



Original Article

Occurrence Rate of Oral *Candida* Species in Edentulous Denture Wearers Dentate SubjectsAbbas Ali Jafari^{1*} Ph.D., Abbas Fallah-Tafti² M.D., Ali Fattahi-Bafghi¹ Ph.D., Benafsha Arzy² M.D.¹ Department of Parasitology and Mycology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.² Department of Prosthodontics, School of Dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

A B S T R A C T

Article historyReceived 30 Sep 2014
Accepted 11 Nov 2014
Available online 15 Dec 2014**Keywords**Denture
Edentulous
Occurrence
Oral *Candida* species**Background and Aims:** Different *Candida* species, especially *Candida albicans* have been known as part of human oral cavity normal flora. Changes in the oral environment resulting from tooth loss or denture application can affect oral microflora. The general purpose of the current study was to determine *Candida species* occurrence rate in the oral cavity of denture wearer patients in comparison with those without denture.**Materials and Methods:** A total 30 edentulous elderly with complete removable denture and 30 dentulous elderly people, who had been admitted for non-prosthetic treatments, were randomly selected in Yazd dentistry department. Their oral rinse samples were collected for mycological examination, and cultured on CHROMagar *Candida* plates. Frequency and density of *Candida species* isolated from both groups were compared using SPSS software with T-test, and differences were considered significant at $p < 0.05$.**Results:** Oral *Candida* species were isolated from 63.3% of edentulous people with dentures in comparison with 33.3% dentulous elderly persons ($p = 0.001$). Non-*albicans Candida species* were isolated more frequently in denture wearers compared with the dentulous group ($p = 0.03$). There was no significant difference between both groups in case of *Candida albicans* isolation ($p = 0.09$).**Conclusion:** The findings of the current study show that long-term use of dentures in edentulous denture users can result in a wide *Candida species* colonization, causing denture stomatitis.***Corresponding Author:** Department of Parasitology and Mycology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran, **Tel:** +983537250808, **E-mail address:** jaabno@gmail.com

Introduction

Candida species, particularly *Candida albicans* have been recognized as commensal human normal flora isolated from different parts of human body. There are a wide variety of microorganisms in the normal human oral cavity covering 300 to 400 species including 20 species of *Candida*.

Candida species are present in the oral cavity of almost half of the population. Some alterations such as tooth loss, and application of the complete removable denture associated with poor oral hygiene in edentulous patients may cause their overgrowth [1, 2].

Although, oral *Candida* is not harmful in normal healthy hosts, but several predisposing factors such as old age, immunosuppression, diabetes, leukemia, acquired immunodeficiency syndrome and neoplasia (particularly head or neck cancer) may cause opportunistic candidiasis infection. Oral candidiasis has been reported to be associated with candidiasis in the lung and also deglutition pneumonia [3]. Literature studies revealed a causal relationship between denture wearing and persistent or even recurrent denture stomatitis as a result of the initial adhesion of *Candida* species to the denture base materials such as acrylic resin and polymethyl methacrylate [4, 5].

Denture stomatitis is usually a chronic inflammatory disorder of palatal and alveolar mucosa covered with removable dental prostheses, and is associated with burning, bleeding, an unpleasant taste, or halitosis in complete removable denture users [6].

However, the prevalence of denture stomatitis is ranged from 15 to 65% in edentulous community, while in institutionalized denture wearing population it may reach at up to 72% [7, 8]. However, multi-factorial etiology for this mucosal infection has long been reported, including trauma caused by ill-fitting dentures, microbial biofilm, poor denture hygiene, continuous denture wearing habits and several other systemic factors. Numerous studies highlighted the main role of *Candida albicans* in the development of denture stomatitis [1, 9, 10].

The aim of this analytical-descriptive study was to compare the frequency and density of different *Candida* species isolated from oral cavity of edentulous denture wearer and elderly subjects wearing their own dentition.

Materials and Methods

In the current cross-sectional study, 30 edentulous elderly people having complete removable denture and 30 edentulous patients, who referred to Yazd dentistry department for non prosthetic treatments, were randomly selected. In order to reach minimum 5 unit differences in the average microbial colony counts between the groups, the significant level of 5%, $\alpha=5\%$, $\beta=2\%$, $S=80\%$ using the formula, 30 subjects were opted for each group.

The elderly subjects with the history of underlying diseases such as malignancies, diabetes, neoplasm, immunosuppression, and current antibiotic users were excluded from the

present study. All subjects were in the same age range and matched based on sex. All subjects completed an informed consent form to participate before being accepted for oral sample collection.

Sample collection and detection of *Candida* species

only subjects passing more than two hours from their last eating and brushing were included in the present study. The patients were requested to rinse their mouth with 10 ml of sterile phosphate-buffered saline (PBS, 0.1 M, pH 7.2) for one minute (denture had to be removed prior to sampling in denture users) and then to return the oral rinse to a sterile universal container. All oral rinse samples were concentrated by centrifugation (at 1700g for 10 min), and the supernatant was discarded. The deposit was re-suspended in 1 ml of sterile PBS, and a 10 µl sample was inoculated on the CHROMagar *Candida* plates (CHROMagar, France) by the method of Beighton et al. [12], and incubated for 48 to 72 hrs at 37°C. The plates were then assessed for

identification and enumeration of isolated *Candida* species based on the color of *Candida* colonies and the media chart. The colony forming unit (CFU) was multiplied by the dilution factor to yield the CFU per milliliter of original oral rinse sample for each individual [13].

The study was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences.

Statistical Analysis

Frequency and density of recovered *Candida* Spp. colonies (CFU/ml) isolated from both groups were compared using SPSS software with T test, and differences were considered significant at p<0.05.

Results

Positive oral rinse samples with *Candida* species were more frequently seen in edentulous denture wearers compared with dentulous subjects (Table 1).

Table 1. Subjects variables and the frequency of positive *Candida* samples in edentulous denture wearers and edentulous subjects

Variables	Edentulous denture wearer	Elderly edentulous subjects	P _{value}
Sex	18 male, 12 female	15 male, 15 female	0.341
Age	57.5±12	53.5±15.5	0.106
<i>Candida</i> positive oral sample	19	10	0.001
Positive samples with <i>C. albicans</i>	9	6	0.08
Non-albicans positive samples	10	4	0.03

There was a statistically different higher positive culture in denture users in comparison with subjects without denture ($p=0.001$). However there was no statistically significant difference between the frequency of isolated samples with *Candida albicans* ($p=0.12$) between both groups, but non-albicans *Candida* species were mostly seen (Fig. 1) In edentulous

denture users compared with the elderly people with normal dentition ($p=0.03$). In case of *Candida* density in oral samples, a higher density of *Candida* spp. colonies was recovered from edentulous denture users in comparison with edentulous elderly persons except for *C.glabrata*(Table 2).

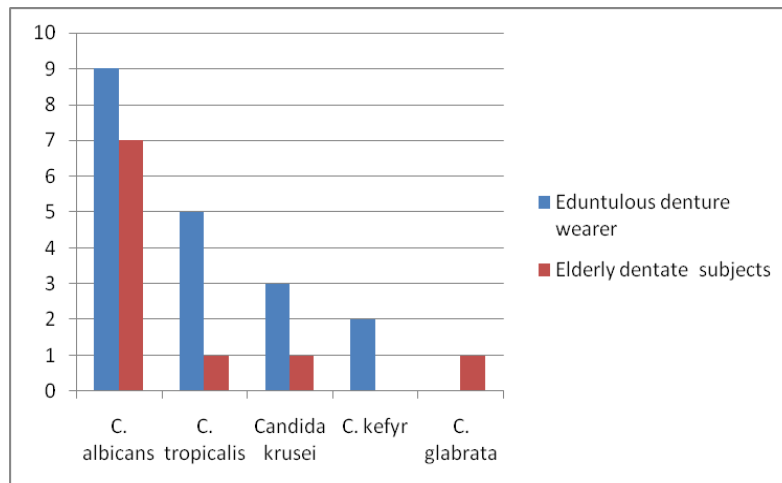


Fig. 1. Isolation frequencies of oral *Candida* species in edentulous denture wearers and dentate subjects

Table 2. Density (Mean±SD, CFU/ml) of recovered *Candida* species in edentulous denture wearers and dentulous subjects

<i>Candida</i> species	Edentulous denture wearer	Elderly edentulous subjects
<i>C. albicans</i>	57.5±18.3	44.6±11.9
<i>C. tropicalis</i>	32.4±12.7	27.2±11.3
<i>Candida krusei</i>	18±8.3	19.8±9.1
<i>C. kefyr</i>	24.5±7.8	0
<i>C. glabrata</i>	0	13.2±2.8

Discussion

In Iran, the size of elderly population has increased, and this requires corresponding improvements in their health and medical care. Citizens who were older than 65 years of age represented 3.1% of the overall Iranian population in 1986, 5.1% in 2006, and are expected to reach 10% in 2035 [14]. Prevention of elderly oral diseases and improving their oral health care is a prerequisite for better quality of life in this population. Preserving of 20 teeth or more is ideally needed to warranty an acceptable mastication and food intake even up to the age of 80 years [15].

Candida species are reported as the most frequent opportunistic fungi, causing candidiasis in different areas of human body including oral cavity.

Since these fungi are commensally presented in human oral cavity, their colonization can promote oral candidiasis, with its different manifestations [16]. Denture stomatitis, as a mucosal manifestations of oral candidiasis in elderly denture users, results from attachment and colonization of *Candida* species on denture hard surface.

The present study was conducted in order to show the role of denture on the frequency and density of oral *Candida* colonization in denture users and normal edentulous subjects [17]. Aging has been reported as the cause of progressively increasing incidence of *Candida* in the human oral cavity. However, in the current study, an increase in positive oral *Candida* particularly non-*albicans Candida*

species was seen in edentulous elderly denture wearers in comparison with dentate people [15]. The results of the present study showed a higher frequency of positive *Candida* species in oral rinse specimens of edentulous denture than samples of those without denture. Oral *Candida* species were isolated from 63.3% of edentulous people with dentures, a finding supported by Budetz-jorgencen [18] study, who isolated them in 65% of denture users. Our results differ greatly from 85% found by Arirachakaran study [10]. The differences may be explained by different diagnostic criteria or differences in the groups including age, institutionalized versus non-institutionalized subjects, or even the influences of other factors such as diseases or drugs.

Candida albicans was the most commonly isolated species isolated from oral rinse culture in the present study, which is similar to that HE's et al. study [19].

However, a statistically significant higher frequency and density of non-*albicans Candida* species also were recovered from oral rinse in denture wearer samples in comparison with those without denture. Recently a shift from *Candida albicans* towards non-*albicans* species recovered from oral candidiasis and denture stomatitis lesions has been observed [20, 21].

Unfortunately, these species are more resistant to common antifungal drugs than *C. albicans*. This further attention to oral and denture hygiene of denture users in order to control possible oral candidiasis and involvement of adjacent organs like lung and gastrointestinal tissues in asthenia immunosuppressed elderly

people [22, 23]. A high prevalence of colonization of *Candida* spp. is often documented in elderly denture wearers [6, 23]. *Candida* species adhere to acrylic surfaces of dentures which plays an important role for the colonization and in turn in the pathogenesis of oral candidiasis [6, 24].

Conclusion

Findings from the present study indicated more frequent and density of *Candida species* especially non-albicans *Candida species* in patients with dentures in comparison with non-denture wearers highlighting the role of denture in denture stomatitis and the need for

better oral as well as denture hygiene in edentulous denture wearers.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Acknowledgement

This article resulted from a part of dentistry student thesis, and was financially supported by Vice Chancellor for Research and technology of Shahid Sadoughi University of Medical Sciences. The authors would like to thank Ms Mahin Ghafoorzadah for her kind help in the laboratory works.

References

- [1]. Daniluk T, Tokajuk G, Stokowska W, Fiedoruk K, Sciepek M, Zaremba M.L, et al. Occurrence rate of oral *Candida albicans* in denture wearer patients. *Adv Med Sci* 2006; 51 (1):77-80.
- [2]. de Foggi CC, Machado AL, Zamperini CA, Fernandes D, Wady AF, Vergani CE. Effect of surface roughness on the hydrophobicity of a denture-base acrylic resin and *Candida albicans* colonization. *J Investig Clin Dent* 2014; 20.
- [3]. Honda E, Mutoh T, Maeda N. Study on oral microbial flora of elderly people – Comparison of elderly people living in nursing homes and in their own homes. *J Gerodont* 2000; 14:297-306.
- [4]. Pereira-Cenci T, Fernandes FS, Skupien JA, Mesko ME, Straioto FG, Del Bel Cury AA. Can new dentures decrease *Candida* levels? *Int J Prosthodont* 2013; 26(5):470-477.
- [5]. Mendonca e, Bertolini Md, Cavalcanti YW, Bordin D, Silva WJ, Cury AA. *Candida albicans* biofilms and MMA surface treatment influence the adhesion of soft denture liners to PMMA resin. *Braz Oral Res* 2014; 28(1):61-66.
- [6]. Radford D.R, Challacombe S.J, Walter J.D. Denture plaque and adherence of *Candida albicans* to denture-base materials in vivo and in vitro. *Crit Rev Oral Biol Med* 1999; 10(1):99-116.
- [7]. Honda E, Mutoh T, Maeda N. Study on oral microbial flora of elderly people– Comparison of elderly people living in nursing homes and in their own homes. *J Gerodont* 2000; 14:297-306.
- [8]. Espinoza I, Rojas R, Aranda W, Gamonal J. Prevalence of oral mucosal lesions in elderly people in Santiago, Chile. *J Oral Pathol Med* 2003; 32(10): 571-575.
- [9]. Altarawneh S, Bencharit S, Mendoza L, Curran A, Barrow D, Barros S, et al. Clinical and histological findings of denture stomatitis as related to intraoral colonization patterns of *Candida albicans*, salivary flow, and dry mouth. *J Prosthodont* 2013; 22(1):13-22.
- [10]. Arirachakaran P, Piboonratanakit P, Kiatkroekkrui P, Sornmai M, Srimart N. Prevalence of oral *Candida* carriage in denture wearers. *CU Dent J* 2009; 32:101-12.
- [11]. Johnson C.C, Yu A, Lee H, Fidel P.L, Noverr M.C. Development of a contemporary animal model of *Candida albicans*-associated denture stomatitis using a novel intraoral denture system. *Infect Immun* 2012; 80(5):1736-1743.
- [12]. Beighton D, Ludford R, Clark D.T. Use of CHROMagar *Candida* medium for

- isolation of yeasts from dental samples. *J Clin Microbiol* 1995; 33:3025-3027.
- [13]. Canay S, Erguven S, Yulug N. The function of enzymes in removing *Candida* accumulated on denture plaque. *J Islamic Acad Sci* 1991; 4 (1):87-89.
- [14]. Moti-Haghshenas N. Sociological aspects of the aging population and the challenge of active aging in Iran. *Journal of Sociological Studies* 2011; 1(2): 133-147.
- [15]. Mizuno T, Nakagaki H, Murakami T. Dental Health Surveys at 80 years old in Tokoname City. *J Dent Hlth* 1994; 44:161-169.
- [16]. Webb B.C, Thomas C.J, Willcox M.D, Harty D.W, Knox K.W, Webb B.C, et al. *Candida*-associated denture stomatitis. Aetiology and management: a review. Part 1. Factors influencing distribution of *Candida* species in the oral cavity. *Aust Dent J* 1998; 43:45-50.
- [17]. Mizugai H, Isogai E, Hirose K, Chiba I. Effect of Denture Wearing on Occurrence of *Candida* Species in the Oral Cavity. *The Journal of Applied Research* 2007; 7(3): 27-30.
- [18]. Budtz-Jorgensen E. The significance of *Candida albicans* in denture stomatitis. *Scand. J. Denmt. Res* 2005; 82: 151-190.
- [19]. He X.Y, Meurman J.H, Kari K, Rautemaa R, Samaranayake L.P. (2006). In vitro adhesion of *Candida* species to denture base materials. *Mycoses* 1974; 49:80-84.
- [20]. Krcmery V, Barnes A.J. Non-*albicans* *Candida* spp. causing fungaemia: pathogenicity and antifungal resistance. *J Hosp Infect* 2002; 50:243-60.
- [21]. Samaranayake L.P. *Candida krusei* infections and fluconazole therapy. *Hong Kong Med J* 1997; 3:312-314.
- [22]. Salerno C, Pascale M, Contaldo M, Esposito V, Busciolano M, Milillo L, et al. *Candida*-associated denture stomatitis. *Med Oral Patol Oral Cir Bucal* 2011; 1; 16(2): 139-143.
- [23]. Sherman R.G, Prusinski L, Ravenel M.C, Joralmon R.A. Oral candidosis. *Quintessence Int* 2003; 33 (7):521-32.
- [24]. Lockhart S.R, Joly S, Vargas K. Natural defenses against *Candida* colonization breakdown in the oral cavities of the elderly. *J Dent Res* 1999;78:857-868.