Candida albicans Isolation from Buccal Mucosa of Patients with HIV Wearing Removable Dental Prostheses

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> **Purpose:** The aim of this study was to evaluate the influence of complete or partial removable dental prostheses (RDPs) on the frequency of Candida albicans isolated from the mouth and the presence of oral candidiasis in human immunodeficiency virus-positive (HIV+) patients correlated with CD4 levels. Materials and Methods: One hundred ninety-three HIV+ patients were evaluated; 68 had RDPs and 125 did not. CD4 cell count was obtained after blood sampling and performed on the day of clinical examination. The material was collected from the buccal mucosa for isolation of yeasts with a sterile swab and seeded onto Sabouraud dextrose agar with chloramphenicol. C albicans strains were identified by testing germ tubes and chlamydospore formation and biochemical (zymogram, auxanogram) characteristics. The results were subjected to the Fischer exact test and chi-square tests. Results: C albicans were isolated from 45 (66.17%) patients who had RDPs and 48 (38.4%) who did not (P = .0003). The presence of oral candidiasis was observed in 14 patients (7.25%), and 10 of the 14 (71.43%) were RDP users. The absence of candidiasis occurred in 121 (67.59%) nonusers and 58 (32.40%) users of RDPs (P = .0065). The mean CD4 cell count was lower in patients with oral candidiasis regardless of the use of RDPs. Conclusion: The use of RDPs was an important factor in the isolation of C albicans among HIV+ patients, and CD4 level seems to play a role in the presence of oral candidiasis. Int J Prosthodont 2012;25:127-131.

The most recent Joint United Nations Programme on HIV/AIDS (UNAIDS) estimate is that some 36 million people worldwide are living with human immunodeficiency virus (HIV), not counting the 25 million who have already died since AIDS was recognized. HIV mortality has overtaken that of malaria and is superseded only by tobacco.¹ According to the World Health Organization, 1.8 million people died from AIDS in 2009.² People with HIV who have not

taken antiretroviral therapy and have a CD4 count greater than 350 cells/ μ L have a raised risk of death compared with the general uninfected population.³ In Brazil, the probability of death from AIDS in the first 12 months of HIV care is 16.5%.⁴

In patients with AIDS, it is known that among the opportunistic infections, oral candidiasis is the most frequent. The manifestations of candidal infections in HIV disease are usually restricted to superficial candidiasis with varying degrees of severity; the major clinical variants are similar to patients not infected with HIV, characterized as pseudomembranous, ery-thematous, and hyperplastic forms.⁵ The infection affects the mucous membranes of the oral cavity, mainly the surfaces of the tongue, palate, cheeks, and lips.⁶

Candidiasis is most commonly caused by *Candida albicans*,⁷ and this yeast exists in the oral flora of healthy individuals with or without teeth.⁸ The yeasts that colonize the oral cavity are the main source for oral candidiasis. Thus, these microorganisms are a potential source for disseminated candidiasis.⁹

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Fig 1a Pseudomembranous candidiasis under the removable prosthesis, characterized by white patches on the hard palate permeated by an erythematous area.



Fig 1b White patches were scraped and removed.



Fig 1c After removal of the patches, an erythematous base was revealed.

There are several factors leading to the presence of *C albicans* in oral cavities, their increase in number, and their gaining of pathogenicity. Some of these factors are diabetes mellitus, AIDS, long-term antibiotic or corticosteroid use, radiation therapy resulting from head/neck cancer, and nutritional disorders. In addition, the use of prostheses increases candidal colonization and thus leads to denture-related stomatitis.¹⁰

In patients suffering from AIDS, it is known that nonspecific oral immunity is affected, contributing to the frequent diagnosis of candidiasis.^{9,11-13} Also, the study of *C albicans* is very important in assessing the evolutionary behavior of the disease.^{9,14-18}

Some changes in oral flora of denture wearers have led to an increase in *C albicans* colonization.^{19,20} The accumulation of microbial plaque (bacteria or yeasts) on and in the fitting surface of the denture and the underlying mucosa is the most frequent cause of the generalized simple and granular types of dentureinduced stomatitis.⁵ The frequency of isolation of *C albicans* was 10.53% in individuals with a healthy mucosa and 67.65% in those who experienced prosthesis stomatitis.⁸ Smoking was associated with an increase in the frequency and density of yeast in denture wearers.¹⁰

It is important to identify patients with increased risk for colonization of dental and denture plaque by pathogenic organisms to prevent associated diseases.²⁰ Besides, the knowledge of predisposed factors for *Candida spp* colonization in denture wearers and AIDS patients are extensively discussed, and the association of these conditions can bring new perspectives in the prevention of yeast infections.

The aim of this study was to evaluate the influence of complete or partial removable dental prostheses (RDPs) in the frequency of *C albicans* isolated from the mouth and the presence of oral candidiasis in HIV+ patients correlated with the CD4 levels.

Materials and Methods

One hundred ninety-three HIV+ patients of both sexes, in both treatment and follow-up at the AIDS home (Fundação Zerbini, São Paulo, Brazil), were recruited to participate in this study. All patients agreed to participate in the research by signing a consent form approved by the research ethics committees of the University of São Paulo Dental and Medical Schools, São Paul, Brazil; AIDS home; and Adolfo Lutz Institute, São Paulo, Brazil.

Clinical Criteria and Sample Collection

The clinical examination was carried out by a single examiner, a specialist in stomatology, seeking to assess the presence of oral lesions compatible with pseudomembranous candidiasis, characterized by white patches on the surfaces of the oral mucosa, tongue, and elsewhere in the mouth that can be scraped off, revealing an erythematous base (Figs 1a to 1c). In cases in which the clinical diagnosis was positive, material was collected from the lesion for cytologic testing using a sterile swab to collect material, which was sampled into glass slides, fixed in alcohol, and forwarded to the laboratory for periodic acid Schiff staining and microscopic examination. Cytologic positivity (Figs 2a and 2b), combined with a clinical diagnosis, confirmed the presence of candidiasis.

After a complete oral examination with the culture techniques, the material was collected from the oral mucosa by the same single examiner using a sterile swab and was seeded close to the lamp flame onto Sabouraud dextrose agar (Difco) and 100 μ g/mL chloranfenicol (Chloromycetin, Parke-Davis). The plates were then incubated at 25°C for the yeasts to be isolated later, and in cases of negativity, plates were maintained for 30 days.

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Fig 2a Hyphal growth interspersed among epithelial cells.

The CD4 cell count was obtained by means of blood collection performed on the same day as stomatologic examination, and results were later noted on the patients' file records. CD4 count levels were considered as follows: (1) \geq 500 cells/mm³, (2) 500 cells/mm³ > number of CD4 cells \geq 200 cells/mm³, and (3) < 200 cells/mm³.

Isolation of Yeasts

C albicans strains were identified in accordance with the identification protocol of the Mycology Center of the Microbiology Department of the Biomedical Sciences Institute of the University of São Paulo, which included microculture on a slide (cornmeal agar in addition to Tween 80, BD–Becton, Dickinson, and Co) and examination of germ tubes at optical microscopy (200×) between the glass and cover slide after 3 hours of incubation of the isolates in test tubes with 1 mL bovine fetal serum (Gibco) at 37°C. Also, carbon hydrate fermentation tests and assimilation tests for carbon and nitrogen sources were performed.

Clinical Record Readings

Patients' files enabled recording of the results of the CD4 cell counts done on the day of collection as well as notation of patients who were prosthesis wearers after oral examination. With regard to the use of RDPs, the patients were classified as nonusers and users.

Statistical Analysis

Qualitative data were analyzed using the Fisher exact test for the comparison of presence of isolates of *C albicans* \times use of RDPs and the use of RDPs \times absence of candidiasis. To analyze CD4 levels and presence of candidiasis, the chi-square test was performed. A chi-square test for trend was made to study



Fig 2b Hyphae form *(arrow)* showing a reddish color in the periodic acid Schiff staining (original magnification ×40).

the influence of RDPs and CD4 levels in patients with candidiasis. A significance level of .05 was set for all tests.

Results

From the 193 patients, 137 (70.98%) were men with a mean age of 40 years and 56 (29.02%) were women with a mean age of 37 years.

C albicans was isolated from 45 (66.17%) patients who used RDPs and 48 (38.4%) nonusers (P = .0003). The lack of colonization occurred in 77 (61.6%) nonusers and 23 (33.82%) RDP users.

The presence of oral candidiasis was observed in 14 patients (7.25%), and 10 (71.43%) of these patients were RDP users. The absence of candidiasis occurred in 121 (67.59%) nonusers and 58 (32.40%) users (P = .0065).

Mean CD4 cell counts in patients with and without oral candidiasis, regardless of the use of RDPs, are presented in Table 1. Of the 14 patients with oral candidiasis, when patients were allocated into groups by CD4 level distribution, 2 patients (1 RDP user, 1 nonuser) were in group 1, 4 patients (all RDP users) were in group 2, and 8 patients (5 RDP users, 3 nonusers) were in group 3, with no statistical significance in the trend of these variables assessed together (P = .8165).

Discussion

Epidemiologic data emphasize the high prevalence of oral candidiasis in HIV-infected individuals,²¹ and *C albicans* is the most prevalent of the *Candida* species.^{22,23} This fungal infection plays a significant role in predicting the evolution of HIV infection and AIDS.²⁴ The presence of oral candidiasis in "controlled" HIV+ individuals may be indicative of patient nonadherence to therapy or possible failure.²⁵

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CD4 group*	Without oral candidiasis ($n = 179$)	With oral candidiasis ($n = 14$)
1	64	2
2	80	4
3	35	8
P [†]	.0046	

 Table 1
 CD4 Cell Count Levels and Correlation with the Presence of Oral Candidiasis

*Group 1 = \geq 500 cells/mm³; group 2 = 500 cells/mm³ > number of CD4 cells \geq 200 cells/mm³; group 3 = < 200 cells/mm³.

[†]Significant difference (P < .05), chi-square test.

The frequency of oral candidiasis in patients with HIV varies among different studies. However, the prevalence of this infection in these patients is decreasing, especially because of the increasing immunologic response associated with highly active antiretroviral therapy.²⁶ The present data corroborate this statement since the prevalence of oral candidiasis found was 7.25%, which can be considered low when compared to values of 65.65% found by Ceballos-Salobreña et al¹⁶ and 37.8% found by Arendorf et al.²⁷ Also, another study in a Brazilian population found oral candidiasis in 28.6% of patients.²⁸ In addition, there might be an influence of the sampling method on the percentage of patients with colonization or infection. Cerqueira et al²⁴ evaluated oral sites for the assessment of Candida spp growth, and positivity was observed in 95.8% of all samples, including 95.7% of tongue samples, 87.0% of saliva samples, 56.6% of hard palate mucosa samples, and 47.8% of right jugal mucosa samples. The present results found that the jugal mucosa tended to have less or an absence of colonization.

In this study, *C albicans* was isolated in 66.2% of HIV+ patients who used an RDP, which is in agreement with other authors who studied the prevalence in normoreactive users of prostheses.^{8,21,29} However, in healthy patients without HIV infection, 25% of complete denture wearers and 58% of removable partial denture wearers presented colonization by *Candida spp.*⁸ These data suggest that the colonization in patients who are HIV+ and HIV- occurs in similar proportions despite the systemic health status, and the major factor to colonization seems to be pointing to some local conditions.

Wearing a complete or removable partial denture was determined as an important factor in increasing the Candidal carrier rate.¹⁹ The adhesion of fungi to hot polymerization basic materials used in removable denture making was the most prominent factor.³⁰

The CD4 cell count was correlated with the presence of clinical candidiasis, and infected patients had low CD4 count levels compared to patients without candidiasis (P = .046), which is in agreement with other authors.^{23,31} Significant differences were also observed in the low CD4 lymphocyte count group during the third and fifth months following denture insertion.³² Host defenses to C albicans in general and oral candidiasis in particular are believed to be dependent on a combination of innate and acquired immune responses. Variations in the susceptibility to oropharyngeal candidiasis between individuals with low CD4 T-cell counts suggest an additional important role for innate immune responses.³³ However, other authors found that a patient's immune status, mean CD4 count, and HIV viral load did not contribute to the presence of yeast,²² although a tendency for yeast growth related to a decrease in CD4 count was observed.24

C albicans lives as a saprophyte in the oral cavity but is capable of causing infection if there are predisposing factors related to the host, such as individuals infected with HIV. Use of removable prostheses may cause these microorganisms to gain pathogenicity.⁸

Conclusion

Use of RDPs was an important factor in the isolation of *C albicans* among HIV+ patients, and the CD4 level seems to play a role in the prevalence of oral candidiasis.

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