ECRONICON OPEN ACCESS

EC DENTAL SCIENCE
Research Article

Identifying the Different Kinds of Oral *Candida* Species in Denture Wearing Patients

Yasser Khaled[1]* and Bindiya K Pahuja[2]

[1]Vice-Chair, Marquette University Academic Senate, Member, Canadian Oral Health research Group, Assistant Professor, Department of General Dental Sciences, Oral Pathology, Oral Medicine and TMD/Orofacial Pain, Marquette University, School of Dentistry, USA

[2]Periodontology and Implantology, Senior Resident, AIIMS, New Delhi, India

***Corresponding Author:** Yasser Khaled, Vice-Chair, Marquette University Academic Senate, Member, Canadian Oral Health research Group, Assistant Professor, Department of General Dental Sciences, Oral Pathology, Oral Medicine and TMD/Orofacial Pain, Marquette University, School of Dentistry, USA.

Received: May 17, 2019; **Published:** June 17, 2019

Abstract

Oral candidiasis is a common opportunistic infection in denture wearing patients. Presence of dentures in the oral cavity of many patients can promote the *Candida* colonization and results in higher incidence of oral and systemic candidiasis. This study was undertaken to assess the various species of fungus *Candida* associated with patients of denture stomatitis (both diabetic and non-diabetic) and also in healthy denture wearing patients.

Keywords: *Candida albicans; Denture Stomatitis; Dental Plaque; Antifungal; Oral Yeast*

Introduction

The oral cavity has a very complex environment and has a rich variety of *Candida* species [1]. *C. albicans* represents the most common causative agent of oral candidiasis; however, other species of *Candida*, like *C. glabrata, C. tropicalis, C. krusei* and *C. parapsilosis* have been isolated with relative frequency from patients with denture stomatitis [2].

Oral candidiasis in the form of *Candida*-associated denture stomatitis, is a common disease in a large percentage of denture wearers, which means the *Candida* species has an association with dentures and other oral prosthesis [3]. Yeast cells adhere and colonize oral surfaces including mucosa and acrylic dentures and have the ability to co-aggregate with oral bacteria. The prevalence of *Candida* species also significantly increases with the degree of inflammation and it plays an important role in progression of denture stomatitis [4,5].

Aims and Objectives

1) To find the most common kind of *Candida* species associated with denture stomatitis.
2) To assess the *Candida* species associated with non-diabetic denture stomatitis.
3) To assess the *Candida* species associated with diabetic denture stomatitis.
4) And also, to assess the *Candida* species found in healthy denture wearers without denture stomatitis.

Materials and Methods

A total of 60 denture wearing patients between the age of 50 - 69 years were taken. Out of which 20 (10 males and 10 females) patients were known diabetics and had denture stomatitis. Another group of 20 (10 males and 10 females) patients had denture stomatitis without debilitating systemic illnesses and the last group of 20 (13 males and 7 females) denture wearing individuals were having healthy oral mucosa under their dentures. All the 3 groups were selected from the department of Oral medicine and Radiology, V.S. Dental college and

hospital, Bangalore. None of the above patients were receiving any treatment for stomatitis for last 3 months including steroids, antibiotics, antifungals or antiseptic mouthwashes. Patients with co-morbidities and on immunosuppressive drugs were also excluded. patients wearing the dentures for a duration of more than 30 years were also excluded from the study. The comparative study was completed in a total of 18 months.

After obtaining the written study consent by the patient a thorough personal, medical and dental history was taken and recorded. Blood sugar levels of each patient at baseline (including random and fasting blood sugar) were recorded.

Each patient is asked to remove their denture before intraoral examination. Careful intraoral examination was done, if denture stomatitis encountered, it was been diagnosed and classified according to Newton.

Type I	A localized simple inflammation or pin point hyperemia
Type II	An erythematous or generalized simple type is seen as more diffuse erythema involving a part or the entire denture covered mucosa
Type III	A granular type (inflammatory papillary hyperplasia) commonly involving the central part of the hard palate and the alveolar ridge.

A sample is collected from the mucosal surface of denture using cotton oral swab. Cotton swab is placed in sterile swab tube, 5ml of sterile normal saline is added to the tube and vertexed for 1 minute. Container is sent to laboratory for microbiologic analysis. Immediately after collection of the samples it was transferred to the Department of Microbiology, Kempegowda Institute Of Medical Science Hospital, Bangalore for further evaluation.

Firstly, in a freshly collected sample, a gram stain was done and also inoculated onto Sabouraud's Dextrose Agar media at 37°C. cream color smooth, pasty growth was appeared in 48 - 72 hrs Sometimes growth may be observed within an overnight incubation.

Sabouraud's agar tube was prepared by dissolving 20 mg glucose, 10 mg peptone, 15 mg agar, and 1 litre of water with steam. After adjusting Ph to 5.4 the agar was autoclaved at 115°C for 15 minutes, 20 ml amounts was dispensed into each test tube. Growth was identified by the colony characteristics and the growth of *Candida* was confirmed by Gram's staining as gram positive budding yeast like cells and germ tube was put up.

Secondly the sample is plated on CHROMagar and incubated at 37°C for 48 hrs. Colonies will be presumptively identified by colony color. This media contains chromogenic substrates that react with specific enzymes secreted by yeast producing colonies with various pigmentations. These enzymes are species specific, allowing detection of yeast to species level. The chromogenic substance is a- glucosaminidase substrate, which is metabolized to give the colored colonies of different *Candida* species.

***Candida* species**	**Colour and colonies on CHROM agar medium**
C. albicans	Light green colour (Apple green colonies)
C. dubliniensis	Dark green
C. tropicalis	Blue with purple pigment diffusion
C. glabrata	Dark pink-purple
C. krusei	Large, fuzzy, rose coloured colonies with white edge
C. parapsilosis	Off white to pale - pink colonies
C. guilliermondii	Small pink - purple colonies
Others	Small, creamy colonies that are white, pale pink, light lavender

Thirdly *Candida* species were also identified by carbohydrate assimilation test using *Candida* identification kit. KB006 is a standardized test system that can be used for identification of *Candida* species. It can also be used for validating known laboratory strains. The complete list of organisms that can be identified with this system is given in the identification index provided with the kit. The tests are based on the principle of pH change and substrate utilization on incubation; organisms undergo metabolic changes which are indicated

by a spontaneous color change in the media. KB006 cannot be used directly on clinical specimens. The organisms to be identified were first isolated and purified on a common medium like Sabourauds dextrose agar. The inoculums was prepared by picking 2 - 4 well isolated colonies and homogenous suspension was made in 2 - 3 ml sterile saline. The density of the suspension was adjusted to 0.50D at 620 nm. The kit was opened aseptically by peeling off the sealing foil. Then each well was inoculated with 50 µl of above inoculums using surface inoculation method above inoculums was incubated at a temperature of 22.5°C ± 2.5°C for duration of 24 to 48 hours. Results were interpreted as per the standards given in the identification index.

	Urase	Melbose	Lactose	Maltose	Sucrose	Galactose	Cellobiose	Inositol	Xylose	Dulcitol	Raffinose	Trehalose
C. albicans	-	-	-	+	-	+	-	-	+	-	-	+
C. catenulata	-	-	-	-		-	-	-	+	-	-	-
C. dubliniensis	-	-	-	+	-	+	-	-	+	-	-	+
C. famata	-	+	-	-	+weak	-	+	-	+	+	+	+weak
C. glabrata	-	-	-	-	-	-	-	-	-	-	-	+
C. guilliermondii	-	+	-	-	+	+	+	-	+	+	+	+
C. kefyr	-	-	+	-	+	+	+	-	+	-	+	-
C. krusei	+	-	-	-	-	-	-	-	-	-	-	-
C. lambica	-	-	-	-	-	-	-	-	+	-	-	-
C. lipolytica	+	-	-	-	-	-	-	-	-	-	-	-
C. lusitaniae	-	-	-	-	+	+	+	-	+	-	-	+
C. parapsilosis	-	-	-	-	-	-	-	-	+	-	-	-
C. pintolopesii	-	-	-	-	-	-	-	-	-	-	-	-
C. rugosa	-	-	-	-	-	-	-	-	+	-	-	-
C. tropicalis	-	-	-	+	+	+	+	-	+	-	-	+
C. zeylanoides	-	-	-	-	-	-	-	-	-	-	-	-
C. pseudotropicalis	-	-	+	-	+	+	+	-	+	Nd	+	-
C. stellatoidea	-	-	-	+	-	+	-	-	+	Nd		

Interpretation of results

Results were interpreted as per the standards given in the identification index.

The data was then analyzed by turkey post hoc test, kruskal wallis test followed by duns multiple comparisons test, fisher exact test and x analysis.

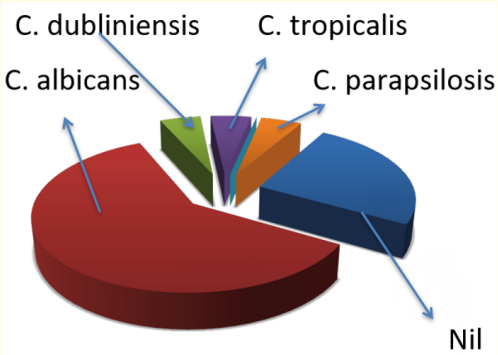
Results

Among a total of 60 patients comprising study groups of 20 patients in group I (diabetic), 20 patients in group II (non-diabetic) and patients in group III (healthy denture wearers), a sum of 20 patients were negative for *Candida* organism. Out of 40 positive cases, 30 (50%) cases had *C. albicans*, 4 (6.6%) cases had *C. dubliniensis*, 2 (3.3%) cases had *C. tropicalis* and 3 (5%) cases had *C. glabrata* species and 1 (1.6%) cases had *C. parapsilosis.*

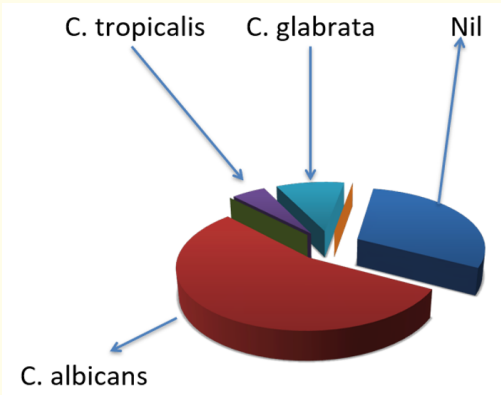
Candida species distribution in 20 group I diabetic patients: 4 types of *Candida* species were identified. *C. albicans* was seen in 12 (60%) patients, *C. dubliniensis* was seen in 1 (5%) patient, *C. tropicalis* was seen in 1(5%) patient and *C. parapsilosis* was seen in 1 (5%) patient.

Candida species distribution in 20 group II non-diabetic patients: *C. albicans* was seen in 11 (55%) patients, *C. tropicalis* was seen in 1(5%) patient, and *C. glabrata* was seen in 2 (10%) patients. Prevalence of *C. glabrata* was higher in group II following *C. albicans.*

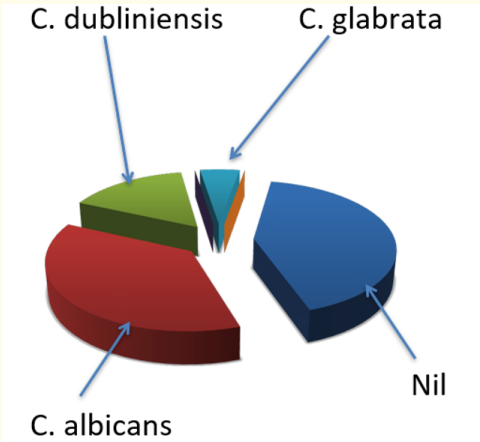
Candida species distribution in 20 group III healthy denture wearers: *C. albicans* was seen in 7 (35%) patients, *C. dubliniensis* was seen in 3 (15%), and *C. glabrata* was seen in 1 (5%) patient. Prevalence of *C. dubliniensis* was higher in control group next to *C. albicans.*



Graph 1: *Candida species identified in group I patients.*



Graph 2: *Candida species identified in group II patients.*



Graph 3: *Candida species identified in group III patients.*

Discussion

The presence of a denture in the oral cavity, associated with the local alterations of the oral mucosa and the systemic complications, may render the denture wearer patient with diabetes even more prone to candidal infection [2,5,7,8]. A significantly higher incidence of *Candida* infection and increased levels of *Candida* spp. were found in diabetic patients wearing removable dentures. The presence of a removable denture may decrease the salivary pH and saliva flow rate and impede the mechanical cleaning of the soft tissue surfaces by the tongue [3,5,7]. In addition, denture induced trauma may reduce tissue resistance against infection because of the increase in permeability of the epithelium to soluble candidal antigens and toxins [3]. Moreover, the tissue surface of the acrylic resin denture acts as a reservoir that harbors microorganisms, enhancing their infective potential and aggravating a previously existing condition. For this reason, both systemic and local predisposing factors might promote an increase in the number of microorganisms and therefore the risk of oral candidiasis in diabetics, especially in those patients wearing removable dentures [5,7].

The relationship between dentures and oral candidiasis has been extensively studied in the literature [1-6], particularly because diabetic patients are more susceptible to fungal infections than those without diabetes [1-3].

Yeast adhesion to epithelial cell surfaces is recognized as an essential first step in the process of candidal colonization and subsequent infection. Salivary glucose levels in diabetic patients favors yeast growth owing to increased numbers of available receptors for *Candida*. Consequently, buccal cells from diabetic patients have shown an increased adherence of *C. albicans* compared with buccal cells from non-diabetics [7].

Different *Candida* species have been frequently isolated from the oral cavities of patients with diabetes. *Candida albicans* is the most commonly recovered species in diabetic patients, with a prevalence of up to 80%. This oral fungal pathogen is the most virulent of the *Candida* species and is able to grow as biofilm, which consists of a complex community of cells embedded in a matrix of extracellular polysaccharide [1-3,9,11].

The epidemiology of *Candida* infections has changed with emergence of non albicans species which have been increasingly described in both compromised and non-compromised hosts. Non-albicans species have been consistently observed in diabetes patients and in those with denture stomatitis [1,3,5,6,8,9].

Although *C. albicans* is still the most frequently isolated species from patients with *Candida* infections [1,2,5-7,13-17], the growing prevalence of non-albicans species is clearly a concern. In the present study, *C. glabrata* was isolated from 5% and 15% in healthy and denture stomatitis patients respectively. *C. tropicalis* was isolated from 5% in denture stomatitis patients.

Vanden Abbeele., *et al.* also observed that *C. albicans* was the commonest yeast found on patients' dentures, followed by *C. glabrata* and *C. tropicalis*. In another study, *C. albicans, C. glabrata* and *C. tropicalis* represented _80% of isolates from clinical infections. In terms of frequency distribution, some studies have shown that *C. tropicalis* was the second most prevalent species identified [2,5,14,15]. However, contrasting results have been found in other studies, in which *C. glabrata* was the most common yeast after *C. albicans* [7,13,14,17].

Suárez B., *et al.* observed in his study that *C. albicans* were the largest isolated species followed by *C. parapsilosis* in uncontrolled diabetic patients [18].

In the present study, *C. albicans* (50%) was the most prevalent species identified and second most prevalent was *C. dubliniensis* species (6.6%), followed by *C. glabrata* (5%), *C. tropicalis* (3.3%) and *C. parapsilosis* (1.6%).

The differences in findings among studies are likely to be related to a combination of factors, such as sample techniques and culture media used. Conventional sampling techniques used in various studies include oral rinses [3,8,13,17]. Although this sampling technique provides adequate qualitative information, it can be argued whether adherent biofilm cells or loosely adherent cells residing at the peripheries of the biofilm are removed during this procedure. To overcome this limitation, swabbing of the denture fitting surfaces was used for sampling in the present investigation [5,13,15,16].

Because Chromagar *Candida* is generally more sensitive than other media in detecting mixed populations of yeasts, this improved culture medium was used in the present study [1,5,8,13,15,16] and provided differential staining and highlighted colonial morphologic variations between *Candida* species, thus increasing the sensitivity of detection of yeasts.

A review of the literature published to date, on the relationship between DM and oral candidal infection reveals a continuing debate. Studies on candidal colonization are often contradictory, which may be a result of the variety of sampling techniques employed [1,4,10,18]. The present study comprehensively investigated microbiologically oral yeast colonization and evaluated the effect of diabetes and denture stomatitis, which could potentially influence the candidal carriage rate and species variation.

Conclusions

Based on the observation of present study to compare the Candida species in diabetics with denture stomatitis, non-diabetics with denture stomatitis and healthy denture wearing individuals the following conclusions can be drawn:

1) *Candida* species were most prevalent in denture stomatitis patients than in patients with healthy oral mucosa below dentures, but among the two stomatitis groups the prevalence was equal.
2) Most commonly identified *Candida* species was *C. albicans* among all the groups.
3) In group I, i.e. diabetics with denture stomatitis, most common species identified was *C. albicans,* followed by *C. dubliniensis, C. tropicalis* and *C. parapsilosis*.
4) In group II, i.e. nondiabetics with denture stomatitis, most common species was *C. albicans*, followed by *C. glabrata, C. tropicalis*.
5) In group III, i.e. denture wearing individuals without diabetes and healthy mucosa, most common species was *C. albicans* followed by *C. dubliniensis* and *C. glabrata*.
6) The prevalence of *C. glabrata* is higher in denture stomatitis i.e. group II, indicates higher prevalence with degree of inflammation.
7) The isolation of four different types of species in group I suggests that the greater diversity of *Candida* species was present in diabetic patients.

Thus, it can be inferred that both albican and non-albican species of *Candida* can be identified in denture stomatitis with or without diabetes. But more diversity of *Candida* species were observed in patients with diabetes and denture stomatitis which indicates there is influence of diabetes on *Candida* species variation. Even in healthy denture wearing individuals also showed presence of non albican *Candida*. Most common species was *C. albicans* as supported by various studies. Prevalence of *C. glabrata* was higher in the presence of inflammation. Presence of *C. parapsilosis* was seen in patients with diabetes mellitus.

Species variation was not that significant but still different species were identified among groups, which indicates the influence of diabetes and denture stomatitis on species of *Candida* occurrence. The knowledge of prevalence species distribution, rapid species identification, antifungal susceptibility testing and the development of new antifungal drugs are mandatory to achieve a decrease in *Candida* infections and an increase in quality of life of denture-wearing individuals with and without diabetes mellitus. Our study included small group of 60 patients, so further studies are required to identify more species associated with diabetes and denture stomatitis so that better treatment plan can be provided.

Bibliography

1. Belazi M., *et al*. "Candidal overgrowth in diabetic patients: potential predisposing factors". *Mycoses* 48.3 (2005): 192-196.
2. Fisher BM., *et al*. "Carriage of Candida species in the oral cavity in diabetic patients: relationship to glycaemic control". *Journal of Oral Pathology* 16.5 (1987): 282-284.
3. Miranda ET., *et al*. "Species diversity of yeast in oral colonization of insulin-treated diabetes mellitus patients". *Mycopathologia* 162.2 (2006): 83-89.

4. Kadir T., *et al*. "Mycological and cytological examination of oral candidal carriage in diabetic patients and nondiabetic control subjects: thorough analysis of local aetiologic and systemic factors". *Journal of Oral Rehabilitation* 29.5 (2002): 452-457.

5. Lotfi-Kamran MH., *et al*. "Candida colonization on the denture of diabetic and nondiabetic patients". *Dental Research Journal* 6.1 (2009): 23-27.

6. Motta-Silva AC., *et al*. "Erythematous oral candidiasis in patients with controlled type II diabetes mellitus and complete dentures". *Mycopathologia* 169.3 (2010): 215-223.

7. Dorocka-Bobkowska B., *et al*. "Noninsulin-dependent diabetes mellitus as a risk factor for denture stomatitis". *Journal of Oral Pathology and Medicine* 25.8 (1996): 411-415.

8. Khosravi AR., *et al*. "Factors affecting the prevalence of yeasts in the oral cavity of patients with diabetes mellitus". *Journal de Mycologie Médicale* 18.2 (2008): 83-88.

9. Budtz-Jorgensen E. "Histopathology, immunology and serology of oral yeast infections. Diagnosis of oral candidosis". *Acta Odontologica Scandinavica* 48.1 (1990): 37-43.

10. Manfredi M., *et al*. "The isolation, identification and molecular analysis of Candida spp. isolated from the oral cavities of patients with diabetes mellitus". *Oral Microbiology and Immunology* 17.3 (2002): 181-185.

11. Calderone RA and Fonzi WA. "Virulence factors of Candida albicans". *Trends in Microbiology* 9.7 (2001): 327-335.

12. Willis AM., *et al*. "Isolation of C. dubliniensis from insulin-using diabetes mellitus patients". *Journal of Oral Pathology and Medicine* 29.2 (2000): 86-90.

13. Coco BJ., *et al*. "Mixed Candida albicans and Candida glabrata populations associated with the pathogenesis of denture stomatitis". *Oral Microbiology and Immunology* 23.5 (2008): 377-383.

14. De Resende MA., *et al*. "Prevalence and antifungal susceptibility of yeasts obtained from the oral cavity of elderly individuals". *Mycopathologia* 162.1 (2006): 39-44.

15. Thomas CJ and Whittle T. "A 2-year study of Candida associated denture stomatitis treatment in aged care subjects". *Gerodontology* 22.3 (2005): 168-176.

16. Paula Volpato Sanita., *et al*. "Candida spp. prevalence in well controlled type 2 diabetic patients with denture stomatitis". *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 111.6 (2011): 726-733.

17. Mosca CO., *et al*. "Isolation of Candida dubliniensis in a teenager with denture stomatitis". *Medicina Oral Patologia Oral y Cirugia Bucal* 10.1 (2005): 25-31.

18. Al-Karaawi ZM., *et al*. "Molecular characterization of Candida spp. isolated from the oral cavities of patients from diverse clinical settings". *Oral Microbiology and Immunology* 17.1 (2002): 44-49.

Volume 18 Issue 7 July 2019