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Oral frailty and carriage of oral *Candida* in community-dwelling older adults (Checkup to discover Health with Energy for senior Residents in Iwamizawa; CHEER Iwamizawa)

Running Title: Oral frailty and carriage of oral *Candida*

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Ethics Approval: This study was conducted in accordance with the Declaration of Helsinki and was approved by the Hokkaido University Faculty of Dental Medicine Clinical/Epidemiological Research Institutional Review Board (2020 no. 9).

Patient Consent: The principal investigator explained the nature of the study to the participants in advance, both orally and in writing, and all participants provided written consent.

Permission to Reproduce Material from Other Sources: Not applicable

Clinical Trial Registration: Not applicable

Abstract

Objective: To examine the association between oral frailty and oral *Candida* carriage as a general indicator of deteriorating oral function in older adults.

Background: Older adults exhibit an elevated risk of oral candidiasis caused by *Candida*. Although many studies have identified factors associated with oral *Candida* carriage, none have evaluated its relationship with oral function.

Materials and Methods: This study included 210 community-dwelling older adults aged ≥ 60 years who participated in wellness checks. Fungal flora expression in saliva samples was evaluated to identify oral *C. albicans* and *C. glabrata.* Participants were categorized by detection of neither strain (group 1), either one of the strains (group 2), or both strains (group 3). The relationship between oral *Candida* carriage and oral frailty was evaluated by multinomial logistic regression analysis.

Results: The participants included 58 men and 152 women with a mean age of 74.2 ± 6.1 years. A total of 88 (41.9%), 94 (44.8%), and 28 (13.3%) participants were assigned to groups 1, 2, and 3, respectively. In the multinomial logistic regression analysis, significant associations were observed between group 1 and group 2 for "Have you choked on your tea or soup recently?" and the number of applicable oral frailty items. Between group 1 and group 3, significant associations were observed for the number of remaining teeth, masticatory performance, and the number of applicable oral frailty items.

Conclusion: We obtained basic data useful for intervention studies aimed at verifying whether oral function management prevents deterioration of the oral bacterial flora.

Keywords: community-dwelling older adults, cross-sectional study, *Candida albicans, Candida glabrata*, internal transcribed spacer 2 (ITS2), oral frailty.

Introduction

Oral *Candida* are normal fungi present in the oral cavity in 3–75%[1-3] of healthy individuals. Systemic deterioration in the host's condition; however, can cause oral *Candida* to propagate, leading to an opportunistic infection called oral candidiasis. Oral candidiasis, triggered primarily by *Candida albicans (C. albicans)*, presents with symptoms such as redness and pain of the oral mucosa and dysgeusia. *C. albicans* has little pathogenic potential on its own, even in comparison with normal oral bacteria, and oral candidiasis is rare in healthy individuals. However, older adults have an elevated risk for developing candidiasis due to factors such as oral hygiene deterioration, denture use, reduced salivary secretion, undernutrition, and deteriorated immunity. Oral *Candida* strains are also a cause of aspiration pneumonia in older adults. In addition, some studies have reported that the pathology of oral candidiasis has recently changed.[4,5] In addition to *C. albicans*, an increase in another strain of *Candida*, *C. glabrata*, has been reported in patients with oral candidiasis.[5-7] Infection by *C. glabrata* is believed to occur as a mixed infection with *C. albicans* rather than a single infection.[7-9] According to some studies, an increase in resistance to the azole class of antifungals in cases of mixed infections leads to treatment failure and repeated relapse.[9-11] Although clinical studies on oral *Candida* have been conducted in patients with oral candidiasis, few have investigated its expression in healthy, asymptomatic, older adults.

In contrast, there has been an increased focus on the evaluation of oral frailty, which is assessed comprehensively based on the number of missing teeth, diminished tongue movement and strength, reduced masticatory performance, and impaired swallowing. Older adults with oral frailty were found to have significantly higher rates of physical frailty and sarcopenia over a two-year period, as well as significantly higher numbers of deaths and increased long-term care requirements over approximately four years.[12] However, the mechanism by which oral frailty is related to the onset of these events is not well understood. Compared with other oral microflora, such as periodontopathic bacteria, *Candida* strains are associated with greater deterioration of the oral environment and the

patient's systemic condition, including a heightened risk of undernutrition, deteriorated immunity, and aspiration pneumonia.

The aim of this cross-sectional study was to determine the association between the carriage of oral *Candida* and oral frailty in Japanese community-dwelling older adults.

Methods

Study design

This was a cross-sectional study of community-dwelling older adults.

Participants

Citizens of Iwamizawa City in Hokkaido in northern Japan, aged ≥ 60 years, were recruited to participate in a wellness check. Local government employees visited the social clubs of senior citizens in the city to describe the nature of the study and recruit potential participants. In addition, the city newsletter ran an article to recruit participants, and recruitment flyers were posted on the walls of major facilities in the city. The recruited participants were evaluated in October 2020, and those who had undergone previous wellness checks were selected for inclusion. The principal investigator explained the nature of the study to the participants in advance, both orally and in writing, and obtained written consent. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Hokkaido University Faculty of Dental Medicine Clinical/Epidemiological Research Institutional Review Board (2020 no. 9).

Assessment of outcomes (oral Candida carriage)

The participants chewed a special gum used for salivary tests (Saliva Gum α; Tokyo Shizaisha, Tokyo, Japan) for 1 min, and the saliva that flowed during that time was collected in sterilized plastic tubes and weighed. The collected saliva was subsequently centrifuged at the Oral Microbiome Center (Kagawa, Japan) to yield pellets, which were cryopreserved until DNA extraction.

After the salivary pellets were homogenized with MORA-EXTRACT (Cosmo Bio Co., Ltd., Tokyo, Japan) and a genomic DNA purification kit (Genfind V2; Nippon Genetics Co., Ltd., Tokyo, Japan) was used to extract and purify the DNA. An internal transcribed spacer 2 (ITS2) analysis was conducted using MiSeq (Illumina, Inc.; Tokyo, Japan) based on Meta-16S analytical techniques. Polymerase chain reaction (PCR) amplification of the ITS2 in each sample was performed using the following ITS2 amplification primers[13]: F gITS7 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGN GTGARTCATCGARTCTTTG-3' and R ITS4 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNTCCTCCGCTTATTGATATGC-3'. The PCR cycle was as follows: 95 ° C for 3 minutes, 95 ° C for 30 seconds, 55 ° C for 30 seconds, 72 ° C for 30 cycles of 30 seconds, 72 ° C for 5 minutes. PCR was performed twice to generate an Illumina sequencing library, and two 301 bp fragments were sequenced using MiSeq V3 reagent to obtain the FASTQ sequences. Data were analyzed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline. Fungal compositions were calculated based on 97% clustering and referring to the UNITE_ITS database (12.11). Based on these analyses, the participants were classified into the following three groups: neither *C. albicans* nor *C. glabrata* detected [*C. alb* (−) & *C. glab* (−) group], either strain detected [*C. alb* (+) or *C. glab* (+) group], or both strains detected [*C. alb* (+) & *C. glab* (+) group].

Endpoints

Basic demographic characteristics and dental health parameters, including assessments of oral function and removable denture use, were compared among the groups. Oral microflora measurements were based on the saliva collected at the time its volume was being measured.

Quantification Methods

Basic characteristics

Body mass index (BMI) was calculated based on the participants' height and weight, which were measured at the health checkup site. Information related to age, sex, diabetes, smoking status, medication, and physical frailty was collected using self-administered questionnaires.

Grip strength was measured with a Jamar hydraulic dynamometer (Model #MG-4005NC, Patterson Medical Holdings, Inc., Bolingbrook, IL, USA) by an investigator who had practiced grip strength measurements in advance. Based on the Asian Working Group for Sarcopenia (AWGS) 2019 consensus[14], grip strength was measured in the present study as follows. The participant was seated, with his or her elbow close to the trunk and the elbow bent at a 90° angle. Grip strength in the left and right hand were measured in a manner that prevented the participant from feeling the weight of the dynamometer. The maximum values for each side were recorded for analysis.

Gait speed was determined as the time required for each participant to walk a 5-meter segment of a track; the distance traveled per second was calculated based on the 3 m before and after the target segment while walking with a normal gait.

Assessment of physical frailty

Physical frailty was assessed using the Japanese Cardiovascular Health Study (J-CHS) criteria.[15] Specifically, unintentional weight loss was defined as a reduction of ≥ 2−3 kg in body weight over the previous six months. Reduced muscle strength was defined as grip strength < 28 kg in men and < 18 kg in women. Exhaustion was defined as feeling tired for no known reason over the previous two weeks. Slow gait speed was defined as a normal gait speed < 1.0 m/s, and low physical activity was defined as not engaging in light or regular exercise or sports at least once per week. Participants who met at least three of the five J-CHS criteria were classified as being physically frail.

Assessment of oral health indicators

Oral function and removable denture use were assessed using the same criteria by ten dentists who had undergone prior training. In this study, all dentures were removable dentures with

mucosal coverage. The participants confirmed that the dentures were used at mealtimes and removed at bedtime. The following parameters were measured: number of remaining teeth, masticatory performance, tongue pressure, and oral diadochokinesis (ODK), which represents tongue dexterity. The number of remaining teeth was defined as the number of teeth that had erupted in the oral cavity minus the number of stumps and teeth with severe periodontitis. Masticatory performance was assessed using color-changeable chewing gum (Masticatory Performance Evaluating Gum xylitol; Lotte Co., Ltd., Tokyo, Japan). The a* parameter was measured at five sites with a color-difference meter (CR-20 Color Reader; Konica Minolta, Tokyo, Japan) after 1 min of chewing, and the mean of these five measurements was defined as the masticatory performance. Tongue pressure was assessed using a JMS tongue pressure device (JMS Co., Ltd., Hiroshima, Japan). Participants pressed the tongue pressure probe between their tongue and palate and applied tongue pressure with their maximum voluntary muscle strength for approximately 7 s. Tongue pressure was measured three times, and the maximum of these measurements was used. ODK was assessed using an oral function measurement device (Kenkokun Handy; Takei Scientific Instruments Co., Ltd., Niigata, Japan). Participants were asked to repeat the syllable "ta" as many times as possible over a 5-second period, and the number of sounds per second was recorded.

Based on a study by Tanaka et al.,[12] the cut-off values for each component were defined as follows: number of remaining teeth < 20, masticatory performance < 14.2 for men and < 10.8 for women, tongue pressure < 27.4 kPa for men and < 26.5 kPa for women, and an ODK < 5.2 times/s for men and < 5.4 times/s for women. Subjective assessments consisted of two questions: an affirmative response to the question, "Do you have any difficulty eating tough foods compared to 6 months ago?" was considered to be caused by reduced masticatory performance, whereas answering "yes" to the question, "Have you choked on your tea or soup recently?" was considered to be indicative of impaired swallowing. Oral frailty was determined according to the results of the aforementioned six objective and subjective items. Participants presenting with three or more of the above items were classified as experiencing oral frailty.

Statistical Analysis

A previous study reported the relationships between oral *Candida* and age,[16] sex,[17] BMI,[18] physical frailty, smoking status,[18] number of medications, diabetes,[19] denture use,[18] and secreted saliva volume.[20] In this study, continuous variables were compared among the three groups using the Kruskal-Wallis test, whereas categorical variables were compared using the χ^2 test. Similarly, the six individual oral frailty items, the number of applicable items, and the overall oral frailty data were analyzed, with the continuous and categorical variables compared among the three groups using the Kruskal-Wallis and χ^2 tests, respectively.

Correlation analysis of oral frailty test items and related factors was then conducted, as well as a multinomial logistic regression analysis with age,[16] sex,[17] BMI,[18] physical frailty, smoking status, number of medications, diabetes,[19] denture use,[18] and saliva secretion[20] as independent variables, and oral *Candida* carriage as the dependent variable. In the present study, multinomial logistic regression analysis was also conducted with oral *Candida* carriage as the dependent variable, the six individual oral frailty items, the number of applicable items, and the overall frailty assessment as independent variables, and age, sex, BMI, physical frailty, smoking status, number of medications, diabetes, denture use, and salivary secretion volume as covariates. Nine independent variables were selected for the final multivariate analysis model. Therefore, the sample size needed for the smaller category of dependent variables was 90. Previous studies reported that the *C.alb* (+) & *C.glab* (+) group accounted for 52% of the participants.[20] Therefore, the smaller category was estimated to account for 48% of the participants, implying that a total sample size of at least 188 participants was required. Finally, six covariates were entered, which were selected using a directed acyclic graph (DAG) (Figure 1). All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) Statistics Version 27 (IBM Corp., Armonk, NY, USA) with the level of statistical significance set at $p < 0.05$. The DAGitty software (Theoretical Biology & Bioinformatics Group, University of Utrecht, http://dagitty.net) was used to perform the DAG.

Results

This study initially included 232 participants. Of these, 22 were excluded due to incomplete questionnaires, receiving an antibiotic prescription within the preceding month, or absence of an oral function assessment; thus, 210 participants (58 men and 152 women, mean age 74.2 ± 6.1 years) were included in the analysis.

In 88 participants (41.9%), neither *C. albicans* nor *C. glabrata* were detected, one or the other was detected in 94 participants (44.8%; *C. alb* only in 69 participants and *C. glab* only in 25 participants), and both were detected in 28 participants (13.3%). Seventeen participants (8.1%) were classified as having oral frailty. In the three-group comparisons of factors associated with oral *Candida* carriage, the *C. alb* (−) & *C. glab* (−) group significantly differed from the *C. alb* (+) or *C. glab* (+) group and the *C. alb*(+) & *C. glab* (+) group in terms of age and the use of dentures (Table 1).

In the three-group comparisons of the six individual oral frailty items, the number of applicable items, and the overall oral frailty assessment, significant differences were observed for the following variables: the number of remaining teeth, masticatory performance, number of applicable oral frailty items, and subjective choking impairment assessed by the “I have choked on my tea or soup recently” item of the questionnaire (Table 2).

Table 3 shows the correlation of oral frailty test items. The number of remaining teeth showed negative correlations with age, sex, and removable denture use. Masticatory performance showed negative correlations with age and removable denture use and showed a positive correlation with saliva secretion. ODK for the “ta” syllable showed negative correlations with age and removable denture use. Tongue pressure showed a negative correlation with age and positive correlations with BMI and medications. Difficulties eating tough foods showed positive correlations with physical frailty and removable denture use. Difficulties swallowing tea or soup showed positive correlations with physical frailty and medications. Oral frailty (number of applicable items) showed positive

correlations with age, sex, physical frailty, diabetes, and removable denture use. Oral frailty also showed positive correlations with age, physical frailty, and removable denture use.

Table 4 shows the multinomial logistic regression analysis with oral *Candida* carriage as the dependent variable and factors reportedly associated with oral *Candida* carriage as independent variables. Significant differences were observed between the *C. alb* (−) & *C. glab* (−) group and the *C. alb* (+) or *C. glab* (+) group in terms of age (odds ratio [OR]: 1.08; 95% confidence interval [CI]: 1.02−1.14; p = 0.007) and denture use (OR: 2.13; 95% CI: 1.10–4.11; p = 0.025). The *C. alb* (−) & *C. glab* (−) group and the *C. alb* (+) & *C. glab* (+) group also differed significantly in terms of age (OR: 1.17; 95% CI: 1.06–1.29; p = 0.002) and denture use (OR 51.30; 95% CI 6.38–412.41; p < 0.0001).

Table 5 shows the multinomial logistic regression analysis with oral *Candida* carriage as the dependent variable and the six individual oral frailty items, the number of applicable items, and the overall oral frailty assessment as independent variables. Six covariates were entered: age, sex, removable denture use, saliva secretion, medications, and physical frailty, which were selected using the DAG (Figure 1). Significant associations were observed between the *C. alb* (−) & *C. glab* (−) group and the *C. alb* (+) or *C. glab* (+) group for an affirmative response to the question, “Have you choked on your tea or soup recently?” (OR: 2.66; 95% CI: 1.31–5.39; p = 0.007) and the number of applicable oral frailty items (OR: 1.75; 95% CI: 1.18–2.59; p = 0.006). Between the *C. alb* (−) and *C. glab* (−) groups and the *C. alb* (+) & *C. glab* (+) groups, significant associations were observed in terms of the number of remaining teeth (OR: 0.91; 95% CI: 0.85–0.99; p = 0.021), masticatory performance (OR: 0.86; 95% CI: 0.76–0.97; p = 0.018), and the number of applicable oral frailty items (OR: 1.81; 95% CI: 1.03–3.17; p = 0.039).

Discussion

The present cross-sectional study demonstrated that the presence of *C. albicans* and/or *C. glabrata* in saliva samples is associated with oral frailty. Similarly, a large number of applicable oral frailty items, a small number of remaining teeth, and impaired masticatory performance were

associated with the presence of *C. albicans* and/or *C. glabrata*. To the best of our knowledge, there is no published evidence on the use of next-generation sequencing-based comprehensive analyses of fungal flora in community-dwelling older adults, rather than patients with oral candidiasis, or focused on factors related to the presence of oral *C. albicans* and/or *C. glabrata*. The present study is the first to demonstrate the relationship between oral function and oral *Candida* carriage. The prevalence of oral disease increases with age and is interrelated with diminished oral function. Thus, older adults face an ever-increasing likelihood of oral function deterioration as they age. The present findings suggest that the exacerbation of oral bacterial flora associated with the presence of oral *Candida* is related to the deterioration of oral function that is observed in older adults. Deterioration of the oral function may affect *Candida* carriage according to the following pathway. Oral *Candida* is a superficial indigenous bacterium that resides on the surface of the oral mucosa. When the oral function is good, *Candida* adhering to the mucous membrane is easily washed away due to the self-cleansing action of the saliva; therefore, only a small amount of *Candida* remains adherent. However, as the oral function declines, the self-cleansing effect of the saliva decreases, and antifungal substances present in the saliva also decrease, making it easier for *Candida* to adhere to the mucous membranes. Accordingly, the amount of *Candida* increases and is more easily detected. Although further research is necessary to verify these conclusions, these findings indicate that the testing and management of the deteriorated oral function can contribute to the early detection and prevention of degradation in oral microflora colonies in older adults.

The present study has several limitations. First, factors such as dental caries, periodontal disease, and specific aspects of dentures, including size and usage status, were not considered. These factors need to be investigated further in future studies on *Candida*. Furthermore, this study did not assess mucosal coverage. However, since the extent of mucosal coverage is related to the number of remaining teeth, its effect on our results is considered to be limited. Future research on *Candida* should quantify and investigate the extent of mucosal coverage. In addition, *Candida* is considered an indigenous microorganism, but it is not detected in all people, even after culturing. It is difficult to

evaluate the number of colonies on a flat plate because the number of colonies varies greatly depending on host factors, and the host conditions are not uniform[21]. Therefore, future studies should take these potential confounders into consideration, as they affect oral function, and aim to overcome the limits intrinsic to our approach based on convenience sampling. Second, difficulty in swallowing tea or soup was not associated with the *C. alb* (+) & *C. glab* (+) group but were associated with the *C. alb* (+) or *C. glab* (+) group. The lower masticatory performance may have been related to the duplication of *Candida*, although participants with swallowing problems but who do not have oral problems may not have had both *C. alb* (+) & *C. glab* (+) in their microflora. Third, the participants were highly health-conscious Japanese individuals who participated voluntarily, which may limit the generalizability of the study's findings. It is difficult to recruit residents who normally avoid health assessments; therefore, strategies for recruiting such residents should be examined.

Oral candidiasis has long been considered a fungal infection caused by *C. albicans,*[4,5] whereas *C. glabrata* has attracted little attention, likely owing to its relatively weak virulence. According to recent studies, the isolation of both *C. albicans* and *C. glabrata* from patients with oropharyngeal candidiasis has become common.[22] An increasing number of cases showing resistance to antifungals has been observed in immunosuppressed patients, such as those with head and neck cancers[23] and HIV,[24] as well as in those with *Candida*-associated denture stomatitis.[8] *C. glabrata* has also been implicated in the increased resistance to antifungals.[9-11] In a study using a model of the oral mucosal epithelium, *C. albicans* demonstrated a high capacity to form colonies and invade the epithelium, whereas *C. glabrata* demonstrated only mild colony formation and no invasive capacity. However, mixed infections have been reported to enhance the invasive capacity of *C. glabrata,*[25] and, conversely, genes that encode proteins that form the cell wall in *C. glabrata* are crucial for the adhesion of *C. albicans* hyphae to the mucosae.[26] Thus, the detection of both *C. albicans* and *C. glabrata* in the oral cavity is a risk factor for the manifestation of intractable oral candidiasis. Understanding the deterioration of oral function related to oral candidiasis may provide new insight into combating this infection.

Although several studies have reported the deterioration of oral function and oral hygiene,[27,28] no studies have investigated the relationship between oral *Candida* and oral function. Intriguingly, the present study found that the number of remaining teeth and masticatory performance were significantly associated with single and mixed manifestations of oral *Candida*. The absence of teeth is a factor in the deterioration of oral hygiene in older adults.[29] Oral *Candida* is strongly associated with the use of dentures, which supplement lost teeth; thus, a large number of remaining teeth may be associated with a low level of single and mixed manifestations of oral *Candida*. Similarly, the remaining teeth greatly affect masticatory performance. In individuals with deteriorating masticatory function due to oral frailty, bacteria are removed from the teeth and oral mucosa by the physical act of eating and drinking. Deterioration of the oral bacterial flora is also correlated with the growth of oral candidiasis; therefore, the maintenance of teeth is essential to preventing the deterioration of oral microflora related to oral *Candida* infection.

In the present study, the *C. alb* (+) & *C. glab* (+) group included more older individuals, denture users, and participants with fewer remaining teeth compared with the other groups. These findings corroborate those of previous studies. Of particular note is the finding that 96.4% of participants presenting with both *C. glabrata* and *C. albicans* were denture users. The cell surface of *C. glabrata* is four times more hydrophobic than that of *C. albicans*, and *C. glabrata* adheres twice as strongly to acrylic resins, which explains why *C. glabrata* is reported to adhere to dentures more easily than *C. albicans.*[4] According to a study measuring biofilms on the surfaces of dentures produced by single or mixed strains of oral *Candida*, the combination of *C. albicans* and *C. glabrata* yielded the largest volume of biofilm.[8] Therefore, reducing oral *Candida* strains that adhere to denture surfaces is important to improve the health of community-dwelling older adults.

In a previous study of this population in Japan, *C. albicans* alone was detected in 33.3% of the saliva samples, *C. glabrata* alone was detected in 0.0% of samples, and both were detected in 52.4% of samples.[20] The rate of detection of *C. albicans* alone in the present study was 32.9%, which is consistent with the previous findings. In contrast, the rates of detection of *C. glabrata* alone and

with *C. albicans* were 11.9% and 13.3%, respectively, representing a total of 25.2% of participants. While this total was only half that found in the study cited above, the participants in the previous study were on average five years older and had a higher rate of denture use. This suggests that the present study had fewer participants with *C. glabrata* alone and more participants with both *C. glabrata* and *C. albicans*.

In the present study, four strains of *Candida* were detected in the saliva, including *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*. *C. tropicalis* and *C. parapsilosis* infections were not included in the analysis because they were detected (either alone or with *C. albicans* and/or *C. glabrata*) in only 3.3% and 6.7% of the saliva samples, respectively. In addition, neither strain was detected in the *C. alb* (−) & *C. glab* (−) group, and few studies have reported on the relationship of these strains with oral candidiasis. However, a previous study of older adults in a different region of Japan reported that *C. dubliniensis* and *Malassezia restricta* were detected, whereas a Brazilian study did not confirm the presence of either of these strains.[30] However, unlike the present study, neither of these studies comprehensively analyzed fungal flora populations using next-generation sequencing, and it is possible that their findings may have been affected by differences in accuracy related to cases involving multiple strains.

Although previous studies have reported that sex,[17] BMI,[18] physical frailty, smoking, number of medications, diabetes[19], and salivary secretion[20] are associated with oral *Candida*, the present study did not identify any of these factors being associated with oral *Candida* carriage. This may be due to the fact that the participants consisted of community-dwelling older adults lacking the above risk factors. Risk factors including stroke, ischemic heart disease, and depression, all of which are associated with oral *Candida,* were present in less than 4% of participants and were therefore, excluded from the analysis.

In two previous studies conducted in other regions of Japan,[27,28] the prevalence of oral frailty was 15.9% and 22.5%; thus, the prevalence of 8.1% found in the present study was comparatively low. Although the mean age of the participants in these previous studies (73.0 ± 5.5 years and 76.3 ±

6.5 years) was approximately the same as that in the present study (74.2 ± 6.1 years), the inclusion criterion for age differed. The reason behind the difference in the prevalence of oral frailty is that participants in the previous studies were ≥ 65 years while participants in the present study were ≥ 60 years. In addition, the absence of an association between oral frailty and oral *Candida* carriage in the present study may have been due to the low number of participants who met the criteria for oral frailty. Therefore, the interpretation of these findings must account for the high degree of oral function among participants in this study.

Longitudinal studies on oral frailty in community-dwelling older adults have shown that oral frailty is associated with overall frailty, sarcopenia, the need for long-term care, and all-cause mortality[31]. In addition, the degradation of the oral microflora is associated with undernutrition, immunosuppression, and the risk of experiencing aspiration pneumonia. This association suggests that the degradation of the oral microflora may represent a mechanism through which oral frailty leads to overall frailty, sarcopenia, the need for long-term care, and all-cause mortality[12].

The present study was cross-sectional in design, which precludes us from drawing conclusions about causal relationships. However, it is more likely that the deterioration of the masticatory performance was the cause of oral *Candida* carriage rather than the effect. Future interventional follow-up studies should focus on improving masticatory performance in relation to tooth loss and frailty, the need for long-term care, death, and other oral frailty-related outcomes. Due to the recent coronavirus pandemic, the number of participants in the study has decreased, and the number of samples has become insufficient for analysis.

Conclusion

The findings of the present study suggest that oral frailty is associated with the degradation of the oral microflora in community-dwelling older adults. We obtained basic data that will be useful

for interventional studies aimed at verifying whether oral function management prevents degradation of the oral bacterial flora.

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TABLE1 Comparison of *C. alb*(-) & *C. glab*(-), *C. alb*(+) or *C. glab*(+), and *C. alb*(+) & *C. glab*(+) groups and related factors

Variables	Overall (n=210)	*C. alb*(-) & *C. glab*(-) (n=88)	*C. alb*(+) or *C. glab*(+) *C. alb*(n=69), *C. glab*(n=25), total(n=94)	*C. alb*(+) & *C. glab*(+) (n=28)	p-value
	mean±SD n (%), Median (Q1, Q3)				
Age, years	74.2±6.1	72.2±6.3	75.2±5.7	77.5±4.7	<0.001[a,***]
Sex, male, n(%)	58 (27.6)	22 (25)	24 (25.5)	12 (42.9)	0.153[b]
BMI, kg/m^2	22.9±3.4	22.6±3.2	23.0±3.6	23.1±3.0	0.714[a]
Physical frailty, n(%)	18 (8.7)	8 (9.1)	9 (9.6)	1 (3.6)	0.593[b]
Smoker, n(%)	10 (4.8)	4 (4.5)	4 (4.3)	2 (7.1)	0.686[b]
Medications	1 (0, 2)	1 (0, 2)	1 (0, 2)	1 (0, 1.8)	0.426[a]
Diabetes, n(%)	25 (11.9)	6 (6.8)	16 (17)	3 (10.7)	0.185[b]
Removable denture use, n(%)	105 (50)	27 (30.7)	51 (54.3)	27 (96.4)	<0.001[b,***]
Upper denture use, n(%)	18 (8.6)	8 (9.1)	5 (5.3)	5 (17.9)	
Lower denture use, n(%)	21 (10)	6 (6.8)	10 (10.6)	5 (17.9)	<0.001[b,***]

Upper and lower denture use , n(%)	66 (31.4)	13 (14.8)	36 (38.3)	17 (60.7)	
Saliva secretion, mg/min	2.8 (1.9, 3.8)	2.9 (1.9, 4.4)	2.6 (1.7, 3.5)	2.8 (2, 3.5)	0.188[a]

Categorical variables are shown as number (percentage); continuous variables that are normally distributed are shown as mean and standard deviation, and those that are not normally distributed as median (Q1, Q3). BMI, body mass index; *C. alb*., *Candida albicans, C. glab., Candida glabrata*; Q1, first quartile; Q3, third quartile; SD, standard deviation. [a]Kruskal-Wallis test, [b]χ^2 test.; ***p<0.001.

TABLE 2. Comparison of *C. alb*(-) & *C. glab*(-), *C.alb*(+) or *C. glab*(+), and *C. alb*(+) & *C. glab*(+) groups and oral frailty test items

Variables	Overall (n=210)	*C. alb*(-) & *C glab*(-) (n=88)	*C. alb*(+) or *C. glab*(+) *C. alb*(n=69), *C. glab*(n=25), total(n=94)	*C. alb*(+) & *C. glab*(+) (n=28)	p-value
	Median (Q1, Q3)				
Number of remaining teeth, n	22 (15, 26)	25 (22, 27)	21 (13, 25)	12 (7, 16)	<0.001[a]***
Masticatory performance	22.7 (19.7, 25.6)	25.1 (21.9, 27.1)	22.2 (19.9, 24.3)	19.3 (16.5, 22.0)	<0.001[a]***
ODK for “ta” syllable, times/s	6.4 (5.8, 6.7)	6.4 (6, 6.8)	6.2 (5.8, 6.6)	6.4 (5.7, 6.6)	0.102[a]
Tongue pressure, kPa	35 (30.6, 38.8)	34.6 (30.1, 38.9)	35.4 (30.8, 38.7)	35.7 (31.4, 40.3)	0.689[a]
Oral frailty (number of applicable items)	1 (0, 2)	0 (0, 2)	1 (1, 2)	1 (1, 2)	<0.001[a]***
	n (%)				
Difficulties eating tough foods (yes)	34 (16.2)	13 (14.8)	14 (14.9)	7 (25)	0.397[b]
Difficulties swallowing tea or soup (yes)	69 (32.9)	23 (26.1)	40 (42.6)	6 (21.4)	0.024[b]*
Oral frailty	17 (8.1)	4 (4.5)	9 (9.6)	4 (14.3)	0.201[b]

Continuous variables are expressed as median (Q1, Q3). Categorical variables are shown as number (percentage).

C. alb., *Candida albicans, C. glab., Candida glabrata*; CI, confidence interval; ODK; oral diadochokinesis; OR, odds ratio; Q1, first quartile; Q3, third quartile. [a]Kruskal-Wallis test. [b]χ^2 test.; *p<0.05 ***p<0.001

TABLE 3 Correlation of oral frailty test items and related factors

Variables	Age	Sex	BMI	Physical Frailty	Smoker	Medications	Diabetes	Removable denture use	Saliva secretion
Number of remaining teeth, n	-0.265**	-0.147*	-0.067	-0.078	-0.034	-0.009	-0.058	-0.596**	0.007
Masticatory performance	-0.170**	-0.007	-0.043	-0.049	-0.069	-0.002	-0.021	-0.362**	0.271**
ODK for “ta” syllable, times/s	-0.213**	-0.036	-0.067	-0.045	-0.047	-0.065	-0.100	-0.141*	0.028
Tongue pressure, kPa	-0.100*	-0.022	0.126**	-0.064	-0.058	0.110*	0.017	0.064	0.057
Difficulties eating tough foods (yes)	0.030	0.104	-0.013	0.218**	-0.038	0.002	-0.082	0.223**	0.012
Difficulties swallowing tea or soup (yes)	-0.044	-0.001	0.035	0.341**	-0.014	0.144*	0.087	-0.071	0.000
Oral frailty (number of applicable items)	0.213**	0.126*	-0.003	0.289**	-0.010	0.068	0.137*	0.387**	0.685
Oral frailty	0.118*	0.090	0.093	0.242**	0.098	0.096	-0.001	0.192**	0.052

ODK; oral diadochokinesis, BMI; body mass index. *p<0.05, **p<0.01, Kendall's Tau-b test

TABLE 4. Relationship between *C. alb*(-) & *C. glab*(-), *C. alb*(+) or *C. glab*(+), C. alb(+) & *C. glab*(+) groups and age, sex, and systemic disease factors

Variables	*C. alb*(+) or *C. glab*(+)				*C. alb*(+) & *C. glab*(+)			
	ref. *C. alb*(-) & *C. glab*(-)							
	OR	95% CI		p-value	OR	95% CI		p-value
Age, years	1.08	1.02	1.14	0.007**	1.17	1.06	1.29	0.002**
Sex, male	0.74	0.35	1.57	0.437	1.35	0.45	4.02	0.589
BMI, kg/m^2	1.03	0.94	1.14	0.512	1.05	0.88	1.26	0.565
Physical frailty	0.71	0.23	2.17	0.547	0.16	0.02	1.69	0.129
Smoker	0.96	0.19	4.79	0.959	0.60	0.07	5.50	0.651
Medications	0.95	0.67	1.33	0.754	0.68	0.38	1.20	0.182
Diabetes	2.52	0.82	7.72	0.105	1.91	0.35	10.41	0.454
Removable denture use	2.13	1.10	4.11	0.025**	51.30	6.38	412.41	<0.001 ***
Saliva secretion, mg/min	0.84	0.67	1.05	0.126	0.98	0.69	1.39	0.904

BMI, body mass index; *C. alb*., *Candida albicans, C. glab., Candida glabrata*; CI, confidence interval; OR, odds ratio. **p<0.01, ***p<0.001

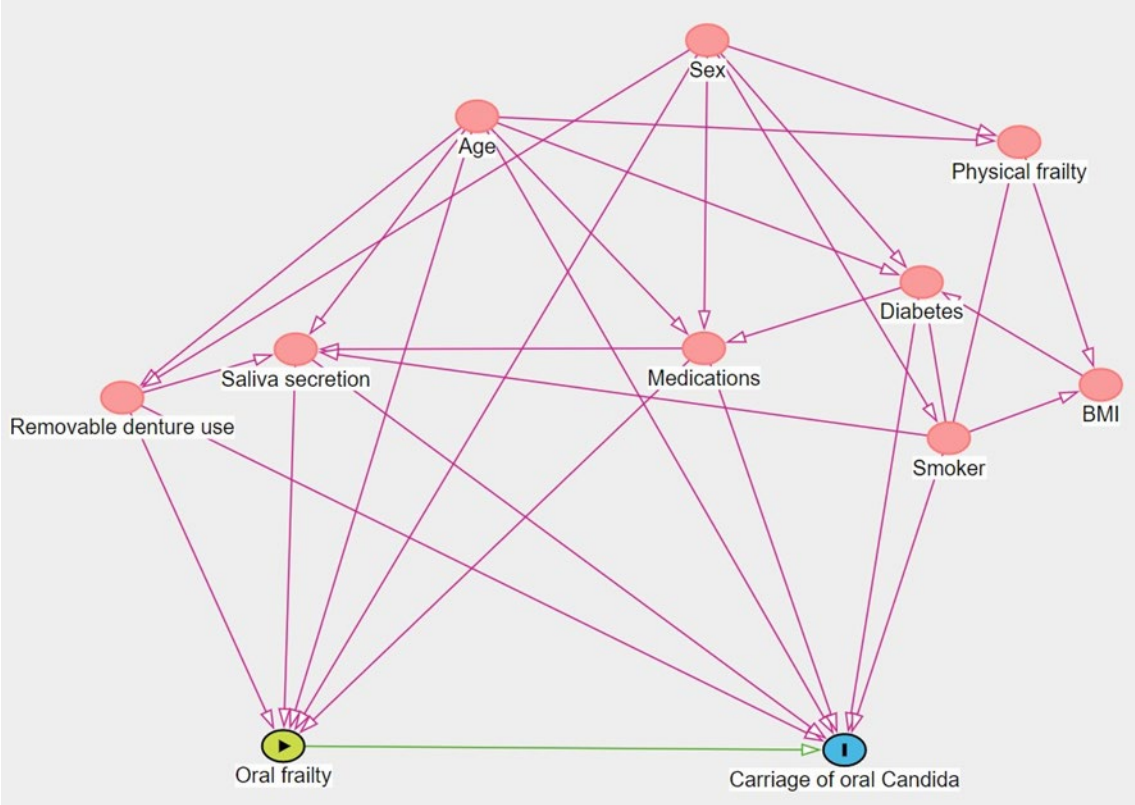
TABLE5. Relationship between *C. alb*(-) & *C. glab*(-), *C. alb*(+) or *C. glab*(+), *C. alb*(+) & *C. glab*(+) groups and oral function

Variables	*C. alb*(+) or *C. glab*(+)				*C. alb*(+) & *C. glab*(+)			
	ref. *C. alb*(-) & *C. glab*(-)							
	OR	95% CI		p-value	OR	95% CI		p-value
Number of remaining teeth, n	0.95	0.89	1.01	0.081	0.91	0.85	0.99	0.021*
Masticatory performance	0.92	0.84	1.01	0.067	0.86	0.76	0.97	0.018*
ODK for “ta” syllable, times/s	0.94	0.59	1.49	0.791	0.82	0.46	1.84	0.818
Tongue pressure, kPa	1.01	0.97	1.06	0.691	1.02	0.95	1.09	0.649
Difficulties eating tough foods (yes)	0.77	0.32	1.89	0.572	0.94	0.28	3.18	0.916
Difficulties in swallowing tea or soup (yes)	2.66	1.31	5.39	0.007**	1.17	0.35	3.92	0.794
Oral frailty (number of applicable items)	1.75	1.18	2.59	0.006**	1.81	1.03	3.17	0.039*
Oral frailty	1.72	0.44	6.70	0.435	2.07	0.36	11.96	0.416

C. alb., *Candida albicans, C. glab., Candida glabrata*; CI, confidence interval; ODK, oral diadochokinesis; OR, odds ratio. *p<0.05 **p<0.01,

Dependent variable: age, sex, removable denture use, saliva secretion, medications, and physical frailty

Figure 1


Sex
Age
Physical frailty
Diabetes
Saliva secretion
Medications
BMI
Removable denture use
Smoker
Oral frailty
Carriage of oral Candida