Candida spp. prevalence in well controlled type 2 diabetic patients with denture stomatitis

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Objective. The aim of this study was to compare the prevalence of *Candida* spp. in diabetics and nondiabetics with and without denture stomatitis (DS).

Study design. Mycologic samples were taken from the dentures of 90 healthy subjects (control group [CG]), 80 denture stomatitis nondiabetics (DSND), and 40 denture stomatitis diabetics (DSD; well controlled type 2) for identification of *Candida* spp. Results were analyzed by Fisher exact test, Bonferroni-corrected confidence interval, and χ^2 test ($\alpha = .05$).

Results. *Candida albicans* was the predominant species isolated (81.9%; $P < .016$), with *C. tropicalis* and *C. glabrata* demonstrating similar prevalence (15.71% and 15.24%, respectively). The prevalence of *C. albicans* and *C. tropicalis* in the DS groups were significantly higher ($P < .01$) than in the CG. The prevalence of *C. tropicalis* significantly increased with the highest degree of inflammation ($P < .05$).

Conclusions. The prevalence of *Candida* spp. was similar between diabetic and nondiabetic patients with DS. *Candida tropicalis* may play a role in the progression of DS. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:726-733)**

Diabetes mellitus is a chronic metabolic disorder that is becoming one of the most important chronic diseases worldwide. It has been estimated that the number of adults with diabetes in the world will rise to 300 million by the year 2025.[1] The relationship between diabetes and oral candidiasis has been extensively studied in the literature,[2-13] particularly because diabetic patients are more susceptible to fungal infections than those without diabetes.[2-4,10] Yeast adhesion to epithelial cell surfaces is recognized as an essential first step in the process of candidal colonization and subsequent infection.[14] Salivary glucose levels in diabetic patients favors yeast growth owing to increased numbers of available receptors for *Candida*.[15] Consequently, buccal cells from diabetic patients have shown an increased adherence of *C. albicans* compared with buccal cells from nondiabetics.[3,4] The microvascular degeneration found in histologic examination of diabetic patients may also predispose to *Candida* colonization,[16] making them more susceptible to infections. Another host factor that may promote the oral carriage of *Candida* in diabetics is the possible defects in candidacidal activity of neutrophils, particularly in the presence of glucose.[17] Reduced salivary flow, associated with diabetes, may also play a role in *Candida* colonization and, consequently, in the pathogenesis of oral candidiasis in these patients.[8]

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.[4,5,7,10,11] A significantly higher incidence of *Candida* infection and increased levels of *Candida* spp. were found in diabetic patients wearing removable dentures.[7,10,11] The presence of a removable denture may decrease the salivary pH and saliva flow rate and impede the mechanical cleaning of the soft

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tissue surfaces by the tongue.[18] 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.[19] 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.[14,18] 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.

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%.[2,3,5,11] This oral fungal pathogen is the most virulent of the *Candida* species[20] and is able to grow as biofilm, which consists of a complex community of cells embedded in a matrix of extracellular polysaccharide.[21] These cells exhibit distinctive phenotypic properties from planktonic cells and increased resistance to antimicrobial agents.[21,22] The epidemiology of *Candida* infections has changed with emergence of non-*albicans* species which have been increasingly described in both compromised and noncompromised hosts.[2-7,9-13,23-27] Non-*albicans* species have been consistently observed in diabetes patients[2-7,9-13] and in those with denture stomatitis.[23-27] Multiple isolations of *Candida* species, including *C. albicans*, *C. glabrata*, *C. tropicalis, C. famata, C. krusei*, *C. kefyr*, *C. colliculosa*, *C. parapsilosis*, *C. guillermondii*, and *C. rugosa* were recorded in diabetes patients.[2,4-7,9,11,12] *Candida dubliniensis* has also been isolated from the oral cavities of diabetes patients.[11,13] In addition, diabetes patients with dentures had more non-*albicans Candida* species isolated than dentate diabetes patients.[6,9-11] Similar findings have been observed in nondiabetic denture-wearing patients with denture stomatitis, where *C. tropicalis* and *C. glabrata* were the non-*albicans* species most often isolated.[23,25,27]

A number of yeast-related factors, such as adhesion to host cells and acrylic surfaces,[20,28-31] cell-surface hydrophobicity,[28,31-33] and secretion of several degradative enzymes,[12,20,29,34] are recognized to promote the virulence of *Candida* spp. and may explain the changes in *Candida* infection epidemiology. Another important factor related to these different species of *Candida* is the development of drug resistance. Along with this species diversity and long-term administration of medications, strains with elevated virulence and acquired resistance against antifungal drugs might colonize the oral cavity.[35,36] Studies have demonstrated that the widespread use of medications, especially azoles, has promoted selection of resistant species by shifting colonization to more naturally resistant *Candida* species, such as *C. glabrata*, *C. dubliniensis*, and *C. krusei*.[35,36] The emergence of resistance among *Candida* isolates to currently available antifungal drugs, associated with epidemiologic changes in *Candida* flora, has important implications for mortality.[37-44] It has been demonstrated that >90% of fungemia cases are attributable to *Candida* species[37,38] and that the number of deaths as a result of fungemia has ranged from ≥40% to almost 80% in immunocompromised hosts.[37-43] In addition, a high crude mortality was also observed among nonimmunocompromised patients (60%)[40] and those with diabetes (67%).[39] *Candida tropicalis* has been reported to be one of the leading *Candida* species other than *C. albicans* to cause fungemia.[38,39,41,43,44] Therefore, accurate identification and the evaluation of the clinical prevalence of *Candida* spp. in specific groups of patients are essential to determine strategies for control and management of oral candidal infection and thus to improve the mortality rate associated with this infection. Although there have been numerous studies evaluating the prevalence of *Candida* spp. in different groups of patients,[2,3,5-11,13,14,23-27] there have been only a few studies[4,12] that compared the prevalence in denture wearers meeting specific criteria for type 2 diabetes and denture stomatitis with that in nondiabetic denture wearers with denture stomatitis. Therefore, the aim of the present cross-sectional study was to compare the prevalence of *Candida* spp. in well controlled type 2 diabetic and nondiabetic denture-wearing patients with and without denture stomatitis.

SUBJECTS AND METHODS

Subjects

In the present study, subjects were selected either from staff and patients of the São Paulo State University, Araraquara Dental School, or recruited from the general population of the Araraquara metropolitan area. Individuals who had received or were currently receiving treatment with antibiotics, antifungals, or steroids in the past 3 months; patients with anemia, immunosuppression or cancer therapy (radio- or chemotherapy); and those wearing the same denture for >30 years were excluded. A total of 210 voluntary patients were selected to participate in the study. The protocol of the project was approved by the Ethics Committee of the Araraquara Dental School (06/2006—0004.0.199.174-06 SISNEP), and each subject signed an informed consent form. Personal, medical, and dental histories of the patients (age and gender of the patients, medication use, smoking habit, and denture age) were recorded. Two risk factors for denture stomatitis were considered

Table I. Summary of recommendations for adults with diabetes[48]

Test	*Goal value*
Fasting blood glucose level	90-130 mg/dL
Postprandial capillary plasma glucose	<180 mg/dL
Glycosylated hemoglobin level	<7%
Serum lipids	
Low-density lipoprotein	<100 mg/dL
Triglycerides	<150 mg/dL
High-density lipoprotein	>40 mg/dL
Serum creatinine	0.4-1.3 mg/dL
Urine	
Protein	Absent
Glucose	Absent
Nitrite	Absent
Leukocytes	<10 U/mm^3

in this study: the age of the dentures[45] and smoking habit.[6,46]

A comprehensive oral examination of the 210 patients was performed by the same investigator, who was blinded to all clinical information and the diabetic state of the subjects, and their mucosal characteristics were classified according to the criteria proposed by Newton[47]: 0, absence of palatal inflammation; 1 (type I), petechiae dispersed throughout all or any part of palatal mucosa in contact with the denture (localized simple inflammation); 2 (type II), macular erythema without hyperplasia (generalized simple inflammation); and 3 (type III), diffuse or generalized erythema with papillary hyperplasia (inflammatory papillary hyperplasia).

According to the systemic condition of the patients and the characteristics of their palatal mucosa, the 210 volunteers were divided into 3 groups of study: control group (CG), 90 individuals without diabetes and with healthy palatal mucosa; 80 nondiabetic patients diagnosed with denture stomatitis according to Newton's criteria[47] (DSND); and 40 diabetic patients with denture stomatitis according to Newton's criteria[47] (DSD).

Diabetic patients were evaluated in relation to their medical care of diabetes, and only those with well controlled type 2 diabetes were selected. Based on the recommendations of the "Standards of Medical Care in Diabetes" (2007),[48] 4 clinical chemistry tests were used to assess the degree of diabetic control: fasting blood glucose level, postprandial capillary plasma glucose, glycosylated hemoglobin level, and serum lipids. Serum creatinine and urine tests were also assessed to evaluate the systemic health condition of the diabetic individuals. All tests were taken before clinical and mycologic procedures. Only patients fulfilling the recommended goals for the tests (Table I) were selected.

Clinical and mycologic procedures

Oral swab samples were collected from the tissue surface of the upper denture of all patients.[9,23,25,26] Each swab was placed into a test tube containing 5 mL of 0.9% sterile saline solution and vortexed for 1 minute to suspend the organisms from the swab. An aliquot (50 μL) from this suspension was spread-plated on Chromagar *Candida*[2,9,10,23,25,26] and incubated at 30°C for 5 days. Colonies were presumptively identified by colony color. Thereafter, biochemical tests were performed to confirm all identifications. One colony of each color type on Chromagar *Candida* was transfered onto fresh Sabouraud dextrose agar for purity. After 48 hours at 37°C, yeast isolates were identified by using the following biochemical tests: carbohydrate assimilation pattern using the API ID32C system (Biomérieux, Marcy-l'Etoile, France) and morphologic characteristics produced on corn meal agar with Tween-80. In addition, green colonies on Chromagar *Candida* were submitted to hypertonic Sabouraud broth test for discriminating *C. albicans* and *C. dubliniensis*.[49]

Statistical analyses

Demographic characteristics of the patients and the risk factors were statistically analyzed to ensure homogeneity between the groups by means of 1-way analysis of variance followed by Tukey post hoc test (age of patients), Kruskal-Wallis followed by Dunns multiple-comparisons test (age of dentures), and Fisher exact test (gender and smoking habit). Bonferroni-corrected confidence interval, Fisher exact test, and χ^2 analysis of several proportions were used to compare the percentage of different species of *Candida* among experimental groups, the percentage of diabetics and nondiabetics in relation to the Newton types of denture stomatitis, and the distribution of *Candida* spp. in relation to Newton classification. Differences were considered to be statistically significant at $P < .05$.

RESULTS

The demographic characteristics of the 210 complete denture wearer patients and the risk factors for denture stomatitis are described in Table II. The mean age of the patients was 62.5 years (range 59.6-65.5 years) at the time of the initial evaluation. In all groups, the number of female patients was more than 3 times higher than that of male patients, with no significant differences among the groups ($P = .247$). Patients in CG had their dentures placed more recently than those in DSND and DSD (Dunn multiple-comparisons test: $P < .0001$). Few smokers participated in this study and no significant differences were found in their distribution among the 3 groups of study ($P = .910$).

Table II. Descriptive analysis of demographic characteristics and risk factors

	Demographic characteristics		*Risk factors*	
	Mean age (y)	*Gender (% female)*	*Mean age of dentures (y)*	*% nonsmokers*
CG (n = 90)	65.5[a]	72.3[a]	4.5[a]	86.7[a]
DSND (n = 80)	59.6[b]	77.5[a]	15.1[b]	85.0[a]
DSD (n = 40)	62.4[ab]	87.5[a]	13.7[b]	87.5[a]
P value	<.0002*	.247‡	<.0001†	.910‡

CG, healthy control group; *DSND,* denture stomatitis without diabetes; *DSD,* denture stomatitis with diabetes.
*One-way analysis of variance; †Kruskal-Wallis test; ‡Fisher exact test.
[a,b]In columns, values with the same letter were not statistically different ($P > .05$).

Considering all of the patients together, *C. albicans* was the predominant yeast, isolated from 81.9% of the participants ($P < .016$ [after Bonferroni correction]). *Candida glabrata* and *C. tropicalis* were isolated from 15.71% and 15.24% of the 210 participants, respectively, with no statistical differences between them ($P > .05$; Bonferroni-corrected 95% confidence intervals: *C. albicans* 0.75-0.88; *C. tropicalis* 0.08-0.19; and *C. glabrata* 0.09-0.20). When the distribution of *Candida* spp. within each group was analyzed (Table III), *C. albicans* was also the predominant yeast in all groups ($P < .016$ [after Bonferroni correction]). Regardless of the group, *C. glabrata* and *C. tropicalis* were the non*albicans* species most frequently isolated, with no significant differences between them ($P > .05$). The percentage frequencies of *C. albicans* and *C. tropicalis* in the denture stomatitis groups (DSND and DSD) were significantly higher than those in CG (χ^2 test: $P < .01$), whereas there were no significant differences in the percentage of frequency of *C. glabrata* observed in the 3 groups of patients ($P > .05$).

Twenty percent of DSND and 38% of DSD patients had Newton type I, 61% and 53%, respectively, had Newton type II, and 19% and 10% had Newton type III, with no significant differences between groups of patients (Fisher exact test: $P > .09$). Because no significant differences were found between the 2 groups of patients with denture stomatitis, regarding either *Candida* spp. prevalence or Newton types, patients were pooled and the frequency distribution of *Candida* spp. in the different types of denture stomatitis was evaluated. The χ^2 test for the comparison of several proportions revealed that the frequency distribution of *C. tropicalis* significantly increased ($P < .01$) relative to the highest degree of inflammation (Table IV). The prevalences of *C. albicans* ($P = .4015$) and *C. glabrata* ($P = .2939$) were not influenced by the degree of inflammation.

Several study participants had >1 species of yeast on the denture surfaces. For the 210 patients sampled, yeast mixtures isolated were *C. albicans* + *C. glabrata* (40.4%), *C. albicans* + *C. tropicalis* (36.5%), and *C. albicans* + *C. glabrata* + *C. tropicalis* (19.2%). The frequency distribution of mixed species populations observed in CG (14.4%) was significantly lower ($P < .01$) than that observed in the 120 patients with denture stomatitis (32.5%).

Table III. Frequency distribution (%) of *Candida* spp. in relation to the groups of study

	CG	*DSND*	*DSD*
C. albicans	64[a]	93[b]	100[b]
C. tropicalis	2[a]	28[b]	20[b]
C. glabrata	14[a]	20[a]	10[a]

Values for *C. tropicalis* and *C. glabrata* were not significantly different ($P > .05$; Bonferroni-corrected 95% confidence intervals: CG: *C. albicans* 0.51-0.76, *C. tropicalis* 0.001-0.09, and *C. glabrata* 0.07-0.25; DSND: *C. albicans* 0.82-0.98, *C. tropicalis* 0.16-0.41, and *C. glabrata* 0.10-0.33; and DSD: *C. albicans* .89-01.0, *C. tropicalis* 0.07-0.40, and *C. glabrata* 0.02-0.27).
Abbreviations as in Table II.
[a,b]In rows, values with the same letter were not statistically different (χ^2 test: $P > .05$).

DISCUSSION

A higher percentage of female patients was observed in the present investigation, and this result agrees with previous studies.[7,10,24] It has been found that elderly women presented more oral lesions than men[50] and that the hormonal factor and the great incidence of iron deficiency in women could be responsible for that disparity.[18,51] In addition, this difference can be explained by the fact that women seek dental treatment at a higher rate than men.[52] The mean age of the dentures, in CG, was considered to be satisfactory (4.5 years old) and was lower than in DSND (15.1 years old) and DSD (13.7 years old). The age of dentures has been related to the occurrence of denture stomatitis.[45] Moreover, tissue trauma, frequently present in patients with poorly fitting dentures and nonbalanced occlusion, can affect the occurrence of this infection.[51] Old dentures are also more difficult to keep clean because of the greater

Table IV. Frequency distribution (%) of *Candida* spp. in different types of denture stomatitis

	Type 1	*Type 2*	*Type 3*	*P value*
C. albicans	100	94.3	94.7	.4015
C. tropicalis	25.8	18.6	57.9	<.01
C. glabrata	9.7	15.7	26.3	.2939

Significant difference only between types 2 and 3 for *C. tropicalis*.

tendency to porosities in the denture base,[45] favoring *Candida* colonization. Therefore, it is reasonable to suppose that patients of DSND and DSD were more susceptible to denture stomatitis than those of CG. Smoking habit has also been found to be an important local factor in oral candidiasis.[6,46] Tobacco smoking associated with denture friction on the oral mucosa alters the mucosal surface, leading to contamination by *Candida* spp.[46] However, the percentage of nonsmokers in all groups of this study was considerably high; therefore, it is difficult to ascertain the influence of this risk factor on the outcomes determined in this study.

Not surprisingly, of the 210 patients evaluated, 81.9% (172 patients) harbored *C. albicans*, which was by far the predominant yeast isolated. This finding is in agreement with earlier reports.[2,3,5,9,11,12,23,26] *Candida albicans* was also isolated from 64% of the patients without denture stomatitis, which was increased to 93% and 100% in the groups of denture stomatitis patients (DSND and DSD, respectively). In agreement with these results, Coco et al.[23] demonstrated that *C. albicans* was isolated from 75% of patients without denture stomatitis and from 81% of those with clinical signs of the infection. *Candida albicans* expresses several virulence factors that contribute to its pathogenesis and very high prevalence. These factors include host recognition biomolecules (adhesins), morphogenesis (the reversible transition between unicellular yeast cells and filamentous growth forms), and aspartyl protease and phospholipase production.[20,29] Phenotypic switching is accompanied by changes in antigen expression, colony morphology, and tissue affinities in *C. albicans*, which might provide cells with a flexibility that results in the adaptation of the organism to the hostile conditions imposed by the host and treatment modality.[20] In addition, *C. albicans* has the ability to adhere to mucosal and denture surfaces, which is considered to be the first step in the pathogenesis of denture stomatitis.[14]

Although *C. albicans* is still the most frequently isolated species from patients with *Candida* infections,[2-6,9,11-13,23-27] the growing prevalence of non-*albicans* species is clearly a concern. In the present study, *C. glabrata* and *C. tropicalis* were isolated from 15.71% and 15.24% of healthy and denture stomatitis patients, respectively. Vanden Abbeele et al.[26] 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.[10] In terms of frequency distribution, some studies have shown that *C. tropicalis* was the second most prevalent species identified.[5,9,24,25] However, contrasting results have been found in other studies, in which *C. glabrata* was the most common yeast after *C. albicans*.[4,6,23,26,27] In the present investigation, there were no significant differences between the prevalence of *C. tropicalis* and *C. glabrata* in all patient groups. The differences in findings among studies are likely to be related to a combination of factors, such as sample techniques[10,23-27] and culture media[53] used. Conventional sampling techniques used in various studies include oral rinses.[7,10,23] 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.[9,23,25,26] Because Chromagar *Candida* is generally more sensitive than other media in detecting mixed populations of yeasts,[53] this improved culture medium was used in the present study[2,9,10,23,25,26] and provided differential staining and highlighted colonial morphologic variations between *Candida* species, thus increasing the sensitivity of detection of yeasts.

Furthermore, a large variation in species frequency has been reported in different regions of the world.[54,55] Although several investigators reported that *C. tropicalis* is one of the most common non-*albicans* species isolated in Brazil and South America,[38,39,41,54,55] the literature contains substantial data indicating that *C. glabrata* is found much more frequently in North America.[54,55] Although these non-*albicans* species are in general less virulent than *C. albicans*,[20,30,32-34] it has been reported that *C. tropicalis* and *C. glabrata* have the ability to cause fungemia in humans[41,43,44] and that they are associated with a higher mortality rate than *C. albicans*.[38,41,43] Therefore, the pathogenicity of these species cannot be underestimated and more attention has to be paid to their appearance. It is important to mention that, in the present investigation, the species *C. dubliniensis* was not detected among any *Candida* isolates. This result agrees with other studies in which this species was not found in elderly individuals,[24,26] in denture wearers with and without denture stomatitis,[23,27,34] or in diabetic patients.[2,4,5,7-9,12] However, *C. dubliniensis* has been isolated in both diabetic[10,11,13] and nondiabetic[25] patients. This disparity may be related to problems with identification techniques, be-

cause *C. dubliniensis* and *C. albicans* have similar phenotypic characteristics, making distinction between them difficult for conventional mycology laboratory testing. In the present investigation, the fact that only the hypertonic Sabouraud broth test[49] was performed for the discrimination between these 2 species may be considered as a limitation of this study. The isolates of both species produce a green color on Chromagar *Candida* and chlamydoconidia on corn meal agar with Tween-80. Therefore, if present, *C. dubliniensis* isolates could have been misidentified as *C. albicans*. In further studies, polymerase chain reaction should be conducted to facilitate the differential diagnosis between these 2 species.

In the present study, no significant differences in *Candida* spp. prevalence were found between denture stomatitis patients with and without diabetes. Contrasting results have been reported in earlier studies, in which diabetic patients were found to be more likely to have a higher prevalence of *Candida* spp. than nondiabetics.[2-4,8-10] However, it is important to mention that either poorly controlled diabetic individuals were included in these studies (e.g., patients with glycosylated hemoglobin level >7% or fasting blood glucose level >130 mg/dL)[3,4,9] or the degree of diabetic control was not reported.[2,8,10] In the present investigation, only well controlled type 2 diabetes patients were included, and this may help explain the results. All patients with denture stomatitis were pooled, and the distribution of the oral yeasts was analyzed in relation to Newton classification of the infection.[47] The results showed that the frequency distribution of *C. tropicalis* significantly increased relative to the highest degree of inflammation (Table IV). These results, combined with the evidence for enhanced frequency distribution of *C. tropicalis* in DSND and DSD groups (Table III), suggest that the higher frequency of this species in denture surfaces may play a role in the progression of denture stomatitis. It has been suggested that species more resistant to therapeutic approaches may emerge in patients with candidiasis, especially those with recurrent infections.[35,36] In addition, *C. tropicalis* has been demonstrated to display higher potential for dissemination and mortality rates than *C. albicans* and other species.[38,39,41,43,44] Several studies showed that *C. tropicalis* is one of the most commonly isolated non-*albicans* species in denture wearers,[9,25,26] in patients with denture stomatitis,[25,27] and in diabetes patients with[4,12] and without[5,7,9,10] denture stomatitis. Although the reason for the increased incidence of *C. tropicalis* infections is not completely understood, it could be attributed to some virulence factors associated with this species. Different *C. tropicalis* strains have demonstrated a high cell-surface hydrophobicity, which is involved in the adherence of microorganisms to different surfaces and considered to be an important pathogenic attribute of yeasts.[32,33] It has been demonstrated that *C. tropicalis* obtained from the oral cavity of denture wearers with denture stomatitis were more adherent to buccal ephitelial cells than those obtained from patients without signs of disease.[30] *Candida tropicalis* strains seem to grow rapidly in the human body, because of their high sucrose assimilation ability.[56] Other important virulence factor of this species is its capability to produce degradative enzymes, such as phospholipase[12] and proteinase,[12,34] which are directly correlated to the invasion and destruction of host tissue.[20,29] The ability of *C. tropicalis* strains to form biofilm on different surfaces[57,58] is another potential virulence trait, which may increase their resistance to antifungal treatment.[21,22] Although these specific virulence factors related to *C. tropicalis* may help explain the results obtained in the present study, further investigations will be required to examine and better understand the mechanism of pathogenicity of this *Candida* species.

Several study participants had >1 species of yeast on the denture surfaces. The frequency distribution of mixed species populations observed in CG (14.4%) was significantly lower than that observed in the 120 patients with denture stomatitis (32.5%). The yeast associations noted in the present study were *C. albicans* together with either *C. tropicalis* and/or *C. glabrata*, confirming trends found at other institutions.[2,5,27] Whether these combinations of yeasts contribute to enhanced pathogenicity remains to be elucidated. The majority of patients with denture stomatitis had a chronic disease process that required prolonged and multiple treatments, which favors the emergence of other non-*albicans* species and formation of mixed biofilms.[35,36] Biofilm-associated resistance[21,22] may explain the high recurrence rates that are often associated with this type of infection. Furthermore, several virulence factors have been associated with non-*albicans Candida* strains, such as the ability to adhere and colonize different substrates (host cells and acrylic surfaces), forming mixed biofilm, secretion of degradative enzymes, and development of drug resistance.[28,35,36,57,58] The complex interactions between these 3 species are not well defined, but the findings from the present study suggest that a synergistic relationship may be involved, with the enhanced pathogenic potential of these combinations facilitating the onset of the infection.

In conclusion, non-*albicans* species were identified in both healthy and denture stomatitis patients with and without diabetes. *Candida albicans* and *C. tropicalis* strains were more prevalent in diabetic and nondiabetic patients with denture stomatitis than in the healthy

ones. In patients with severe denture stomatitis, *C. tropicalis* was more frequent. 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 type 2 diabetes mellitus.

REFERENCES

1. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: Prevalence, numerical estimates, and projections. Diabetes Care 1998;21:1414-31.
2. Belazi M, Velegraki A, Fleva A, Gidarakou I, Papanaum L, Baka D, et al. Candidal overgrowth in diabetic patients: potential predisposing factors. Mycoses 2005;48:192-6.
3. Darwazeh AMG, Lamey PJ, Samaranayake LP, Mac-Farlane TW, Fisher BM, MacRury SM, et al. The relationship between colonisation, secretor status and in vitro adhesion of *Candida albicans* to buccal epithelial cells from diabetics. J Med Microbiol 1990;33:43-9.
4. Dorocka-Bobkowska B, Budtz-Jorgensen E, Wloch S. Noninsulin-dependent diabetes mellitus as a risk factor for denture stomatitis. J Oral Pathol Med 1996;25:411-5.
5. Fisher BM, Lamey PJ, Samaranayake LP, MacFarlane TW, Frier BM. Carriage of *Candida* species in the oral cavity in diabetic patients: relationship to glycaemic control. J Oral Pathol 1987; 16:282-4.
6. Fongsmut T, Deerochanawong C, Prachyabrued W. Intraoral *Candida* in Thai diabetes patients. J Med Assoc Thai 1998;81: 449-53.
7. Gonçalves RH, Miranda ET, Zaia JE, Giannini MJ. Species diversity of yeast in oral colonization of insulin-treated diabetes mellitus patients. Mycopathologia 2006;162:83-9.
8. Kadir T, Pisiriciler R, Akyuz S, Yarat A, Emekli N, Ipbuker A. Mycological and cytological examination of oral candidal carriage in diabetic patients and nondiabetic control subjects: thorough analysis of local aetiologic and systemic factors. J Oral Rehabil 2002;29:452-7.
9. Lotfi-Kamran MH, Jafari AA, Tafti-Falah A, Tavakoli E, Falahzadeh MH. *Candida* colonization on the denture of diabetic and nondiabetic patients. Dent Res:J2009:6:23-7.
10. Khosravi AR, Yarahmadi S, Baiat M, Shokri H, Pourkabireh M. Factors affecting the prevalence of yeasts in the oral cavity of patients with diabeter mellitus. J Mycol Med 2008;18:83-8.
11. Manfredi M, McCullough MJ, Al-Karaawi ZM, Hurel SJ, Porter SR. The isolation, identification and molecular analysis of *Candida* spp. isolated from the oral cavities of patients with diabetes mellitus. Oral Microbiol Immunol 2002;17:181-5.
12. Motta-Silva AC, Aleva NA, Chavasco JK, Armond MC, França JP, Pereira LJ. Erythematous oral candidiasis in patients with controlled type II diabetes mellitus and complete dentures. Mycopathologia 2010;169:215-23.
13. Willis AM, Coulter WA, Sullivan DJ, Coleman DC, Hayes JR, Bell PM, et al. Isolation of *C. dubliniensis* from insulin-using diabetes mellitus patients. J Oral Pathol Med 2000;29:86-90.
14. Davenport JC. The oral distribution of *Candida* in denture stomatitis. Br Dent J 1970;129:151-6.
15. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N Engl J Med 1988;318:1315-21.
16. Farman AG, Nutt G. Oral *Candida*, debilitating disease and atrophic lesions of the tongue. J Biol Buccale 1976;4:203-26.
17. Wilson RM, Reeves WG. Neutrophil phagocytosis and killing in insulin-dependent diabetes. Clin Exp Immunol 1986;63:478-84.
18. Budtz-Jorgensen E. Etiology, pathogenesis, therapy, and prophylaxis of oral yeast infections. Acta Odontol Scand 1990;48:61-9.
19. Budtz-Jorgensen E. Histopathology, immunology and serology of oral yeast infections. Diagnosis of oral candidosis. Acta Odontol Scand 1990;48:37-43.
20. Calderone RA, Fonzi WA. Virulence factors of *Candida albicans*. Trends Microbiol 2001;9:327-35.
21. Ramage G, Saville SP, Thomas DP, López-Ribot JL. *Candida* biofilms: an update. Eukaryot Cell 2005;4:633-8.
22. Ramage G, Tomsett K, Wickes BL, López-Ribot JL, Redding SW. Denture stomatitis: a role for *Candida* biofilms. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;98:53-9.
23. Coco BJ, Bagg J, Cross LJ, Jose A, Cross J, Ramage G. Mixed *Candida albicans* and *Candida glabrata* populations associated with the pathogenesis of denture stomatitis. Oral Microbiol Immunol 2008;23:377-83.
24. de Resende MA, de Sousa LV, de Oliveira RC, Koga-Ito CY, Lyon JP. Prevalence and antifungal susceptibility of yeasts obtained from the oral cavity of elderly individuals. Mycopathologia 2006;162:39-44.
25. Marcos-Arias C, Vicente JL, Sahand IH, Eguia A, De-Juan A, Madariaga L, et al. Isolation of *Candida dubliniensis* in denture stomatitis. Arch Oral Biol 2009;54:127-31.
26. Vanden Abbeele A, de Meel H, Ahariz M, Perraudin JP, Beyer I, Courtois P. Denture contamination by yeasts in the elderly. Gerodontology 2008;25:222-8.
27. Webb BC, Thomas CJ, Whittle T. A 2-year study of *Candida*-associated denture stomatitis treatment in aged care subjects. Gerodontology 2005;22:168-76.
28. Luo G, Samaranayake LP. *Candida glabrata*, an emerging fungal pathogen, exhibits superior relative cell surface hydrophobicity and adhesion to denture acrylic surfaces compared with *Candida albicans*. APMIS 2002;110:601-10.
29. Lyon JP, Resende MA. Correlation between adhesion, enzyme production, and susceptibility to fluconazole in *Candida albicans* obtained from denture wearers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:632-8.
30. Lyon JP, Resende MA. Evaluation of adhesion to buccal epithelial cells in *Candida* species obtained from denture wearers after exposure to fluconazole. Mycoses 2007;50:21-4.
31. Samaranayake YH, Wu PC, Samaranayake LP, So M. Relationship between cell surface hydrophobibity and adherence of *Candida krusei* and *Candida albicans* to epithelial and denture acrylic surfaces. APMIS 1995;103:707-13.
32. Klotz SA, Drutz DJ, Zajic JE. Factors governing adherence of *Candida* species to plastic surfaces. Infect Immun 1985;50: 97-101.
33. Minagi S, Miyake Y, Fujioka Y, Tsuru H, Suginaka H. Cellsurface hydrophobicity of *Candida* species as determined by the contact-angle and hydrocarbon-adherence methods. J Gen Microbiol 1986;132:1111-5.
34. Pinto E, Ribeiro IC, Ferreira NJ, Fortes CE, Fonseca PA, Figueiral MH. Correlation between enzyme production, germ tube formation and susceptibility to fluconazole in *Candida* species isolated from patients with denture-related stomatitis and control individuals. J Oral Pathol Med 2008;37:587-92.
35. Arevalo MP, Arias A, Andreu A, Rodriguez C, Sierra A. Fluconazole, itraconazole and ketoconazole in vitro activity against *Candida* spp. J Chemother 1994;6:226-9.
36. Martinez M, López-Ribot JL, Kirkpatrick WR, Coco BJ, Bachmann SP, Patterson TF. Replacement of *Candida albicans* with *Candida dubliniensis* in human immunodeficiency virus–

infected patients with oropharyngeal candidiasis treated with fluconazole. J Clin Microbiol 2002;40:3135-9.
37. Abelson JA, Moore T, Bruckner D, Deville J, Nielsen K. Frequency of fungemia in hospitalized pediatric inpatients over 11 years at a tertiary care institution. Pediatrics 2005;116:61-7.
38. Costa SF, Marinho I, Araújo EA, Manrique AE, Medeiros EA, Levin AS. Nosocomial fungaemia: a 2-year prospective study. J Hosp Infect 2000;45:69-72.
39. Colombo AL, Nucci M, Salomão R, Branchini ML, Richtmann R, Derossi A, et al. High rate of nonalbicans candidemia in Brazilian tertiary care hospitals. Diagn Microbiol Infect Dis 1999;34:281-6.
40. Dimopoulos G, Karabinis A, Samonis G, Falagas ME. Candidemia in immunocompromised and immunocompetent critically ill patients: a prospective comparative study. Eur J Clin Microbiol Infect Dis 2007;26:377-84.
41. Goldani LZ, Mário PS. *Candida tropicalis* fungemia in a tertiary care hospital. J Infect 2003;46:155-60.
42. Fraser VJ, Jones M, Dunkel J, Storfer S, Medoff G, Dunagan WC. Candidemia in a tertiary care hospital: epidemiology, risk factors, and predictors of mortality. Clin Infect Dis 1992; 15:414-21.
43. Meunier-Carpentier F, Kiehn TE, Armstrong D. Fungemia in the immunocompromised host. Changing patterns, antigenemia, high mortality. Am J Med 1981;71:363-70.
44. Wingard JR, Merz WG, Saral R. *Candida tropicalis*: a major pathogen in immunocompromised patients. Ann Intern Med 1979;91:539-43.
45. Budtz-Jorgensen E. Oral mucosal lesions associated with the wearing of removable dentures. J Oral Pathol 1981;10:65-80.
46. Arendorf TM, Walker DM, Kingdom RJ, Roll JR, Newcombe RG. Tobacco smoking and denture wearing in oral candidal leukoplakia. Br Dent J 1983;155:340-3.
47. Newton AV. Denture sore mouth. A possible etiology. Br Dent J 1962;112:357-60.
48. American Diabetes Association. Standards of medical care in diabetes. Diabetes Care 2007;30(Suppl 1):S4-41.
49. Alves SH, Milan EP, de Laet-Sant'Ana P, Oliveira LO, Santurio JM, Colombo AL. Hypertonic Sabouraud broth as a simple and powerful test for *Candida dubliniensis* screening. Diagn Microbiol Infect Dis 2002;43:85-6.
50. Espinoza I, Rojas R, Aranda W, Gamonal J. Prevalence of oral mucosal lesions in elderly people in Santiago, Chile. J Oral Pathol Med 2003;32:571-5.
51. Figueiral MH, Azul A, Pinto E, Fonseca PA, Branco FM, Scully C. Denture-related stomatitis: Identification of aetiological and predisposing factors—a large cohort. J Oral Rehabil 2007; 34:448-55.
52. Dorey JL, Blasberg B, MacEntee MI, Conklin RJ. Oral mucosal disorders in denture wearers. J Prosthet Dent 1985;53:210-13.
53. Pfaller MA, Houston A, Coffmann S. Application of Chromagar Candida for rapid screening of clinical specimens for *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida (Torulopsis) glabrata*. J Clin Microbiol 1996;34:58-61.
54. Colombo AL, Perfect J, DiNubile M, Bartizal K, Motyl M, Hicks P, et al. Global distribution and outcomes for *Candida* species causing invasive candidiasis: results from an international randomized double-blind study of caspofungin versus amphotericin B for the treatment of invasive candidiasis. Eur J Clin Microbiol Infect Dis 2003;22:470-4.
55. Pfaller MA, Jones RN, Doern GV, Sader HS, Messer SA, Houston A, et al. Bloodstream infections due to *Candida* species: SENTRY antimicrobial surveillance program in North America and Latin America, 1997-1998. Antimicrob Agents Chemother 2000;44:747-51.
56. Okawa Y, Miyauchi M, Kobayashi H. Comparison of pathogenicity of various *Candida tropicalis* strains. Biol Pharm Bull 2008;31:1507-10.
57. Mutluay MM, Oğuz S, Orstavik D, Fløystrand F, Doğan A, Söderling E, et al. Experiments on in vivo biofilm formation and in vitro adhesion of *Candida* species on polysiloxane liners. Gerodontology 2010;27:283-91.
58. Paulitsch AH, Willinger B, Zsalatz B, Stabentheiner E, Marth E, Buzina W. In-vivo *Candida* biofilms in scanning electron microscopy. Med Mycol 2009;47:690-6.

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