

IMPACT OF DIFFERENT ORAL HYGIENE AIDS FOR THE REDUCTION OF MORNING BAD BREATH AMONG DENTAL STUDENTS: A CROSSOVER CLINICAL TRIAL

SHAIJAL GODHA, PRALHAD L DASAR, SANDESH N,
PRASHANT MISHRA, SANDEEP KUMAR, SWATI BALSARAF,
UPENDRA SINGH BHADAURIA, SHALEEN VYAS

Department of Public Health Dentistry, Sri Aurobindo College of Dentistry,
Indore, India

Abstract

Background and aims. To assess and compare the effects of different oral hygiene procedures on the reduction of morning bad breath, plaque and gingival status in healthy subjects.

Methods. A four step cross-over trial was performed on 32 study subjects. They were allocated into four groups: Group I: tooth brushing; Group II: tooth brushing and tongue scraping; Group III: tooth brushing and mouth washing; and Group IV: tooth brushing, tongue scraping and use of mouthwash. A washout interval of 7 days was employed. At the beginning and at the end of all intervention periods, breath score was measured by hand held sulfide monitor (Breath Alert) at four time intervals. The Plaque and Gingival status was evaluated using Plaque and Gingival Index.

Results. The highest reduction in mean breath score (2.12 ± 0.65), plaque score (0.75 ± 0.47) and gingival score (0.67 ± 0.41) were found in the Group IV followed by Group II and Group III. A significant positive correlation was observed between plaque scores and gingival scores before intervention ($r=0.443$; p value < 0.001) and after intervention ($r=0.846$; p value < 0.001).

Conclusion. The study findings suggest that mechanical aids in conjunction with chemical regimens are considered as the most effective method for reducing the morning bad breath in healthy subjects and should be incorporated in daily oral hygiene practices.

Keywords: bad breath, dental plaque, gingivitis, mouthwash, sulfide monitor, tongue scraping

Introduction

Halitosis, oral malodor, feter oris, or bad breath are the common terms which are used to depict obnoxious breath emitted from a person's mouth, in spite of whether the malodorous substances in the breath are derived from oral or non-oral sources, and it can have major detrimental impact on normal social interactions [1,2]. The cause of halitosis have been attributed to a number of factors, i.e source could be from oral causes i.e. tongue coating, peri-implant disease, periodontal disease, deep carious lesions,

exposed necrotic tooth pulps, pericoronitis, mucosal ulcerations, healing wounds, impacted food or debris, imperfect dental restorations, unclean dentures and factors causing decreased salivary flow rate [3-5]. Non-oral sources of breath odor are generally related to systemic problems and/or medications including conditions such as diabetes, liver and kidney disorders, and pulmonary disease. Some medications, especially those that reduce salivary flow such as antidepressants, antipsychotics, narcotics, decongestants, antihistamines, and antihypertensive drugs contribute towards non-oral sources of breath odor [6].

The main contributor to halitosis is known to be the volatile sulfur compounds (VSC): hydrogen sulfide, methyl

Manuscript received: 01.01.2016

Received in revised form: 21.01.2016

Accepted: 03.02.2016

Address for correspondence: shaijal1901@gmail.com

mercaptan and dimethyl sulfide). VSCs are produced by the degradation of food debris, desquamated cells, saliva proteins, dental plaque and microbial putrefaction by a variety of oral anaerobic organisms which include *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola*, *Fusobacterium nucleatum*, *Tannerella forsythensis*, *Porphyromonas endodontalis* and *Eubacterium* species [2,6]. These organisms are recoverable in huge numbers from the periodontal pockets, gingival crevicular fluid, tongue, predominantly where coating of tongue is prominent [7]. The dorsum of the tongue provides an appropriate environment for the development of these microorganisms, as deep crypts of tongue provide the favourable redox potentials and offer a best milieu for the initiation of VSCs and other volatile compounds that contribute to bad breath [8,9].

There are various side effects associated with H₂S, such as localized halitosis, periodontal tissue disorders and discoloration and corrosion of metal restorations. A study by Yamaguchi T et al. [10] suggests that H₂S can cause tooth wear by triggering changes to enamel surface structure and crystal morphology, and also bond strength of enamel and composite restorations could be affected by H₂S.

Halitosis exists in various diverse clinical situations. Malodorous breath on arising after a night's sleep is a condition which is commonly known as "morning bad breath". This appears to result from the excessive quantities of volatile gases containing sulphur of bacterial origin and also due to reduced salivation during sleep promotes abundance of bacterial proliferation that release obnoxious gases [11].

Currently there is no accepted protocol intended for the reduction of bad breath. Patients mostly mask bad breath through habitual brushing or with a wide range of methods like mints, chewing gums, liquid drops, and the use of mouthwashes. But most of these simply provide a momentary smell that is able to mask the unfavorable malodor [12].

Both mechanical and chemical methods are available for controlling bad breath. For example, oral malodor can be reduced by diminishing the amount of contributory bacteria present in the oral cavity or by converting VSCs to non-volatile products [13]. Mechanical tooth cleaning aids, such as tooth brushing or interdental flossing, is considered as an important routine oral hygiene practice, but many studies found out that individual tooth brushing will not significantly decrease oral malodorous breath. While, on the other hand, tongue cleaning and mouth rinsing can reduce level of volatile sulphur compounds [14].

Reduction of the contributing oral microorganisms could also be achieved by improving oral hygiene in addition to tongue cleaning. This can be achieved by scraping the tongue dorsum with the help of tongue scrapers to remove trapped bacteria and food from the filiform papillae [8].

To target the anaerobic micro-organisms and thus

reduce bad breath, various topical anti-bacterial agents have been used. Anti-microbial compounds such as chlorhexidine (CHX), cetylpyridinium chloride (CPC), triclosan, chlorine dioxide, essential oils, zinc salts, hydrogen peroxide and sodium bicarbonate have been used, either individually or in different combinations, or collectively with the mechanical aids, for their effectiveness to reduce bad breath [15].

There are various therapies that reduce morning bad breath but they do not imply efficacy in the treatment of halitosis; yet, morning bad breath has often been used as a model to test the efficacy of various therapies on oral malodor as a substitute for working with true halitosis patients [16]. Various studies of tongue cleaning procedures and mouth rinse applications have been conducted. Though there are few studies which have compared the effect of combination of mechanical and chemical oral hygiene procedures on the reduction of bad breath in healthy subjects [17]. Therefore, this study aim at assessing and comparing the effects of different oral hygiene procedures, i.e., tooth brushing, tongue cleaning and mouth washing alone and in combination, on the reduction of morning bad breath, plaque and gingival status in healthy subjects.

Methods

Study subjects

The study was conducted in the Department of Public Health Dentistry at Sri Aurobindo College of Dentistry, from May 2015 to July 2015. The permission was obtained from the Dean of Sri Aurobindo College of Dentistry to carry out the study among dental students. Ethical approval was obtained from the Institutional Ethical Committee of Sri Aurobindo College of Dentistry. All participants volunteered to participate in the study and were given oral and written information about the purpose of the study. All the participants gave informed consent.

A pilot study was conducted and based upon the findings of the pilot study, a statistical software (PASS13) was used to determine the final sample size. Under the assumption that $\alpha=0.05$ and power at 80%, size of 24 in the experimental groups was calculated. Therefore, under consideration of dropout rate a sample size of 32 per group was designed.

Thirty two dental students, 16 females and 16 males (aged 20–28 years) gave their consent to participate in the present study. All subjects underwent an oral examination prior to the start of study, which included whole mouth periodontal probing and caries assessment.

The subjects with good oral hygiene and the subjects willing to participate and provide the informed consent prior to start of the study were included in the study.

The subjects with dental caries, medical disorders, undergoing antibiotic therapy, smokers, pregnant women and those subjects on pre-study screening (screening of individuals before the participants were allocated into the groups), presented a probing pocket depth >4mm were

excluded from the study.

Study Design and Procedures

The present study was designed as a prospective cross over, single-blind study with an examination period of 8 weeks. CONSORT (Consolidated Statement of Reporting Trials) guidelines were used in reporting of the present study.

Study subjects were divided into 4 groups, which underwent four trial periods of 7 days. In each period, every volunteer performed the following oral hygiene procedures:

Group I: tooth brushing only;

Group II: tooth brushing and tongue scraping;

Group III: tooth brushing and use of mouthwash; and

Group IV: tooth brushing, tongue scraping and use of mouthwash.

A coordinator organized a hygiene kit containing toothbrushes, tongue scrapers and mouthwashes and was liable for giving the kits to the volunteers. Investigator was carefully trained. The investigator was responsible for evaluating and recording the breath scores, plaque and gingival scores. The investigator was blinded to treatment assignment for the study duration and was trained in plaque and gingival measurement. The several measurements of plaque and gingival levels concealed an intra-examiner kappa value of 0.80. This cross over trial comprised of two phases that is pre-experimental and experimental phase.

Pre-experimental phase

One week prior the commencement of the study, study subjects underwent motivation sessions in which standard oral hygiene instructions were given. They were not permitted to use any type of mouthwash, perform interdental flossing, and tongue scraping from the week before the first experimental period.

Baseline assessment

Following the pre-experimental phase the study subjects were scheduled in the morning for baseline evaluation of breath scores, in agreement with the following criteria: the night before the appointment, volunteers were asked to avoid the consumption of foods which produce a strong odor like ginger, garlic, onions, eggs, and cabbages as well as intake of alcoholic beverages. They were instructed to refrain from oral hygiene measures for 20 hours before commencement of the experiments. In the morning, subjects should 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.

According to cross-over design, the subjects received one of assigned experimental group that they were allowed to use during the following 7 days period.

Experimental phase

Each experimental phase was for one week duration

followed by 7 days wash out interval. In between the experimental periods the study subjects maintained the standard oral hygiene that was practiced by them throughout pre-experimental phase. Compliance was assessed by calling the subjects after 7th day of each experimental phase by an investigator.

Evaluation Criteria

Breath evaluations

At the start and at the end of all experimental periods, breath scores were measured by asking the volunteers to expel air through their mouths into a hand-held sulfide monitor, Breath Alert™ (Tanita Corporation®-Japan), according to the manufacturer's instructions as performed previously in a study done by Pedrazzi et al and Jeronimo M et al. [18,19].

The breath of each volunteer was examined at the following time intervals:

- T_{00} = before using the product (baseline data)
- T_0 = immediately after using the product.
- T_1 = after 1 hour
- T_3 = after 3 hours on day 1 and day 7

Oral health examination

Following the breath evaluation, the Plaque and Gingival status were evaluated with the use of Plaque Index by Silness.P and Loe.H and Gingival Index by Loe and Silness respectively.

Clinical Assessment was done on 1st day before the intervention used and on day 7th, after one week of each experimental period, Plaque Index and Gingival Index were reassessed.

Measurement tools

A structured proforma was developed consisting of socio-demographic details and oral hygiene practices of study participants. It also included the formats for recording breath scores day 1 and day 7 at each time interval (T_{00} , T_0 , T_1 , T_3), Plaque index 1967 and Gingival index 1963 to record the plaque status and gingival status respectively. Hand held sulfide monitor (Breath Alert) was used to measure breath scores at four time intervals. The mouth mirror, a light source, dental explorer, William's periodontal probe and air drying of the teeth and gingiva were used for the scoring of Plaque and Gingival index. The trial was performed under the strict sterilization protocol.

Statistical analysis

Statistical analyses was performed using the Statistical Package for Social Sciences for Windows (SPSS, IBM Version 20.0). According to the cross over design, the mean breath scores, plaque and gingival scores were compared within the groups using Paired sample t test and between the groups applying the ANOVA (Analysis of variance) with Post hoc Tukey's test. The relationship between the plaque scores and gingival scores day 1 and 7th day was obtained by Pearson's correlation. Level of significance was set at $p \leq 0.05$.

Results

All the selected subjects (n=32) completed the study so response rate obtained was 100%. The distribution of study participants with respect to the age, gender and education and there oral hygiene practices is shown in Table I. Mean Age of study subjects was found to be 23.65 ± 1.94 and there was an equal distribution of females and males among the study subjects.

Table II shows within group comparison of mean values of breath scores and difference in scores before and after for each intervention group. The highest mean values of breath scores were found in groups 1 and 3 in which tongue scraping was excluded (Fig 1). While the highest reduction in mean values of breath scores was found in groups 4 and 2 in which tongue scraping was performed. Group 1 did not differ significantly from baseline i.e. (T_{00}) to each time interval i.e. (T_0 , T_1 , T_3) before and after the intervention used.

Table III shows group comparisons of mean values of breath scores before intervention (day 1) and after intervention (day 7). Prior to the intervention, there were no significant differences ($p > 0.05$) for breath scores between the four groups from baseline i.e. (T_{00}) to each time interval i.e. (T_0 , T_1 , T_3). Additionally, considering the washout periods, it was noticed that no carryover effect occurred between the interventions. The comparison of the

interventions after 7 days revealed that breath scores at each time interval were most inhibited in Group 4 ($p < 0.001$) followed by Group 2 ($p < 0.001$) followed by Group 3 and Group 1. Post Hoc analysis showed significant difference between Group 4 and Group 1, Group 2 and Group 3 at each time interval i.e. T_{00} , T_0 , T_1 , T_3 .

The comparison of mean plaque and gingival scores on day 1 and day 7 within and between the groups is presented in Table IV and V respectively. The highest reduction in mean plaque value (0.75 ± 0.47) and mean gingival value (0.67 ± 0.41) was found for Group 4 which shows that Group 4 was significantly ($p < 0.001$) more effective than Groups 1, 2 and 3 in reducing plaque, while scores recorded on day 7 i.e. after the intervention used and Post Hoc analysis also showed a significant difference between Group 4 and Groups 1, 2 and 3. The results also demonstrated that no significant difference was observed in the plaque scores (p value=0.146) and gingival scores (p value=0.850) recorded on day 1 between the groups. A significant reduction ($p < 0.001$) was observed in the plaque and gingival scores recorded on day 7 between the groups.

Furthermore, a significant positive correlation was observed between plaque scores and gingival scores before intervention (day 1) ($r = 0.443$; p value < 0.001) and after intervention (day 7) ($r = 0.846$; p value < 0.001).

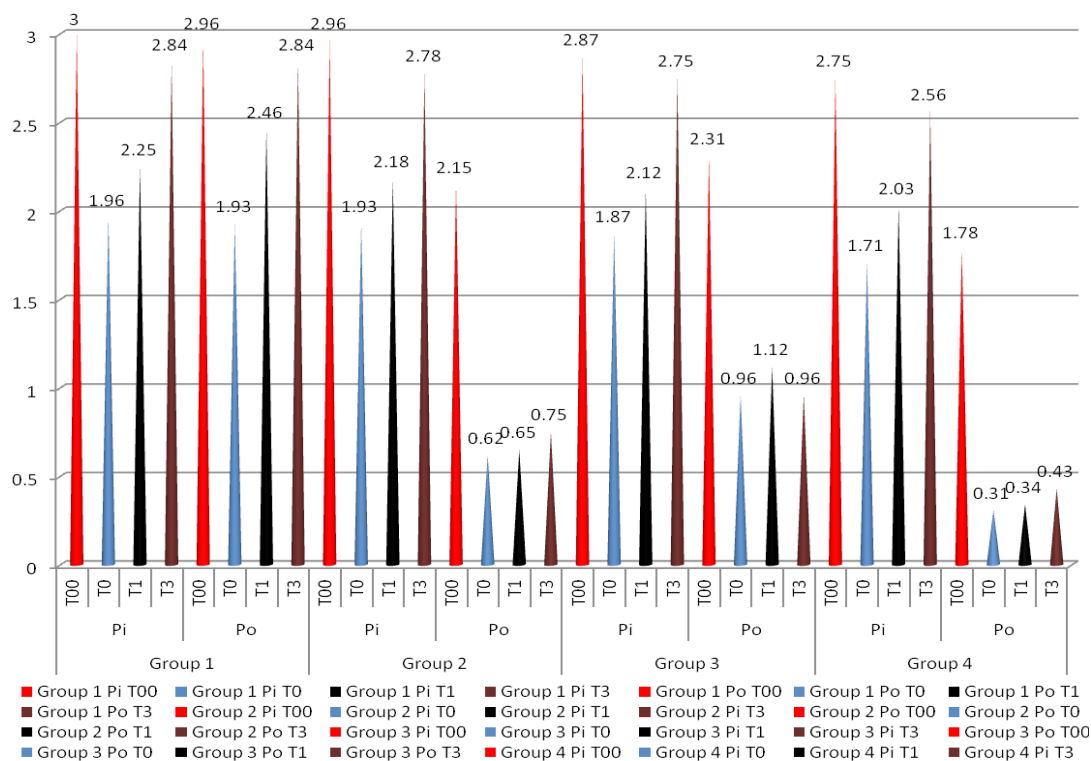


Figure 1. Mean Breath scores before the intervention (Pi) and after the intervention (Po) of Group 1, 2, 3, 4 at four time intervals.

Table I. Demographic characteristics and Oral hygiene practices of participants (N=32).

FACTORS	N (%)
Age	
Mean Age	23.65±1.94
Gender	
Male	16 (50%)
Female	16 (50%)
Education	
B.D.S II nd Year	9 (28.1%)
Interns	12 (37.5%)
Post-Graduates	11 (34.4%)
Tooth Brushing Method	
Horizontal-scrub	5 (15.6%)
Horizontal+ Circular	18 (56.2%)
Horizontal+ Vertical	3 (9.4%)
Other Technique	6 (18.8%)
Dentifrice	
Yes	32 (100%)
No	0 (0%)
Other Oral Hygiene Practices Used	
Yes	5 (15.6%)
No	27 (84.4%)

Table III. Inter group comparison of mean values of breath scores before intervention (day 1) and after intervention (day 7) (mean±SD; n=32).

Groups	Pre-Intervention (day 1)				Post-Intervention (day 7)			
	T ₀₀	T ₀	T ₁	T ₃	T ₀₀	T ₀	T ₁	T ₃
Group 1	3.00±0.43	1.96±0.47	2.25±0.56	2.84±0.57	2.96±0.47	1.93±0.50	2.46±0.62	2.84±0.57
Group 2	2.96±0.40	1.93±0.43	2.18±0.59	2.78±0.55	2.15±0.44	0.62±0.49	0.65±0.48	0.75±0.43
Group 3	2.87±0.42	1.87±0.42	2.12±0.60	2.75±0.50	2.31±0.47	0.96±0.40	1.12±0.42	1.96±0.30
Group 4	2.75±0.50	1.71±0.52	2.03±0.53	2.56±0.56	1.78±0.42	0.31±0.47	0.34±0.48	0.43±0.50
P-value	.111	.145	.477	.206	<0.001*	<0.001*	<0.001*	<0.001*

ANOVA and Post Hoc Tukey's test; *p value ≤0.05

Table II. Intra group comparison of mean values of breath scores before intervention (day 1) and after intervention (day 7) and difference in changes (Δ scores) before and after for each intervention (mean \pm SD; n=32).

Groups	T_{00}				T_0				T_1				T_3			
	P_i	P_o	$P_i - P_o$	P-value	P_i	P_o	$P_i - P_o$	P-value	P_i	P_o	$P_i - P_o$	P-value	P_i	P_o	$P_i - P_o$	P-value
Group 1	3.00 \pm 0.43	2.96 \pm 0.47	0.03 \pm 0.17	0.325	1.96 \pm 0.47	1.93 \pm 0.50	0.03 \pm 0.07	0.662	2.25 \pm 0.56	2.46 \pm 0.62	-0.21 \pm 0.79	0.129	2.84 \pm 0.57	2.84 \pm 0.57	0.00 \pm 0.35	1.000
Group 2	2.96 \pm 0.40	2.15 \pm 0.44	0.81 \pm 0.39	<0.001*	1.93 \pm 0.43	0.62 \pm 0.49	1.31 \pm 0.47	<0.001*	2.18 \pm 0.59	0.65 \pm 0.48	1.53 \pm 0.56	<0.001*	2.78 \pm 0.55	0.75 \pm 0.43	2.03 \pm 0.53	<0.001*
Group 3	2.87 \pm 0.42	2.31 \pm 0.47	0.56 \pm 0.50	<0.001*	1.87 \pm 0.42	0.96 \pm 0.40	0.90 \pm 0.39	<0.001*	2.12 \pm 0.60	1.12 \pm 0.42	1.00 \pm 0.56	<0.001*	2.75 \pm 0.50	1.96 \pm 0.30	0.78 \pm 0.42	<0.001*
Group 4	2.75 \pm 0.50	1.78 \pm 0.42	0.96 \pm 0.30	<0.001*	1.71 \pm 0.52	0.31 \pm 0.47	1.40 \pm 0.55	<0.001*	2.03 \pm 0.53	0.3 \pm 0.48	1.68 \pm 0.73	<0.001*	2.56 \pm 0.56	0.43 \pm 0.50	2.12 \pm 0.65	<0.001*

Paired t test; *p value <0.05; P_i =Pre-intervention; P_o =Post-intervention

Table IV. Intra and Inter Group comparison of mean plaque scores before intervention (day 1) and after intervention (day 7).

Groups	Plaque score day 1 (Mean± SD)	Plaque score day 7 (Mean± SD)	Mean Difference in plaque scores at 1 st and 7 th day	P value
Group 1	1.75±0.38	1.66±0.35	0.08±0.27	0.079
Group 2	1.68±0.26	1.45±0.26	0.22±0.14	<0.001*
Group 3	1.60±0.51	1.07±0.20	0.53±0.49	<0.001*
Group 4	1.51±0.47	0.76±0.14	0.75±0.47	<0.001*
P value	0.146	<0.001*	—	—

Paired t test, ANOVA and Post Hoc Tukey's test; *p value ≤0.05

Table V. Intra and Inter group comparison of mean gingival scores before intervention (day 1) and after intervention (day 7).

Groups	Gingival score day 1 (Mean± SD)	Gingival score day 7 (Mean± SD)	Mean Difference in Gingival scores at 1 st and 7 th day	P value
Group 1	1.50±0.15	1.40±0.14	0.09±0.12	<0.001*
Group 2	1.47±0.18	1.33±0.20	0.14±0.11	<0.001*
Group 3	1.44±0.32	0.97±0.18	0.46±0.36	<0.001*
Group 4	1.44±0.44	0.77±0.45	0.67±0.41	<0.001*
P value	0.850	<0.001*	—	—

Paired t test, ANOVA and Post Hoc Tukey's test; *p value <0.05

Discussion

In this cross-over trial, the contributions of various oral hygiene procedures in reducing morning bad breath like tooth brushing, tongue scraping, use of mouthwash and combined use of all these oral hygiene aids in healthy subjects were investigated.

In this study, intra group analysis showed significant difference in reduction of breath scores in groups 2, 3 and 4 from baseline (T00) to each time interval (T0, T1, T3) Group 1 showed no significant difference from baseline (T00) to each time interval (T0, T1, T3). This could be attributed to the fact that in Group 1 (tooth brushing) was performed by the participants which may have disrupted the formation of plaque biofilm present on the tooth surface thereby increasing the breath scores at each time interval. Similar findings were reported in previous studies carried out by Jeronimo M et al. [19] and Newby et al. [20].

The Post Intervention analysis (day 7) revealed significant difference in reducing the breath scores from baseline (T00) to each time interval (T0, T1, T3) in every group. The highest reduction in breath scores at each time interval was observed in Group 4 involving combined use of brushing, tongue scraping and mouthwash followed by

Group 2 involving use of tongue scraping method followed by group 3 and group 1. The findings are in the accordance with the findings of the previous study by Aung Ei et al. [14]. They reported that individual mechanical oral hygiene aid was able to immediately reduce bad breath, but for short duration, whereas combined use of Chlorhexidine with mechanical aids like brushing and tongue scraping significantly reduced breath for longer period of time. This suggests that the chemical action of a mouthwash reduces bad breath by reaching those areas that are difficult to be accessed by tongue cleaning procedure, in particular the posterior one-third of the tongue and are suggestive of the beneficial impact of combined use of mechanical and chemical oral hygiene aids on morning breath and should therefore be integrated into daily oral hygiene procedure.

In the present study the results also demonstrated that the subjects who performed tongue cleaning showed significantly lower mean breath values compared with Groups 3 and 1. This could be explained on the basis that the tongue has a larger surface area and its papillary structure represents an anaerobic environment in the oral cavity, favoring the accretion of oral debris and growth of microorganisms which are in turn responsible for the formation of VSC.

The results of the present study are in agreement with other studies performed by Faveri et al. [11] and Pedrazzi et al. [18] that have demonstrated that the tongue is considered as the major site for the initiation of VSCs and removal of tongue coating improves oral malodour. In contrast to our results a study done by Carvalho et al. [16] and Jeronimo M et al. [19] reported that use of Chlorhexidine mouth rinse offered the best results in reducing morning bad breath for the prolonged duration of the time.

Intra and inter group analysis of mean plaque scores recorded before the intervention (day 1) and after the intervention (day 7) showed significant reduction in group 2 , group 3 and group 4. The highest reduction in mean plaque scores was obtained in group 4, suggestive of the fact that the use of mouthwash in conjunction with mechanical aids is useful in improving the oral hygiene of the individual. Similar findings were reported in previous studies carried out by Bhopale D [21] and Yates et al. [22]. The present study results also demonstrated that Group 3 (involving the individual use of mouthwash) was also found to be effective in reducing the mean plaque scores from baseline to each time interval. This finding is in agreement with the previous studies by Grundemann et al. [23], Francetti et al. [24] and Waghmare et al. [25]. This may be due to the property of substantivity and anti-plaque property of Chlorhexidine which suggest that individual use of Chemical oral hygiene aids i.e. use of mouthwash is superior to the use of mechanical aids in improving the oral hygiene status of the individual.

The present study compared the mean gingival scores on the 1st and 7th days. Significant reduction was observed between the gingival scores recorded on day 1 and day 7 in all the groups. Group 4 showed significantly higher reduction in gingival scores compared to other groups. This can be explained by the fact that combined use of mechanical and chemical oral hygiene aids is useful in reducing gingival bleeding as well as gingival inflammation. Group 3 was also found to be effective in reducing gingival scores in the individuals which is similar to the findings of Najafi M et al. [26], Neto C et al. [27] and J.L Lyes et al. [28]. This is suggestive of the fact that the chemical agents are superior to mechanical means in reducing gingival inflammation.

Pearson's correlation coefficient test was performed to observe correlation between the Plaque scores and Gingival Scores before intervention (day 1) and after the intervention (day 7) and a positive correlation of plaque scores with gingival scores on days 1 and 7 was observed. This shows that the individuals with a higher plaque score at baseline also had more marked gingival inflammation. Thus, the present study suggests an association between plaque and gingival status. This finding is in agreement with the study by Neto C et al. [27]. In which they found that increase in plaque score is associated with increased gingival inflammation.

The limitation of the present study could be that Volatile sulphur compound monitors (i.e. Halimeter) were not used for the present study due to the financial and time constraints. Although every effort has been taken by the authors to include and standardize the factors affecting bad breath, there might be other confounding factors that could have influenced the study results. Also, the small number of the study subjects 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 indicate that both tongue cleaning as the mechanical method and use of mouth wash as chemical method significantly reduce bad breath. However, combining both mechanical and chemical regimens are the most effective way in the reduction of morning bad breath in healthy subjects and should be considered as part of daily oral hygiene practices. This study also demonstrated that use of mouthwash in conjunction with mechanical aids are effective in reducing plaque and gingival scores and also the individual use of Chemical oral hygiene aids, i.e. use of mouthwash is superior to the use of mechanical aids in reducing plaque status and gingival inflammation.

Acknowledgements

We gratefully thank the study subjects for participating in the study.

References

1. Cortelli JR, Barbosa MD, Westphal MA. Halitosis: a review of associated factors and therapeutic approach. *Braz Oral Res.* 2008;22:44-54.
2. Hughes FJ, Rod McNab R. Oral malodour--a review. *Arch Oral Biol.* 2008;53:S1-S7.
3. 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.
4. Verran J. Malodour in denture wearers: an ill-defined problem. *Oral Dis.* 2005;11:24-28.
5. van den Broek AM, Feenstra L, de Baat C. A review of the current literature on management of halitosis. *Oral Dis.* 2008;14:30-39.
6. Setia S, Pannu P, Gambhir RS, Galhotra V, Ahluwalia P, Sofat A. Correlation of oral hygiene practices, smoking and oral health conditions with self perceived halitosis amongst undergraduate dental students. *J Nat Sci Biol Med.* 2014;5(1):67-72.
7. Outhouse TL, Al-Alawi R, Fedorowicz Z, Keenan JV. Tongue scraping for treating halitosis. *Cochrane Database Syst Rev.* 2006;(2):CD005519.
8. Quirynen M, Avontrodt P, Soers C, Zhao H, Pauwels M, van Steenberghe D. Impact of tongue cleansers on microbial load and taste. *J Clin Periodontol.* 2004;31(7):506-510.
9. Tonzetich J. Oral malodour: an indicator of health status and oral cleanliness. *Int Dent J.* 1978;28(3):309-319.
10. Yamaguchi T, Hanabusa M, Hosoya N, Chiba T, Yoshida T, Morito A. Enamel surface changes caused by hydrogen sulfide. *J*

Conserv Dent. 2015;18(6):427–430.

11. Faveri M, Hayacibara MF, Pupio GC, Cury JA, Tsuzuki CO, Hayacibara RM. A cross-over study on the effect of various therapeutic approaches to morning breath odour. *J Clin Periodontol*. 2006;33:555–560.

12. Roldán S, Herrera D, Santa-Cruz I, O'Connor A, González I, Sanz M. Comparative effects of different chlorhexidine mouthrinse formulations on volatile sulphur compounds and salivary bacterial counts. *J Clin Periodontol*. 2004;31:1128–1134.

13. Yaegaki K, Coil JM, Kamemizu T, Miyazaki H. Tongue brushing and mouth rinsing as basic treatment measures for halitosis. *Int Dent J*. 2002;52:192–196.

14. Aung EE, Ueno M, Zaitzu T, Furukawa S, Kawaguchi Y. Effectiveness of three oral hygiene regimens on oral malodor reduction: a randomized clinical trial. *Trials*. 2015;16:31.

15. Winkel EG, Roldan S, van Winkelhoff AJ, Herrera D, Sanz M. Clinical effects of a new mouthrinse containing chlorhexidine, cetylpyridinium chloride and zinc-lactate on oral halitosis. A dual-center, double-blind placebo-controlled study. *J Clin Periodontol*. 2003;30:300–306.

16. Carvalho MD, Tabchoury CM, Cury JA, Toledo S, Nogueira-Filho GR. Impact of mouthrinses on morning bad breath in healthy subjects. *J Clin Periodontol*. 2004;31:85–90.

17. Donaldson AC, Riggio MP, Rolph HJ, Bagg J, Hodge PJ. Clinical examination of subjects with halitosis. *Oral Dis*. 2007;13:63–70.

18. Pedrazzi V, Sato S, de Mattos Mda G, Lara EH, Panzeri H. Tongue-cleaning methods: a comparative clinical trial employing a toothbrush and a tongue scraper. *J Periodontol*. 2004;75:1009–1012.

19. Oliveira-Neto JM, Sato S, Pedrazzi V. How to deal with morning bad breath: A randomized, crossover clinical trial. *J Indian Soc Periodontol*. 2013;17(6):757–761.

20. Newby EE, Hickling JM, Hughes FJ, Proskin HM, Bosma MP. Control of oral malodour by dentifrices measured by gas

chromatography. *Arch Oral Biol*. 2008;53 Suppl 1:S19-S25.

21. Bhopale D. Effectiveness of the chlorhexidine containing dentifrice on reduction of plaque and gingival inflammation - A controlled clinical trial. *Global Journal of Medicine and Public Health*. 2014;3(1):1-7.

22. Yates R, Jenkins S, Newcombe R, Wade W, Moran J, Addy M. A 6-month home usage trial of a 1% chlorhexidine toothpaste (1). Effects on plaque, gingivitis, calculus and toothstaining. *J Clin Periodontol*. 1993;20:130-138.

23. Gründemann LJ, Timmerman MF, Ijzerman Y, van der Weijden GA, van der Weijden GA. Stain, plaque and gingivitis reduction by combining chlorhexidine and peroxyborate. *J Clin Periodontol*. 2000;27(1):9-15.

24. Francetti L, del Fabbro M, Testori T, Weinstein RL. Chlorhexidine spray versus chlorhexidine mouthwash in the control of dental plaque after periodontal surgery. *J Clin Periodontol*. 2000;27:425-430.

25. Waghmare PF, Chaudhari AU, Karhadkar VM, Jamkhande AS. Comparative evaluation of turmeric and chlorhexidine gluconate mouthwash in prevention of plaque formation and gingivitis: a clinical and microbiological study. *J Contemp Dent Pract*. 2011;12(4):221-224.

26. Najafi MH, Taheri M, Mokhtari MR, Forouzanfar A, Farazi F, Mirzaee M, et al. Comparative study of 0.2% and 0.12% digluconate chlorhexidine mouth rinses on the level of dental staining and gingival indices. *Dent Res J (Isfahan)*. 2012;9(3):305-308.

27. Franco Neto CA, Parolo CC, Rösing CK, Maltz M. Comparative analysis of the effect of two chlorhexidine mouthrinses on plaque accumulation and gingival bleeding. *Braz Oral Res*. 2008;22(2):139-144.

28. Leyes Borrajo JL, Garcia VL, Lopez CG, Rodriguez-Nuñez I, Garcia FM, Gallas TM. Efficacy of chlorhexidine mouthrinses with and without alcohol: a clinical study. *J Periodontol*. 2002;73:317-321.