The Effects of Tongue Coating on Volatile Sulfur Compounds Production in the Oral Malodor Patients

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Ⅰ. INTRODUCTION

Oral malodor or halitosis has been a serious problem among the population, and now oral malodor is a public concern and a social and economic problem.[1,2] An estimated US$850 million mouthwash industry exists in the United States.[1] At least 50 percent of the population suffer from chronic oral malodor, and approximately half of these individuals experience a severe problem that creates personal discomfort and social embarrassment.[3] Ninety-two percent of dentists surveyed at the ADA's 1995 annual session reported that they had patients with chronic bad breath. Almost half reported seeing six or more patients weekly with unpleasant breath.[2] So there are increasing needs for researches on halitosis and proper care of oral environment to reduce oral malodor.[4]

When the oral malodor is to be studied and the patients who are complaining oral malodor are to be evaluated, the measurement of its severity is an essential element. Even though there are many ways in estimating oral malodor, it has been usually assessed by direct nasal sniffing at the expelled mouth air. This is often referred to as 'organoleptic' or 'hedonic' assessment.[5] Although the organoleptic measurement is still used in the clinical field because of its simplicity, it has many problems. First, malodorous severity is measured subjectively by judges on the ranking of the unpleasantness or severity of air samples. Unfortunately individuals differ in their sensitivities to smells, and perceive oral malodor differently.[3] Moreover there is not any good standard about ordinal scale which can be used in grading oral malodor. And the mouth air is largely diluted by lung air as the subject rapidly breathes out.[5] Therefore, more accurate and reproducible objective methods for oral malodor measurement was needed. But objective measurement of oral malodor was not easy until gas chromatographic analysis had been introduced in this field. Mass spectrometric analysis of oral air sample had found that some volatile sulfurous components were present in human oral air sample.[6,7] And later it was

revealed by gas chromatography that these components were volatile sulfur compounds(VSC).[8] From then, gas chromatographic studies of human oral malodor have had a great advance in researching oral malodor. And many authors[6,8-14] who used gas chromatography found that human oral malodor was composed of mainly volatile sulfur compounds(VSC), especially hydrogen sulfide H_2S, methyl mercaptan CH_3SH, and dimethyl sulfide $(CH_3)_2S$ which arise from breakdown of cysteine, cystine, methionine[15-17] and peptides[18] by microbial putrefaction within the oral cavity.[19-21] The reliability of gas chromatography was tested and it was announced that there was a statistically significant positive correlation between the perceived oral malodor and the concentration of VSC(H_2S and CH_3SH) emitted by individual subjects.[11] And in most malodorous persons, it is believed that hydrogen sulfide and methyl mercaptan constitute over 90 % of total volatile sulfur compound in intra-oral air.[8,14]

Using this gas chromatographic method, many investigators researched the cause of oral malodor and its influences on malodor formation. Human oral malodor has many causes[4,6] and these causes are usually divided into intra-oral and extra-oral ones.[22,23] Intra-oral causes are generally thought to play a major role in halitosis.[24,25] Among the many intra-oral causes, periodontal involvements were extensively investigated. And it was revealed that bad breath from periodontally diseased persons could not be reduced by tongue scraping, but could be reduced by elimination of periodontal pockets.[9] Furthermore VSC production and methyl mercaptan/hydrogen sulfide ratio appeared to increase with the extent of periodontal disease.[26] Tonzetich[27] presented that putrefactive activity, odor intensity, and VSC content were all significantly elevated in mouths exhibiting inflammatory or periodontal involvement. Miyazaki *et al.*[28] announced that there was a significant correlation between the VSC value and periodontal conditions and tongue coating status, and also suggested that oral malodor might be caused mainly by tongue coating in the younger generation and by periodontal diseases together with tongue coating in older cohorts in the general population. Through these studies, it was widely accepted that oral malodor was related with periodontal destruction. But Bosy *et al.*[29] announced that there was no significant association between the presence of halitosis and periodontitis. Tonzetich and Ng[30] found that the dorsoposterior surface of the tongue was the predominant site of putrefaction. Quirynen *et al.*[31] announced that both the organoleptic ratings and the VSC scores correlated well with the presence of tongue coating. In fact, there are many people who complain of oral malodor but do not have periodontal diseases.[32] With these results in mind, in the present study, the individual VSC concentrations were investigated more precisely in the oral air of the periodontally healthy persons by using gas chromatography, and the effect of tongue coating on oral malodor formation was reevaluated.

Ⅱ. MATERIALS AND METHODS

Among the patients who visited the oral malodor clinic of Seoul National University Dental Hospital, subjects were chosen from them who were non-smokers and periodontally healthy persons (pocket depth ≤ 4 mm) with no history of antibiotic medications for the last 3 months and did not exhibit a cleft or fissured tongue. All of the subjects had not any sign of systemic disorders which could affect oral malodor.

The 18 subjects(mean age 31.4 years, ranging from 15 to 49 years: 8 males, 10 females) were instructed to abstain from eating, chewing, oral hygiene procedures and any other oral activities from night to the morning on the test day. The oral air samples of each subject were collected prior to and after tongue coating removal and the results were compared.

1. Apparatus and experimental conditions

Gastight syringe(10 ml vol. with SampleLock valve,

Hamilton Co. Reno Nevada USA) was used in sample collection and also in sample injection. Six centimeter-long and 1/8-inch width teflon tube was made which could be connected to the syringe. Prior to sample collection, all subjects were informed that they should close the mouth for 3 min. And then Gastight syringe(10 ml) with 6 cm-long teflon tube was inserted into the mouth. The teflon tube's end was located in the intraoral area at a distance of 4 cm inward from the mandibular anterior teeth and 1 cm above the tongue surface and the subject's lip was apart to approximately 2 cm. During sample collection, the subjects were instructed to hold his or her breath in order to avoid lung air interruption. After 10 ml of oral air was collected, 6 cm teflon tube was changed with an injection needle for sample injection. In sample injection, so-called direct injection method[8,9] was used. This method has many advantages compared with sample loop system. Although many authors used this 6 or 8 way valve system, that is sample loop,[10-12,14,26,35] in order to avoid strong back pressure in studying human oral malodor, sampling valve system is complex and has many junctions which intra-oral air samples must pass through. So sample loss is more likely to occur. In direct injection, however, test samples can be directly injected to the column inlet portion, so sample loss can be minimized.

The gas chromatography(HP5890, Hewlett-Packard Co. Avondale PA USA) was used equipped with flame photometric detector with 393 nm(3930 Å) wavelength for the sulfur mode optical filter, and 60/80 mesh size Chromosil 330 column(Supelco Co. Bellforte PA USA) which Steudler[33] and Hatton[34] used in the previous studies. Chromosil 330 is a treated silica gel designed to separate ppb levels of hydrogen sulfide, mercaptans, alkyl sulfides and disulfides. The column was customized with 2 meter length and 1/4 inch diameter in which the Chromosil 330 packing should be void at 1 feet in inlet portion and 1/2 feet at outlet portion. Usually the Chromosil 330 column has been made into 1 feet void at each ends. But in this study, the 1/2 feet void column in outlet portion was used in order to reduce the peak tailing appearance which may be shown in chromatogram.

The chromatographic run conditions are as follows: Initial time and initial oven temperature is 2 min at 40℃. and the oven temperature was raised at rate 8℃/min, final time and final oven temperature is 10 min. at 100℃, attenuation of gas chromatograph is 4, nitrogen carrier flow rate is 20 ml/min, hydrogen flow rate is 75 ml/min(27 psig), air flow rate is 100 ml/min(56 psig). The temperature of injector and detector was 100℃ and 200℃ each.

The peaks were recorded at integrator HP3394A (Hewlett-Packard Co. Avondale PA USA). The integrator run condition are as follows: chart speed is 0.5 cm/min, attenuation is 2, threshold is 3, peak width is 0.16, area rejection is 500.

2. Tongue coating removal

Regarding tongue cleaning procedures, it was reported that there was no statistically significant difference between tongue brushing and tongue scraping in plaque accumulation or gingivitis.[37] So the tongue scraping instead of tongue brushing was used because of its convenience in tongue coating isolation.

Before tongue coating removal, the tongue surface was lightly blown out by air syringe to remove saliva, then tongue coating in wet state was carefully scraped.

For tongue coating removal, the cellulose strip (15 cm×1 cm) was made and bended at two points in middle area to scrape easily. And the tongue coating was carefully scraped from circumvallate papilla area to the apex of the tongue, then oral malodor sample was recollected and analyzed again by gas chromatography.

3. Standard calibration for sulfur compounds

For standard calibration, gas permeater(Gastec Co. Japan) and standard permeation tubes(H_2S, CH_3SH, $(CH_3)_2S$, Gastec Co. Japan)were used. Because standard permeation tubes can generate

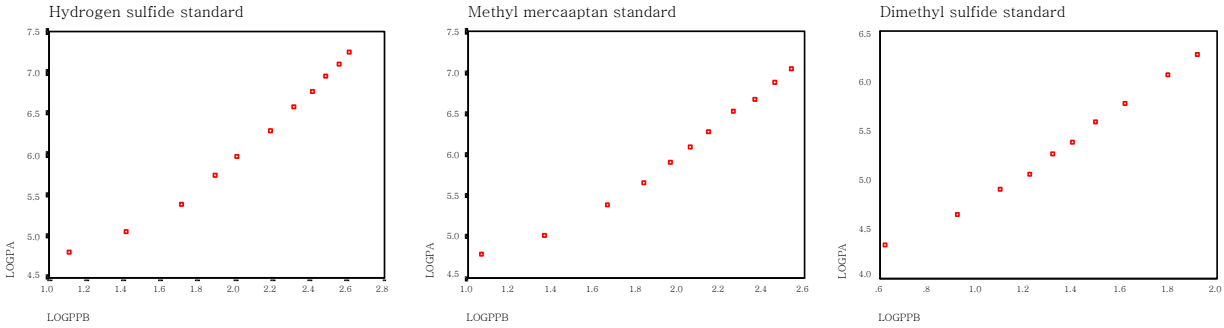

Fig. 1. Standard calibration of each sulfur compounds(PA means peak area)

exact concentrations of the three VSCs in gaseous form, thereby it is possible to calibrate the FPD detector accurately for the three VSCs.

With respect to standard calibrations, the previous studies used peak area[8,35,36] or peak height.[9,11] Preliminary study had found that the peak area was more reliable than peak height. So, in this study, the peak area was used in standard calibration.

Ⅲ. RESULTS

By using permeater and permeation tubes, the standard calibration curves could be obtained. Fig. 1 represents the standard calibration curves of each VSC.

During gas chromatographic run, the three volatile sulfur compounds were easily separated and it was possible to calculate each concentration of these compounds through the calibration curves. Fig. 2 represents the individual volatile sulfur compounds which are eluted during gas chromatographic run in the same subject before and after tongue coating removal.

Total VSC concentration(in part-per-billion) in the oral air was compared between before and after tongue coating removal. Statistically significant ($p<0.01$) reduction of total VSC was observed after the tongue coating had been removed(Table 1). And the concentration of each component was also compared between before and after tongue coating removal(Table 2, 3, 4).

Fig. 2. Typical chromatographic recording of oral malodor between before and after tongue coating removal in the same subject.(1. hydrogen sulfide, 2. methyl mercaptan, 3. dimethyl sulfide)

Table 1. Concentration of total VSCs before and after tongue coating removal(n=18).

	before removal(mean±SD, ppb)	after removal(mean±SD, ppb)	difference(mean±SD, ppb)
Total VSC	304.80±406.22	178.86±285.86	125.94±168.64**

**: p< 0.01

Table 2. Concentration of hydrogen sulfide before and after tongue coating removal(n=18).

	before removal(mean±SD, ppb)	after removal(mean±SD, ppb)	difference(mean±SD, ppb)
H_2S	191.88±253.57	113.73±189.57	78.15±119.00**

**: p< 0.01

Table 3. Concentration of methyl mercaptan before and after tongue coating removal(n=18).

	before removal(mean±SD, ppb)	after removal(mean±SD, ppb)	difference(mean±SD, ppb)
CH_3SH	84.73±139.04	48.12±76.73	36.60±67.93*

*: p< 0.05

Table 4. Concentration of dimethyl sulfide before and after tongue coating removal(n=18).

	before removal(mean±SD, ppb)	after removal(mean±SD, ppb)	difference(mean±SD, ppb)
$(CH_3)_2S$	28.20±33.99	17.01±24.33	11.19±15.37**

**: p< 0.01

Table 5. The reduction rate(%) of total and individual components of VSC

	H_2S	CH_3SH	$(CH_3)_2S$	Total VSC
Reduction(%)	43.62**	38.88*	30.21**	41.71**

**: p< 0.01

Table 6. The proportion(%) of each volatile sulfur compounds before and after tongue coating removal(n=18)

	H_2S(%)	CH_3SH(%)	$(CH_3)_2S$(%)
Before removal	59.96±22.27	25.08±13.12	14.96±22.39
After removal	59.52±22.11	24.85±16.03	15.63±23.12
p-value	0.917	0.952	0.680

Table 5 represent the reduction percentages of individual and total volatile sulfur compounds after tongue coating removal. It can be found that hydrogen sulfide was more reduced than other two components.

Considering the statistical interpretations and the reduction amount of hydrogen sulfide together, it can be postulated that the reduction of total VSC is mainly due to the decrease in hydrogen sulfide concentration.

As can be seen in the Table 6, hydrogen sulfide and methyl mercaptan together constituted about 85 % of total volatile sulfur compounds before tongue coating removal, and this proportion was not markedly altered after tongue coating removal ($p>0.05$).

Ⅳ. DISCUSSION

In this study, the measurement of oral malodor was carried out by gas chromatography. Although there are simple ways to assess oral malodor such as organoleptic and portable sulfide monitor measurement, the gas chromatographic analysis has many advantages over these simple measurements. First it is possible to separate and measure individual gases, and extremely low concentrations of gases can be detected.[5] Moreover, objective results can be obtained. But its cumbersome nature and high cost hindered the clinician from adopting this methods in oral malodor measurement.

Despite its accurate and reproducible analysis, there was no standard procedure about gas chromatographic run. For example, in the past studies, the oven temperature was usually 70℃ [8,9,26,36] or 58 to 72℃[10-12] or 80℃,[13] which is called isothermal run condition. But more than 99% of human oral expired air is composed of CO_2 and H_2O.[8] So oven temperature must be at least 100℃ during gas chromatographic run, otherwise column conditioning will be required at each analysis. So temperature programming run condition was used in this study in order to obtain more reliable and precise results. Regarding the column preparations, in the previous studies,[8-12,14,26,33,35,36] teflon columns were used. But the teflon column has many disadvantages for studying human oral malodor. Repeated temperature changes make the teflon column more rigid and as a result gas leakage is more prone to occur. And polymer packing in teflon column is difficult. Moreover teflon column has low thermal stability and low dimensional stability onto the pressure of carrier flow. To avoid these defects, the glass column was used. Fig. 2 is the typical chromatographic recording of oral malodor by this method.

The results of the present study using this method was that total VSC concentration was decreased by 41.71% after tongue coating removal in periodontally healthy persons. The tongue, as a primary factor in malodor formation, has large amounts of bacteria which are present in fissures and between mucosal papillae. These fissures and crypts may create an environment where the microorganisms are protected from the flushing action of the saliva.[32] The tongue coating is composed of blood components and other nutrients, large amounts of desquamated epithelial cells and bacteria. Therefore it can be suggested that tongue coating has its own putrefactive capacity to produce large amounts of volatile sulfur compounds and other malodorous molecules.[32] But, despite tongue coating removal, the rest 58.29% of VSC were remained in intra-oral air. This suggests that there are many sites in which the VSC emanates in oral area. These sites may be the oral mucosa, plaques adhering to the teeth and interproximal area, saliva, and so on.

Yaegaki and Sanada[26] had found that immediately after tongue cleaning, total sulfur production decreased by 51.8% in the group with a probing depth of less than 4 mm, and by 49.0% in patients with periodontal disease with a probing depth of 4 mm or more. But in that study, the tongue dorsal surface was cleaned with cotten pellets immersed in physiologic saline after tongue coating removal. Therefore the difference between the results of these two studies can be explained by the difference in the procedures of tongue coating scraping. Moreover instead of direct injection, sample loop was used in that study and oven temperature was also different. So the modifications of gas chromatographic run might also play a part in this differences.

In the present study, all subjects had healthy periodontium, and the results were as follows; approximately 59% of total VSC was the hydrogen sulfide and the reduction of total VSC was mainly due to hydrogen sulfide decrease in concentration. This results agree with the previous findings in which Yaegaki *et al.*[38] announced that methyl mercaptan was the main component of VSC in patients with periodontal involvement and hydrogen sulfide was the main constituent in orally healthy subjects.

As previously described, it has been believed that hydrogen sulfide and methyl mercaptan constitute over 90% of total VSC in human oral malodor.[8,14]

But the results of this study are somewhat different. Hydrogen sulfide and methyl mercaptan were found to constitute about 85% of total VSC before tongue coating removal in this experiment. This difference is thought to arise from the modifications of gas chromatographic run conditions. That is, the previous studies used isothermal oven temperature condition, especially below 100℃ in gas chromatographic analysis. And the column was packed with different polymers between these studies. Moreover, sample injection were performed mainly by sample loop method. That is, there was no consensus in gas chromatographic analysis for oral malodor. Considering some other volatile sulfur compounds which can be eluted in later retention times(data not shown), this proportion will be more lowered. Furthermore this proportion was not altered by tongue scraping in the periodontally healthy persons. It can be conjectured from the finding that the superficial and deep layer of tongue coating is not different in VSC composition.

Although the subjects were confined to periodontally healthy persons in this experiment, more studies about oral malodor in periodontal patients are also required.

The preliminary study revealed that the VSC with higher molecular weight(ex. diethyl sulfide and dimethyl disulfide) were usually detected in intra-oral air in moderate to severe malodorous patients which was sampled especially early in the morning. These compounds has been rarely described in early studies. So further researches for the role of these compounds in malodor formation are needed.

In clinical standpoint, people perceive oral malodor of other persons at some distance from them. But previous studies including this experiment confined oral air collection in intra-oral space. Therefore the researches for more accurate and precise methods in sample collection from the around of the mouth will be required in future.

V. CONCLUSIONS

Eighteen subjects were involved in the present study, who were periodontally healthy but complained of oral malodor. To estimate the effect of tongue coating on oral malodor formation, total and each concentrations of volatile sulfur compounds were compared between before and after tongue coating removal by gas chromatographic analysis.

The obtained results were as follows;

1. Total volatile sulfur compounds were composed of hydrogen sulfide(59.96%), methyl mercaptan (25.08%) and dimethyl sulfide(14.96%). Among these components, hydrogen sulfide(about 60%) was the main VSC in oral malodor patients with healthy periodontium.
2. The total concentrations of VSC were significantly reduced by 41.71% after tongue coating removal($p<0.01$).
3. After tongue coating removed, the concentration of hydrogen sulfide was reduced by 43.62% ($p<0.01$), and that of methyl mercaptan and dimethyl sulfide were reduced by 38.88%($p<0.05$) and 30.21%($p<0.01$) each.
4. The proportions of VSC was not altered after tongue coating removal($p>0.05$).

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국문초록

구취 환자에서 설태가 휘발성 황화합물의 생성에 미치는 영향에 관한 연구

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본 연구에서는 구강 내 공기 중 설태 제거 전후의 휘발성 황화합물 농도를 gas chromatography를 이용하여 비교 분석하였다. 피검자로는 서울대학교 치과병원 구취클리닉에 내원한 환자 중에서 치주 건강 상태가 양호하며 구취를 호소하는 환자 18 명(평균연령 31.4세; 남자 8명, 여자 10명)을 대상으로 하였으며 구취를 측정하기 전에 모든 피검자들은 실험 전날 취침 전부터 실험 당일 실험시작 전까지 음식 섭취나 양치질 등의 모든 구강 활동을 금지하였다. 구취 시료는 채취 전에 피검자로 하여금 3분간 입을 다물게 한 후 입을 약 2cm정도 벌린 상태에서 시행하였으며 시료 채취 후 설태를 제거하였다. 설태 제거 후에 구강 내 공기를 다시 채취한 후 gas chromatography를 통하여 휘발성 황화합물의 각 성분별 농도를 분석하였다.

분석과정에서는 과거에 휘발성 황화합물의 검출 시 사용되어진 sampling loop와 isothermal run condition 대신 좀더 효율적인 직접표본주입방법과 oven temperature programmed analysis를 시행하였다.

1. 전체 휘발성 황화합물은 Hydrogen sulfide (59.96%), Methyl mercaptan (25.08%), Dimethyl sulfide (14.96%)로 구성되었다. 이 중 Hydrogen sulfide는 전체 휘발성 황화합물중 약 60%를 차지하여 치주상태가 양호한 구취환자에서의 주요한 구취 구 성 성분이었다.
2. 설태 제거 후 전체 휘발성 황화합물의 농도감소는 제거 전에 비하여 41.71%로 유의 하게 감소하였다($p<0.01$).
3. 설태 제거 후에 Hydrogen sulfide의 농도감소는 43.62% ($p<0.01$), Methyl mercaptan 의 농도감소는 38.88% ($p<0.05$), 그리고 Dimethyl sulfide의 농도감소는 30.21% ($p<0.01$)로 각각 유의하게 감소하였다.
4. 전체 휘발성 황화합물의 구성비율은 설태 제거 전후에 유의한 차이가 없었다 ($p>0.05$).

주요어 : 구취, 가스 크로마토그래피, 설태, 휘발성 황화합물