

Frequency of Candida Spp. On the Anterior and Posterior Surfaces of Tongue Among Healthy Denture and Non-denture Wearers

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Research Article

Keywords: Candida Albicans, colonization, Age, denture, gender, Elderly

Posted Date: February 25th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-246905/v1>

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Abstract

Background: oral Candidiasis is the most common opportunistic 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.

Objectives: hence, this study was aimed to compare the Candida spp. on the anterior and posterior surfaces of tongue among healthy denture and non-denture wearers.

Methods: in current cross-sectional study, oral specimens were collected from anterior and posterior tongue dorsa by swabbing for mycological examination. Samples were collected from 26 healthy denture wearers (DW) and 10 non-denture wearers (NDW). All samples were cultured on Sabouraud's dextrose agar (S) and chloramphenicol (SC) and chloramphenicol and cycloheximide (SCC) mediums. Isolated colonies were counted. Germ Tube test was performed to determine the albicans species. Data was collected and entered in SPSS software version 17. Mann-Whitney and Wilcoxon nonparametric tests were used for statistical comparison of data due to non-compliance with normal distribution.

Results: the higher density of isolated colonies were seen in denture wearer group comparing to others (P Value = 0.031). There was a significant difference between candida colonization of anterior and posterior of tongue (P Value = 0.006). *C. Albicans* was the most common isolated candida species.

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

Introduction

Candidiasis is the most common opportunistic yeast infection that affects the oral mucosa. In most cases, these lesions are caused by *Candida albicans*(1). This organism can overlap with bacteria in dental biofilms, attach to epithelial cells, and invade oral tissues, that could lead to candidiasis (2).

Denture stomatitis is a common form of oral candidiasis presents as diffuse inflammation on the palatal surface. It is known as candida-associated lesion. *C. albicans* is the main pathogen beside the other pathogens (3–5). *C. albicans* is normal commensal organisms in 40 to 60% of healthy individuals (6). This microorganism could be detected as Candidiasis in 70–80% healthy individuals after wearing prosthetic dentures (6–8).

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(9).

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 past literatures, the number of *Candida* colony count on the dorsal surface of the tongue has been reported to be more than 10,000 CFU (10).

Correctly incorporated posterior palatal seal into the prosthesis has an important role in preventing reflux and food accumulation beneath the denture. Presence of ill-fitting denture could be the cause of transfer betrayed candida to oral space (11). Difference in candida colonization on the anterior and posterior of the tongue can impact on choosing the best form of medicine management of candidiasis (mouth washes, tablets, pastilles or troches). Al-Dossary et al., found 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 (12). 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(13). None of the previous studies compared different parts of tongue regarding candida colony counts. Therefore, this study, was aimed to compare the mean colony counts of *C. albicans* and *non-albicans* on the anterior and posterior of tongue.

Methods And Materials

This was a cross-sectional study conducted in Yazd during 2020. The study was approved by the Ethics Committee of Yazd Shahid Sadoughi University of Medical Sciences.) IR.SSU.REC.1399.179 (The study was carried out on 36 subjects (21 men and 15 women). The age range was in 32 to 85 years. It included a group comprising 26 DW with age range of 53.1 ± 14 years and 10 NDW with age range of 31.2 ± 8.9 years. DW had been using removable complete dentures for a minimum of 1-year. The mean length of time wearing denture in all patients was 13.6 ± 14 months. All of them referred to the Department of Oral and Maxillofacial medicine of Yazd Dental School in during 2020. All methods were carried out in accordance with relevant guidelines and regulations.

Inclusion and exclusion criteria

No one in either groups had received antibiotics, steroids or immune therapy, or used any antiseptic mouthwash since a week ago. Having a history of any systemic disease, malignancy or smoking lead not to enter the study. Patients who ignored instructions for denture health, such as removing it over nights or frequently cleaning it, were also excluded. Persons using defective or damaged dentures also were excluded from the study.

First, a questionnaire 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 took 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 of

the tongue, 10 NDW were sampled. After Explaining the purpose of the study and obtaining written consent, samples were taken with sterile cotton swabs from the anterior of tongue and with another swab from the posterior one-third of the tongue. 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 1 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 0.05 mg / mL and cycloheximide, respectively) then they were incubated at 37 ° C for two days. After incubation, all isolated colonies were counted. 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(3).

Statistical analysis

Data was collected and entered in SPSS software version 17. Required tables and indicators for statistical comparison was prepared. First the normality of the data (distribution of *C. albicans* colony counts on the anterior and posterior of tongue) was evaluated by the Shapiro-Wilk test. Mann-Whitney and Wilcoxon tests were used for data with non-normal distribution. A significant level of 5% was established for all analysis.

Results

21 men and 15 women entered to the study. Totally 72 samples were taken from anterior and posterior surface of tongue. 20 samples (27.7%) were taken from NDW and 52 samples (72.3%) were taken from DW. The mean age DW and NDW were 53.1 ± 14 years and 31.2 ± 8.9 years respectively.

From the samples analyzed 76.9% ($n = 20$) in DW and 30% ($n = 3$) in NDW were *Candida* positive. and *Candida albicans* was the most frequently isolated species, accounting for 75% and 66.6% of all the species identified in DW and NDW respectively.

There was a significant difference in the *Candida* species isolated from different parts of tongue. Positive culture of *Candida* species on the anterior surface was seen in 76.9% of DW. DW harbored a mixed species of *Candida* (80.8%) which was predominantly *C.albicans* (57%), while in NDW a mixed species of *Candida* (30%) was seen with 20% for *C.albicans*. In posterior surface, a significantly greater proportion of DW had higher colonization of *Candida* compared to NDW. The difference was statistically significant ($P = 0.031$). (Table 1).

Table 1
Distribution of *Candida* species isolated from the anterior and posterior parts of tongue in DW and NDW

Study groups	Denture wearers (DW)	Non-denture wearers (NDW)	Total
Culture results	number (n)	number (n)	number (n)
Negative culture (anterior)	6 (23.1)	7 (70)	13 (36.1)
Negative culture (posterior)	6 (23.1)	7 (70)	13 (36.1)
<i>C. albicans</i> (anterior)	15 (57.7)	2 (20)	17 (47.2)
<i>C. albicans</i> (posterior)	15 (57.7)	2 (20)	17 (47.2)
<i>Non-candida spp</i> (anterior)	5 (19.2)	1 (10)	6 (16.7)
<i>Non-candida spp</i> (posterior)	5 (19.2)	1 (10)	6 (16.7)
Total	26 (100)	10 (100)	36 (100)
P Value = 0.031			

In NDW, positive culture for *C. albicans* in anterior surface of tongue was positive in posterior culture, too and vice versa (P Value = 0.003). this finding was repeated in DW, too. (P Value = 0.001) (Table 2). Spearman correlation coefficient between the results of anterior and posterior surface was $r = 1$, which indicates the results are the same, which showed a positive and significant correlation between candida colonization and different parts of tongue in DW (P Value = 0.001). (Table 2)

Table 2

Distribution frequency of culture results of *Candida* species of different parts of tongue in NDW and DW

NDW	Anterior culture	Negative culture	<i>C. albicans</i>	Other <i>Candida</i> spp.	Total	P Value = 0.003
	Posterior culture	number (n)	number (n)	number (n)	number (n)	
	Negative culture	7 (70)	0 (0)	0 (0)	7 (70)	
	<i>C. albicans</i>	0 (0)	2 (20)	0 (0)	2 (20)	
	Other <i>Candida</i> spp.	0 (0)	0 (0)	1 (10)	1 (10)	
	total	7 (70)	2 (20)	1 (10)	10 (100)	
DW	Anterior culture	Negative culture	<i>C. albicans</i>	Other <i>Candida</i> spp.	Total	P Value = 0.001
	Posterior culture	number (n)	number (n)	number (n)	number (n)	
	Negative culture	6 (23.1)	0 (0)	0 (0)	6 (23.1)	
	<i>C. albicans</i>	0 (0)	15 (57.7)	0 (0)	15 (57.7)	
	Other <i>Candida</i> spp.	0 (0)	0 (0)	5 (19.2)	5 (19.2)	
	Total	6 (23.1)	15 (57.7)	5 (19.2)	26 (100)	
(Fisher's Exact Test)						

In contrast to NDW, the prevalence of *C. albicans* was higher in posterior part of tongue in DW (P Value = 0.006). (Table 3)

Table 3

Mean and standard deviation of the *C. albicans* colony counts in anterior and posterior parts of tongue according to the culture site

Studied groups	Mean \pm standard deviation of <i>C. albicans</i> colony count in anterior part of tongue	Mean \pm standard deviation of <i>C. albicans</i> colony count in posterior part of tongue	P value
NDW	220 \mp 42	630 \mp 98	0.18
DW	886 \mp 1625	2324 \mp 3424	0.006
(Wilcoxon test)			

In DW group, although a higher proportion of *C. albicans* was isolated from subjects in the 50-85-year-old group, there is no significant difference between age and prevalence of *C. albicans*. (Table 4)

Table 4
Distribution of *C. albicans* among denture wearers in different age groups

Culture region	Age group	Number (%)	Mean ± Standard deviation	Minimum	Maximum	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	
(Mann-Whitney Test)						

A correlation of gender with each part of tongue has been made. The results are shown in Table 5. A statistically significant difference between males and females in the intensity of yeast growth was found. females showed higher rate of yeast growth in both parts of tongue ($P < 0.05$). (Table 5)

Table 5
Yeast growth in each part of tongue between genders in denture wearers

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)						

As shown in Table 6, however, the average of *C. albicans* decreased with increasing the time of denture use, there was not a significant correlation between time of denture use and increased oral *C. albicans* isolated from different parts of tongue;

Table 6

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 (months)

Culture area	time of denture use (months)	Number (%)	Mean \pm Standard Deviation	Minimum	Maximum	P Value
Anterior culture	1–10	7 (46.7%)	1270 \mp 2223.8	40	6200	0.451
	11–36	8 (53.3%)	550 \mp 973.5	10	2750	
	Total	15(100)	886 \mp 1652.7	10	6200	
Posterior culture	1–10	7 (46.7%)	3133.5 \mp 4481.5	110	12500	0.297
	11–36	8 (53.3%)	1615.6 \mp 2236.8	35	5500	
	Total	15(100)	2324 \mp 3424	35	12500	

Discussion

In this study, 52 samples from DW and 20 ones 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. According to the authors said, to date, no similar study has been performed on comparing the colony counts of Candida spp. of the anterior and posterior surfaces of tongue, and other researchers have examined other oral sites .(13, 14)

In the present study a higher colonization of Candida species was seen in DW compared with NDW. This finding showed statistical significance when compared with NDW (P = 0.031). A similar trend was seen by some researchers that Candida colonization was higher among DW(12, 14). In the study of Prakash et al. (15) oral samples were taken from palatal mucosa of DW and NDW in the present study samples were obtained from anterior and posterior surfaces of tongue. In both study higher rate of candida colonization was seen in DW. Denture insertion accelerates the colonization and biofilm formation. 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(16). Tissue contact surface of the denture increases the easy colonization of acidogenic microorganisms and Candida. the preparation 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* (17). 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 the efficient ways to control yeast colonization in denture wearers.

The posterior surface of the tongue showed higher rate of candida colonization than anterior area, although only significant correlation was seen in DW ($P = 0.006$), but 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(11). 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 management of oral candidiasis in DW and NDW.

In all of participants, when the candida culture of the anterior surface of tongue was negative, also it was negative in the posterior region. 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 assessment of colony counts of candida species.

In this study like previous literatures *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*(10, 14, 16, 18, 19) .on both surfaces of tongue *C. albicans* was isolated more in females than males in DW with statically significant power.

Females revealed significantly the higher distribution of Candida colonies in different parts of tongue. In a similar study by Loster et al.(20), 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(20). 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 (1) 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(21).

There was not a statistically significant correlation between candida colonization and the duration of using denture in anterior and posterior of tongue. ($P > 0.05$). It's worth mentioning resorption of alveolar bone is a serious and common clinical problem lead to reduction of retention and stability of dentures(22). Future studies with large sample size More samples may be shown a significant relationship between age and length of time of wearing denture and the average Candida colony counts.

It should be mentioned that one of the limitations of this study was lack of previous research studies on the topic and another one was the high cost of mycological findings due to sanction.

Conclusion

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

Declarations

Ethics approval and consent to participate

Informed consent was obtained from all subjects or, if subjects are under 18, from a parent and/or legal guardian to access to their document. This research project (ref no: IR.SSU.REC.1399.179) was approved by the ethics committee of Yazd Shahid Sadoughi University of medical science, Yazd, Iran.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article (and its supplementary information files)

Competing interests

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

Funding

The Vice Chancellor for Research and Technology of Yazd Shahid Sadoughi University of Medical Sciences.

Authors' contributions

FO Developed the initial concept of study. FO and SH contributed to the study design and wrote the manuscript. FKH collected the data and FO oversaw the data collection. FO contributed the methodology and analysis and interpretation of data. FO and FKH contributed to data analysis. All authors revised and approved the final manuscript.

Acknowledgement

This study has been financially supported by Yazd Shahid Sadoughi University of Medical Sciences Vice Chancellor for Research and Technology.

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