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The Relation of Oral Function(s) and the Levels of Oral Bacteria in Community-Dwelling Older Adults Pilot Study

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ABSTRACT

Background: Daily management of oral hygiene reduces mortality risk in older adults. This study aimed to investigate whether oral function(s) are related to the levels of oral bacteria in community-dwelling older adults.

Methods: A cross-sectional pilot study was conducted. Oral functions, including chewing ability, maximum labial closing force, and maximum tongue pressure, were evaluated. Oral bacterial level was assessed using a rapid quantification system based on a dielectrophoresis impedance measurement method. The correlation between oral bacterial level and each variable was evaluated using Pearson correlation coefficient and multiple regression analysis. Multiple comparisons of variables according to oral bacterial levels were performed by analysis of covariance to adjust for confounders.

Results: All participants were women. Among oral functions, only maximum tongue pressure was inversely correlated with oral bacterial level, according to Pearson correlation coefficient($r = -0.69$, $p = 0.005$) and multiple regression ($B \pm SE = 0.081 \pm 0.026$, $p = 0.014$) analyses. The maximum tongue pressure in subjects with $10^{6.5}$ to 10^{7}colony-forming units (CFU)/ml of oral bacteria (39.2 ± 3.9) was significantly higher than in those with 10^{7} to $10^{7.5}$CFU/ml (30.2 ± 5.1) ($p = 0.004$) and $10^{7.5}$ to 10^{8}CFU/ml level (25.3 ± 2.3) ($p = 0.001$), adjusted for age.

Conclusion: Within the limitations of this pilot study, increasing tongue pressure was significantly associated with lower oral bacterial levels in community-dwelling older adults.

Keywords: Chewing ability, Labial closing force, Older adults, Oral bacteria, Tongue force

INTRODUCTION

As aging in Japanese society progresses, the proportion of individuals ≥65 years of age has reached 27.3%. Aging elicits sarcopenia, including characteristics of loss of muscle mass, strength and function, which leads to frailty in older individuals [1,2]. Sarcopenia is associated with multilayered physiological problems such as reduction in physical activity, neurological decline, hormonal changes, inflammatory pathway activation, fatty infiltration, and poor nutrition[2]. In addition, loss of muscle function increases the risk for fall-related injuries, which may lead to hospitalization and/or a bedridden state [3]. Masticatory functions consist of complicated, inter-related orofacial muscle movements, including coordinated movement of the tongue, buccal, and lips. These masticatory muscles are also influenced by aging-associated sarcopenia [4]. Loss of masticatory muscle function leads to less efficiency in reducing the mass of food bolus, and tends to result in the avoidance of hard-texture diets containing high fiber and protein [5], which in turn may induce protein-energy malnutrition, such as kwashiorkor and marasmus symptoms, in older individuals, resulting in sarcopenia. In addition, impaired swallowing function is closely related to malnutrition [6], A vicious circle of sarcopenia and malnutrition, which is common in older individuals, exacerbate the risk for frailty. A recent study demonstrated that weakness or impairment in oral function (i.e., "oral frailty") was defined as the co-existence of between 3 and 6 factors including: the number of natural teeth; chewing ability; articulatory oral motor skill; tongue pressure; and subjective difficulties in eating and swallowing [7]. Oral frailty

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appears with the initial symptoms of sarcopenia, and increases the risk for disability and mortality [7].

Aging is positively correlated with the presence of opportunistic infections, including methicillin-resistant *Staphylococcus aureus*, *Haemophilus influenza*, and *Candida albicans,* in the oral cavity [8]. Recent increasing number of remaining teeth cause a detrimental effect on the prevalence of periodontitis in older adults in Japan. A high prevalence of periodontitis in older adults increases the risk for carotid intima media thickness, hypertension, and atherosclerotic plaque formation, leading to cardiovascular diseases [9-11]. A relationship between periodontitis and pneumonia and/or mortality risk from pneumonia in older adults has also been identified [12,13]. Poor oral hygiene and/or ineffective denture cleaning are possible risk factors for pneumonia [14,15]. Multiple lines of evidence have suggested that aging-related growth of oral pathogens increase the risk for systemic disease among older adults.

The purpose of this study, therefore, was to investigate whether deterioration of oral function(s) is correlated with the levels of oral bacteria in community-dwelling older adults. Our hypothesis was that oral frailty appearing in the lips, tongue, and chewing ability are impacted by levels of oral bacteria.

METHODS

Study design and participants

Fifteen individuals who attended a seminar addressing food and health were surveyed in this cross-sectional study, which was performed in July 2017. Oral bacterial level and oral functions were assessed at 09:00-10:00 am. This study was conducted with the approval of the Medical Ethics Committee of Kyushu Dental University (No.17-1). Informed consent for participation was obtained from all subjects after written explanation of the study was provided.

Oral bacterial levels

Bacterial levels were measured using a rapid oral bacteria quantification system (Panasonic Healthcare Co. Ltd., Osaka, Japan), based on a dielectrophoresis and impedance measurement method [16,17]. The detection limit of this equipmentwas10^5colony-forming units (CFU)/ml. Definition of bacterial level conformed to manufacturer's instructions as follows: level 1, $<10^5$CFU/ml; level 2, 10^5 to 10^6CFU/ml; level 3, 10^6 to $10^{6.5}$CFU/ml; level 4, $10^{6.5}$ to 10^7CFU/ml; level 5, 10^7 to $10^{7.5}$CFU/ml; level 6, $10^{7.5}$ to 10^8CFU/ml; and level 7, $>10^8$CFU/ml.

Chewing ability test

Chewing ability was evaluated using Masticatory Performance Evaluating Gum XYLITOL (Lotte Co., Ltd., Tokyo, Japan).The test chewing gum contains citric acid, and red, yellow and blue dyes that change color when chewed [18]. The color of the chewing gum changes according to a scale from 0 to 5printed on its package, and was determined visually after chewing. The color of the chewed gum was assessed by a single registered dental hygienist.

Labial closing force measurement

Labial closing force was evaluated using Lipplekun (Shofu Co., Kyoto). This newly developed medical device consists of a measuring apparatus and spindle connected to a disposable button-type intraoral piece by dental floss. Labial closing force is measured with a range from 0 N to 19.9 N. Measurement was performed with subjects in a relaxed sitting position, who were asked to grasp the button type piece with their lips as tightly as possible. The measurement was performed 3 times, and the maximum value was considered to represent the labial closing force.

Tongue pressure measurement

Tongue pressure was evaluated using a specified tongue pressure measurement device (JMS Co, TPM-01) [19]. The TPM-01 is a newly developed, handheld manometry device consisting of a small balloon-type disposable oral probe. At zero calibration, the probe is inflated with air at a pressure of 19.6 kPa [20]. Measurement was performed with subjects in a relaxed sitting position, who were asked to squash the balloon-formed probe interposed between the tongue and palate with as much force as possible. The measurement was performed 3 times, and the maximum value represented tongue pressure.

Statistical Analysis

Experimental data are expressed as mean ± standard deviation (SD). Multiple regression analysis was used to analyze factors correlated with each variable. Analysis of covariance was performed for multiple comparisons adjusted for confounders. Comparison with each pair was analyzed by generalized linear model. Statistical analysis was performed using SPSS version 22 (SPSS Japan Inc., Tokyo Japan). Two-tailed p-values were calculated in all analyses; the alpha level for statistical significance was set at 0.05.

RESULTS AND DISCUSSION

Age and oral-related variables of the participants are summarized in **Table 1**. The age of the participants ranged from 55 to 84 years (mean age, 73.0 ± 7.0 years), and all were women. Oral bacterial level ranged from 4 to 6, corresponding to $10^{6.5}$ to 10^7CFU/ml to $10^{7.5}$ to 10^8CFU/ml, respectively. Oral function was assessed according to chewing ability, labial closing force and tongue pressure. Chewing ability was assessed using a colorimetric method involving chewing gum and scored 4 or 5 on a color scale. The mean value of the maximum labial closing force and maximum tongue pressure was 10.8 ± 2.8 N and 31.3 ± 6.2 kPa, respectively.

Table 1. Characteristics of participants

Variables	N	(%)
Age		
<70	6	40
71-80	6	40
>81	3	20
Bacterial Level		
4	3	20
5	10	66.7
6	2	13.3
Masticatory Test Gum		
4	8	53.3
5	7	46.7
Maximum Labial Pressure		
<10	5	38.5
>10	8	61.5
Maximum Tongue Pressure		
<30	5	33.3
>31	10	66.7

The correlation between oral function and oral bacterial level was assessed using Pearson mutual correlation coefficient (**Table 2).** Age had no correlation with any oral functions or oral bacterial level. Among the three oral functions, only maximum tongue pressure was inversely correlated with oral bacterial level ($r = -0.69$, $p = 0.005$).

Table 2. Correlation between bacterial level and oral functions

	Variables	1	2	3	4	5
1	Age	–				
2	Masticatory Test Gum	−0.27	–			
3	Maximum Labial Pressure	−0.01	0.17	–		
4	Maximum Tongue Pressure	0.05	0.46	0.32	–	
5	Bacterial Level	−0.17	-0.41	0.02	−0.69**	–

***: $p < 0.01$ analyzed by Pearson mutual correlation coefficient*

Subsequently, the correlation between oral functions and oral bacterial level was evaluated using multiple regression analysis (**Table 3).** Only maximum tongue pressure was significantly correlated with oral bacterial level ($p = 0.014$). The beta value (standard partial regression coefficient) of maximum tongue pressure was −0.921, indicating a higher weight than other two oral functions and age.

Considering these results, the maximum tongue pressure among three levels of oral bacteria (i.e., CFU/ml), adjusted according to age using generalized linear model, were compared (**Figure 1).** The maximum tongue pressure in participants at level 4 ($10^{6.5}$ to 10^{7}CFU/ml; 39.2 ± 3.9kPa) was significantly higher than in participants at level 5 (10^{7} to $10^{7.5}$CFU/ml; 30.2 ± 5.1kPa) ($p = 0.004$) and level 6 ($10^{7.5}$ to 10^{8}CFU/ml; 25.3 ± 2.3kPa) ($p = 0.001$).

Results of the present pilot study demonstrated that tongue strength is inversely correlated with oral bacterial level determined using a dielectrophoresis impedance measurement method. Age was not correlated with oral bacterial level or oral functions. Whereas many studies have reported a relationship between aging and oral frailty [21-23], there was no such correlation between age and oral function (chewing ability, labial closing force, and tongue pressure) in this study. Participants in this study reside independently in a community, and engage in a high level of social activities, evidenced by their attendance at this

seminar. In addition, the participants walked up a steep path to gather at the seminar site, suggesting the presence of sound systemic muscle strength, which may have a positive influence on the oral muscles. Age had no correlation with oral bacterial levels. The proportion of interdental brush usage has been reported to be 49.1% in community-dwelling older adults in Japan [24], suggesting that oral hygiene activities among individual older adults may be at a higher level.

Table 3. Multiple regression analysis on correlation between bacterial level and oral functions

Variables	B	SE	beta	p value
Constant	4.450	2.146		0.072
Age	0.007	0.020	0.091	0.744
Masticatory Test Gum	0.398	0.290	0.373	0.207
Maximum Labial Pressure	0.063	0.053	0.320	0.266
Maximum Tongue Pressure	−0.081	0.026	−0.921	**0.014**

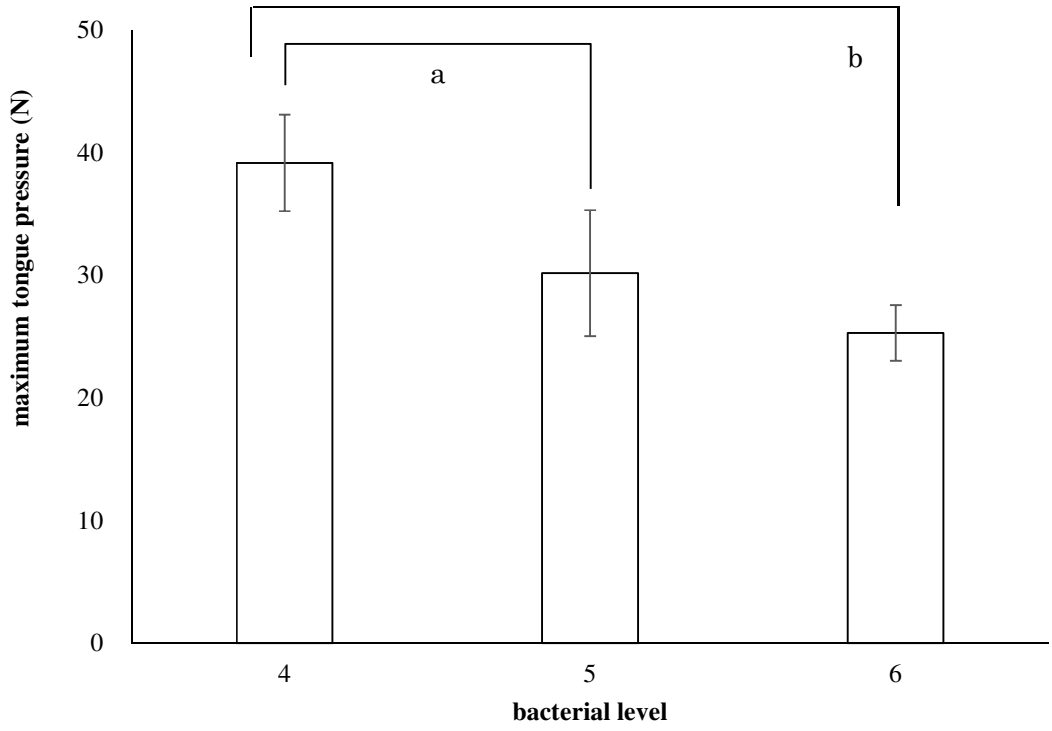

Figure 1. Multiple comparison of maximum tongue pressure by bacterial level adjusted by age analysis of variance: p = 0.016, a: p = 0.004, b: p = 0.001 analyzed by generalized linear model. Each bacterial level indicated as follow; $10^{6.5}$ to 10^{7} CFU/ml for level 4, 10^{7} to $10^{7.5}$ CFU/ml for level 5, and $10^{7.5}$ to 10^{8} CFU/ml for level 6.

During the swallowing process, the tongue continues to move with intricate maneuvers, and changes its shape and position. The sampling position of oral bacteria was the tongue dorsum, and results reflecting a correlation between maximum tongue pressure and oral bacterial level appear to be reasonable. Multiple studies have reported that age is inversely related to maximum tongue pressure in older adults [25-28]. Other studies have demonstrated that a prolonged swallowing phase in older subjects is related to changes in the dynamics of tongue movement compared with younger subjects [29]. In contrast, maximum tongue pressure had no correlation with age in this study. Given that

participants had high activity levels, such attending the seminar, our results suggest that preservation of active and independent living in older adults may require sound oral function, including adequate tongue pressure.

The labial closing force plays an important role in holding the food bolus in the oral cavity, and maintaining negative pressure during swallowing. Previous studies have reported that labial closing force is positively correlated with hand grip force in older adults [30], suggesting a close relationship with frailty. Labial closing force is a critical factor for swallowing capacity in stroke patients with subclinical facial paresis; however, these two factors have no relationship in healthy subjects [31]. That study also demonstrated that labial closing force had no correlation with age.[31]In the present study, all participants were healthy, which may explain why labial closing force was not correlated with other oral functions or bacterial levels. In addition, our results also indicated no relationship between labial closing force and age, which is consistent with previous studies.

This pilot study had several limitations, the first of which was the small number of subjects and its preliminary nature. Whereas neither chewing ability nor maximum labial closing force were correlated with bacterial levels, other outcome(s) may be revealed with a larger sample size. Second, we could not assess sex-based differences in oral bacterial levels and oral functions because all of our subjects were women. Previous studies have reported sex-based differences in the prevalence ratio of periodontitis [32] and the risk for frailty [33]. Third, the effect of the number of teeth, occlusal support, or denture wearing on oral functions and/or oral bacterial levels was not assessed because oral examinations were not performed in this study. Fourth, not all potential confounding factors, such as education level, socioeconomic status, or health-related behavior, were collected. A previous study reported a significant relationship between socioeconomic status and oral hygiene habits, including tooth brushing frequency and periodical dental examination, in residential homes for older adults [34].

CONCLUSIONS

This pilot study demonstrated that increased maximum tongue pressure had the potential to lower the levels of oral bacteria in community-dwelling older adults. The relationship between oral bacterial level and other oral functions, including chewing ability and maximum labial closing force, should be further investigated with a larger sample size and possible confounders. Nevertheless, our results potentially indicate that tongue strength training interventions in the community could reduce mortality risk caused by increasing levels of oral pathogens due to unfavorable oral hygiene.

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