

ORIGINAL ARTICLE

FACTORS RELATED TO ORAL CANDIDIASIS IN ELDERLY USERS AND NON-USERS OF REMOVABLE DENTAL PROSTHESES

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SUMMARY

This study examined the association between oral candidiasis in elderly users and nonusers of prosthesis and its predisposing factors. To this end, we performed a cross-sectional study where saliva samples from 48 patients who used prosthesis and 43 patients (control group) who did not use. Among the 91 patients, *Candida* spp were isolated in 40 (83.3%) who used prosthesis and in 23 (53.5%) in the control group. A statistically significant association was determined between the two groups, the isolation of yeasts and dental prosthesis ($p < 0.05$, OR = 4.3). The most common etiological agent was *Candida albicans* (37 isolates), with 23 (62.2%) in the denture group and 14 (37.8%) (control group). Among patients who presented clinical manifestations of oral candidiasis ($n = 24$), 83.3% ($n = 20$) belonged to the group that wore dentures, while only 16.7% ($n = 4$) belonged to the control group. Elderly patients with diabetes had 4.4 times higher estimated risk of developing oral candidiasis when compared with individuals without this condition. There was no statistically significant association between being user prostheses and have diabetes with the onset of candidiasis. No statistically significant association was determined between xerostomia, use of prosthesis and oral candidiasis. The use of prosthetics and poor oral hygiene in elderly patients predisposes to the development of oral candidiasis.

KEYWORDS: Candidiasis; Dental prosthesis; Xerostomia; Elderly.

INTRODUCTION

Proportionally, the fastest growing segment of the population is the elderly population, composed of individuals aged 60 and over. The oral cavity is considered to be of prime physiological and metabolic importance, and undergoes numerous changes with the aging process, including: shrinkage of periodontal tissues due to reduced cellularity; reduction in the thickness of oral mucosa due to progressive tissue dehydration; reduction of the papillae; and systemic changes that have a direct effect on oral tissues and physiological processes¹.

The high frequency of partial or complete absence of the teeth, and the need for improvements in the health and quality of life of elderly persons requires the use of dental prostheses. The presence of oral devices like prostheses and orthodontic appliances can alter the oral environment, leading to modifications in the physical and biological characteristics of the saliva and other oral structures, often causing an imbalance in the local microbiota².

Denture can interfere with, or contribute to, the detection of clinical or subclinical disease processes resulting from the interaction between

the prosthesis and microorganisms; moreover, denture is often related to the appearance of oral lesions, such as traumatic ulcers, mucogingival hyperplasias, and oral candidiasis³.

Numerous epidemiological studies have been conducted on elderly institutionalized patients who use health services and are members of senior citizens centers in various regions throughout Brazil. Denture stomatitis affects two-thirds of removable prosthesis wearers, resulting in patient injury, including bleeding, pain, and discomfort, which can make the use of prosthesis unviable, thereby directly compromising the overall health and quality of life of these individuals⁴⁻⁵.

This study aimed to determine possible correlations between oral candidiasis in elderly wearers and non-wearers of denture, and the presence of yeasts of the *Candida* species, together with their interrelation with predisposing factors to this pathology, such as diabetes, hyposalivation and oral hygiene.

METHODS

This work was approved by the Ethical Committee under the case

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number 242715/CEP/HUJM/2013. Participants in this study attended the *Padre Firmo Pinto Duarte Filho* Senior Citizens Center, run by the city government; the philanthropic foundation *Abrigo do Bom Jesus de Cuiabá*; and the private institutions: Dentistry Clinic of the School of Dentistry of the University of Cuiabá (UNIC), and *Três Américas* Clinic Ltd., all located in the City of Cuiabá, MT, Brazil.

In this epidemiological cross-sectional study, the collection of biological material was performed from December 2012 to April 2013. Written consent was obtained from each participant in the study. The study population consisted of 91 elderly patients (over 60 years old) and selection was not performed according to sex or ethnicity. None of the participants were being prescribed antibiotic therapies nor used antiseptic mouthwash in the 10 days preceding saliva collection. No special care was taken regarding guidance on their diet and tooth-brushing routines.

Two groups were studied. Group A, consisting of 48 patients who wore complete removable dentures; 10 men aged 66 to 83 years old, and 38 women aged 62 to 84 years old; and Group B (the control group), consisting of 43 patients who did not wear complete removable dental prostheses; 18 men aged 66 to 93 years old and 25 women aged 63 to 89 years old. The exclusion criteria included patients with altered cognition (memory impairment, dementia, disorientation), as they would not be able to answer to the questions and could cause problems for the spontaneous saliva collection; patients with infectious diseases that were undergoing treatment (tuberculosis and leprosy); patients on antibiotics or immunosuppressants; and frequent mouthwash users. Saliva was collected from the participants without stimulation, using disposable plastic collectors (Exalab®). The samples were immediately plated in duplicate on sterile Petri dishes containing Sabouraud dextrose agar (Difco™) plus chloramphenicol at a concentration of 100 mg/L of medium, and in duplicate on sterile Petri dishes containing Mycosel™ agar (Difco™). Media pH was adjusted to between 5.6 and 6.0⁶. On each plate, a 100 µL sample of pure saliva was inoculated with the aid of a calibrated micropipette. The plates containing the seeded culture media were then forwarded to the Mycology Research Laboratory of the Faculty of Medicine of the Federal University of Mato Grosso (FM-UFMT). Incubation was performed in a biochemical oxygen demand (BOD) incubator for 48 to 72 h at 25 °C.

Following incubation, characteristic colony forming units (CFUs) of yeasts of the genus *Candida* were counted and the number of CFUs/mm of saliva was calculated. When colonies presenting macroscopic characteristics consistent with yeasts were observed, smears were taken and stained using the Gram method, in order to observe the morphological staining characteristics. Isolated colonies were tested for purity using plating on CHROMagar™ chromogenic medium (DIFCO™). *Candida* species were identified using VITEK®2 Compact System

equipment (BioMérieux Inc.), in accordance with the manufacturer's recommendations⁷.

The strains used as controls were *Candida glabrata* (ATCC MYA 2950), *Candida lusitanae* (ATCC 34449), *Candida utilis* (ATCC 9950), *Geotrichum capitatum* (ATCC 28576), *Kloeckera apis* (ATCC 32857), *Prototheca wickerhamii* (ATCC 16529), and *Trichosporon mucoides* (ATCC 204094)⁷.

RESULTS

Yeasts isolates of the genus *Candida* were obtained from the samples of 40 patients (83.3%) who wore denture, while in the control group (non-wearers), samples of 23 patients (53.5%) also contained these fungal organisms (Table 1). The presence of characteristic clinical signs and symptoms of oral candidiasis were verified in 24 (26.4%) participants of the study population. A statistically significant association was verified between the yeast isolates of the genus *Candida* and the presence of oral candidiasis ($p = 0.009$).

Elderly wearers of removable prostheses presented a 4.4-fold greater chance of *Candida* spp isolation than elderly non-wearers (95% CI = 1.65-11.4). A statistically significant association was verified between the presence of a complete prosthesis and the isolation of yeasts of the genus *Candida* ($p = 0.004$).

Among patients with clinical manifestations of oral candidiasis ($n = 24$), 83.3% ($n = 20$) belonged to the group who wore removable dental prostheses, while only 16.7% ($n = 4$) belonged to the control group. A statistically significant association was determined between the use of removable dental prostheses and the presence of oral candidiasis ($p = 0.001$). Elderly individuals who wore removable dental prostheses showed a 6.9-fold higher chance of developing oral candidiasis compared with elderly non-wearers.

Figure 1 shows the different levels of oral/removable dentures hygiene and the origin of the samples. At the time of collection, elderly individuals who attended the *Padre Firmo* Senior Citizens Center were 3.2 times more likely to present regular oral hygiene (Grade 2) compared to those who resided at the Shelter *Bom Jesus* ($p = 0.05$, OR = 3.2; 95% CI = 1.1-9.4). In contrast, at the time of collection, elderly individuals who resided at the Shelter *Bom Jesus* were 5.8 times more likely to present poor oral hygiene (Grade 3) compared with those who attended the *Padre Firmo* Senior Citizens Center. Thus, a statistically significant association was verified for this variable ($p = 0.05$, OR = 5.8; 95% CI = 1.7-19.0).

Elderly patients with diabetes showed a 4.4-fold higher chance of developing oral candidiasis ($p = 0.004$; OR = 4.4; 95% CI = 1.6-11.9)

Table 1
Correlation between the isolation of yeasts of the genus *Candida* in the oral cavity and the presence of candidiasis

	Presence of oral candidiasis		Absence of oral candidiasis		p value	OR	IC95%
	n	%	n	%			
Patient samples with yeast isolates	23	37	40	63	0.009	15.5	1.9 – 121.9
Patient samples without yeast isolates	01	04	27	96			

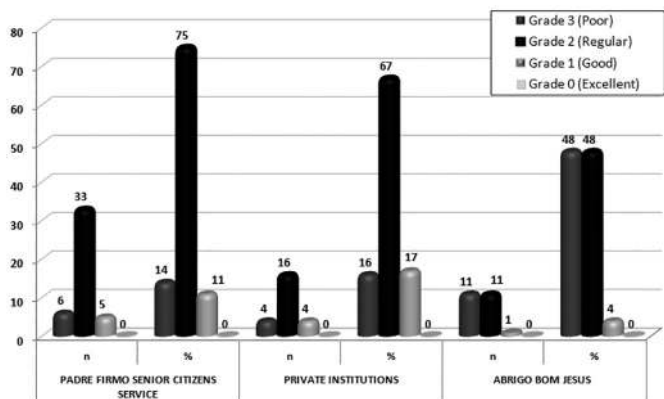


Fig. 1 - Correlation between the presence of candidiasis and the isolation of genus *Candida* yeasts in the oral cavity.

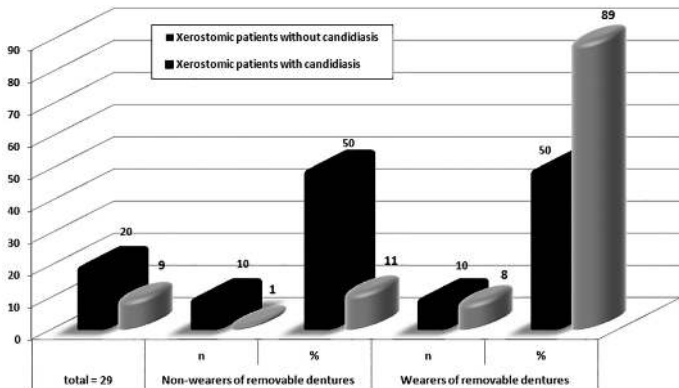


Fig. 2 - Association between the different levels of oral hygiene, or the removable dentures hygiene, and the origin of the samples.

compared to individuals without diabetes. However, no statistical association was verified between prosthesis exposure and outcome in elderly diabetic patients with candidiasis.

No statistically significant association was determined between the presence of hyposalivation and an outcome of oral candidiasis in this sample. Given the data analyzed, no statistically significant association was determined between prosthesis exposure and this outcome in elderly patients with hyposalivation and candidiasis (Table 2) (Fig. 3).

DISCUSSION

The results obtained here indicate that the use of removable dental

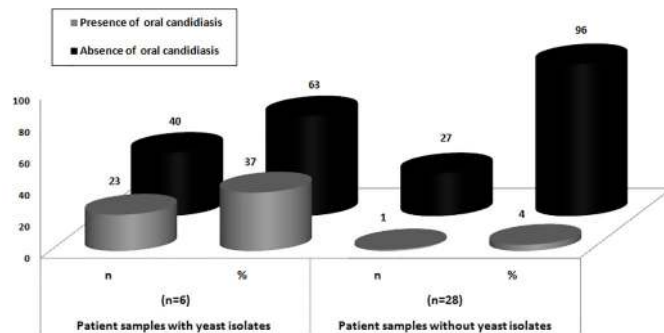


Fig. 3 - Association between oral candidiasis and the use of removable dentures in patients with hyposalivation.

prostheses and deficient hygienization of the prostheses among elderly patients predisposes them to a candidiasis infection⁸⁻¹⁰.

Denture stomatitis is an entity that is associated with those who wear removable dental prostheses and oral candidiasis. The etiology of this fungal infection is multifactorial and has been associated with the use of removable dental prostheses, hyposalivation and *Candida albicans*^{8,11}. It is known that *Candida albicans* exerts an important role in the development of denture stomatitis, since it can initiate, maintain, and exacerbate this condition. However, an iatrogenic factor is required (poor adaption wear due to prolonged use) or poor removable dental prostheses hygienization¹¹⁻¹².

In this study, the mean patient age was 72.7 years old. PEREIRA¹³ studied adults who were 50 years old or over, analyzing data for two age groups: 50-74 years old, and 75 years old and over. SALIBA¹⁴ examined individuals aged 42 to 102 years old, dividing the data obtained into age groups. FRARE¹⁵ also grouped the data of the individuals evaluated in their study and they were aged 55 years old or over. Thus, important variations in the grouping of data according to age exist in the samples studied.

Different methods of saliva collection are also verified among such studies. Our group opted for whole saliva collection, since this permits identification and quantification of yeast isolates with a high degree of confidence. As expected, the isolation of yeasts in patients who wore removable dental prostheses (83.3%) was clearly much higher than among non-wearers (53.5%).

Although several groups¹⁶⁻¹⁷ claim that the identification of genus *Candida* yeasts using the CHROMagar technique is highly reliable, in this study, the technique was used to verify the degree of purity of the

Table 2
Correlation between the isolation of yeasts of the genus *Candida* in the oral cavity and the presence of candidiasis

	Xerostomic patients with candidiasis		Xerostomic patients without candidiasis		p value	OR	IC95%
	n	%	n	%			
Patients wearers of removable dentures	08	89	10	50	0.07	8.0	0.8 – 76.3
Patients non-wearers of removable dentures	01	11	10	50			

yeast colonies isolated. For the final classification of the species obtained, an automated and classical microculture plate method was used¹⁸. *C. albicans* was the most frequently isolated among elderly participants from the public and private institutions evaluated. This finding confirms the results published by a number of other research groups¹⁹⁻²⁰.

Concerning the distribution of species in the genus *Candida*, variations exist in percentages reported in the literature, from 30%²¹ to approximately 80%²². Regarding the presence of non-*albicans* species, the most frequently reported isolates are *C. glabrata*, *C. dubliniensis*, *C. tropicalis*, and *C. parapsilosis*²¹⁻²².

The presence of removable dental prostheses increases the number of genus *Candida* yeasts, predisposing patients to the development of candidiasis. This finding is justified due to the excellent adhesion of these microorganisms to acrylic prostheses a finding in agreement with the majority of previous reports²³⁻²⁴.

The prevalence of denture stomatitis varies between 11% and 67% of removable dental prostheses wearers²⁵, with the use of dentures during sleep considered a contributing factor^{8,22,26-30}.

In this study, patients who wore removable dental prostheses were 6.9 times more likely to develop oral candidiasis than elderly non-wearers. This fact could be explained by the precarious conditions of the dental prostheses (poor adaption and fabrication) or high porosity due to prolonged use. All of these are factors associated with an increase in colony counts of genus *Candida* yeasts^{1,21}. The majority of researchers conclude that denture hygienization is inadequately performed by most patients who wear removable dental prostheses and that this fact is strongly related to the increased prevalence of *Candida* spp³¹.

Of the 91 patients evaluated in this study, 31.9% (n = 29) wore removable dental prostheses and had diabetes. Diabetic patients who wore dentures showed a 4.4-fold greater chance of developing oral candidiasis. Numerous systemic medical conditions (candidiasis, leukemia, AIDS) show a well-defined relationship with effects in the oral cavity. However, definitive associations between diseases like diabetes mellitus and the oral manifestations observed in this study are considered controversial³².

SOUSA²⁴ investigated the prevalence of oral mucosal alterations in patients affected by type 2 diabetes mellitus. This author analyzed heredity factors, blood glucose control, and local factors (use of prostheses and dry mouth). The results obtained showed that in 68.8% of diabetic patients (n = 66) ($p < 0.001$), heredity factors were significant. Reduced salivary flow in diabetic patients was 49% (n = 47), while among non-diabetic patients, it was 34% (n = 34). The presence of candidiasis was 30.5% among diabetic patients (n = 29), similar to that obtained in this study, and 36% among non-diabetic patients (n = 36). This group concluded that oral alterations were not associated with diabetes mellitus, and considered the influence of other factors, including the use of prostheses. SHULMAN³³ and YAMASHITA³⁴ also reported no association between diabetes and alterations in oral soft tissues and the use of complete removable dental prostheses.

In this study, the clinical condition of hyposalivation affected 31.8% (n = 29) of the studied population. Common causes of this disorder, that should be highlighted include local cancer therapy; psychological

disorders; autoimmune diseases; and use of certain medications, such as antidepressants, antihypertensives, and diuretics. The use of medications is a major cause of xerostomia, but is rarely associated with irreversible damage to the salivary glands.

IACOPINO & WATEN³⁵ and ARTICO³⁶ reviewed the literature concerning infection by genus *Candida* yeasts and denture stomatitis. The authors described the clinical lesions and affirmed that the main predisposing factors to *Candida* spp. infection were deficiencies in the host immune system, xerostomia, and the use of denture.

Abundant fungal structures are observed in specimens obtained from the surface of complete removable dental prostheses. This fact allows us to infer that inadequate use of prostheses, together with inadequate hygienization favor the growth of genus *Candida* yeasts, particularly *C. albicans*^{9,10,12}.

The most important preventive measure, specific to the elderly population, is the control of the oral environment, particularly regarding the prevention of dental caries (coronary, root, and periodontal)³⁷.

These actions will tend to diminish the need for complete dentures, which should reduce the incidence of oral candidiasis. Daily hygienization and disinfection of removable dental prostheses is necessary to promote health and conservation of the oral tissues. The maintenance of healthy mucosa is related to the degree of cleanliness of the prostheses in contact with oral tissues.

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