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**Research Paper** 

# Association of tongue brushing with the number of fungiform taste buds and taste perception: A preliminary study using confocal laser scanning microscopy in combination with a filter-paper disc method



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## ABSTRACT

*Objective:* The aim of this study was to investigate the association of tongue brushing with the number of fungiform taste buds and taste perception using a confocal laser scanning microscopy in combination with a filterpaper disc method (FPDM).

*Methods*: Twenty-four subjects with or without a habit of tongue brushing (11 males and 13 females, 20–46 years old) participated in this study. Nine of the 24 subjects had no habit of tongue brushing (Group 1, n = 9). Fifteen subjects had a habit of tongue brushing, and the brushing regions of the tongue were as follows: central region (Group 2, n = 7), or entire region (Group 3, n = 8) of the tongue dorsum. Using confocal laser scanning microscopy, the average number of taste buds per fungiform papilla (FP) was counted. Taste perception was evaluated using an FPDM. These observations were performed in the midlateral region of the tongue since the distribution of fungiform papillae is large in the midlateral region compared to that in the central region. *Results*: The subjects in Group 3 showed a significantly decreased number of fungiform taste buds compared to Group 1 and Group 2. Group 3 also showed significantly higher FPDM scores than the other two groups. *Conclusions*: Excessive tongue brushing of the entire tongue dorsum, including the midlateral region, may have

an association with the decreased number of FP and taste buds and decreased taste sensation. To avoid these conditions, instituting proper tongue brushing methods, such as limiting it to the central region of the tongue and using a light touch, is suggested and is important for the subjects who are eager to participate in tongue brushing.

#### 1. Introduction

Tongue brushing has been practiced since ancient times (Christen & Swanson, 1978), and this tongue cleaning method is usually performed to remove tongue coating, which is known to consist of blood debris, nutrients, large amounts of desquamated cells and bacteria, as these are causes of oral malodor (Calil et al., 2009; Hughes & McNab, 2008; Lu, Tang, Chen, Wong, & Ye, 2014). White and Armaleh (2004) recommended tongue brushing to reduce the number of bacteria in the oral cavity. Matsui, Chosa, Minami, Kimura, and Kishi (2014) reported that tongue cleaning reduced the amount of bacteria in the tongue coating based on real-time PCR analyses. In another study,

Gross, Barnes, and Lyon (1975) reported that tongue brushing reduced tongue coating as well as dental plaque formation, suggesting that tongue coating is one of the reservoirs of dental plaque.

In contrast, tongue brushing may have potential for influencing fungiform papillae (FP) and taste buds. For example, it is noted that a hard tongue cleaner or brush is possibly abrasive to the soft, sensitive structures of the tongue. During a pilot study, one of the authors (T. S.) noticed a glazed tongue, with confocal microscopy, in a middle-aged woman who had the habit of vigorously brushing her tongue 4–5 times a day for many years. Since taste buds open onto the surface of papillae (Miller & Reedy, 1990) and gustatory receptors exist in taste pores on the surface of taste buds, we thought that vigorous tongue brushing

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could have an association with taste buds number and taste disturbances. However, to date, there have been no reports concerning the association of tongue brushing with FP and taste buds.

Many studies have looked at FP noninvasively in relation to taste function (Webb, Bolhuis, Cicerale, Hayes, & Keast, 2015). Recently, confocal laser scanning microscopy has been utilized as a non-invasive observation method. This method was first developed to observe the corneal surface and was then applied to observe the tongue surface (Just, Stave, Pau, & Guthoff, 2005). It enables researchers and clinicians to observe surface morphology of FP and taste buds in vivo (Just, Stave et al., 2005; Just, Pau et al., 2005; Just, Srur, Stachs, & Pau, 2009; Saito et al., 2014, 2015; Saito, Ito, Ito, Manabe, & Sano, 2016; Saito, Ito, Ito, & Manabe, 2016; Saito et al., 2016). To clarify the association of tongue brushing with fungiform taste buds and taste perception, we investigated the number of fungiform taste buds using confocal laser scanning microscopy and taste perception using a filter-paper disc method (FPDM) in the subjects with or without a habit of tongue brushing.

## 2. Materials and methods

#### 2.1. Subjects

Twenty-four subjects were recruited for this study (Table 1). Nine of the 24 subjects had no habit of tongue brushing (Group 1, n = 9). Fifteen had a habit of tongue brushing and filled out a questionnaire about their method of tongue brushing, such as duration and brushing region on the tongue (central region (Group 2, n = 7) or entire region (Group 3, n = 8) of the tongue dorsum). After explaining the significance of this study, the subjects agreed to participate in this study and gave informed consent. The study was approved by the Institutional Research Board (Ethical Committee of the University of Fukui, Faculty of Medical Sciences; No. 20150087).

#### 2.2. Confocal laser scanning microscopy

A confocal laser scanning microscope (Heidelberg Engineering, Heidelberg, Germany), consisting of the Heidelberg Retina Tomograph HRT II and Rostock Cornea Module, was employed to observe FP and taste buds. For illumination, a diode laser at 650 nm was used. A water immersion lens ( $\times$ 65) was used to take images. The resolution depth and field of view were approximately 1 to  $2 \,\mu m$  and  $400 \times 400 \,\mu m$ , respectively. For each subject, 10 FP on the right and left midlateral regions of the tongue as shown in Figs. 1A and 2A (elliptic open circle), were stained with 0.5% methylene blue solution under an operating microscope ( $\times 10$ ). The size of the counted area was not measured. The subjects were allowed to relax between scans and no subjects had trouble holding still by pressing their tongue dorsum on the objective lens. After the dorsum of the tongue was pressed on the objective lens, the stained FP were observed using a confocal laser microscope. By using the z-scan technique, taste pores and taste buds in the FP could be observed. The images of taste pores and taste buds were saved as image files and video sequences (Saito et al., 2014, 2015; Saito et al., 2016; Saito, Ito, Ito, & Manabe, 2016; Saito, Ito, Ito, Manabe, & Sano, 2016). The scan of a single papilla took 30 s and 20 scans took approximately 30 min. Then, the number of taste buds with taste pores per FP was counted, and the average number of taste buds per FP was calculated for quantitative analyses. One of the authors (T.I.) operated the confocal laser microscope.

#### 2.3. Filter-paper disc method (FPDM)

Immediately before observing with the confocal laser microscope, taste perception was evaluated using the FPDM. FPDM was developed for qualitative and quantitative clinical gustometry using filter-paper discs (Tomita, Ikeda, & Okuda, 1986). A commercial assay kit (Taste Disc<sup>\*</sup>, Sanwa Chemicals, Nagoya, Japan) was used. Taste solutions consisted of sucrose (sweet), sodium chloride (salty), tartaric acid

Table 1

Average number of taste buds per fungiform papilla and average filter-paper disc method (FPDM) score in the 24 subjects with or without tongue brushing.

| Group    | Case        | Age         | Sex        | Duration of tongue brushing | Average number of TB per FP | Average FPDM score |       |      |        |               |
|----------|-------------|-------------|------------|-----------------------------|-----------------------------|--------------------|-------|------|--------|---------------|
|          |             |             |            |                             |                             | Sweet              | Salty | Sour | Bitter | Total average |
| Group 1: | the subject | s without a | a habit of | tongue brushing             |                             |                    |       |      |        |               |
|          | 1           | 38          | F          | 0 y                         | 0.3                         | 6                  | 6     | 5.5  | 6      | 5.9           |
|          | 2           | 42          | Μ          | 0 y                         | 0.5                         | 6                  | 6     | 4.5  | 5.5    | 5.5           |
|          | 3           | 25          | F          | 0 y                         | 4.3                         | 3.5                | 3     | 1    | 1.5    | 2.3           |
|          | 4           | 26          | F          | 0 y                         | 2.9                         | 4.5                | 2.5   | 4    | 3.5    | 3.6           |
|          | 5           | 26          | М          | 0 y                         | 4.1                         | 2                  | 4.5   | 3.5  | 5      | 3.8           |
|          | 6           | 26          | М          | 0 y                         | 4.7                         | 4.5                | 4.5   | 4    | 3.5    | 4.1           |
|          | 7           | 35          | F          | 0 y                         | 2.3                         | 3                  | 3     | 3    | 3.5    | 3.1           |
|          | 8           | 38          | М          | 0 y                         | 3.9                         | 4.5                | 5     | 4    | 3.5    | 4.3           |
|          | 9           | 33          | Μ          | 0 y                         | 5.4                         | 6                  | 3.5   | 3    | 4      | 4.1           |
| Group 2: | the subject | s who brus  | h central  | region of the tongue dorsum |                             |                    |       |      |        |               |
| -        | 1           | 27          | F          | 10 y                        | 1.2                         | 2.5                | 5.5   | 4.5  | 2.5    | 3.8           |
|          | 2           | 26          | F          | 2 y                         | 0.3                         | 5.5                | 6     | 6    | 6      | 5.9           |
|          | 3           | 39          | М          | 20 y                        | 8.2                         | 4                  | 4     | 3    | 4      | 3.8           |
|          | 4           | 36          | М          | 16 y                        | 3.0                         | 6                  | 6     | 5.5  | 5.5    | 5.8           |
|          | 5           | 32          | М          | 15 y                        | 3.7                         | 5.5                | 6     | 5.5  | 6      | 5.8           |
|          | 6           | 20          | М          | 10 y                        | 2.4                         | 3                  | 2.5   | 3    | 4.5    | 3.3           |
|          | 7           | 27          | F          | 10 y                        | 3.2                         | 3                  | 1.5   | 3.5  | 1.5    | 2.4           |
| Group 3: | the subject | s who brus  | h entire r | egion of the tongue dorsum  |                             |                    |       |      |        |               |
| -        | 1           | 26          | F          | 6 y                         | 0.8                         | 6                  | 6     | 6    | 6      | 6             |
|          | 2           | 25          | F          | 4 y                         | 0.5                         | 5.5                | 6     | 5.5  | 6      | 5.8           |
|          | 3           | 24          | F          | 2 y                         | 0.7                         | 6                  | 6     | 6    | 6      | 6             |
|          | 4           | 46          | F          | 3 y                         | 0.5                         | 6                  | 6     | 6    | 6      | 6             |
|          | 5           | 31          | М          | 15 y                        | 0.3                         | 6                  | 6     | 5.5  | 6      | 5.9           |
|          | 6           | 39          | М          | 10 y                        | 1.2                         | 6                  | 6     | 6    | 6      | 6             |
|          | 7           | 35          | F          | 25 y                        | 0.5                         | 6                  | 6     | 6    | 6      | 6             |
|          | 8           | 35          | F          | 10 y                        | 0                           | 6                  | 6     | 6    | 6      | 6             |

Abbreviations: F: female, M: male, FP: fungiform papilla, FPDM: filter-paper disc method, TB: taste bud, y: year.

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