Effects of Tooth Brushing, Mouth Washing and Tongue Cleaning on Three Volatile Sulfur Compounds – A Randomized Clinical Trial –

Ei Ei AUNG¹,², Masayuki UENO², Takashi ZAITSU³, Sayaka FURUKAWA² and Yoko KAWAGUCHI²

1) International Health Policy Program, Ministry of Public Health, Nonthaburi, Thailand
2) Department of Oral Health Promotion, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

Abstract: Objective: The purpose of this study was to assess the effects of tooth brushing, mouth washing and tongue cleaning on three volatile sulfur compound (VSC) concentrations. Methods: Thirty male volunteers were randomly assigned into group A and B. Both groups brushed their teeth for the first one week. During the next three weeks, group A used chlorine dioxide (ClO₂) mouthwash and group B performed tongue cleaning in addition to tooth brushing. Both groups practiced the combination of tooth brushing, mouth washing and tongue cleaning for the final one week. Clinical oral health status was examined, and concentrations of three VSC gases (ng/10mL): hydrogen sulfide (H₂S), methyl mercaptan (CH₃SH) and dimethyl sulfide ((CH₃)₂S), were measured. Results: At the 1st week examination, three VSC gases were decreased compared with the baseline in both groups, but there were no significant differences. In group A, H₂S concentration was significantly decreased from 5.59 ± 4.38 at the baseline to 1.01 ± 0.83 at the 4th week and to 0.91 ± 0.84 at the 5th week examinations (p < 0.05). CH₃SH and (CH₃)₂S did not show significant reductions from the baseline. A similar trend was observed in group B. H₂S concentration was significantly decreased at the 4th week and 5th week examinations compared with the baseline. CH₃SH was also significantly reduced from the baseline to the 4th week examination, but (CH₃)₂S was not. Conclusion: Mouth washing or tongue cleaning decreased VSC levels, especially H₂S. Further, the combination of mouth washing and tongue cleaning brought the greatest reduction of VSCs.

Key words: hydrogen sulphide, methyl mercaptan, dimethyl sulphide, oral malodor, halitosis

Introduction

Oral malodor, also called halitosis, is a common problem in many countries. Oral malodor affects our daily life by causing emotional stress, embarrassment and social disharmony. According to previous epidemiological studies on oral malodor, 30% to 50% of the study populations had oral malodor. The putrefaction of protein from epithelial cells by anaerobic bacteria produces the volatile sulphur compounds (VSCs) that cause the odor. The three main gases of VSCs are hydrogen sulphide (H₂S), methyl mercaptan (CH₃SH) and dimethyl sulfide ((CH₃)₂S). These VSCs can be measured by using sulphide monitoring devices such as Halimeter, Breathtron, Oral Chroma and gas chromatography as well as the organoleptic test.

Multiple factors influence oral malodor. The origins of oral malodor are oral-health related problems and systemic diseases. Among these causes, 80% to 90% originate from the oral cavity. The main causal factors are periodontal diseases, tongue coating, poor oral hygiene, xerostomia and dental caries. The remainder is from systemic diseases such as upper respiratory tract problems, digestive diseases, chronic liver diseases, renal failure and metabolic disorders. Many studies found a strong association between tongue coating and oral malodor level. The tongue, especially its dorsal surface, is the area where microorganisms, desquamated epithelium cells and food debris easily accumulate because of its distinct anatomical structure.

People try to use a variety of medications and products to improve their oral breath. In some cases, people
decrease the frequency of meals and increase the consumption of mints, gums, candy and even tobacco and betel quid. However, there are scientifically proved ways to manage oral malodor, for example, removal of dental plaque by tooth brushing, reduction of tongue coating by tongue cleaning, and decreasing of oral bacteria counts by mouth washing.

There are very few studies comparing the effects of tooth brushing, tongue cleaning and mouth washing on individual VSC gas concentrations. Therefore, this study aimed to assess the effect of tooth brushing, tongue cleaning and mouth washing as well as the combination of these methods on the reduction of three main VSC gases.

Material and Methods

1. Study design and procedure

This was a randomized clinical trial with a single blind, 5-week parallel study design. The study was carried out in Yangon, Myanmar from September to October in 2013. Originally, forty-eight male volunteers signed a consent form to participate in this study. After screening by using the inclusion criteria, eighteen subjects were excluded. The inclusion criteria employed were: no known systemic diseases, no current use of antibiotics, no severe dental caries, no periodontal pocket more than 3 mm in depth, no history of allergy to any kind of mouthwash and no habit of smoking or betel quid chewing. The final number of the study subjects was 30.

Subjects were randomly divided into two groups: group A (n=15) and B (n=15). For the first one week, both groups were instructed to brush their teeth with a scrubbing method using their own toothbrush. From the 2nd to 4th week, group A used 12 mL of chlorine dioxide (ClO2) Fresh® mouthwash (Bio-Cide International, Inc., Oklahoma, USA and Pine Medical Co., Tokyo, Japan) for 30 seconds twice daily, and group B performed tongue cleaning twice daily with a small toothbrush, in addition to daily tooth brushing. Subjects were instructed to practice mouth washing or tongue cleaning after waking up in the morning and before going bed at night. For the final (5th) week, all subjects were requested to practice all three oral hygiene methods.

2. Oral malodor measurement

Oral malodor was evaluated between 2 pm and 5 pm. Subjects were requested to refrain from any kind of drinking and eating as well as oral hygiene practice at least 2 hours before the measurement. The three VSC gas concentrations were measured using an Oral Chroma® (FIS Inc., Hyogo, Japan). The air inside the subjects’ oral cavity was collected with a 1 mL syringe after closing the mouth for 3 minutes. A 0.5 mL air sample was then injected into the machine. The concentrations of the three VSC gases were calculated in units of ng/10 mL. The threshold values for each gas concentration were: 1.5 ng/10 mL H2S, 0.5 ng/10 mL CH3SH and 0.2 ng/10 mL (CH3)2S.

3. Clinical oral health status

At the baseline, dentition status such as numbers of present teeth, decayed teeth (DT), filled teeth (FT), and missing teeth (MT), excluding third molars, was determined. The amount of plaque was evaluated by using the debris index (DI) scores of the Oral Hygiene Index (OHI).

Gingival bleeding on probing (BOP) was recorded if bleeding was detected after examination with a periodontal probe.

Tongue coating was evaluated by a modified Winkel tongue-coating index: 0 = no tongue coating, 1 = thin tongue coating (visible papillae), 2 = thick tongue coating (invisible papillae). The tongue surface was divided into nine areas and the tongue coating index was calculated by adding the scores of all nine areas, producing a range from 0 to 18.

For saliva characteristics, subjects were requested to spit all the saliva pooled in the oral cavity into a collecting paper cup for 5 minutes. The flow rate of saliva was calculated as mL per minute and the pH of the saliva was determined by using a bromothymol blue test paper.

Dentition status and saliva characteristics were examined only at the baseline, and the VSC gas concentrations, debris index, bleeding on probing and tongue coating were recorded at the baseline as well as every examination interval. All the examinations were performed by an investigator who was blinded to subjects’ assigned group.

4. Ethical approval

The ethical committee for human research at Tokyo Medical and Dental University approved this clinical study (No.850) and the study protocol was also approved by University of Dental Medicine (Yangon) in Myanmar.

5. Data analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS 16 SPSS Japan Inc., Tokyo, Japan). The independent sample t-test was used to determine significant differences of means between the
Results

1. Baseline characteristics of subjects

Table 1 shows the baseline characteristics of the subjects in groups A and B. There were no significant differences in any variables, including age, clinical oral health status: numbers of present teeth, decayed teeth, missing teeth, filled teeth, the debris index, bleeding on probing, saliva flow rate and pH, tongue coating and the three VSC gas concentrations between the two groups.

2. Changes of clinical oral health status

Table 2 shows the changes of the mean debris index (DI), bleeding on probing (BOP) and tongue coating scores at the baseline and each examination interval. Compared to the baseline, there was a significant reduction of the DI score at every examination interval in both groups (p<0.05). Similarly, BOP was significantly decreased at each examination interval compared with the baseline (p<0.05). There were no significant differences in DI or BOP between groups A and B at any examination interval.

In comparison with the baseline, no significant decrease of tongue coating was observed at the 1st week examination in either group. Tongue coating scores were significantly decreased from baseline to the 4th and 5th week examinations in both groups (p<0.05). At the 4th week examination the tongue coating score in group B (0.92 ± 0.39) was significantly lower than that in group A (1.01 ± 0.83) at the 4th week and 0.91 ± 0.84 at the 5th week examinations (p<0.05). A similar trend was observed in group B; H2S concentration was significantly decreased from 3.44 ± 2.61 at the baseline to 1.97±1.34 at the 4th week (p<0.05) and 0.77±0.81 at the 5th week examinations (p<0.01).

There were no significant differences in CH3SH and (CH3)2S concentrations between the baseline and examination intervals, except the 4th week examination in group B. In group A, CH3SH changed from 1.27 ± 1.24 at the baseline to 0.52 ± 0.72 at the 1st week, 0.48 ± 0.39 at the 4th week and 0.93 ± 1.34 at the 5th week examinations. (CH3)2S was changed from 0.72 ± 0.62 at the baseline to 0.22 ± 0.34 at the 1st week, 0.36 ± 0.35 at the 4th week and 0.42 ± 0.50 at the 5th week examinations. However, these changes did not differ significantly from the baseline values.

In group B, CH3SH showed a significant reduction from the baseline (1.63 ± 1.38) to the 4th week examination (0.53 ± 0.38). No significant changes from baseline were observed at other examination intervals. On the other hand, (CH3)2S showed no significant changes from the baseline (0.53 ± 0.58) to the 1st week (0.35 ± 0.31), 4th week (0.34 ± 0.33), or 5th week (0.35 ± 0.39) examination.

3. Changes of the three volatile sulphur compounds

gas concentrations

Figure 1 shows the changes of the three VSC gas concentrations. H2S concentration was decreased from baseline to the 1st week examination in both groups, although the differences did not reach statistical significance. In group A, H2S concentration was significantly decreased from 5.59 ± 4.38 at the baseline to 1.01 ± 0.83 at the 4th week and 0.91 ± 0.84 at the 5th week examinations (p<0.05). A similar trend was observed in group B;

Table 1  Baseline characteristics of the subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group A</th>
<th>Group B</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>19.8 ± 2.90</td>
<td>21.1 ± 3.50</td>
<td>0.27</td>
</tr>
<tr>
<td>Present teeth</td>
<td>27.6 ± 0.51</td>
<td>27.9 ± 0.35</td>
<td>0.11</td>
</tr>
<tr>
<td>DT</td>
<td>0.13 ± 0.35</td>
<td>0</td>
<td>0.16</td>
</tr>
<tr>
<td>MT</td>
<td>0.07 ± 0.26</td>
<td>0</td>
<td>0.33</td>
</tr>
<tr>
<td>DI</td>
<td>0.40 ± 0.51</td>
<td>0.13 ± 0.35</td>
<td>0.11</td>
</tr>
<tr>
<td>BOP</td>
<td>12.5 ± 7.62</td>
<td>12.1 ± 8.97</td>
<td>0.90</td>
</tr>
<tr>
<td>Tongue coating</td>
<td>12.4 ± 4.19</td>
<td>11.5 ± 5.21</td>
<td>0.59</td>
</tr>
<tr>
<td>Saliva flow rate</td>
<td>0.58 ± 0.19</td>
<td>0.48 ± 0.17</td>
<td>0.14</td>
</tr>
<tr>
<td>(mL/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saliva pH</td>
<td>7.00 ± 0.39</td>
<td>7.05 ± 0.40</td>
<td>0.75</td>
</tr>
<tr>
<td>Hydrogen sulphide</td>
<td>5.59 ± 4.38</td>
<td>7.07 ± 5.80</td>
<td>0.44</td>
</tr>
<tr>
<td>(ng/10 mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl mercaptan</td>
<td>1.27 ± 1.24</td>
<td>1.63 ± 1.38</td>
<td>0.46</td>
</tr>
<tr>
<td>(ng/10 mL)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Dimethyl sulphide</td>
<td>0.72 ± 0.62</td>
<td>0.53 ± 0.58</td>
<td>0.38</td>
</tr>
<tr>
<td>(ng/10 mL)</td>
<td></td>
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</table>

*p value for mean differences between group A and group B.
Discussion

This study examined the effect of chemical and mechanical procedures on changes in VSC gas concentrations for five weeks. All three VSC gases were reduced by applying these procedures. To minimize the influence of systemic diseases and menstrual cycle on VSCs, healthy young male adults were recruited in the study 21. All the subjects lived in the same place for professional monk training where they had the same frequency and content of meals, therefore, the effect of different dietary patterns on the oral malodor was negligible. The VSC concentrations were measured using an Oral Chroma® that has been widely used in epidemiological studies because of its portability, reliability and ability to assess three VSC gas concentrations separately 16.

A scrubbing method was chosen for tooth brushing because it was easy and effective for subjects to remove dental plaque 20. Although the kind of toothpaste used was not specified for tooth brushing, the subjects were instructed how to brush their teeth with a scrubbing method prior to the study so that they could perform tooth brushing uniformly.

Tongue cleaning is a mechanical method to remove debris on the tongue surface, and its effectiveness depends on how well and how skillfully the subject performs tongue cleaning 21. A previous study showed that around 43% of Myanmar people practiced tongue cleaning 22. A tongue brush is not popular in Myanmar but a toothbrush is available everywhere. Thus, tongue cleaning was performed using a small toothbrush in this study.

Mouthwashes have been widely used as an oral malodor remedy around the world 18, 23-25. Although some mouthwashes are reported to give harmful side effects such as mucosa staining, irritation, and taste alteration in long-term use, ClO₂ has no such side effects 26. Therefore, in this study a ClO₂ mouthwash was used to evaluate the chemical effect on VSCs.

The debris index, bleeding on probing and tongue coating scores at the baseline indicated that the oral hygiene of subjects was not good enough. After one week of tooth brushing, DI and BOP scores were significantly reduced. The tooth brushing effectively improved the oral hygiene condition and gingival health, but there was no significant improvement on tongue coating. This is compatible with the result that tooth brushing can reduce oral malodor to some extent but that each VSC gas concentration remains above the threshold level 27. At the 4th and 5th week examinations after adding mouth washing or tongue cleaning to tooth brushing, low levels of tongue coating scores in addition to lower DI, BOP scores were achieved.

For the change of individual VSC gas concentrations, both mouth washing and tongue cleaning significantly reduced the H₂S level. H₂S mainly originates from oral debris, in particular from tongue coating 28, 29. Mouthwash reduced H₂S by a bactericidal action and a chemical action of converting VSCs to non-volatile substances 29.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Baseline Mean ± SD</th>
<th>1st week Mean ± SD</th>
<th>4th week Mean ± SD</th>
<th>5th week Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI</td>
<td>A</td>
<td>0.83 ± 0.20</td>
<td>0.18 ± 0.15</td>
<td>0.24 ± 0.20</td>
<td>0.15 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.89 ± 0.24</td>
<td>0.26 ± 0.17</td>
<td>0.27 ± 0.20</td>
<td>0.13 ± 0.12</td>
</tr>
<tr>
<td>p value*</td>
<td></td>
<td>0.47</td>
<td>0.20</td>
<td>0.74</td>
<td>0.70</td>
</tr>
<tr>
<td>BOP</td>
<td>A</td>
<td>12.5 ± 7.62</td>
<td>2.07 ± 5.11</td>
<td>0.53 ± 1.60</td>
<td>0.80 ± 3.09</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>12.1 ± 8.97</td>
<td>4.20 ± 4.92</td>
<td>0.89 ± 0.24</td>
<td>0.27 ± 0.70</td>
</tr>
<tr>
<td>p value*</td>
<td></td>
<td>0.90</td>
<td>0.25</td>
<td>0.90</td>
<td>0.52</td>
</tr>
<tr>
<td>Tongue coating</td>
<td>A</td>
<td>12.4 ± 4.19</td>
<td>9.00 ± 2.95</td>
<td>5.60 ± 4.41</td>
<td>0.20 ± 0.56</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>11.5 ± 5.21</td>
<td>11.9 ± 4.63</td>
<td>0.92 ± 1.80</td>
<td>0.27 ± 0.79</td>
</tr>
<tr>
<td>p value*</td>
<td></td>
<td>0.59</td>
<td>0.05</td>
<td>&lt;0.01</td>
<td>0.79</td>
</tr>
</tbody>
</table>

*p value for mean differences between group A and group B. Bold letter means a significant difference between groups.
On the other hand, tongue cleaning mechanically reduces the total bacteria count on the tongue but it has no gas conversion mechanism like ClO₂ mouthwash has.

Further, it was reported that among the bacteria on the tongue surface, 75% are Veillonella parvula, Actinomyces odontolyticus, Streptococcus intermedius and Clostridium innocuum, and most of them produce H₂S \(^{28}\). Tongue cleaning reduces H₂S by removing these H₂S-producing bacteria. A previous study demonstrates that ClO₂ can reduce tongue coating by decreasing the total bacteria counts as well as specific bacteria in saliva and other oral debris like Fusobacterium nucleatum that can also produce H₂S \(^{29}\).

At the baseline, CH₃SH and (CH₃)₂S levels were not high compared with H₂S. CH₃SH and (CH₃)₂S are associated with periodontal-disease-related bacteria and systemic conditions. A former study proved that periodontal pathogens were particularly responsible for CH₃SH

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*Fig. 1 Changes of mean H₂S, CH₃SH, (CH₃)₂S gas concentrations in groups A and B

* \(p < 0.05\), ** \(p < 0.01\)
gas production, and the proportion of periodontal pathogens on tongue coatings was higher in subjects with periodontitis than those without. Because current subjects were free from periodontitis, it seems plausible that little CH$_3$SH was produced. Systemic conditions often related with (CH$_3$)$_2$S production are liver cirrhosis, metabolic disorders, anti-allergenic and suplatast tosilate drug use. Current subjects did not have such systemic diseases or take any medications, thereby (CH$_3$)$_2$S was considered to be synthesized from methylation of methyl mercaptan.

Similar to H$_2$S, both CH$_3$SH and (CH$_3$)$_2$S showed a decreasing pattern by mouth washing or tongue cleaning, but the changes were not prominent due to low concentrations at the baseline. At the final 5th week examination, CH$_3$SH in the mouth-washing group indicated a slightly increasing trend. Some bacteria, especially periodontal pathogens, might become resistant to ClO$_2$ mouthwash after 4 weeks of use, but a further research will be needed to confirm this hypothesis.

One of the limitations of this study was that the oral microorganisms were not investigated. An examination of the changes in oral bacteria could reveal more about the relationship of mouth washing and tongue cleaning with the three VSC gases. Another limitation was the length of the study. This was a 5-week study, therefore the long-term change of VSCs by continuous use of mouth washing and tongue cleaning remain unknown.

Nonetheless, there have been very limited studies that assessed the effect of tooth brushing, mouth washing and tongue cleaning on individual VSC gas concentrations. The current study gives useful information about how different methods affect individual VSC gas concentrations. Tongue cleaning and mouth washing mainly affect the H$_2$S concentration and they would be beneficial for the treatment of halitosis patients whose primary VSC gas is H$_2$S.

**Conclusion**

Both mouth washing, as a chemical method, and tongue cleaning, as a mechanical method, decreased VSCs, especially H$_2$S. Mouth washing and tongue cleaning had a similar effect on VSCs reduction. Further, the combination of mouth washing and tongue cleaning brought the best reduction of VSCs.

**Competing Interests**

The authors declare that there are no conflicts of interest in this study.


