

Original Article**Evaluation of Presence of Candida in Complete Denture Wearer in Tissue and Denture Surfaces Using Smear Method**

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ABSTRACT

Background: Candidiasis is the most frequent fungal infection of the human oral cavity. *Candida albicans* is an opportunistic fungal microorganism and normal flora of the oral cavity. While different *Candida* species can cause infection, 85% of all oral cavity fungal infections are caused by *Candida albicans*; other types can cause infection less frequently.

Aim: This study aims to determine *Candida* count as an index of fungal contamination in dentures. The results would determine the level of patients' oral hygiene and can be useful in infection control programs which consequently would assist in the prevention of opportunistic fungal infections, particularly, in elderly people.

Methods: In this experimental study, 100 patients with complete denture randomly selected from the city of Ardabil. Before completing the study questionnaires, consent of patient taken by orally and the necessary demographic data were collected to all patients. Samples were collected two times with two-month interval. The samples were stained with Giemsa using the direct smear method and were studied under light microscope. Data were analyzed by statistical methods such as table and graph and Chi-Square test using SPSS.16 software.

Results: Out of 100 studied individuals, 53 had *Candida* (with different frequency), which was directly associated with cigarette smoking (duration of usage and the number of cigarettes used), and also the oral hygiene maintenance.

Conclusion: Oral hygiene maintenance, patient's training, ceasing the smoking habit and controlling systematic diseases can decrease the amount of *Candida* in the oral cavity.

Keywords: Direct smear, complete denture, *Candida*, prevalence, Smear Method

INTRODUCTION

Candidiasis is the most frequent fungal infection of the oral cavity in human beings. *Candida albicans* is an opportunistic microorganism and microflora of the oral cavity, which is observed in approximately 60% of healthy adults and 45-65% of healthy children [1,2,3]. Different *Candida* species are able to cause infection, however, *Candida albicans* accounts for 85% of all oral cavity fungal infections. Other *Candida* species can produce infection less frequently [4,5].

Candidiasis is most prevalent in diabetic patients, elderly people, denture wearers, patients treated with antibiotics (or corticosteroids, chemotherapy and radiotherapy), xerostomia, deficient immune system

(such as HIV/AIDS), malnutrition and poor oral hygiene [1-3,5]. A study conducted in Kerman, Iran (2002), revealed a significant correlation between smoking cigarettes and the prevalence of candidiasis [6].

Candidiasis is categorized to: acute pseudomembranous, acute atrophic, chronic hyperplastic, chronic atrophic, median rhomboid glossitis and angular cheilitis [1,5-6].

Pseudomembranous Candidiasis or thrush is defined as the white or yellow papules or plaques that can be removed by scraping with a blunt instrument such as a tongue blade on it. An erythematous or hemorrhagic area will remain when the surface area is removed.

These papules include desquamous epithelial cells, fibrin, and fungal hyphae. This type of candidiasis is observed on the buccal and labial mucosa, soft palate, hard palate, tongue and oropharynx region.

The risk factors for developing this type of candidiasis are aging, diabetes mellitus, AIDS, leukemia and consumption of corticosteroids and antibiotics. The differential diagnosis of this lesion would be lichen planus, squamous cell carcinoma (SCC), leukoplakia and lichenoid reactions [1,6]. The etiology of Acute Atrophic Candidiasis is the consumption of wide-spectrum antibiotics, which is usually associated with burning mouth or tongue. The tongue might become brightly colored or become similar to those with vitamin B12 or Folate deficiency [6,7].

Chronic hyperplastic candidiasis is typically presented as white or red plaques on buccal mucosa or the border of the tongue. They are usually caused by smoking and disappear when quitting the smoking; but if persistent, they will turn out to dysphagia or even malignancy [5].

Chronic atrophic candidiasis, also called denture stomatitis, is observed as localized erythematous patches on parts of the oral mucosa covered by the denture. This lesion usually appears on the palatal mucosa; however, it might also be detected on the mandibular mucosa [1]. For diagnosis, the denture must be removed and the underlying tissue requires careful examination. It might be helpful to employ a sterile cotton swab to confirm the diagnosis.

Median Rhomboid Glossitis is detected as a symmetrical area in the anterior region of circumvallate papilla; and is made of atrophic filiform papillae. A biopsy from this area reveals the presence of Candida in 85% of cases [1, 6]. Angular Cheilitis usually occurs as erythematous fissures in one, or more commonly both, corners of the mouth, usually observed in those with oral Candidial infection. Other microorganisms such as staphylococcus and streptococcus can also have a contributory role. Regarding the presence a persistent wet area, this condition is produced in wrinkles around the corners of the mouth and along the nasolabial folds, particularly in elderly people. Moreover, the condition is also observed in denture wearers with bone loss and reduced vertical dimension (mandibular over closure and loss of lower face height). Other possible etiologic factors are iron-deficiency anemia and vitamin B12 deficiency [5].

MATERIALS AND METHODS

In this experimental study, 100 patients with complete denture randomly selected from the city of Ardabil. Before completing the study questionnaires, consent of patient taken by orally and then patient were asked to remove the denture and demographic data collected from patients. Sterile swab was prepared and delivered to the operator. When doing the biopsy, without touching either the tip of the cotton swab or the pathology slide, the cotton swab was scrapped over the palatal mucosa in a circular pattern. The biopsy was also performed from any suspicious lesion or any erythematous area present on the denture-covering regions. In order to examine the denture, cotton swab was scrapped over the inner surface of the maxillary denture [8-10]. If the patient has recently started wearing the denture, only the mucosal biopsy was taken. The swab was smeared on the microscope slide in a zigzag pattern and the slides were labeled M (for mucosa) or D (for the denture), regarding the biopsy site. The slides were dried, then fixed (by alcohol burner) and sent to the laboratory with requisite patients' information. The slides (after fixation) were packed up to be prevented from any exposure to air or hand.

After two months, the study was repeated and the smears were taken from both mucosa and the inner surface of the denture. The samples were sent to the laboratory labeled with the patients' record number.

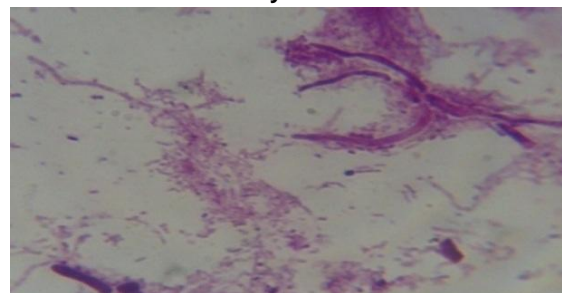
Preparing the sterile cotton swabs

The cotton swabs were sterilized by the laboratory autoclave under the pressure of 1kps/cm, in 121 °C for 20 minutes.

Staining material

The smear slides were stained using Giemsa Stain diluted 1:20 with tap water for 30 minutes (Figure 1).

Figure 1: Stained smears to detect the hyphae and myceliums



This staining method includes a combination of materials with different proportions of methylene blue and eosin. The above mentioned method was proposed by Lillie (1943) that brought about acceptable results. Giemsa staining, as used in this study, is now available as Giemsa commercial dye and consists of the following components(9) : Azure A Eosinate, Azure B Eosinate, Methylene blue Eosinate, solvent (made up of equal amount of methyl alcohol and glycerol) and diluting agent (made up of neutral phosphate solution with pH=7).

Staining method

The slides were fixed after being moved over the heat, twice or three times. The diluted Giemsa solution was spread over the slides and the slides were left under the dye for 30 minutes. The slides were then washed under the tap water and were left to be dried in outside air [8].

The evaluation and report of the slides

After being stained and dried, the slides were inspected using the 100× oil immersion objective lenses of light microscope. The presence of fungal elements was scaled and reported as negative, rare, few, moderate and many.

The microscopic evaluation of slides showed the round or oval yeast cells (blastoconidia) with 3-5 μm diameter, as well as mycelium or pseudomycelium on the surface or inside the epithelium. In superficial lesions or new lesions, the blastoconidias (yeast phase) were generally more frequent and in deeper lesions the pseudomyceliums (pseudohyphae) were more often observed. With direct scrutiny of the stained samples, the frequency of Candida was reported in following basis [9]:

- Negative: (HPF) 0 in each 40 Fields,
- Rare: (HPF) 2 in each 40 fields,
- few :(HPF) 10 in each 40 fields,
- Moderate :(HPF) 20 in each 40 fields,
- Many: (HPF) >20 in each 40 fields.

Statistical Analysis

Collected data analyzed by descriptive and analytic statistical methods such as table, graph and Chi-Square test using SPSS.16 software. The significant level was kept at $p < 0.05$.

RESULTS

Most of patients with 58% did not have Candida in the mucosa (Figure 2). 47% of patients did not have

Candida in their denture (Figure 3).

Figure 2: Frequency of Candida in mucosa of patients

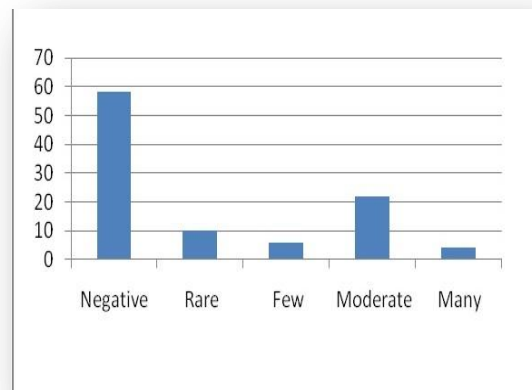
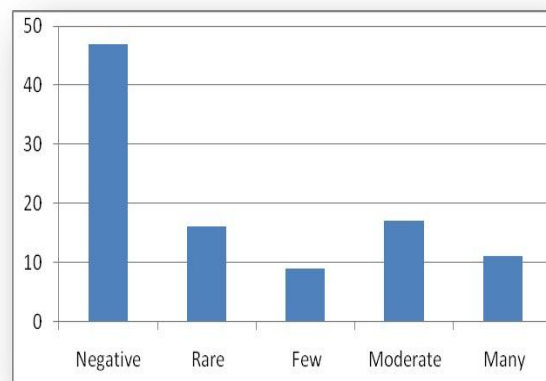


Figure 3: Frequency of Candida in Denture of patients



31% of patients were female and 69% male. 14 patients (70%) of all smokers and 28 patients (35%) of all non smokers have infection.

According to table 1, Candida was detected less frequently in the mucosa of the patients who were trained about oral hygiene maintenance by their dentists. There was a statistically significant difference in mucosal Candida counts among those who were trained about oral hygiene and those who were not ($P=0.009$).

Table 2 shows that Candida is less frequent in the mucosa of the patients who clean their denture with salt water. There was a statistically significant difference in mucosal Candida counts among denture wearers based on their denture cleaning method ($P=0.011$).

Table 1: Rate of Candida in mucous of patients by receive health education by the dentist

Health Education Candida Rate	Yes	No	Total
Negative	28	19	47
	62.2%	34.5%	47.0%
Rare	8	8	16
	17.8%	14.5%	16.0%
Few	3	6	9
	6.7%	10.9%	9.0%
Moderate	3	14	17
	6.7%	25.5%	17.0%
Many	3	8	11
	6.7%	14.5%	11.0%
Total	45	55	100
	100.0%	100.0%	100.0%
Chi-Square test results	Chi-Square ratio	Degree of freedom	Significance level
	13.6	4	0.009

Table 2: Rate of Candida in mucous of patients by using denture cleaning devices

Candida Counts	Never removed	Brush	Salt water	Water	Total
Negative	24	17	12	5	58
	58.5%	56.7%	70.6%	41.7%	58.0%
Rare	3	2	0	5	10
	7.3%	6.7%	.0%	41.7%	10.0%
Few	5	1	0	0	6
	12.2%	3.3%	.0%	.0%	6.0%
Moderate	9	8	3	2	22
	22.0%	26.6%	17.6%	16.6%	22.0%
Many	0	2	2	0	4
	.0%	6.7%	11.8%	.0%	4.0%
Total	41	30	17	12	100
	100.0%	100.0%	100.0%	100.0%	100.0%
Chi-Square test results	Chi-Square ratio	Degree of freedom	Significance level		
	25.9	12	.011		

Table 3: Rate of Candida in mucous of patients by uses smoking

Candida Counts	Yes	No	Total
Negative	6	52	58
	30.0%	65.0%	58.0%
Rare	2	8	10
	10.0%	10.0%	10.0%
Few	4	2	6
	20.0%	2.5%	6.0%
Moderate	7	15	22
	35.0%	18.7%	22.0%
Many	1	3	4
	5.0%	3.8%	4.0%
Total	20	80	100
	100.0%	100.0%	100.0%
Chi-Square test results	Chi-Square ratio	Degree of freedom	Significance level
	13.529	4	.009

Table 3 represents that Candida is more frequent in smokers compared with non-smokers. The results of the Chi-Square test showed a significant correlation between the presence of mucosal Candida in denture wearers and smoking individuals (P=0.009).

The results of table 4 indicate that absence of Candida was only seen in non-smoker patients and the highest rate of Candida was among those who smoked more than 10 cigarettes per day. There was a statistically significant correlation between the amount of mucosal Candida in denture wearers and the number of cigarettes they smoke (P=0.001).

The results illustrated in table 5 shows that the Candida count is higher in patients who had no mucosal lesion. The statistical analysis revealed that Candida counts in patients with mucosal lesion was significantly different comparing to those with no mucosal lesions (P=0.004).

DISCUSSION

Denture stomatitis is the most common form of oral candidiasis (25%-60%) with more predilections for palatal mucosa. If the condition is left untreated, it may cause bone loss, denture loose-fitting and patient's dissatisfaction [1].

Table 4: Rate of Candida in mucous of patients by number of uses cigarettes (per day)

Candida Counts	Never	1-5	6-10	11-15	16-20	Total
Negative	52 65.0%	3 60.0%	0 .0%	0 .0%	3 37.5%	58 58.0%
Rare	8 10.0%	1 20.0%	1 25.0%	0 .0%	0 .0%	10 10.0%
Few	2 2.5%	0 .0%	2 50.0%	0 .0%	2 25.0%	6 6.0%
Moderate	15 18.7%	0 .0%	1 25.0%	3 100.0%	3 37.5%	22 22.0%
Many	3 3.8%	1 20.0%	0 .0%	0 .0%	0 .0%	4 4.0%
Total	80 100.0%	5 100.0%	4 100.0%	3 100.0%	8 100.0%	100 100.0%
Chi-Square test result	Chi-Square ratio 42.135		Degree of freedom 16		Significance level .0001	

Table 5: Rate of Candida in mucous of patients by mucosal lesion

Candida Counts	With lesion	Without lesion	Total
Negative	0 .0%	58 61.1%	58 58.0%
Rare	0 .0%	10 10.5%	10 10.0%
Few	0 .0%	6 6.3%	6 6.0%
Moderate	4 80.0%	18 18.9%	22 22.0%
Many	1 20.0%	3 3.2%	4 4.0%
Total	5 100.0%	95 100.0%	100 100.0%
Chi-Square test results	Chi-Square ratio 15.311	Degree of freedom 4	Significance level .004

In a study enrolled by Nadoushan et al, in Yazd (2006) [2], concluded that out of 50 elderly people, 29 cases (58%) had elevated colonization of Candida albicans. In their study, the frequency of Candida was 52% and 48% in males and females respectively while in the current study this frequency was found to be 44.9% and 35.5% in males and females respectively.

The frequency of Candida prevalence in this study is fewer than its prevalence in Yazd, based on the aforementioned study. The current study, performed on 100 patients referred to private and public clinics of Ardebil, 42% exhibited Candida in the mucosa and

53% in their denture; which 5% of these infected dentures were accompanied by mucosal lesion. The Candida counts in the mucosa and denture surface was reported to be "Moderate" and "Many" in these studied people.

In 2002, Chamani et al studied the frequency of oral Candidal infection in Kerman dental clinics and found that the smears were positive in 87.4% of patients, but suspicious clinical signs of candidiasis were observed only in 23.9% of the studied patients [6]. They found no statistically significant difference between the presence of Candida in males and females.

In a different research enrolled by Chamani et al. (2005), 318 patients (170 males and 148 females) were studied; 68 patients (21.4%) were suspected to have Candidiasis and 278 cases (87.4%) turned out to be positive to have the infection.

Infection was seen in 50 male (72.4%) and 18 female (58.1%). From all patients 20% smoked cigarettes. The incidence of infection in smokers with 70% (14/20) is twice that of non-smokers with 35% (28/80). One of the reasons might be the difference in men and women's uses habits; for instance, our study found that Candida was more frequently detected in males (14.5%). In addition, it is reported that males are more infection carriers (of Candida) than females [5].

Moreover, a significant relation exists between the presence of Candida and the duration of smoking habit and the highest rate was observed in patients who have smoked for more than 20 years. However, this study showed the highest rate of Candida in the mucosa and dentures was in patients who have smoked more than 10 cigarettes per day.

Another study by Chamani and colleagues in Kerman reported a meaningful relation between smoking and the prevalence of Candidiasis. Therefore, it can be concluded that smoking could probably be a crucial factor for prevalence of candidiasis in men [6]. Concerning the relation between the presence of Candida and systematic diseases, the current study showed no significant correlation between the presence of Candida in the mucosa and dentures of diabetic patients compared with other patients. A study by Pires et al [11] revealed a meaningful association between having denture stomatitis and presence of Candida in the oral cavity. Likewise, our study showed a significant relation between the presence of Candida and mucosal lesions.

This study also showed that Candida was much less frequent in patients who received oral hygiene and denture care trainings by their dentists. A higher colonization of Candida was observed in elderly denture wearers. There is a sort of protein in saliva that separates the Candida from plastic surfaces and the acrylic resin. This protein is less active in aged people who confirm why daily cleaning of denture is essential and vital for these denture wearers [2]. In this study the presence of Candida fungus was assessed twice; at the time of insertion and two months after wearing the denture, respectively. The presence of Candida at the time of insertion was seen

in 28% of samples. This number reached 40% after two months which means it increased 12% approximately.

In the study of Usman Muneer et al., the Candidal infection was evaluated in 40 patients at the time of denture delivery and one month later and the results showed a 35% increase in the occurrence of infection [12].

Regarding the potential complications reported for Candida infections, any increase in prevalence of infection should be considered critical. Reviewing the literature and studying the experimental and clinical epidemiologic researches depicts that Candida species could probably contribute to oral cancer and consequently, should not be considered as a risk-free harmless infectious condition. Based on some studies, certain species of Candida can produce nitrosamine and carcinogenic chemical agents. Some species may be able to promote oral cancer. Moreover, the significant association of certain Candida species with white and red dysplastic lesions (speckled leukoplakia), and the in vitro effects of hyperplastic Candida on the epithelium, declares that Candida can perform as a carcinogen or cancer promoting agent [1,13].

It should be considered that different risk factors in the studied population and the difference in sensitivity and specificity of laboratory tests that were employed in the diagnosis of Candidiasis might be the reasons for dissimilar values reported, regarding the incidence of Candidiasis in different studies. The diagnostic approach for candidiasis is varies in different studies. Some researchers rely only on culturing methods while others benefit from the smear method only [6]. Candida is reported to be a part of the normal flora of at least one-third of people. Those researches that consider the cultures as the only diagnostic criterion might mistakenly be involved with overestimation of infection since the culture result could be false positive due to the proliferation of normal flora. In order to solve this dilemma, some researchers consider the number of colonies as the differential index for discriminating the proliferation of normal flora from pathogenic microorganisms. If the number of colonies were few, it would be reported as negative, although the use of this criterion has also been allied with errors [6].

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Ethical Approved:

This experimental research only done on Denture samples and the patients not necessary to be in the study because of this reason the ethical approve or confirmation no need.

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