided in the present study.

In this study, postintervention intragroup and intergroup analysis showed Group 4, combination of all undertaken oral hygiene regimen presented the highest reduction in mean values of breath scores from baseline to all time intervals as well as found to be highly significant from other Groups 1, 2, and 3 comprised using toothbrush, mouthwash, and tongue cleaning in alone, respectively. This result was found to be in concordance with the study done by Aung et al. and Oliveira-Neto et al., who also demonstrated that a combination of mechanical and chemical measures has the strongest effect of VSCs and thereby helps in reducing bad breath within the 1st h of using both mechanical and chemical measures and lasted up to 3 h. The results again found to be similar with the study done by Patil et al. on children all three oral hygiene measures had an additive effect as it acted on all the etiologic niches in the oral cavity from tooth surface to tongue to microbial load.

This study also demonstrated that after Group 4, the participants who performed tongue cleaning in Group 3 showed significantly lower mean breath values followed by Group 2 and Group 1, who used mouthwash and only tooth brushing, respectively, as an oral hygiene measure. It may be due to the fact that dorsal and posterior surface of tongue exhibited a very irregular surface topography and aggregated anaerobic microbiota of the tongue biofilm is one of the main reasons for the release of sulfur compounds and cleaning the tongue significantly reduced concentrations of VSCs, such as CH$_3$SH; and to a lesser extent, hydrogen sulfide. Thus, in isolation, tongue cleaning was found to be superior than tooth brushing and mouth rinsing in our study similarly to the studies done by Faveri et al. and Patil et al. and in contrast to studies done by van Steenbergh et al. and Blom et al. (2012), who reported that chemical action of a mouthwash reduces bad breath by reaching those areas that are difficult to be accessed by tongue cleaning procedure.

Apart from all groups, Group 1 comprised only tooth brushing showed no significant difference in the mean value of breath score, this could be attributed to the fact tooth brushing may disrupt the formation of plaque biofilm and

**Table 2: Intergroup comparison of mean value of breath scores at different time intervals after intervention (day 7) (mean±standard deviation; n=40)**

| Groups | $T_0$ | $T_1$ | $T_4$ |
|--------|-------|-------|-------|
| Group 1 | 3.00±0.71 | 2.48±0.31 | 2.78±0.46 |
| Group 2 | 2.70±0.46 | 1.78±0.42 | 1.83±0.47 |
| Group 3 | 2.50±0.50 | 1.40±0.49 | 1.50±0.50 |
| Group 4 | 2.30±0.56 | 0.70±0.46 | 0.65±0.40 |

ANOVA and post hoc Tukey’s test; *P≤0.05. $T_0$=Before using oral hygiene measure, $T_1$=Immediately after using oral hygiene measure, $T_4$=After 2 h using oral hygiene measure

**Table 3: Intragroup comparison of mean value of breath scores on different time intervals before intervention (day 1) and after intervention (day 7) (mean±standard deviation; n=40)**

| Groups | $T_0$ | $T_1$ | Difference | $T_0$ | $T_1$ | Difference | $T_0$ | $T_1$ | Difference |
|--------|-------|-------|------------|-------|-------|------------|-------|-------|------------|
| Group 1 | 3.15±0.73 | 3.00±0.71 | 0.15±0.86 | 0.27 | 2.60±0.44 | 2.48±0.31 | 0.12±0.48 | 0.37 | 2.90±0.37 | 2.78±0.46 | 0.12±0.52 | 0.28 |
| Group 2 | 3.08±0.57 | 2.70±0.46 | 0.38±0.34 | 0.003* | 2.55±0.48 | 1.78±0.42 | 0.77±0.52 | <0.001* | 2.85±0.36 | 1.83±0.47 | 1.02±0.53 | <0.001* |
| Group 3 | 2.98±0.27 | 2.50±0.50 | 0.48±0.50 | 0.001* | 2.43±0.59 | 1.40±0.49 | 1.03±0.56 | <0.001* | 2.78±0.42 | 1.50±0.50 | 1.28±0.59 | <0.001* |
| Group 4 | 2.88±0.33 | 2.30±0.56 | 0.58±0.59 | <0.001* | 2.28±0.64 | 0.70±0.46 | 1.58±0.81 | <0.001* | 2.68±0.52 | 0.65±0.40 | 2.03±0.69 | <0.001* |

Paired t-test; *P<0.05. $P=$Before intervention, $P =$After intervention, $T_0$=Before using oral hygiene measure, $T_1$=Immediately after using oral hygiene measure, $T_2$=After 2 h using oral hygiene measure
Table 4: Intragroup comparison of mean plaque scores before intervention (day 1) and after intervention (day 7) (mean±standard deviation; n=40)

| Groups | Plaque score day 1 | Plaque score day 7 | Mean difference | P      |
|--------|-------------------|-------------------|----------------|--------|
| Group 1| 2.07±0.18         | 1.98±0.22         | 0.09±0.07      | <0.001*|
| Group 2| 2.03±0.14         | 1.41±0.20         | 0.62±0.17      | <0.001*|
| Group 3| 1.97±0.09         | 1.79±0.11         | 0.18±0.11      | <0.001*|
| Group 4| 1.91±0.12         | 1.12±0.14         | 0.79±0.19      | <0.001*|

Paired t-test; *P<0.05

Table 5: Intragroup comparison of mean gingival scores before intervention (day 1) and after intervention (day 7) (mean±standard deviation; n=40)

| Groups | Gingival score day 1 | Gingival score day 7 | Mean difference | P      |
|--------|----------------------|----------------------|----------------|--------|
| Group 1| 1.57±0.15            | 1.50±0.17            | 0.07±0.17      | 0.02*  |
| Group 2| 1.54±0.16            | 1.08±0.16            | 0.46±0.19      | <0.001*|
| Group 3| 1.52±0.16            | 1.33±0.19            | 0.19±0.22      | <0.001*|
| Group 4| 1.50±0.15            | 0.96±0.15            | 0.54±0.23      | <0.001*|

Paired t-test; *P<0.05

Table 6: Intergroup comparison of mean plaque scores and mean gingival scores before Intervention (day 1) and after intervention (day 7) (mean±standard deviation; n=40)

| Groups | Plaque score day 1 | Plaque score day 7 | Gingival score day 1 | Gingival score day 7 | P       | ANOVA test and post hoc: Tukey’s test; *P<0.05 |
|--------|-------------------|-------------------|---------------------|---------------------|---------|---------------------------------------------|
| Group 1| 2.07±0.18         | 1.98±0.22         | 1.57±0.15           | 1.50±0.17           | 0.07    | 0.29                                       |
| Group 2| 2.03±0.14         | 1.41±0.20         | 1.54±0.16           | 1.08±0.16           | <0.001  | 0.433**                                    |
| Group 3| 1.97±0.09         | 1.79±0.11         | 1.52±0.16           | 1.33±0.19           | <0.001  | **Correlation is significant at the 0.01 level (two-tailed). P = After intervention |
| Group 4| 1.91±0.12         | 1.12±0.14         | 1.50±0.15           | 0.96±0.15           | <0.001  | Plaque score _P_1_, Gingival score _P_1_ |

**Correlation is significant at the 0.01 level (two-tailed). _P_1_ = Before intervention

Table 7: Correlation between mean plaque and mean gingival index scores before intervention (day 1)

| Plaque _P_1_ | Gingival _P_1_ |
|--------------|----------------|
| Pearson correlation | 1 | 0.433** |
| Significant (two-tailed) | 0.000 |
| _n_ | 160 | 160 |

**Correlation is significant at the 0.01 level (two-tailed). _P_1_ = Before intervention

oral hygiene measures to be highly effective in improving oral health status as compared to other groups. The present study also demonstrated that Group 2 comprised chemical oral hygiene measure (CHX mouthwash) to be highly effective in reducing mean values of plaque and gingival scores from baseline to each time interval. Similar findings were reported by various studies contributing to the fact that CHX has properties of antiplaque substantivity as well as anti-inflammatory suggesting chemical oral hygiene aid to be superior than mechanical aid in improving oral hygiene and gingival health status of an individual.

The study also presented a positive correlation between the plaque scores and gingival scores before intervention (day 1) and after the intervention (day 7), suggesting an association between plaque and gingival status interpreting the fact that the individuals with a higher plaque score at baseline also had more marked gingival inflammation. This finding was found to be similar with the study done by Franco Neto et al.[10]

Limitations of the study

- VSC monitors (i.e., Halimeter) were not used for the present study due to the financial and time constraints
- The small number of the study participants in each group limits the generalization of the results; therefore, further studies with larger sample size are warranted.

Conclusion

The results of the present study suggest that tongue scraping (mechanical method) is superior as compared to mouthwashing (chemical method) in the reduction of bad breath. However, combining both mechanical and chemical oral hygiene regimens yielded much more superior results, and therefore, considered as the most effective way in reducing morning bad breath in healthy young adults and should be incorporated in daily oral hygiene practices. This study, on the other hand, also demonstrated that either individual use of mouthwash (Chemical method) or combination of chemical and mechanical oral hygiene regimens are an effective method in the prevention of plaque and gingivitis.
Recommendation

- The formulation of appropriate preventive strategies including screening for bad breath contributes as a step toward highlighting the hidden problem of morning bad breath in the society
- Promote awareness and training to utilize a combination of mechanical and chemical oral hygiene regimen for prevention of this often neglected problem of bad breath.

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Conflicts of interest

There are no conflicts of interest.

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