Original Article

Could Frequency of Candida spp. Be Different on Various Parts of Tongue in Denture and Non-denture Wearers?

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Abstract

Background & Objectives: Oral Candidiasis is the most common opportunistic fungal infection that could affect the oral mucosa. Studies to date did not compare the colony count of candida of the anterior and posterior surfaces of the tongue. This study makes an attempt to compare the Candida spp. on the anterior and posterior surfaces of tongue among healthy denture and non-denture wearers.

Materials & Methods: Participants of the current cross-sectional study were 26 healthy denture wearers (DW) and 10 healthy non-denture wearers (NDW). Oral specimens were collected from anterior and posterior tongue dorsa by swabbing for mycological examination. After 48 h incubation on Sabouraud dextrose agar (S) and chloramphenicol (SC) and chloramphenicol and Cycloheximide (SCC) ™ medium, C. Albicans and non-Albicans were identified by Germ Tube test. Isolated colonies were evaluated. Obtained data were analyzed by SPSS software version 17. Chi-square, Mann-Whitney and Wilcoxon tests were used for statistical comparison of data due to non-compliance with normal distribution.

Results: The higher rate of isolated colonies was seen in DW group compared to NDW group (P-Value = 0.03). There was a significant difference between candida colonization of anterior and posterior surface of tongue (P-Value = 0.006). *C. Albicans* was the most common isolated candida species.

Conclusions: Mycological findings of this study revealed that the presence of denture can increase colonization of candida on the posterior surface of the tongue. It could be an important role in choosing the best form for medical management of oral candidiasis.

Keywords: Candida Albicans, colonization, Age, denture

Introduction

Candida species are normal commensal organisms of the oral mucosa that may lead to candidiasis in immunocompromised patients (1, 2). In most cases, these lesions are caused by *Candida* Albicans (3).

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This organism can overlap with bacteria in dental biofilms, attach to epithelial cells, and invade oral tissues, which could lead to candidiasis (4).

Denture stomatitis is a common form of oral candidiasis that presents as diffuse inflammation on the palatal surface. It is known as a candida-associated lesion. *C. Albicans* is the main pathogen besides the other pathogens (5-7). *C. Albicans* is a normal commensal organism in 40 to 60% of healthy individuals (8).

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This microorganism could be detected as Candidiasis in 70-80% of healthy individuals after wearing prosthetic dentures (8-10).

Adhesion to the epithelial cells of the oral mucosa is known as the first step of oral candida colonization. The properties of epithelial cell receptors are important factors in the adhesion to the epithelial cells. Fibronectin may act as a candida receptor in epithelial surfaces (11). According to studies, candida is detected in higher frequencies in denture wearers rather than others, especially in palatal covering dentures, but studies so far have not compared the colony count of this yeast on the anterior and posterior surfaces of the tongue. In previous studies, the number of Candida colony count on the dorsal surface of the tongue has been reported to be more than 10,000 CFU (12).

Correctly incorporated posterior palatal seal into the prosthesis has an important role in preventing reflux and food accumulation beneath the denture. The presence of an ill-fitting denture could be the cause of the transfer of betrayed candida to oral space (13). The difference in candida colonization on the anterior and posterior surface of the tongue can impact choosing the best form of medical management of candidiasis (mouthwashes, tablets, pastilles, or troches). None of the previous studies compared different parts of tongue regarding candida colony counts. This study makes an attempt to compare the mean colony counts of C. Albicans and non-Albicans on the anterior and posterior surface of tongue.

Materials & Methods

This cross-sectional study conducted in Yazd during 2020. In 2020, from individuals who attended for annual oral health checkups in the oral and maxillofacial department of Yazd dental school, 36 candidates (21 men and 15 women) aged 32 to 85 years were recruited to this study to detect Candida species. The mean age and standard deviation of DW participants were 53.1 and 14 and NDW participants were 31.2 and 8.9, respectively. DW had been using removable complete dentures for a minimum of 1-year. The mean length of time wearing dentures in all patients was 13.6÷14 months. All methods were conducted in accordance with the ethical standards of the declaration of Helsinki.

Inclusion and exclusion criteria

No one in either group had received antibiotics, steroids, or immune therapy, or used any antiseptic mouthwash since one week ago. Having a history of any systemic disease, malignancy or smoking precluded entry into the study. Patients who ignored instructions for denture health, such as removing it overnights or frequently cleaning it, were also excluded. Persons using defective or damaged dentures were also excluded from the study.

First, the checklist containing demographic information about age and sex, duration of denture uses, and oral health status was prepared and completed by participants.

The sample size was 72, which was actually containing 52 samples from DW and 20 from NDW. In each person, one sample was taken from anterior two-third and another from posterior one-third of the tongue. In order to determine the correct coefficient ratio of C. Albicans colonies on the anterior to the posterior surface of the tongue, 10 NDW were sampled. All the candidates gave informed consent to participate in this study after receiving sufficient information. Oral examinations were performed by a skilled dentist. Oral samples were collected by swabbing 10 times vertically from the anterior surface of tongue and with another swab from the posterior one-third of the tongue with a sterile cotton swab. Samples in all patients were collected from the middle of the tongue in the anterior and posterior (anterior foramen cecum) regions. It was immediately placed in a tube containing 2 ml of sterile normal saline and immediately were transferred to the microbial laboratory. Samples in proximity to a flame were cultured on mediums containing Sabouraud's dextrose agar (S, SC, SCC, which simply contains chloramphenicol 50 mg/lit and Cycloheximide 100m mg/lit, respectively). Then, they were incubated at 37 ° C for two days. After incubation, all isolated colonies were evaluated under a light microscope (SE, Nikon, Japan). Germ Tube test (including dissolving the colony in fresh human serum and incubating at 37 ° C for 2 hours and then examining a drop of it under a microscope) was performed to determine the Albicans species (5).

Fatemeh Owlia, et al.

Statistical analysis

Data were collected and entered into SPSS software version 17. Required tables and indicators for statistical comparison were prepared. First, the normality of the data (distribution of C. *Albicans* colony counts on the anterior and posterior surface of tongue) was evaluated by the Shapiro-Wilk test. Finally chi-square, Mann-Whitney and Wilcoxon tests were used for data with non-normal distribution. A significance level of 5% was considered for all analyses.

Results

21 men (19 DW and 2 NDW) and 15 women (7 DW and 8 NDW) entered the study. Totally, 72 samples were taken from anterior and posterior surface of tongue. 10 persons (27.7%) were NDW and 26 persons (72.3%) from DW. The mean age of DW and NDW were 53.1±14 years and 31.2 ±8.9 years respectively.

Culture results of anterior and posterior surface of tongue in DW and NDW were the same. In NDW group, 30% had positive culture (20% Albicans and 10% non-Albicans) and 70 % had negative ones. Positive culture of Candida species was seen in 76.9% of DW group. DW harbored a mixed species of Candida Albicans and non-Albicans which were predominantly C. Albicans (75%). In posterior surface, a significantly greater proportion of DW had higher colonization of Candida compared to NDW. Pearson chi-square test showed a statistically significant difference (P-Value = 0.03).

In NDW, positive culture for C. *Albicans* in anterior surface of tongue was positive in posterior area too and vice versa (P-Value=0.0001). This finding was similar in DW, too. (P Value = 0.001) (Table 1). Spearman correlation coefficient showed a positive and significant correlation between Candida colonization and different parts of tongue in DW (P-Value= 0001, r=1) (Table 1).

Table 1. Distribution frequency of C. Albicans and non-Albicans species of anterior and posterior parts of tongue in studied groups

	Anterior area of tongue posterior area of tongue	Negative culture	C. *Albicans*	Non-Albicans species	Total	
		number (٪)	number (٪)	number (٪)	number (٪)	
NDW*	Negative culture	7 (70)	0 (0)	0 (0)	7 (70)	P Value = 0.0001
	C. *Albicans*	0 (0)	2 (20)	0 (0)	2 (20)	
	Non-Albicans species	0 (0)	0 (0)	1 (10)	1 (10)	
	total	7 (70)	2 (20)	1 (10)	10 (100)	

	Anterior area of tongue posterior area of tongue	Negative culture	*C. Albicans*	Non-Albicans species	Total	
		number (٪)	number (٪)	number (٪)	number (٪)	
DW**	Negative culture	6 (23.1)	0 (0)	0 (0)	6 (23.1)	P Value = 0.0001
	C. Albicans	0 (0)	15 (57.7)	0 (0)	15 (57.7)	
	Non-Albicans species	0 (0)	0 (0)	5 (19.2)	5 (19.2)	
	total	6 (23.1)	15 (57.7)	5 (19.2)	26 (100)	

Chi-square Test
*Non denture wearers
** Denture wearers

Although the frequency of C. Albicans in each group was more in posterior than anterior area of tongue, only in DW group, this relationship was significant (P Value = 0.006) (Table 2).

Table 2. Mean and standard deviation of the C. Albicans colony counts (CFU) in different parts of tongue

Groups	Mean± standard deviation of *CFU*		P value
	Anterior area	Posterior area	
NDW*	220 ± 42.4	630 ± 98.9	0.18
DW**	886 ± 1625.7	2324 ± 3424	0.006

Wilcoxon Test
*Non denture wearers
** Denture wearers

In DW group, although a higher proportion of C. Albicans was isolated from subjects in the 50-85-year-old group, there was no significant difference between age and frequency of C. *Albicans* (Table 3).

Table 3. Distribution of C. Albicans among denture wearers based on gender and different age groups

Culture region	Age group	Number (%)	Mean± Standard deviation	Minimum of CFU	Maximum of CFU	P-value
Anterior culture	32-49	8(53.3%)	563.7 ± 953.1	20	2750	0.685
	50-85	7(46.6%)	1254.2 ± 2239.9	10	6200	
	Total	15(100%)	886 ± 1652.7	10	6200	
Posterior culture	32-49	8(53.3%)	1400.6 ± 1902.5	35	5500	0.463
	50-85	7(46.6%)	3379.2 ± 4549.4	80	12500	
	Total	15(100%)	2324 ± 3424	35	12500	

Culture region	gender	Number	Mean± Standard deviation	Minimum	Maximum	P-value
Anterior culture	Male	11	417.2 ± 845.7	10	2750	0.036
	Female	4	2175 ± 2709.7	300	6200	
	Total	15	886 ± 1652.7	10	6200	
Posterior culture	Male	11	1264.5 ± 1971.7	35	5500	0.037
	Female	4	5237.5 ± 5130.2	800	12500	
	Total	15	2324 ± 3424	35	12500	

Mann-Whitney Test

A correlation of gender with each part of tongue has been made. A statistically significant difference between males and females in the intensity of yeast growth was found. Females showed a higher rate of yeast growth in both parts of tongue (P<0.05). (Table 3). Considering the limitation of positive cultures in NDW group, the correlation could not be evaluated in this group.

As shown in table 4, however, the average of *C. Albicans* decreased as the time of denture use increased.

There was not a significant correlation between time of denture use and increased oral *C. Albicans* isolated from different parts of tongue.

Table 4. distribution frequency of culture results of C. Albicans in the anterior and posterior surface of tongue in denture wearers according to the time of denture use

Culture region	time of denture use (months)	Number (%)	Mean± Standard Deviation	Minimum	Maximum	P Value
Anterior culture	1-10	7 (46.7%)	1270 ± 2223.8	40	6200	
	11-36	8 (53.3%)	550 ± 973.5	10	2750	0.45
	Total	15(100)	886 ± 1652.7	10	6200	
Posterior culture	1-10	7 (46.7%)	3133.5 ± 4481.5	110	12500	
	11-36	8 (53.3%)	1615.6 ± 2236.8	35	5500	0.29
	Total	15(100)	2324 ± 3424	35	12500	

Mann-Whitney Test

Discussion

In this study, 52 samples from DW and 20 samples from NDW were taken. Samples were obtained with sterile cotton swabs from the anterior and posterior surfaces of tongue at the midline site, and the candida spp. were counted. To date, no study was found about comparing the colony counts of Candida spp. of the anterior and posterior surfaces of tongue, and other research-ers have examined other oral sites (14,15).

However, *Candida colonization* varies widely in the oral cavities of healthy persons regarding previous studies (16). Aging, denture wearing, poor oral/denture hygiene, and low salivary flow, in addition to systemic diseases, were reported as the factors associated with Candida colonization in the oral cavities of elderly people (15).

In the present study, higher colonization of Candida species was seen in DW compared with NDW. Al-Dossary et al. found that the ability of

C. Albicans colonization was higher in denture wearers than in healthy people with teeth. There was an increased risk of oral candidiasis in men, older ages, and complete denture wearers (17). Abu-Elteen et al., detected *C. Albicans* in 78.3% of DW and 36.8% NDW. the most common sites of candida colonization in healthy individuals were tongue, palate, and cheek whereas in denture wearers was upper and lower dentures(18). This finding showed statistical significance in comparison with NDW (P = 0.03). A similar trend was seen by some researchers that Candida colonization was higher among DW(14, 17). In the study of Prakash et al. (19) oral samples were taken from palatal mucosa of DW and NDW while in the present study, the samples were obtained from anterior and posterior surfaces of tongue. In both studies, a higher rate of candida colonization was seen in DW. Denture insertion accelerates colonization and biofilm formation. Therefore, with ignoring the number of remaining teeth, dentures have been used as a susceptible factor to colonization by non-Albicans and growth of C. Albicans, at least in our subjects.

The surface porosities of acrylic resin bring about modifications in the physiology and normal flora of the mouth. It led to the allusion of acidogenic microorganisms and Candida. Therefore, oral health improvement in persons who wear removable prostheses has critical importance (20). The tissue contact surface of the denture increases the easy colonization of acidogenic microorganisms and Candida. The preparation of a warm and humid space under the dentures is the suitable condition for Candida colonization. Another issue in this context may be that removable dentures obstruct the salivary flow from minor salivary glands and the free exchange of oxygen. Thus, the resultant low pH level facilitates the growth of *C. Albicans* (21). Concerning the maintenance of denture hygiene, in order to improve oral mucosal health, the participants were informed to regularly clean their dentures and keep them out of mouth overnight. It is an efficient way to control yeast colonization in denture wearers.

The posterior surface of the tongue showed a higher rate of candida colonization than anterior area. Although the only significant correlation was seen in DW, the same trend was followed in NDW, too. There was no significant difference between the yeast growth of *C. Albicans* of posterior and anterior surface of tongue in NDW. It can be concluded that in patients with normal teeth, oral homeostasis can prevent candida colonization. So, in future studies, just the posterior surface of tongue could be sampled. loss of the end seal of the maxillary prosthesis competency may be the main cause (13). Thus, the Candida colonies formed on the tissue surface of the denture's posterior region are more susceptible to spread rather than the colonies from the anterior part from the labial flange of the denture. This result should be the point of attention for choosing the best form of medicine for the management of oral candidiasis in DW and NDW.

In all of the participants, when the candida culture of the anterior surface of tongue was negative, it was negative in the posterior region as well. This result was repeated for positive culture cases. The difference was significant in both groups. It could be concluded the fungal flora on anterior surface of tongue was similar to the posterior surface. It could be suggested that posterior one-third of the tongue is the best location for sampling for the assessment of colony counts of Candida species. Differential counting of other species of candida should be considered in immunocompromised patients in future studies.

In this study, like previous studies, *C. Albicans* was the most common species. In similar studies, the most common type of Candida found in different parts of tongue was *C. Albicans* (12, 14, 20, 22, 23) on both surfaces of tongue *C. Albicans* was isolated more in females than males in DW with statically significant power.

Women revealed significantly the higher distribution of Candida colonies in different parts of tongue. In a similar study by Loster et al.(24), among all subjects, there was a statistically significant relationship between candida spp. growth intensity and gender.

In each age group, the number of infection-free people was higher in men than women (24). This result can be justified by considering the hormonal changes in women and the presence of predisposing factors to anemia in them, which according to available references (25) is one of the important factors leading to candidiasis.

Although aging causes a progressive increase in Candida counts in the oral cavity with higher counts being observed among elderly DW but there was no significant relationship between Candida counts and age. This could be explained by the diminishing protective capacity of the immune system, which often occurs with aging (26). Sato et al stated that some factors related to non-*Albicans* colonization were "over 80 years of age", "number of remaining teeth", and "use of dentures" (15). According to more frequency of non-Albicans species in NDW, this study confirmed it.

There was not a statistically significant correlation between Candida colonization and the duration of using dentures in anterior and posterior of tongue. ($P > 0.05$). It is worth mentioning that the resorption of alveolar bone is a serious and common clinical problem that leads to a reduction of retention and stability of dentures (27).

Future studies with a large sample size may show a significant relationship between age and length of time of wearing dentures and the average Candida colony counts. This survey might be the first study on the comparison of the amount of *Candida* on anterior and posterior part of tongue in DW and NDW, especially in subjects without signs or symptoms of candidiasis. The results of this study could be helpful in the treatment of candidiasis. However, it is unclear whether the colony count of *Candida* is a risk factor for the incidence of candidiasis. It could be suggested in future studies that the colony counts of both *C. Albicans* and non-*Albicans* will be evaluated after adjusting a number of remaining teeth for the confounders.

It should be mentioned that one of the limitations of this study was the high cost of mycological evaluations due to sanctions. Considering the lack of studies in this regard, this survey was designed as a first step for designing future experiments.

Conclusion

Mycological findings of this study revealed that the presence of dentures can increase the colonization of candida on posterior surface of tongue. It could be an important role in choosing the best treatment option for the management of oral candidiasis.

Acknowledgement

Conflict of interest

Authors mention that there is no conflict of interest in this study.

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