Prevalence of *Candida* in oral cavity of patients wearing dental prosthesis

Ocorrência de *Candida* na cavidade bucal de usuários de prótese dentária

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Abstract

A dental prosthesis is a devise used to substitute missing teeth in order to restore the functional and aesthetic factors affected by the total or partial loss of one or more units. However, there is a high prevalence of oral candidiasis in denture user patients. The objective of the present study is to assess the prevalence of microorganisms in the oral cavity of denture users and to identify and determine the frequency of the main *Candida* species present in the oral cavity of these individuals. Eighty one subjects were randomly addressed, oral mucosa samples were collected and a questionnaire was administered to assess the profile of this population. The identification of species was performed using a chromogenic culture medium. Fifty six point eighty one percent of the individuals reported pre-existing diseases, especially hypertension (22.72 %) and diabetes (9.09 %). *Candida* grew in 77.27% of the denture users and in 22.27% of the individuals without prosthesis. *Candida albicans* was the most prevalent species in patients wearing dental prosthesis and in the control sample. A significant correlation between the presence of *Candida* and the use of prosthesis was found. Thus prosthesis users require permanent care from a dentist in terms of hygiene, adaptation and substitution. These attitudes promote oral health and life quality of users reducing the incidence of *Candida* infections.

Keyword: Oral Health. Dental Prosthesis. *Candida*.

Resumo

Prótese dentária é um aparelho utilizado em substituição aos dentes ausentes, visando restabelecer os fatores funcionais e estéticos envolvidos pela perda ou comprometimento dos dentes, entretanto, verifica-se uma alta prevalência de candidíase bucal em pacientes usuários de próteses. O presente estudo visou à avaliação da carga fúngica bucal de indivíduos usuários de prótese dentária e identificação e frequência das principais espécies de Candida na cavidade bucal desses indivíduos. Os indivíduos foram selecionados aleatoriamente através da abordagem direta. Coletou-se uma amostra da mucosa jugal e aplicou-se um formulário visando traçar o perfil dessa população. A identificação das espécies foi realizada utilizando-se o meio de cultura cromogênico. Foram realizadas coletas em 81 indivíduos. Observou-se que 56,81% dos indivíduos amostrados relataram possuir doenças pré-existentes, sendo as mais prevalentes a hipertensão (22,72%) e a diabetes (9,09%). Foi observado crescimento de *Candida* em 34 (77,27%) dos 44 indivíduos usuários de prótese dentária e em 10 (22,27%) dos 37 indivíduos não usuários. A espécie mais prevalente em usuários de prótese e no grupo controle foi *Candida albicans*. Verificou-se estatisticamente que houve correlação entre a presença de *Candida* e uso de prótese. O uso de prótese exige cuidados permanentes com sua adaptação e necessidade de substituição ao término de sua vida útil, sendo necessário o acompanhamento do profissional odontólogo. Essas atitudes favorecem a saúde bucal e a qualidade de vida dos usuários e visam reduzir a incidência de infecções por *Candida*.

Palavras-chave: Saúde Bucal. Prótese Dentária. *Candida*.

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INTRODUCTION

The oral cavity has a microbiological ecosystem that is usually in harmony, because the components remain in good balance. *Candida* is a frequent commensal fungus in the human body, and is known to be the major cause for opportunistic mycosis in the oral cavity such as candidiasis, candidosis, aphthous stomatitis, prosthetic stomatitis and atrophic candidiasis or stomatitis caused by prosthesis[1].

In a healthy oral mucosa the frequency of these microorganisms varies between 60 and 70%, and they normally do not unleash pathological processes. However, the immunological system is crucial for the health of individuals, and when this system is impaired the individual will be more susceptible to infections[2]. The predominant predisposing factors for pathological development are diabetes mellitus, unhealthy diet, long term use of antibiotics and corticoids, leukemia, chemotherapy, transplants, alcohol consumption, smoking and others[3, 4].

Besides those factors, a high prevalence of oral candidiasis is observed in prosthesis users, because dentures can develop an acid and anaerobic environment in the oral mucosa that promotes yeast proliferation[5].

Dental prosthesis is an artificial set of teeth created to replace some absent units in order to reestablish the masticatory and esthetic functions. However, 60% of prosthesis users show clinical evidences or subclinical pathological problems caused by *Candida*. These oral pathologies are the result of poor oral hygiene, poor prosthesis adaptation, uninterrupted use, incorrect cleaning, and use after the expiration date. Those attitudes can lead to biofilm formation that will propitiate the colonization by yeasts and other microorganisms in the mucosa[6, 7].

The identification of the species involved in infectious processes is important to define the right treatment for the patient´s complete recovery. Among the various species of the genus, *Candida albicans* is the isolate most frequently found in infections, and is considered an opportunistic pathogen prevalent in humans. However other species may also be involved in infectious processes such as *Candida parapsilosis*, *Candida krusei*, *Candida tropicalis*, and *Candida glabrata*[2, 7].

The most effective attitude to control biofilm formation by *Candida* in prosthesis users is mechanical and chemical hygiene. The mechanical method consists of the utilization of two toothbrushes (one for the oral cavity and the other for the denture), toothpaste and neutral soap[8]. The chemical method includes the prosthesis immersion in chemical products such as solvents, detergents, fungicides and bactericides, hypochlorides, peroxides, alkalis, earning acids, enzymes, and chlorhexidine[9].

The objectives of this study are: 1. to identify and assess the frequency of *Candida* in the oral mucosa of prosthesis users; 2. to correlate the use of dentures to other risk factors such as nutrition, hygiene, and preexisting diseases.

METHODOLOGY

This research was descriptive, experimental, and qualitative-quantitative. It was carried out with patients wearing dental prosthesis between July and October on 2013 in Cruz das Almas, Bahia, Brazil. The individuals were randomly selected, and the inclusion criteria were: being 18 years old or older, using dental prosthesis, and be willing to participate.

The samples were collected after approval of the Maria Milza College Ethics Committee (CEP-FAMAM 51/2013), in accordance with the guidelines and standards regulating research involving human beings (resolution 466/12 of the National Health Council).

Participants had to sign the Informed Consent Form which describes all the steps of the study. They also answered a structured questionnaire with objective and discursive questions about hygiene, nutrition, pre-existing diseases, and other topics.

Oral mucosa samples were collected with sterile swaps. The material was inoculated on chromogenic culture medium CHROMagar™Candida (Paris, French). Plates were incubated for 3 to 4 days at 98.6°F. The identification of *Candida* species was based on coloring in accordance with the manufacturer's specification.

The results were compared using the nonparametric chi-square (X^2) test at a significance level of 5% (p< 0.05).

RESULTS AND DISCUSSION

Age

Forty-four individuals wearing dental prosthesis were evaluated, 65.91% female and 34.09% male. The average age was 62.13 years (varying from 36 to 86 years old) and the majority of the participants were seniors (Table 1). The predominance of females was also reported by Paraguassu et al.[10] who found that 78.6% of the individuals studied were females and their average age was 56.25. Also Bomfim et al.[11] observed that 58.5% of the patients wearing dental prosthesis were females and 83.1% of them had oral lesions.

In a research carried out at the Prosthesis Service of the Oral Specialty Center of Cataguases, Minas Gerais, Brazil, the authors verified that the average age of prosthesis users was 60.32 (between 40 and 80 years old). They also found that 77.30% of the lesions occurred in females[12].

Silva et al.[13] observed that oral mucosa lesions related to prosthesis use were more common among women over 40 years old. This result suggests that hormonal deficit and/or factors related to age can facilitate lesion development.

Preexisting diseases

In the present research 56.81% of the participants had a preexisting disease. The most frequent was hypertension (22.72%) followed by diabetes (09.09%); 45.45% of these individuals made daily use of medicines to control their pathologies (Table 1).

According to Alves[3], 74.30% of denture users had some systemic pathology such as prosthetic stomatitis associated to the genus Candida and traumatic ulcers that were also associated to preexisting diseases, as for example hypertension, diabetes, and respiratory diseases. The author reported that elderly people are more susceptible to infections caused by opportunist microorganisms due to the reduced capacity of the immune system, systemic pathologies, and drug association.

As reported by Terra et al.[14], individuals with not controlled diabetes may suffer from xerostomia, burning mouth syndromes, and periodontal diseases. In addition, oral flora modifications leading to candidiasis and angular cheilitis can be observed. In a study with elderly people, Soares et al.[15] observed that their saliva was less efficient to eliminate pathogens probably due to pre-existing diseases, age, and diet and hygiene changes.

Nutrition

In general the population tested had healthy habits: 72.73% never drank alcohol or smoked. In terms of diet, 68.18% ate fruits and/or vegetables daily, and 31.81% did it 4 to 6 times a week. In terms of fat and/or fried foods, 52.27% of the participants reported eating them once or twice a week, and 20.45% never ate this type of food (Table 1).

The dietary habits of these individuals is adequate, and are in accordance with Brasil[16] that states that the weekly frequency of healthy food intake (beans, vegetables, fruits and milk) must be equal to five or more days a week, while unhealthy food such as fried foods, sausages, biscuits, cookies, and soda must be consumed within a maximum of 2 times a week.

Species Occurrence and Identification

Candida growth was observed in 34 out of 44 denture users, and in 10 out of 37 no users (Table 2). The Chi-square test indicated that the association between the presence of Candida and prosthesis use was significant at p<0.05, with a calculated value of 18.37> x^2 tag= 10.83. This result indicates that the presence of the yeast is not random, and thus denture users tend to be more liable to Candida colonization.

C. albicans was the most frequent species, occurring in 11.36% of prosthesis users and in 10.81% of non-users. C. tropicalis (11.36%) and C. krusei (02.27%) were isolated just in individuals wearing dentures. Candida spp. was isolated in both groups, with a frequency of 09.09% in individuals wearing prosthesis and 05.40% in non-users.

Table 1. Population data obtained between July and October of 2013 in Cruz das Almas, Bahia, Brazil.

POPULATION CHARACTERISTICS	NUMBER (%)
SEX	
Male	15 (34.09%)
Female	29 (65.91%)
AGE GROUP	
18 – 30	00 (00.00%)
31 – 40	01 (02.27%)
41 – 60	28 (63.63%)
61 – 80	11 (25.00%)
81 – 90	04 (09.10%)
PREEXISTING DISEASES	
Diabetes	04 (09.09%)
Hypertension	10 (22.72%)
Cardiovascular disease	06 (13.63%)
Cholesterol above recommended levels	05 (11.36%)
No diseases	19 (43.18%)
DAILY USE OF MEDICINES	
Yes	20 (45.45%)
No	24 (54.54%)
SMOKER	
Yes	00 (00.00%)
No	44 (100.0%)
INGESTION OF ALCOHOLIC BEVERAGES	
No ingestion	32 (72.73%)
Maximum once a week	10 (22.72%)
Ingestion 2 to 3 times a week	02 (04.55%)
Ingestion 4 or more times a week	00 (00.00%)
HEALTHY DIET (FRUITS AND VEGETABLES)	
Daily	30 (68.18%)
4 to 6 times a week	14 (31.81%)
1 to 3 times a week	00 (00.00%)
Not at all	00 (00.00%)
INGESTION OF FATS AND FRIED FOOD	
Daily	00 (00.00%)
3 to 6 times week	02 (04.55%)
1 to 2 times week	23 (52.27%)
Do not eat fats or fried food	09 (20.45%)

When considering prosthesis users, an association between species was observed: *C. albicans* and *Candida* spp. (34.09%); *C. albicans* and *C. tropicalis* (92.27%), *C. krusei* and *Candida* spp. (02.27%); *C. krusei* and *C. tropicalis* (02.27%); *C. krusei*, *C. albicans* and *Candida* spp. (2.27%). In non-users the concomitant presence of *C. albicans* and *C. tropicalis* (05.40%); *C. albicans* and *Candida* spp. (5.40%); *C. albicans*, *C. tropicalis* and *Candida* spp. (02.70%) was verified (Table 3).

Coutrim et al.[17] state that although the genus *Candida* comprises 163 species, 60% of the infections are caused by *C. albicans*. This is also the most frequent species and 97.7% of the individuals wearing dental prosthesis present colonization by this yeast. According to Alves[3], *C. albicans* may respond to changes in the host by increasing the number of colony forming units, and invade tissues causing infections that require especial care due to their prevalence in association with dental prosthesis.

Our data are in accordance with those of Melo et al.[7] who reported the presence of 3 species of *Candida* in patients with lesions positive for this yeast. *C. albicans* and *C. tropicalis* were the most frequent species, followed by *C. krusei*. The association between two species in the same lesion was frequent, mainly *C. albicans* and *C. tropicalis* that were observed in 36% of the samples. Figueiral et al.[18] also report *C. albicans* as the most frequently isolated species, with values between 30% and 80%. Other prevalent species were *C. glabrata*, *C. dubliniensis*, *C. tropicalis* and *C. parapsilosis*. The literature review by Melo and Guerra[5] reports that *C. tropicalis* is the second most frequent type, regardless of the site of infection.

Darwazeh et al.[19] reported that *C. tropicalis* was the most isolated species in his study, observed in 80.00% of the individuals; *C. albicans* was the second most isolated species (22.17%) in individuals wearing dental prosthesis, and *C. krusei* was isolated just in 02.27% of the individuals wearing prosthesis.

In a study carried out by Figueiral et al.[18] with 140 dental prosthesis users the authors report that 07.10% did not have yeasts, 81.40% hosted

C. albicans; 07.10% presented *C. glabrata*, and an association between *C. albicans* + *C. glabrata* and *C. tropicalis*; *C. tropicalis* + *C. glabrata*; and *C. albicans* + *C. tropicalis* were verified in 01.40% each. In the control sample 52.90% of the participants did not host the yeast, and 27.20% had *C. albicans*.

In another study carried out with patients wearing dental prosthesis the authors observed a statistically significant prevalence of *C. albicans* over other species. Although all *Candida* species can cause similar clinical manifestations, their therapeutic treatment varies from one species to another[12].

Table 2. Yeast presence (percentage) in individuals with and without dental prosthesis (control sample). Data sampled between July and October 2013 in Cruz das Almas, Bahia, Brazil.

	With prosthesis Number (%)	Without prosthesis Number (%)
% positive individuals to *Candida*	34 (77.27%)	11 (29.72%)
% negative individuals to *Candida*	10 (22.72%)	26 (70.27%)

Table 3. Percentage of *Candida* species growth in individuals with and without dental prosthesis (sample control.) obtained between July and October 2013 in Cruz das Almas, Bahia, Brazil.

Candida specie	With Prosthesis Number (%)	Without Prosthesis Number (%)
Candida albicans	05 (11.36%)	04 (10.81%)
Candida tropicalis	05 (11.36%)	-
Candida krusei	01 (02.27%)	-
Candida spp.	04 (09.09%)	02 (05.40%)
C. albicans and *Candida* spp.	15 (34.09%)	02 (05.40%)
C. albicans and *C. tropicalis*	01 (02.27%)	02 (05.40%)
C. krusei and *C. tropicalis*	01 (02.27%)	-
C. albicans, C. tropicalis and *Candida* spp.	-	01 02.70%)
C. krusei, C. albicans and *Candida* spp.	01 (02.27%)	-
C. krusei and *Candida* spp.	01 (02.27%)	-
Total	*34 (100%)*	*11 (100%)*

Legend: - = not observed

Dental Prosthesis Hygiene

Eighty four point zero nine percent of the participants cleaned their dental prosthesis using toothbrush and toothpaste, followed by chemical products such as soap, vinegar, mouthwash, baking soda and alcohol; 52.27% stated they cleaned the dentures three times a day (Table 4).

Our results are in accordance with those of Castellucci-Barbosa et al.[20] who observed that

76% of the individuals cleaned their prosthesis just with toothpaste; 08.00% used plain water and 16% preferred disinfectants.

Bomfim et al.[11] and Castellucci-Barbosa et al.[20], studying users of dental prosthesis, verified that 100% and 98%, respectively cleaned their dentures daily, while 62.7% did it three or more times a day, fact that both authors considered satisfactory.

Gonçalves et al.[21] claims that mechanical removal of biofilm caused by *Candida* is one of the most accepted methods when combined with additional chemicals. In a research carried out by Catão et al.[9] the authors reported promissory results with sodium perborate (alkaline peroxide) for biofilm in dental prosthesis, reducing the number of microorganisms in 50% to 60% of the samples.

Ferreira et al.[22] used three disinfectant solutions to clean dental prosthesis and found that NaOCl at 0, 5% was the most effective in the adhesion control of *Candida* yeasts.

Individuals with poor oral hygiene host a higher quantity of yeasts, varying between 02% and 69%. Tongue, palate, and oral mucosa are the preferential sites for yeast multiplication. In addition, individuals wearing dental prosthesis had 62% prevalence of oral flora alterations caused by poor hygiene. However, simple measures such as correct orientation on oral mucosa

and prosthesis hygiene can significantly reduce this frequency[23].

According to Alves[3], poor hygiene can result in prosthesis structure alterations. He recommends chemical disinfectants such as vinegar, with potential action against this yeasts, and sodium hypochlorite which is capable of removing all *Candida* colonies.

The correct prosthesis hygiene orientation must be given by the dentist. He must also raise the patient's awareness of the prosthesis as a reservoir for pathogenic microorganisms that must be removed at night for proper, safe and efficient hygienization[24].

According to Goiato et al.[25] the patients must be oriented to remove the dental prosthesis during sleep, because of the relaxation and rest of the tissues, including the cleaning action executed by the saliva, tongue and cheeks are fundamental to maintain the oral mucosa healthy.

Table 4. Materials for and frequency of prosthesis hygiene obtained between July and October of 2013 in Cruz das Almas, Bahia, Brazil.

MATERIAL TO THE PROSTHESIS HYGIENE	NUMBERS (%)
Common toothbrush and toothpaste	37 (84.09%)
Common toothbrush and toothpaste with soap	03 (06.81%)
Common toothbrush and toothpaste with vinegar	01 (02.27%)
Common toothbrush and toothpaste with mouthwash	01 (02.27%)
Common toothbrush and toothpaste with baking soda	01 (02.27%)
Common toothbrush and toothpaste with alcohol	01 (02.27%)
FREQUENCY OF DAILY HYGIENE	
Once	09 (20.45%)
Twice	12 (27.27%)
3 times	23 (52.27%)
More than 3 times	00 (00.00%)

CONCLUSION

The growth of *Candida* species was observed in 11.36% of prosthesis users, and in 10.81% of non-users. Other species isolated in prosthesis users were *Candida krusei* (2.27%) and *Candida tropicalis* (11.36%). *Candida* spp. was identified in 09.09% of dental prosthesis users and in 05.40% of non-users.

Higher isolation of *Candida* species was observed in individuals wearing dental prosthesis. This result can be due to poor oral hygiene and/or poor prosthesis adaptation.

Individuals with lesions caused by removable prosthesis must be offered correct treatment which includes new prosthesis or adjustments of the old one. Periodical visits to the dentist are mandatory to prevent oral health problems.

Research on the pathologies caused by the use of dental prosthesis as predisposing factor for oral microflora alteration are important for diagnosis and reduction of complications.

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