Associations of streptococci and fungi amounts in the oral cavity with nutritional and oral health status in institutionalized elders: a cross sectional study

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Research Article

Keywords: body mass index, oral health assessment tool, oral streptococci, fungi

Posted Date: September 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-857982/v1>

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Version of Record: A version of this preprint was published at BMC Oral Health on November 19th, 2021. See the published version at <https://doi.org/10.1186/s12903-021-01926-0>.

Abstract

Background:

Disruption of the indigenous microflora is likely to relate with frailty caused by undernutrition. However, relationship between undernutrition and oral flora, especially normal indigenous bacteria, is not obvious. The aim of this study was to elucidate the associations of nutritional and oral health conditions with bacteria and fungi prevalence in oral cavity of older individuals.

Methods:

Forty-one institutionalized older individuals with an average age $\pm$ standard deviation of 84.6 ± 8.3 years were enrolled in this study. Body mass index (BMI) and Oral health assessment tool (OHAT) were used to represent nutritional and oral health status. Amounts of total bacteria, streptococci, and fungi in oral specimens collected from the tongue dorsum were determined by quantitative polymerase chain reaction (PCR) assay results.

Results:

There found a significant correlation between BMI and streptococcal amount ($p=0.526$, $p<0.001$). In addition, the undernutrition group (BMI <20) showed a significantly lower average number of oral streptococci ($p=0.003$). In logistic regression models, streptococcal amount was the significant variable accounting for “not undernutrition” [odds ratio 5.68 and 95% confidential interval (CI) was 1.64-19.7 ($p=0.06$)]. On the other hand, participants with poor oral health condition (OHAT ≥ 5) harbored significantly higher level of fungi ($p=0.028$).

Conclusion:

Oral streptococci were associated with systemic nutritional condition and oral fungi were associated with oral health condition. Thus, it is necessary to examine oral indigenous bacteria as well as etiological microorganisms in order to understand the relationship of frailty with oral microflora in older individuals.

Background

Along with the general aging of society worldwide, frailty is gaining attention. This is a common geriatric syndrome that increases the risk of poor health outcomes, including falls, functional disability, hospitalization, and mortality [1–5]. The cycle of frailty generally begins from chronic malnutrition and consequent weight loss [2]. Previous studies have found relationships between malnutrition and poor oral health conditions in elder individuals, in which dental disease status or oral function, including dental caries, periodontitis, tooth loss, and mastication or swallowing disorder, were mainly investigated as malnutrition risks [6–9]. As compared to investigations of oral cavity morphology and functions, microbiological studies are few, though the oral microflora is likely to have a significant relationship with frailty [10]. The indigenous oral microflora, composed of various microorganisms, plays an important role in maintaining homeostasis of oral and systemic health [11]. In the oral cavity, indigenous microorganisms such as streptococci grow and develop a suitable flora for the environment provided by the host. Once that flora is established, it helps to protect the host from invasion by pathogenic microorganisms and subsequent infection [12, 13]. Therefore, when a normal oral microflora is disturbed, the population of pathogenic microorganisms is easily increased, which causes oral and systemic diseases [14]. On the other hand, the oral microbiota can be affected by changes in the oral environment as well as general conditions, such as aging, immunosuppression, and medication [15–17].

Recently, oral fungi have received focus as etiological microorganisms, especially in elders. Older individuals are more prone to colonization by fungi due to such factors as decreased immune response, reduced salivary flow, and denture use [18–20]. *Candida*, which is the most prevalent fungi found in the human oral cavity, are normal commensal and asymptomatic organisms in healthy people conditions [21]. However, in frail older individuals or immunocompromised patients, those can overgrow in oropharyngeal or esophageal mucosa, which causes a burning sensation, taste disorder, severe mucositis, and/or dysphagia, resulting in poor nutrition [22, 23]. Furthermore, even with the absence of such oral symptoms, a relationship of general malnutrition with certain fungi in the oral cavity of elderly individuals has been shown [24]. Fungal growth in the oral cavity as well as systemic malnutrition are thought to be associated with an alteration in prevalence of oral indigenous bacteria. Oral dryness is a condition that can enhance fungal growth, though is not suitable for growth of indigenous oral bacteria such as oral streptococci. In addition, a decrease in oral intake of food, which is generally the start of the frailty cycle, can have a negative effect on growth of oral streptococci, whose main energy source is carbohydrates from staple foods [25–27]. Therefore, assessing oral bacteria including streptococcal amounts in addition to fungi may more accurately reflect malnutrition than assessment of fungi alone.

In this study, the amounts of fungi, total bacteria and commensal streptococci in tongue samples of institutionalized older individuals were determined, and systemic and oral conditions were assessed. From those results, the relationships of systemic nutritional condition and oral health status with oral microbial prevalence were investigated.

Methods

Ethical considerations

The study protocol was approved by The Ethics Committee of Iwate Medical University School of Dentistry (approval no. 01340). Individuals with decision-making ability were given information regarding the study protocol and enrolled as subjects after providing documentation indicating consent. For those who lacked ability to make such a decision due to cognitive decline, study protocol information documentation was given to a closely related family member, who provided consent for participation. Individuals who refused to participate in a study survey or undergo analysis after providing consent were subsequently excluded.

Study design and participants

This was a cross-sectional observational study conducted at a nursing institution for the older people. Consent to participate in the study was received from 42 older individuals or a family member. Later, one rejected participation in the survey, after which no one withdrew their consent for participation. Consequently, 41 elders (8 males, 33 females) with an average age $\pm$ standard deviation (SD) of 84.6 ± 8.3 years (range 70–105 years) completed the present study protocol.

Collection of participant information

Information regarding gender, age, height, weight, application of special medical cares, smoking and drinking habits, intake of antibacterial or anti-fungal medication, and long-term care needs level for each subject was provided by the institution. Long-term care needs level is widely recognized in Japan as it is used in the Japanese long-term care insurance system, which was established in 2000. Following receipt of an application from a community-dweller aged 65 or older, their care needs level is determined by the applicable commission in the administration of their

community based on examinations of mental and physical condition assessed by certification screening personnel, as well as a diagnosis from their primary physician. Long-term care needs consist of 7 levels, including support levels 1 and 2, and care levels 1-5, aside from not applicable. Support level 1 represents the lowest level of care needed and care level 5 the highest [28]. For the present study, the range from support level 1 to care level 5 is referred to as long-term care needs level 1-7. BMI was calculated as follows: weight (kg) / height (cm)².

Oral examinations

Oral examinations were performed by two well-calibrated dentists. Present tooth status including decayed and filled teeth was assessed according to the WHO criteria. Residual roots with or without coping treatment were recorded as a filled or decayed tooth, respectively. The presence of oral cancer and oral potentially malignant disorders (OPMDs), including leukoplakia, oral lichen planus, and erythroplakia, was determined using methods recommended by the WHO [29].

The moisture level of the tongue surface was measured three times using an intraoral moisture meter (Mucus®; Life, Saitama, Japan) at a point 1 cm posterior from the tip of the tongue along the median groove, as noted in a previous study [30]. Tongue coating deposits were evaluated using tongue coating index (TCI). The surface of the tongue dorsum was divided into three sections vertically and three sections laterally (total nine sections). Each section was scored from 0-2 (0: tongue coating not visible, 1: thin tongue coating, papillae of tongue visible, 2: very thick tongue coating, papillae of tongue not visible) and the total score of the 9 sections was recorded as personal TCI [31]. Comprehensive oral health status was assessed using the Oral Health Assessment Tool (OHAT), which is a validated tool used for assessment of oral health and comprises eight domains, including lips, tongue, gums and tissues, saliva, natural teeth, dentures, oral cleanliness, and dental pain, stratified into three grades (healthy, oral changes, unhealthy) [32]. In addition, at the oral examinations, denture use frequency was obtained by oral questioning of the subject or a caregiver. Denture use frequency was categorized into three levels; 1: no use, 2: used for meals, and 3: always worn except at sleeping time.

Collection and genome purification of microbial samples

Microbial samples were collected immediately after the oral examination, as follows. The dorsum of the tongue was swabbed 20 times with a sterile cotton swab, which was then immersed in 1 ml of sterile saline. Collected samples were transferred to the laboratory on ice and stored at -80°C until genome extraction. Genomic DNA was extracted and purified from the collected samples using a Wizard® Genomic DNA Purification Kit (Promega), according to the manufacturer's instructions. The microbial samples were lysed in 50 mM EDTA, 1 mg/ml lysozyme (Thermo Fisher Scientific, Waltham, USA), 0.5 mg/ml lysostaphin (Fujifilm Wako Pure Chemical), and 1 unit/ml lyticase (Sigma-Aldrich, St. Louis, MO, USA) at 37°C for 30 min. In addition, samples were disturbed with an ISOFEAL for Beads Beating device (Nippon Gene, Tokyo, Japan) and a µT-12 beads crusher (TAITEC, Saitama, Japan) at 3200 r/minute for 5 minutes. Purified genomic DNA was dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored at -20°C.

Primer sets for polymerase chain reaction (PCR) assays

A specific primer for amplifying the genome of oral streptococci was designed based on the *S. mutans* ATCC 25175 gene (NCBI Accession No. EF536028) and subjected to Primer-BLAST (basic local alignment search tool) (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/>). The primer sequences (5' to 3') targeting all streptococci elongation factor-Tu were CCAATGCCACAACTCGTGAAC (forward) and GATCACGGATTTCATTTCAACC (reverse).

To test the specificity of a streptococci-specific primer, the following strains were used: *Streptococcus mutans* ATCC 25175, *S. sobrinus* ATCC 27351, *S. salivarius* ATCC 7073, *S. oralis* ATCC 10557, *S. gordonii* ATCC 10558, *S. sanguinis* ATCC 10556, *S. mitis* ATCC 49456, *S. constellatus* ATCC 27823, *S. anginosus* NCTC10713, *S. intermedius* GAI 1157, *S. pyogenes* ATCC 12344, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Staph. epidermidis* ATCC 14990, *Fusobacterium nucleatum* ATCC 25586, and *Escherichia coli* BL21. They were cultured in trypticase soy broth (TSB, Becton Dickinson, Sparks, MD, USA) under an anaerobic condition (80% N₂, 10% CO₂, 10% H₂) at 37°C [33, 34].

One ng of each bacterial DNA sample was confirmed by detection with the streptococci specific primer set using KOD-Plus-Neo DNA polymerase (TOYOBO, Tokyo, Japan). The amplification cycles were as follows: 2 minutes at 98°C for initial heat activation, then one cycle for 10 seconds at 94°C, 10 seconds at 62 °C, and 5 seconds at 68°C, for a total of 30 cycles. These PCR products were subjected to agarose gel electrophoresis using a 3.0% gel and stained with ethidium bromide, with detection performed at 302 nm with ChemiDoc XRS Plus (Bio-Rad Laboratories, Hercules, CA, USA). DNA amplicons were observed in ten oral streptococci, while no PCR products were demonstrated with DNA samples from the other six bacteria species (Figure 1). For amplifying the genome of bacterial 16S rRNA universal and eukaryotic 18S rRNA genes, primer sets reported in previous studies were used [35, 36]. The primer sequences (5' to 3') targeting bacterial the 16S rRNA gene were CGCTAGTAATCGTGGATCAGAATG (forward) and TGTGACGGGCGGTGTGTA (reverse), and those (5' to 3') targeting the 18S rRNA gene were TCTCAGGCTCCYTCTCCGG (forward) and AAGCCATGCATGYCTAAGTATMA (reverse).

Determination of microbial amounts

Stored samples were solved in room temperature. From those samples, genomic DNA was extracted and purified by the same method used for reference microorganisms. To determine microbial amounts in each sample, quantitative PCR assays were performed using a Thermal Cycler Dice Real-Time System II and with the following thermal cycle, as recommended for the TB Green Ex Taq (Takara Bio, Kusatsu, Japan) mixture: 95°C for 30 s, then 40 cycles for 5 s at 95°C and 1 m at 58°C for 16S rRNA and 18S rRNA, and at 62°C for oral streptococcal EF-Tu. Ten ng of genomic DNA from the samples was used as the template. Standard curves for each organism were plotted using *Ct* values obtained from amplification of genomic DNA. Those were extracted from *S. salivarius* ATCC 7073 (1.0×10^2 to 1.0×10^6 CFU) and *C. albicans* SC 5314 (1.0×10^1 to 1.0×10^6 CFU) cells. The numbers of *S. salivarius* and *C. albicans* were determined by plating culture dilutions in trypticase soy agar and YPD agar plates, respectively [35, 37]. The linearity of these assays was correlated between the *Ct* and microbial amounts. Correlation coefficients were 0.969 for 16S rRNA, 0.985 for oral streptococcal EF-Tu, and 0.979 for 18S rRNA. The amounts of the microorganisms were expressed as logarithm number of CFU.

Statistical analyses

A single correlation between measurements was tested by Spearman's rank correlation coefficient analysis. For assessing nutritional condition, participants were classified as BMI < 20 (undernutrition) or ≥ 20 (not undernutrition) according to the criteria for Asian elderly aged 70 years or older in The Global Leadership Initiative on Malnutrition (GLIM) [38]. For OHAT, the participants were divided into low and high OHAT groups based on a cutoff value of 5 (OHAT > 5 and OHAT ≤ 5), after referring to the median of the participants in this study and a previous study [39].

After distributions of all measurements were tested by one-sample Kolmogorov-Smirnov test, measurements with normal distribution were tested by Student's t test in comparison tests between groups of both for BMI and OHAT. In case of that measurements did not show normal distribution, Mann-Whitney's U test or Fisher's exact test was used

in comparison tests. For multivariable analysis to elucidate factors related with BMI or OHAT, multiple logistic regression analyses were performed. SPSS for Windows software, ver. 25.0 (IBM SPSS, Tokyo, Japan), was used for all data analyses.

Results

None of the subjects had either a smoking or drinking habit, and none had a history of antimicrobial or antifungal medication within one month before the examination. Two of the subjects were undergoing special medical care; as one received dialysis treatment three times a week and the other wore a cardiac pacemaker. Neither of those subjects presented outliers in BMI, OHAT, or oral microbial amounts, and their results were included in the analyses. In addition, none of the subjects suffered from oral cancer or OPMD.

Distribution of BMI and OHAT

BMI of participants showed a normal distribution, while OHAT did not. BMI ranged from 15.0 to 36.4 with an average $\pm$ SD of 21.3 ± 4.10 . Median OHAT was 5.0, and the 25th and 75th percentile values were 3.5 and 6.0, respectively (Fig. 2).

Measurements related with microbial amounts

The association between each microbial amount and the other observed values was subjected to Spearman's rank correlation analysis. Table 1 showed correlation coefficients of significantly related measurements with the amount any microorganisms in addition to OHAT score. In the correlation between microbial amounts, there was a significant positive relationship between total bacterial amount and streptococcal amount ($p = 0.512$, $p = 0.001$). On the other hand, fungi showed a negative relationship with both total bacteria and streptococci, although the relationship was slight and not significant. For nutritional condition, only streptococcal amount showed significant correlation with BMI ($p = 0.526$, $p < 0.001$). In oral conditions, TCI score was positively related with total bacterial amount ($p = 0.346$, $p = 0.029$), and was negatively related ($p = -0.331$, $p = 0.037$) with fungal amount. Moreover, fungal amount showed significant negative correlation with Mucus score ($p = -0.383$, $p = 0.018$) which meant lack of oral moisture increased fungal growth. OHAT score was related positively with fungal amount though the relationship was not significant ($p = -0.287$, $p = 0.068$).

Table 1
Correlations between microbial amounts, nutritional and oral conditions.

	Total bacterial amount	Streptococcal amount	Fungal amount	BMI	Eating independence ^a	TCI score	Mucus score	OHAT score
Total bacterial amount	1.00	0.512	-0.205	-0.072	0.150	0.346	-0.011	0.073
		0.001	0.199	0.655	0.348	0.029	0.949	0.651
Streptococcal amount	0.512	1.00	-0.141	0.526	-0.290	-0.053	0.075	-0.171
	0.001		0.379	< 0.001	0.066	0.746	0.654	0.286
Fungal amount	-0.205	-0.141	1.00	-0.055	0.228	-0.331	-0.383	0.287
	0.199	0.379		0.732	0.152	0.037	0.018	0.068
BMI	-0.072	0.526	-0.055	1.00	-0.269	-0.244	0.017	-0.184
	0.655	< 0.001	0.732		0.089	0.130	0.921	0.250
Eating independence ^a	0.150	-0.290	0.228	-0.269	1.00	0.169	-0.157	0.565
	0.348	0.066	0.152	0.089		0.297	0.346	- <0.001
TCI score	0.346	-0.053	-0.331	-0.244	0.169	1.00	0.091	0.010
	0.029	0.746	0.037	0.130	0.297		0.585	0.952
Mucus score	-0.011	0.075	-0.383	0.017	-0.157	0.091	1.00	-0.191
	0.949	0.654	0.018	0.921	0.346	0.585		0.250
OHAT score	0.073	-0.171	0.287	-0.184	0.565	0.010	-0.191	1.00
	0.651	0.286	0.068	0.250	< 0.001	0.952	0.250	
Significantly related measurements with the amount any microorganisms and OHAT were shown in this table.								
Upper value showed correlation coefficient. Lower value showed <i>p</i> -value.								
^a Degree of eating independence was scored as follows. 1: independent, 2: partial assistance needed, 3: full assistance needed.								

Comparisons of health conditions and microbial amounts between participants with and without undernutrition

Fifteen of the 41 participants were determined to have undernutrition based on a BMI cut-off value of < 20 , with the average $\pm$ SD for those 17.5 ± 1.43 , while that was 23.5 ± 3.49 for the non-undernutrition participants. Other than BMI, there were no significant differences between the groups for other systemic or oral health conditions. As for microbial measurements, only streptococcal amounts showed a statistically significant difference between subjects with and without undernutrition (Table 2).

Table 2

Comparisons of health conditions and microbial amounts between participants with and without undernutrition

	With undernutrition (BMI $\geq$ 20, n = 15)	Without undernutrition (BMI < 20, n = 26)	Total	p-value by the comparison test
BMI	17.5 $\pm$ 1.43	23.5 $\pm$ 3.49	21.3 $\pm$ 4.10	< 0.001 ^a
Males/females, %	4/11, 26.7	4/22, 15.4	8/33, 19.5	0.434 ^b
Age in years (average $\pm$ SD)	85.7 $\pm$ 9.70	83.9 $\pm$ 7.47	84.6 $\pm$ 8.28	0.523 ^a
Care needs score (median, range)	6, 1–7	5, 1–7	5, 1–7	0.445 ^c
Eating independence (not independent/independent, %)	6/9, 40.0	7/19, 26.9	13/28, 31.7	0.492 ^b
Mucus score (median, range)	24.3, 0.2–28.1	25.7, 1.5–29.9	25.3, 0.2–29.9	0.315 ^c
TCl score	1, 1–9	2, 1–13	2.0, 1–13	0.173 ^c
Number of present teeth (median, range)	4, 0–26	8.5, 0–25	6, 0–26	0.341 ^c
Number of decayed teeth (median, range)	0, 0–4	0, 0–9	0, 0–9	0.659 ^c
Number of filled teeth (median, range)	2, 0–16	6, 0–21	3, 0–21	0.108 ^c
Edentulous subjects (edentulous/dentate, %)	6/9, 40.0	5/21, 19.2	11/30, 26.8	0.272 ^b
Denture wearers (wearers/non-wearers, %)	8/7, 53.3	16/10, 61.5	23/18, 56.1	0.515 ^b
OHAT score (median, range)	5, 1–13	5, 1–10	5, 1–13	0.512 ^c
Total bacterial amount (average $\pm$ SD)	8.34 $\pm$ 0.50	8.32 $\pm$ 0.81	8.33 $\pm$ 0.62	0.917 ^a

Microbial amounts are shown as logarithm number of CFU.

^a t test, ^b Fisher's exact test, ^c Mann-Whitney U test

	With undernutrition (BMI ≥ 20, n = 15)	Without undernutrition (BMI < 20, n = 26)	Total	<i>p</i> -value by the comparison test
Streptococcal amount (average ± SD)	8.67 ± 0.83	7.80 ± 0.86	8.35 ± 0.93	0.003 ^a
Fungi detection (positive/negative, %)	15/11, 57.7	9/6, 60.0	24/17, 58.5	1.00 ^b
Total fungal amount (median, range)	1.95, 0-4.01	1.71, 0-4.72	1.94, 0- 4.72	0.758 ^c
Microbial amounts are shown as logarithm number of CFU.				
^a t test, ^b Fisher's exact test, ^c Mann-Whitney U test				

Comparisons of health conditions and microbial amounts between participants with and without poor oral health condition

Twenty six of the 41 participants had a high OHAT score (> 5), indicating a poor oral health condition, with the median and range for the high OHAT group 6 and 5–13, respectively, and for the low OHAT group, 2 and 1–4, respectively. As for systemic and oral health conditions, significant differences were found between the groups for care needs score, rate of subjects with eating independence, and number of decayed teeth. In microbial analyses, fungal amount and fungal detection rate were significantly higher in the high OHAT group, while no association with OHAT score was found for total bacterial amount or streptococcal amount (Table 3).

Table 3

Comparisons of health conditions and microbial amounts between participants with and without poor oral health condition

	With poor oral health (OHAT $\geq$ 5, n = 26)	Without poor oral health (OHAT < 5, n = 15)	Total*	p-value by the comparison test
OHAT score (median, range)	6, 5–13	2, 1–4	5, 1–13	< 0.001 ^c
Males/females, %	4/22, 15.4	4/11, 26.7	8/33, 19.5	0.434 ^b
Age in years (average $\pm$ SD)	85.9 $\pm$ 8.49	82.3 $\pm$ 7.62	84.6 $\pm$ 8.28	0.181 ^a
BMI	21.2 $\pm$ 4.65	21.5 $\pm$ 3.04	21.3 $\pm$ 4.10	0.812 ^a
Care needs score (median, range)	6, 2–7	1, 1–6	5, 1–7	< 0.001 ^c
Eating independence (not independent/independent, %)	13/13, 50.0	0/15, 0.0	13/28, 46.4	0.001 ^b
Mucus score (median, range)	24.1, 0.2–29.9	26.5, 18.8–29.9	25.3, 0.2–29.9	0.181 ^c
TCI socre	2, 1–13	2, 1–9	2.0, 1–13	0.825 ^c
Number of present teeth (median, range)	7, 0–26	2, 0–23	6, 0–26	0.583 ^c
Number of decayed teeth (median, range)	0, 0–9	0, 0–1	0, 0–9	0.030 ^c
Number of filled teeth (median, range)	4.5, 0–19	2, 0–21	3, 0–21	0.947 ^c
Edentulous subjects (edentulous/dentate, %)	5/21, 23.8	6/9, 40.0	11/30, 73.2	0.272 ^b
Denture wearer (wearers/non-wearers, %)	12/14, 46.2	11/4, 73.3	23/18, 56.1	0.114 ^b
Total bacterial amount (average $\pm$ SD)	8.34 $\pm$ 0.52	8.31 $\pm$ 0.78	8.33 $\pm$ 0.62	0.917 ^a

Microbial amounts are shown as logarithm number of CFU.

*Same data as shown in Table 3

¹ t test, ² Fisher's exact test, ³ Mann-Whitney U test

	With poor oral health (OHAT ≥ 5 , n = 26)	Without poor oral health (OHAT < 5, n = 15)	Total*	p-value by the comparison test
Streptococcal amount (average $\pm$ SD)	8.26 $\pm$ 0.85	8.51 $\pm$ 1.09	8.35 $\pm$ 0.93	0.407 ^a
Fungi detection (positive/negative, %)	19/7, 73.1	5/10, 33.3	24/17, 58.5	0.021 ^b
Fungal amount (median, range)	2.45, 0-4.72	0, 0-3.23	1.94, 0-4.72	0.028 ^c
Microbial amounts are shown as logarithm number of CFU.				
*Same data as shown in Table 3				
¹ t test, ² Fisher's exact test, ³ Mann-Whitney U test				

Logistic regression analyses

Multiple logistic regression analyses were performed with “not undernutrition” as a dependent variable (BMI ≥ 20 : 1, BMI < 20: 0) and the three related factors (streptococcal amount, eating independence level, TCI) as independent variables. After a stepwise procedure, streptococcal amount was selected for the final model (Table 5). The adjusted odds ratio (AOR) was 6.32, with a 95% confidential interval (CI) of 1.71–23.3 (p = 0.006).

Table 4
Logistic regression models for not undernutrition

	variable	AOR	95% CI	p-value
Full enter model	Streptococcal amount	6.10	1.66–22.4	0.006
	TCI	0.816	0.620–1.07	0.146
	Eating independence	0.744	0.264-2.10	0.577
Final model after stepwise procedure	Streptococcal amount	6.32	1.71–23.3	0.006
	TCI	0.808	0.620–1.05	0.114
Dependent variable: BMI ≥ 20 : 1, BMI < 20: 0				

Similarly, multiple logistic regression for accounting for poor oral health condition was performed. In the regression models, high OHAT was the dependent variable (OHAT ≥ 5 : 1, OHAT < 5: 0), considering the confounding, we excluded number of decayed teeth, and eating independence from the regression models, since those are included in the OHAT criteria or are evaluated for determination of long-term care need. Consequently, long-term care need level, denture use frequency and fungal amount were used as independent variables. After a stepwise procedure, long-term care need level and fungal amount were selected for the final model (Table 6). For fungal amount, the AOR was 2.44, with a 95% confidential interval (CI) of 0.987–6.04 (p = 0.053).

Table 5
Logistic regression models for poor oral health condition

	variable	AOR	95% CI	p-value
Full enter model	Long-term care need level	2.60	1.40–4.82	0.002
	Fungal amount	2.72	1.05–7.05	0.039
	Denture use frequency	0.386	0.107–1.40	0.147
Final model after stepwise procedure	Long-term care need level	2.60	1.52–5.13	0.002
	Fungal amount	2.44	0.987–6.04	0.053
Dependent variable: OHAT > 5: 1, OHAT ≤ 5: 0				

Discussion

In this study, BMI was used as an indicator of systemic nutritional conditions. BMI is widely used to determine whether an individual is underweight or overweight, and also employed in a variety of nutrition assessment tools as a criterion for assessing undernutrition [38, 40, 41]. We found an average BMI of our participants of 21.3 was closed to the average BMI ranging 20.6 to 22.5, reported in previous investigations conducted in Japan of institutionalized elders [42–44]. Thus, the nutritional status of our participant group was considered to be comparable to that of the general institutionalized elders in Japan.

The results showed that oral streptococci amount had a significant association with BMI. Bacteria belonging to the genus *Streptococcus* are the initial inhabitants of the oral cavity, as they are generally acquired right after birth and play an important role in assembly of the oral flora. Moreover, dominance continues until old age [45]. Most of the oral streptococci are not pathogenic and thought to contribute to the oral homeostasis [26]. Conversely, change of eating condition could have an influence on the streptococcus-dominant normal balance of oral microflora. Takeshita et al. reported that dominant genera, such as *Streptococcus* and *Veillonella*, were observed in much lower proportions in tube-fed subjects as compared to those fed orally [46]. Even if the oral-environmental change is not as drastic as tube feeding, a decrease in food intake could have a negative effect on growth of oral streptococci since eating by mouth supplying nutrition to the microbes is necessary for maintenance of indigenous oral flora. Malnutrition represented by low BMI causes frailty in older individuals and it is known that the immune system becomes altered by frailty [47].

We speculate that, in older individuals with low BMI, immune responses in their oral mucosa change, then oral cavity is not a preferential environment for commensal streptococci. The commensal oral streptococci, such as *S. mitis*, *S. oralis*, and *S. sanguinis*, is avoiding the effects of salivary IgA by producing IgA proteases [48, 49]. Not only salivary IgA, but systemic and oral mucosal immune system is also closely associated with commensal oral streptococci [50, 51]. It would explain the significant association between BMI and streptococcal amount observed in this study.

On the other hand, fungal amount was related with oral moisture condition and OHAT. Wu et al. reported that oral care intervention in patients at the end stage of cancer reduced detection of *Candida* on the tongue, accompanied by a decrease in OHAT score [52], which was confirmed by the present results. OHAT criteria includes dryness in assessments for lip, tongue, gums and tissues, and saliva. Oral dryness is one of the significant factors with fungal

colonization in oral cavity [53, 54]. Saliva contains glycoproteins that act to inhibit adhesion of fungi to epithelial cells [55]. Saliva also contains antimicrobial proteins, histatins, defensins et al. those can suppress *Candida* spp. [56]. Thus, dry condition is preferable to colonize for fungi. In contrast with streptococci, fungi have an ability to grow under altered mucosal conditions. These differences in suitable conditions for colonization may allow fungi and streptococci to grow competitively in the oral cavity. However, previous in vitro studies of relationship between fungal and streptococcal colonization have shown contradictory results. Some studies demonstrated oral streptococci contributed to colonization or to modify the virulence of oral fungi [57, 58]. On the contrary, the others reported that a certain streptococcus could inhibit *Candida* colonization in oral cavity [59–61]. In the present study, both fungal and streptococcal amounts in the oral cavity of the same subjects were determined, and the results showed a negative, though not significant, relationship between oral streptococcal and fungal abundance. The actual state of between fungi and streptococcus colonization in the human oral cavity is unknown, and the present results as well did not show an obvious relationship. Elucidation of prevalence of both oral fungi and oral indigenous streptococci in the oral cavity is important for understanding the relationship between frailty and oral health in elderly individuals. It is considered that the oral streptococcus-specific primer established in the present study may be an effective tool for future surveys.

This study has some limitations. First, it is difficult to generalize the results due to the small number of participants and single institution. Furthermore, because of the cross-sectional design, changes in BMI were not recorded. A rapid decrease in BMI is as important as a stable state for assessment of frailty in elders [38]. Thus, additional studies with greater numbers of subjects and institutions that are utilize longitudinal observations are required.

Conclusion

Oral streptococcal levels were found to be associated with the general nutritional status of institutionalized older individuals, while oral fungal levels were associated with oral health status. Therefore, it is necessary to survey oral indigenous bacteria prevalence in oral cavity, including streptococci as well as pathogenic microorganisms to understand the relationship of frailty with oral microflora in older subjects.

Abbreviations

AOR

Adjusted odds ratio; BMI:Body mass index; CFU:Colony forming unit /mL; CI:Confidential interval; OHAT:Oral Health Assessment Tool; SD:Standard deviation; TCI:Tongue coating index; WHO:World Health Organization

Declarations

Declarations

Ethics approval and consent to participate

We obtained informed consent from all participants who took part in our study. This study protocol was approved by the Medical Ethics Committee of Iwate Medical University School of Dentistry (01340).

Consent for publication

Not applicable.

Declarations

Competing interests

The authors have no conflicts of interest to declare.

Funding

A research grant from Iwate Prefecture for Strategic Developmental Research.

Authors' contributions

Hanako Sato was in charge of the sample collection, oral examinations, microbiological analyses including sample preparations, genome purification and polymerase chain reaction analyses of all reference microorganisms and oral specimens, and of writing the manuscript. Akira Yano contributed to the conception of this study and was in charge of planning of microbial analyses in addition to liaison and coordination with cooperating institution in addition to helping draft the manuscript. Yu Shimoyama and conducted microbiological analyses including design of PRC primer, and also determined the optimal conditions for detection of microorganisms in the oral specimens. Toshiro Sato contributed to collection of oral specimens.

Acknowledgements

We would like to express our deep gratitude to the staff members of the Long-Term Care Health Institution as well as Mr. Katsufumi Miyasaka, chairman of the social welfare corporation Sapporo Keiyukai, for their cooperation in this clinical study. It was supported by A research grant from Iwate Prefecture for Strategic Developmental Research.

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Figures

Figure 1

PCR products from 16 strains of streptococcus specific primer designed for this study.

Figure 2

Distribution of BMI (A) and OHAT scores (B) in all subjects. BMI distribution showed normality, while that of OHAT did not. BMI average $\pm$ SD of was 21.3 ± 4.10 . OHAT quartiles were 3.5 for the 25th percentile, 5.0 for the 50th percentile (median), and 6.0 for the 75th percentile.