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# A Study on the Evaluation of Oral Malodor Reduction Effect of Zinc Citrate-Containing Dentifrice on Night Use

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**Objective:** This study was conducted to confirm the oral malodor reduction effect of zinc citrate-containing dentifrice on night use.

**Methods:** This study was designed as a parallel, randomized, double-blind, and control trial for 3 weeks. After 1-week run-in period, the subjects were randomly assigned to the experimental groups (zinc-citrate-containing-dentifrice using group) or the control group. After 3 days, 5 days, 1 week, 2 weeks, and 3 weeks, oral malodors were measured and compared.

**Results:** As a result of measurement of volatile sulfur compounds using Oralchroma, before the experiment, the experimental group 3.01 ng/10 ml and the control group 3.03 ng/10 ml, after 3 days, 2.77 ng/10 ml, 3.01 ng/10 ml, after 5 days, 2.67 ng/10 ml, 2.95 ng/10 ml, after one week, 2.62 ng/10 ml, 2.96 ng/10 ml, and decreased to 2.50 ng/10 ml and 2.92 ng/10 ml after 2 weeks.

**Conclusion:** In case of using the dentifrice for the oral malodor using zinc citrate once before sleeping, It was confirmed that the effective and quick oral malodor reduction effect can be expected compared with the existing dentifrice.

**Keywords:** oral malodor, zinc citrate-containing dentifrice

## Introduction

Oral malodor, which has emerged as an important issue in modern society, is the odor coming from the oral cavity or nasal cavity [1,2]. Oral malodor is an uncomfortable situation that most people will suffer, but it is usually a temporary discomfort. However, 50% of people suffer from chronic oral malodor, and half of them are experiencing problems such as personal discomfort or social discomfort [3]. In the majority of adults, oral malodor is a common problem that has a significant impact on social life [1].

In most cases, oral malodor is caused by the oral itself as a result of bacterial decay of host components and food residues in the mouth [2,4].

The most successful treatment of oral malodor, because it is the main cause of oral malodor microbial mouth is just as important as cleaning the surface of the tongue is a mechanical method using a dentifrice and mouthwashes that the anti-microbial action [5].

Paste-type dentifrice that is commonly used to include a main

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component, a surfactant, a binder, a wetting agent for the prevention of oral diseases. Other ingredients such as water, flavor, sweetener, preservative, or coloring agent or bleach may be added [6,7].

As dentifrice is introduced into modern society, dentifrice has been developed and used so that dentifrice can be used for specific use for better oral health care as interest in oral health increases [8].

Dentifrice has been developed for oral malodor reduction and dentin hypersensitivity treatment. Among them, calcium glycerophosphate for reducing dentin hypersensitivity and zinc citrate for oral malodor reduction are attracting attention [9,10].

The properties of zinc as anti-plaque agents have been known for a long time. Zinc ions in the dental plaque maintain the dental plaque pH high in the presence of sugar to inhibit the metabolism of the dental plaque and prevent tooth surface accumulation in the dental plaque [11-13]. In addition, since long-term use of zinc-containing products does not exhibit destruction of normal bacteria in the oral cavity or other side effects, their effectiveness in suppressing dental plaque accumulation may be more effective in long-term use. In recent laboratory experiments and clinical trials, the inhibitory action of zinc on dental calculus formation has also been reported, which is known to be attributable to the secondary characteristics of zinc ions, namely the crystallization-inhibiting properties of zinc [14,15]. Zinc also inhibits bacterial acid production and metabolism in the dental plaque and ultimately inhibits bacterial growth. These metal ions are retained by the dental plaque and have been observed to bind within and outside the cell [16,17].

In this study, we measured the oral malodor concentration by period and experimented to confirm the reduction effect of the oral malodor concentration by using zinc citrate - containing dentifrice before bedtime.

**Table 1.** Gender and age distribution of subjects

| Gender | Age (yr) | | |
|---|---|---|---|
| | Total | 20s | 30s |
| Total | 60 | 32 | 21 |
| Male | 30 | 15 | 9 |
| Female | 30 | 17 | 12 |

**Table 2.** Principal component combination of experiment dentifrice (S dentifrice)

| Ingredients | Contents (%) |
|---|---|
| Potassium glycyrrhizinate | 0.01 |
| Cetylpyridinium chloride | 0.05 |
| Dental type silica | 15.00 |
| Zinc citrate (Trihydrate) | 0.50 |

# Materials and Methods

## 1. Subject

### 1) Subject recruitment

After the advertisement for the students and ordinary people attending Dankook University, the applicants were confirmed the subject selection criteria and exclusion criteria, and 60 subjects were confirmed.

### 2) Subject distribution

Table 1 shows the gender and age distribution of all subjects.

### 3) Study subjects dentifrice

The study dentifrice proceeded with three dentifrices: experimental dentifrice, control dentifrice and standard dentifrice. Details of each dentifrice are shown in Table 2-4.

(1) Experiment dentifrice (S dentifrice): Experiment dentifrice2 was prepared by using dentifrice, which is composed mainly of potassium glycyrrhizin, cetylpyridinium chloride, dental type silica and zinc citrate (trihydrate).

(2) Control dentifrice (R dentifrice): Dentifrice, which is composed mainly of dental type silica and sodium fluoride, was used.

(3) Standard dentifrice (T dentifrice): The standard dentifrice used dentifrice with the same composition as the control dentifrice.

## 2. Method

### 1) Subject group assignment

This study was designed as a parallel, randomized, double-blind, control experiment for 3 weeks. If an applicant who

**Table 3.** Principal component combination of Control dentifrice (R dentifrice)

| Ingredients | Contents (%) |
|---|---|
| Dental type silica | 19.00 |
| Sodium fluoride | 0.22 |

**Table 4.** Principal component combination of Standard dentifrice (T dentifrice)

| Ingredients | Contents (%) |
|---|---|
| Dental type silica | 19.00 |
| Sodium fluoride | 0.22 |

has signed the test agreement by voluntary registration is registered, Applicants will be assessed for compliance with the selection/exclusion criteria through a visit assessment. After a 1-week run-in period, the selected subjects were randomly assigned to one of the experimental group and the control group according to the registered order so that 30 subjects were distributed per group. And, this study was approved by the IRB of the Dankook University (IRB: DKU IRB 2015-10-012-002).

### 2) How to use and duration of dentifrice

(1) Experiment 1st week, 2nd week

① Using the standard dentifrice (T dentifrice), Brushing should be performed using rolling method for 3 minutes three times a day (after breakfast, after lunch, after dinner).

② One dentifrice applied to the subject one time a day before going to bed should be brushing for 3 minutes using rolling method.

③ Apply dentifrice: The experimental group-Experiment dentifrice (S dentifrice). The control group-Control dentifrice (R dentifrice).

(2) Experimental 3rd week: Using the standard dentifrice (T dentifrice), Brushing should be performed using rolling method for 3 minutes four times a day (after breakfast, after lunch, after dinner).

### 3) Observation test method (by visit)

(1) Visit 1 (Baseline visit, day 0): This visit is held at intervals of one week or more after being assigned to screening. First, we measured the index of oral examination, examined the usual eating pattern, and then instructed the brushing method by rolling method. Then, the applied dentifrice was distributed and then the dentifrice was used to perform brushing.

(2) Visit 2 (after 3 days): This visit was made 3 days after visit 1 (day 0), and the adverse reaction was confirmed and the oral examination index was measured.

(3) Visit 3 (after 5 days): This visit was made 5 days after visit 1 (0 day), and the adverse reaction was confirmed in the same manner as visit 2, and the oral examination index was measured.

(4) Visit 4 (after 1 week): This visit was made 1 week after visit 1 (0 day), and the adverse reaction was confirmed in the same manner as visit 2, and the oral examination index was measured.

**Table 5.** The changes in the results of oral malodor test (unit: Oralchroma, ng/10 ml; BBchecker, BBV)

| Value | Group | Number | | Base | After 3 days | After 5 days | After 1 week | After 2 weeks | After 3 weeks |
|---|---|---|---|---|---|---|---|---|---|
| HS | S | 30 | Mean | 1.20 | 1.05 | 1.03 | 0.84* | 0.89* | 0.96 |
| | | | SD | 0.58 | 0.60 | 0.53 | 0.64 | 0.56 | 0.55 |
| | R | 30 | Mean | 0.91 | 1.07 | 1.07 | 1.06 | 1.14 | 0.91 |
| | | | SD | 0.70 | 0.60 | 0.60 | 0.64 | 0.63 | 0.64 |
| | | p-value | | 0.092 | 0.881 | 0.815 | 0.183 | 0.110 | 0.775 |
| MM | S | 30 | Mean | 0.40 | 0.36 | 0.40 | 0.35 | 0.37 | 0.34 |
| | | | SD | 0.18 | 0.18 | 0.20 | 0.23 | 0.19 | 0.15 |
| | R | 30 | Mean | 0.44 | 0.45 | 0.41 | 0.43 | 0.40 | 0.39 |
| | | | SD | 0.32 | 0.22 | 0.20 | 0.27 | 0.20 | 0.22 |
| | | p-value | | 0.531 | 0.076 | 0.749 | 0.199 | 0.670 | 0.377 |
| DMS | S | 30 | Mean | 1.42 | 1.37 | 1.24 | 1.43 | 1.24 | 1.28 |
| | | | SD | 0.74 | 0.67 | 0.70 | 0.92 | 0.57 | 0.79 |
| | R | 30 | Mean | 1.68 | 1.48 | 1.47 | 1.47 | 1.39 | 1.58 |
| | | | SD | 1.08 | 0.83 | 0.99 | 0.90 | 0.73 | 0.72 |
| | | p-value | | 0.283 | 0.549 | 0.307 | 0.865 | 0.386 | 0.133 |
| VSC | S | 30 | Mean | 3.01 | 2.77 | 2.67* | 2.62* | 2.50* | 2.58* |
| | | | SD | 0.98 | 1.10 | 0.89 | 1.22 | 0.76 | 0.99 |
| | R | 30 | Mean | 3.03 | 3.01 | 2.95 | 2.96 | 2.92 | 2.88 |
| | | | SD | 1.56 | 1.18 | 1.12 | 1.06 | 0.80 | 0.91 |
| | | p-value | | 0.959 | 0.430 | 0.290 | 0.245 | 0.042 | 0.234 |
| BB | S | 30 | Mean | 75.43 | 72.93 | 64.47* | 66.97* | 62.67* | 64.27* |
| | | | SD | 22.75 | 26.61 | 21.76 | 30.10 | 18.74 | 25.04 |
| | R | 30 | Mean | 77.03 | 76.00 | 74.43 | 73.97 | 72.83 | 72.10 |
| | | | SD | 36.86 | 29.13 | 26.59 | 25.10 | 20.43 | 21.54 |
| | | p-value | | 0.840 | 0.672 | 0.118 | 0.332 | 0.049 | 0.199 |

S: experimental group, R: control group, HS: hydrogen sulfide, MM: methyl mercaptan, DMS: dimethyl sulfide, VSC: volatile sulfur compounds, BB: BB checker value by BB checker, SD: standard deviation. p-value: p-value by 2-sample t-test. *$p < 0.05$ by paired t-test between base and after.

(5) Visit 5 (after 2 weeks): This visit was performed two weeks after visit 1 (day 0), and the adverse reaction was confirmed in the same manner as visit 2, and the oral examination index was measured.

(6) Visit 6 (Closing visit, after 3 weeks): The visit was conducted three weeks after visit 1 (day 0), and the adverse reaction was confirmed in the same manner as visit 2, and the oral examination index was measured.

#### 4) Oral malodor test

The oral malodor test was performed using BB Checker (Plustech, Daejeon, Korea) and Oralchroma (Abilit, Tokyo, Japan). The measurement time was measured six times in total, 0 days before the experiment, 3 days after the experiment, 5 days, 1 week, 2 weeks and 3 weeks.

#### 5) Data analysis and statistical methods

The changes in oral malodor measurements were compared using the BB Checker (Plustech) and Oralchromam measurements. At this time, comparisons between groups were made by 2-sample t-test and Mann-Whitney test. Comparisons within the groups were analyzed by paired t-test and Wilcoxon's signed rank test.

## Results

The changes in the results of oral malodor test were shown in Table 5.

## Discussion

Bacteria in the mouth hydrolyze proteins and break down amino acids to produce ammonia, volatile sulfur compounds, lactic acid and other constituents. The final product of odor-causing decay by oral bacteria is mainly Volatile sulfur compounds (VSC), cause of oral malodor [18].

As modernization becomes more important, interpersonal relationships become more important and oral malodor becomes one of the major factors of social and mental disability [19].

Many attempts have been made to control such oral malodor, which usually include the use of appropriate oral care products such as mouthwash and dentifrices for oral malodor, as well as appropriate treatment by specialists and interdental toothbrushes and floss. Products containing cetylpyridinium chloride and zinc chloride as mouthwash have been widely used for oral malodor control [20].

Zinc compounds have been widely used for cosmetics, medicines and dental preparations since ancient times because of their excellent medical utility. In particular, the biological action of zinc in the oral cavity is well known. Zinc influences microorganisms in the oral cavity by various mechanisms, and acts on antibacterial, anti-dental plaque, and anti- dental caries effects [21].

In addition, zinc has been widely used as a treatment for oral malodor because it affects oral anaerobic bacteria and oral volatile sulfur compounds that cause oral malodor [21].

Kim et al. [22] reported that oral malodor can be reduced for 3 hours after brushing with a mouthwash solution containing zinc, It is less toxic than other metals and does not cause tooth staining. Because of its low price, it has many advantages as a preventive and therapeutic medicine for oral infection, dental caries, peridontal disease and oral malodor.

In this study, Oralchroma and BB checker, which are simple gas chromatographs, were used to measure volatile compounds. Oralchroma can be isolated by separating sulfur compounds such as hydrogen sulfide and methyl mercaptan, which are major causes of oral malodor. The BB checker is a measuring instrument that can measure the total amount of gas in the breath.

As a result of this experiment, oral malodor measurement by Oralchroma showed a statistically significant decrease in oral malodor after 5 days in the experimental group. In addition, although the level of volatile sulfur compounds increased in the 3 weeks after discontinuation of the dentifrice for 1 week, the oral malodor reduction effect was maintained.

The results of the oral malodor measurement using the BB checker showed that the experimental group showed a statistically significant decrease in oral malodor after 5 days. After 3 weeks of discontinuation of dentifrice for 1 week, oral malodor was increased but oral malodor reduction was maintained to some extent.

According to Heo et al. [23], Oral malodor was the most severe immediately after sleeping, and thereafter oral malodor was decreased by eating. It is believed that oral residues are retained during the sleeping time without food intake or muscle movements in the oral cavity, resulting in oral malodor. Therefore, the results of this study showed that the effect of dentifrice which can inhibit oral malodor before sleeping was shown to be effective even though it was limited to once a day.

In conclusion, if we develop dentifrice and mouthwash containing zinc citrate and use it well, we can control oral malodor effectively in patients with oral malodor.

## Conclusion

To evaluate the oral malodor reduction effect of the experimental dentifrice containing zinc citrate and the comparative dentifrice for oral malodor and the control dentifrice

except for the main ingredient in the experimental dentifrice, the experimental group of 30 volunteers. The control group of 30 volunteers were asked to use the dentifrice for a total of 60 volunteers for 3 weeks. Before experiment, after 3 days, 5 days, 1 week, 2 weeks, 3 weeks, over a total of six times, as a result of using the Oralchroma and BB checker comparison to record the degree of oral malodor, were as follows.

1. As a result of measurement of volatile sulfur compounds using Oralchroma. Before the experiment, the experimental group 3.01 ng/10 ml and the control group 3.03 ng/10 ml, after 3 days, 2.77 ng/10 ml, 3.01 ng/10 ml, after 5 days, 2.67 ng/10 ml, 2.95 ng/10 ml, after one week, 2.62 ng/10 ml, 2.96 ng/10 ml, after 2 weeks, it decreased to 2.50 ng/10 ml and 2.92 ng/10 ml. The experimental group showed a statistically significant decrease in oral malodor after 5 days. In addition, the value of volatile sulfur compounds increased to 2.58 ng/10 ml after 3 weeks of discontinuation of dentifrice for 1 week, but oral malodor reduction was maintained.

2. The experimental group was 75.43 BBV and the control group was 77.03 BBV before the experiment, 72.93 BBV and 76.00 BBV after 3 days, 64.47 BBV and 74.43 BBV after 5 days, 66.97 BBV and 73.97 BBV after one week, 62.67 BBV and 72.83 BBV after two weeks. And the experimental group showed a statistically significant decrease in oral malodor after 5 days. After 3 weeks of discontinuation of dentifrice for 1 week, the measured value was 64.27 BBV, indicating that oral malodor reduction effect was maintained to some extent although oral malodor level was increased.

As a result of these studies, In case of using the dentifrice for the oral malodor using zinc citrate once before sleeping. It was confirmed that the effective and quick oral malodor reduction effect can be expected compared with the existing dentifrice.

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