

## Microbiological study of *Candida sp* in HIV-seropositive patients with a subclinical diagnosis of oral candidiasis

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**Abstract:** Oral candidiasis is one of the opportunistic diseases most strongly associated with human immunodeficiency virus (HIV) infection. Several epidemiological surveys have demonstrated the prevalence of candidiasis in HIV-positive patients and have emphasized its importance as a marker of disease progression and as a predictor of the increase in immunodepression. The objective of the present study was to evaluate the frequency of *Candida sp* in the oral cavity of HIV-positive patients with low CD4 T lymphocyte count and a subclinical diagnosis of oral candidiasis. Sixty patients routinely seen at the Dental Clinic between August and November 2006 participated in the study. *C. albicans* was the species most frequently isolated, followed by *C. krusei* and *C. tropicalis*. In conclusion, since candidiasis is a highly frequent oral disease in HIV-infected patients, the diagnosis of this infection is of the utmost importance for early treatment, improving the quality of life of these patients.

**Key words:** oral candidiasis; *Candida albicans*; AIDS, HIV infection

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### I. Introduction

Oral candidiasis affects up to 90% of patients infected with human immunodeficiency virus (HIV) and is caused by yeast of the genus *Candida*. Among the 81 known species of the genus, *C. albicans* is the predominant species of the oral microbiota, accounting for 60 to 70% of all isolates, and causes infection under conditions that favor its growth (14, 23). Some of the non-*albicans* species are considered emerging pathogens, including *C. dubliniensis*, *C. glabrata*, *C. tropicalis* and *C. krusei* (1, 2, 13, 15, 16, 30). Studies have reported a reduced survival, discomfort, pain and halitosis in HIV-seropositive patients with oral candidiasis, with some type of treatment being necessary which may compromise even further the immune system of these patients (5, 7, 19, 22).

Infection with HIV and the occurrence of AIDS currently represent one of the major challenges of science (1, 24), a fact supporting the need for the prevention and treatment of diseases, as well as for the promotion and maintenance of oral health, in patients with HIV/AIDS (4, 24, 25). An estimated 30.6 million people are infected with HIV worldwide. There is a predominance of males, with a male to female ratio of about 4:1, except for Central Africa where the gender ratio is practically 1:1. Similarly, young individuals are more frequently affected, with 90% of the patients presenting a mean age of 36.8 years (21).

Numerous oral manifestations have been described for HIV-infected patients and the most common are those resulting from fungal infections, specific and nonspecific bacterial infections, viral infections and neoplasms, and those of unknown etiology (1, 19).

The presence of oral lesions in patients with AIDS has been the subject of various studies since these oral manifestations represent the first signs and symptoms of disease (29). On the basis of these considerations, the objective of the present study was to evaluate the frequency of *Candida sp* in the oral cavity of HIV-infected patients seen at the Dental Clinic, and to compare the results with literature data.

### II. Patients and methods

**Patients** – The sample consisted of 60 HIV-infected patients of both genders (age 20 to 60 years) with a CD4 T lymphocyte count below 400/mm<sup>3</sup>, seen at the Dental Clinic of Uniararas. The gender variable had no influence in the present study since the incidence of AIDS cases was the same among women and men.

**Data collection** – Data were obtained by anamnesis of the patients, analysis of the patients' records, clinical examination, and collection of biological material.

**Material collection and species identification** – Biological material was collected with a sterile swab from the oral cavity of 60 patients seeking routine dental care at the Dental Clinic of Uniararas, and all isolates obtained were identified at the Clinical Microbiology Department, Uniararas. The samples were carefully collected over a period of 4 months (August to November 2006) for the identification of *Candida* species in HIV-seropositive patients with a subclinical diagnosis of candidiasis.

The laboratory diagnosis was initially based on the culture of test tubes containing 2% Sabouraud dextrose agar (SDA; Oxoid, Canada) supplemented with 0.1 mg/mL chloramphenicol, seeded in duplicate. The tubes were incubated at  $35 \pm 1^\circ\text{C}$  and observed daily for 5 days. Smears were then prepared from the colonies grown and submitted to Gram staining (31). After microscopic observation of large, oval, Gram-positive cells, yeast-like elements with multipolar buds or eventual pseudo-hyphae with or without buds suggestive of *Candida*, the colonies were transferred to tubes containing liquid Sabouraud dextrose medium. *C. albicans* was identified by the germ tube test (9, 19).

The colonies grown on SDA were subcultured on Candida Chromagar® (Probac do Brasil®, São Paulo). This differential selective medium was used for the identification of yeast species according to the classical method of Kurtzman and Feelj (10). After incubation, *C. albicans*, *C. krusei*, *C. tropicalis* and other species without clinical importance could be identified.

*Statistical analysis* – The chi-square test was applied to correlate *C. albicans* isolates obtained from the oral cavity of HIV-positive patients with subclinical candidiasis with other *Candida* species, with the level of significance set at  $p < 0.05$ .

### **Ethical issues**

The study was approved by the Research Ethics Committee of Hermínio Ometto University Center - UNIARARAS, Araras, SP, Brazil (protocol 171/2006), according to resolution 196/96 of CONEP.

### **III. Results**

Among the 60 patients from which samples were collected during intraoral examination, intraoral lesions were observed in 13.3% ( $n = 8$ ) and 86.7% ( $n = 52$ ) did not present any type of intraoral lesion (Table I).

Fifty-seven (95%) of the 60 patients studied and diagnosed as HIV seropositive tested positive for yeast of the genus *Candida*, with eight (13.3%) of them presenting clinical signs of candidiasis and 49 (81.7%) subclinical signs. The three remaining patients presented subclinical signs and were colonized with bacteria (Table II). Among the eight patients with intraoral lesions, oral candidiasis was characterized by white plaques or reddish areas located on the tongue, gingiva, hard palate, labial commissures, and cheeks. *C. albicans* was isolated from five of these patients. This species was also isolated from 45 (75%) patients without clinical signs (Table II). Only three of the 60 patients were not on antiretroviral treatment.

*C. albicans* was the most frequently detected species and was isolated from 50 (83.3%) samples. *C. krusei* was isolated from four (6.7%) samples, *C. tropicalis* from two (3.3%), and another *Candida* species was detected in one (1.7%). Bacteria were isolated from three (5%) samples (Table III).

The results obtained were analyzed statistically, discussed and compared with literature data, generating a profile of the frequency of candidiasis caused mainly by *C. albicans* in HIV-seropositive patients (Table III).

### **IV. Discussion**

Studies have demonstrated the installation of a large number of fungal infections in HIV-positive patients as a result of marked alterations in immunological function mediated by T lymphocytes (12, 24). Among these pathogenic fungi, *C. albicans* plays a relevant role, with the frequency of infections caused by this species ranging from 3 to 30% and the mucosal forms being the earliest and most common manifestations (1, 27, 29, 30).

The frequency of oral candidiasis in HIV-positive patients varies among different studies, but the condition may affect up to 94% of infected individuals depending on the stage of infection and the population studied (3, 29). Candidiasis is a reliable marker of HIV disease progression (11).

According to Sonis et al. (28), candidiasis is the most expressive oral fungal infection in HIV-positive patients (four in five patients have candidiasis), with the frequency of oral candidiasis ranging from 20 to 90% in these patients. Greenspan (6) reported that 41% of patients with AIDS had candidiasis during the initial stage of the disease. According to Cavassani et al. (3), candidiasis and hairy leukoplakia were among the most frequent diseases observed in HIV-seropositive patients with a CD4+ count of less than 200 cells/mm<sup>3</sup>.

In the present study, no intraoral lesions were observed in 86.7% of the patients with HIV. This finding was probably due to the maintenance of a strong immune system in the series studied.

According to the Brazilian Ministry of Health (18), the use of potent antiretroviral therapy drastically reduces the occurrence of opportunistic processes due to partial recovery of immunological function after the suppression of viremia and a decline in cell destruction caused by HIV.

In the present study, antiretroviral therapy may have been responsible for the lack of observation of clinical symptoms of candidiasis in some patients even in the presence of a low CD4+ T lymphocyte count, since antiretroviral therapy increases the immunological response and prevents disease progression. Most of

these patients were already undergoing treatment, a fact that might explain the absence of lesions in 52 of the 60 HIV-seropositive patients studied (86.7%).

Since antiretroviral drugs have prolonged the survival of patients, even that of immunosuppressed individuals, the diagnosis and treatment of diseases such as candidiasis represent a challenge that should not be underestimated.

The present results agree with those reported by Crocco et al. (4) who investigated the frequency of different *Candida* species in 100 patients. *C. albicans* was isolated from 76% of the patients, *C. krusei* from 19%, and *C. tropicalis* from 1%, corresponding to a frequency of isolation of 96%. A predominance of *C. albicans* has been reported in the literature among both immunocompetent and immunodepressed patients, with the frequency of isolation and prevalence of non-albicans species varying among different studies (8).

Menezes et al. (17), 80% of HIV-positive patients with or without characteristic lesions of oral candidiasis were positive for *Candida*, with *C. albicans* being identified in 65%, *C. tropicalis* in 27.5%, *C. glabrata* in 2.5%, *C. krusei* in 2.5%, and *C. guilliermondii* in 2.5%. These authors and others suggested *C. albicans* to be the most frequent species in these patients.

A predominance of *C. albicans* was also observed in the present study, with a significant difference when compared to the other *Candida* species isolated. However, statistical comparison of our results with those reported by (17) showed no significant difference ( $p = 0.125 > 0.05$ ) between the different *Candida* species isolated.

*C. albicans* was the predominant yeast in the present study. Similar results have been reported by Silva (26) and Oliveira (20) who isolated *C. albicans* as the predominant species from the oral cavity of patients infected with HIV, as well as *C. tropicalis*, *C. krusei* and *C. glabrata*.

In the present study, the pathogenic *Candida* species found included *C. albicans*, *C. tropicalis*, *C. krusei* and another unidentified species. In addition, a predominance of *C. albicans* was observed in patients with or without candidiasis lesions. Thus, *C. albicans* was the most frequent yeast in patients infected with HIV.

One important fact is that the frequency of colonization with *Candida* species is higher among HIV-positive patients (90.6%) than among healthy individuals (common in up to 50%) as a result of immunosuppression in the former which causes an imbalance in the oral microbiota.

Identification of the species causing candidiasis is important not only because of its epidemiological value but also because of the difference in the sensitivity to antimicrobial agents which may vary among species. In addition, knowledge of healthcare workers about oral manifestations in HIV-positive patients is relevant for the diagnosis, treatment and consequent improvement of the quality of life of these patients.

According to the literature, oral candidiasis is the most prevalent fungal infection among HIV-seropositive patients, thus being an opportunistic infection of high prognostic value. Oral candidiasis may precede systemic manifestations in HIV-infected patients. This fact indicates an important role of the dentist in the prevention and early diagnosis of AIDS.

Since opportunistic fungal infections have been shown to be an important cause of mortality, studies like the present one are justified and essential for a better understanding of the interaction with the human oral environment in an attempt to prevent and control infections caused by these microorganisms.

Oral manifestations are frequently indicative of infection with HIV, thus representing an important diagnostic tool for the early detection of this infection. Professionals always need to be updated in order to fulfill these responsibilities and to be able to perform adequate dental treatment. Changes in this behavior require informative and educational aspects comprising knowledge about HIV infection as a priority in the training of dentistry students so that they become qualified professionals. The dentist plays a fundamental role in the prevention and early diagnosis of AIDS. These patients are potential customers in dental offices because the first manifestations of AIDS normally arise in the mouth. The role of the dentist is to promote and manage the oral health of HIV-seropositive patients in order to guarantee a better quality of life.

The present study shows the importance of interaction between the microbiology laboratory and the dental clinic to confirm the clinical diagnosis, thus providing adequate treatment to the patient.

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