

Oral candidiasis in HIV+ patients under treatment with protease inhibitors

Andréa Lusvarghi Witzel^(a)
**Fernando Ricardo Xavier da
Silveira^(b)**
Maria de Fátima Costa Pires^(c)
Mônica Andrade Lotufo^(d)

^(a)PhD, Professor; ^(b)PhD, Associate Professor
– Department of Stomatology, School of
Dentistry, University of São Paulo.

^(c)PhD, Senior Researcher, Adolfo Lutz
Institute, São Paulo.

^(d)Professor, Dental School, Ibirapuera
University.

Abstract: The purpose of this work was to evaluate the influence of Protease Inhibitors (PI) on the occurrence of oral candidiasis in 111 HIV+ patients under PI therapy (Group A). The controls consisted of 56 patients that were not using PI drugs (Group B) and 26 patients that were not using any drugs for HIV therapy (Group C). The patient's cd4 cell counts were taken in account for the correlations. One hundred and ninety three patients were evaluated. The PI did not affect the prevalence of oral candidiasis ($p = 0.158$) or the frequency of *C. albicans* isolates ($p = 0.133$). Patients with lower cd4 cell counts showed a higher frequency of *C. albicans* isolates ($p = 0.046$) and a greater occurrence of oral candidiasis ($p = 0.036$).

Descriptors: HIV; *Candida albicans*; Protease inhibitors.

Corresponding author:

Andréa Lusvarghi Witzel
R. Prado Valadares, 34, cs 7, Vila Polopoli
São Paulo - SP - Brazil
CEP: 05360-065
E-mail: gandrea@usp.br

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Introduction

Oral candidiasis is the most frequent mycosis produced by yeasts of the genus *Candida*, *Candida albicans* being the most important from the point of view of Public Health, although other species such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* and more recently *C. dubliniensis* have been isolated.^{1,2}

In the acquired human immunodeficiency syndrome (AIDS), it is known that among the opportunistic infections, oral candidiasis is the most frequent one, and *C. albicans*, among others, has been very important in assessing the evolutionary behavior of the disease.³ The first case of acquired human immunodeficiency syndrome (AIDS) related in the literature mentions that the patient was an oral candidiasis carrier.⁴

In human immunodeficiency virus (HIV+) patients, nonspecific oral immunity is reduced, contributing to the frequent appearance of candidiasis.^{5,6}

One of the most critical factors in the control of oral candidiasis in HIV+ patient is the resistance that the yeast develops to various drugs, notably some of the azolic derivatives.⁷

The introduction of protease inhibitor (PI) drugs on the marketplace for the treatment of AIDS has shown promising results in the clinical control of the disease, opening up a new perspective in the efforts made to enhance the systemic conditions of these patients.⁸

Many researchers attribute this improvement to the increase in the number of cd4 lymphocytes and to a reduction in viral load, which restores the patients' specific immunity, providing a decline in the incidence of opportunistic infections.

However, HIV virus carriers submitted to treatment with protease inhibitor drugs manifested a decline in oral candidiasis before there was an increase in the number of cd4 cells, which has led some authors to believe in the action of these drugs in the protease production mechanisms of *C. albicans* as well, leading to a change in its levels of activity.⁹

The *Candida albicans* strains isolated from HIV+ patients were also those least susceptible to the azolic antifungal agents, specifically ketoconazol and fluconazol.¹⁰

The use of PI in the treatment of patients with HIV has diminished the complications related to AIDS, such as Kaposi's sarcoma,¹¹ cryptosporosis¹² and mucosal candidiasis.^{8,13,14}

The PI drugs act directly on the proteases of the HIV, making the enzyme inactive, which favors the formation of non-infecting viral particles, promoting a reduction in viral load and an increase in cd4 cells, reestablishing the immune response.¹⁵

The antifungal effect of the PIs indinavir and saquinavir were demonstrated *in vitro* and *in vivo*.^{10,16}

The aim of this study was to compare the frequency of oral candidiasis in HIV+ patients under treatment with protease inhibitor and non protease inhibitor drugs.

Material and Methods

One hundred and ninety three HIV+ individuals, of both genders, who were having the number of cd4 cells assessed at the AIDs home, Zerbini Foundation, São Paulo, SP, Brazil, were selected for this study.

All the patients agreed to participate in the study by signing an informed consent form approved by the Research Ethics Committees of the School of Dentistry, University of São Paulo protocol n. 18/97), of the AIDs home and of the School of Medicine, University of São Paulo.

- Group A – 111 patients under treatment with protease inhibitors (PI) either associated with other drugs or not.
- Group B – 56 patients under treatment with other non protease inhibitors (NPI).
- Group C – 26 patients that were not using medication for the treatment of AIDS.

The clinical examination was carried out by a single examiner, a specialist in Stomatology, seeking to assess the presence of oral lesions compatible with candidiasis. In the cases in which the clinical diagnosis was positive, material was collected from the lesion for Cytologic testing, using a sterile swab, the smears being fixed in alcohol (70%) immediately and forwarded to the laboratory for staining (Papanicolaou) and Microscopic examination (200 X). Cytologic positivity, combined with a clinical diagnosis, confirmed the presence of candidiasis.

The variables adopted for the present study were as follows:

With regard to the presence of oral candidiasis:

- 0 – Without oral candidiasis
- 1 – Erythematous candidiasis carrier
- 2 – Pseudomembranous candidiasis carrier

The cd4 Cell Count was obtained by means of blood collection performed on the same day as the stomatological examination, and its results were afterwards noted on the patient's case history record. The cd4 count levels were as follows:

With regard to the number of cd4 cells:

- 1 – Number of cd4 \geq 500 cel/mm³
- 2 – 500 cel/mm³ > number of cd4 \geq 200 cel/mm³
- 3 – Number of cd4 < 200 cel/mm³

The material was collected by a single examiner from the oral mucosa using a sterile swab and was seeded, close to the lamp flame, in Sabouraud dextrose agar (Difco®, Detroit, IL, USA), in addition to 100 µg/ml of Chloramphenicol (Park-Davis®, São Paulo, SP, Brazil).

The plates were then incubated at 25°C for posterior yeast isolation. In case of negativity, they were maintained during 30 days.

The samples were identified according to the identification protocol of the Micology Center, Microbiology Department, Biomedical Sciences Institute, University of São Paulo, which included:

- Microculture on Slide (*Corn-meal Agar* in addition to Tween 80)
- Research of germination tubes
- Carbon hydrate fermentation test
- Assimilation tests for carbon and nitrogen sources

Clinical record readings

The patients' clinical records contained information on which medication was used, on the results of the cd4 cell counts done on the day of collection, as well as on the patients that did not use any anti-HIV medication.

The medication therapy administered to HIV virus carriers is very diversified. Generally, a combination of drugs (cocktail) is used. The presence or absence of proteinase inhibitor drugs was considered the delimitation for the groups setting. The drugs

used at the time the patients' records were read considering the delimitation factor of the groups were: indinavir (Merk Sharp Dohme®, São Paulo, SP, Brazil), nelfinavir (Roche®, São Paulo, SP, Brazil), ritonavir (Abott®, São Paulo, SP, Brazil) and saquinavir (Roche®, São Paulo, SP, Brazil).

Statistical analysis

Descriptive analysis was carried out for all the study variables. The Chi-square test was used for the qualitative variables. For the quantitative variables, the homogeneity of the variances was tested by the Levene Test, and the normality of the data, by the Kolmogorov-Smirnov Test with the Lilliefors correction.

The variables that satisfied these two principles were submitted to parametric tests. In order to assess any differences between the medication groups, Analysis of Variance (ANOVA) was used, and when this was statistically significant, the HSD -Tukey (Honest Significant Difference) test was used to assess where the differences were.

Results

Of the 193 patients examined, 70.47% were men (136/193), with a mean age of 40 years, and 29.53% were women (57/193), with a mean age of 37 years.

Analysis of the characteristics of the isolates enabled *C. albicans* to be identified in 47.67% (92/193) of the patients.

With regard to medication, the patients were divided into 3 groups:

- Group A – 111 patients using PI drugs.
- Group B – 56 patients using NPI drugs.
- Group C – 26 patients without medication.

Table 1 shows the biodemographics related to gender and age of the medication groups.

The distribution of *C. albicans* carriers per medication group showed no statistically significant differences (Table 2).

In the clinical intraoral examination, 18.13% (35/193) of the patients presented clinical signs of candidiasis. The erythematous form was predominant, with 60% (21/35) of the cases, and the pseudomembranous form represented 40% (14/35) of the cases. The clinical features of candidiasis in each group are expressed in Table 3.

Table 1 - Biodemographics related to gender and age of the medication groups.

Variable		Group A N = 111	Group B N = 56	Group C N = 26	Test
Gender	Male	82 (73.87)	37 (66.07)	17 (65.38)	Chi-square $\chi^2_2 = 1.462$ p = 0.482
	Female	29 (26.13)	19 (33.93)	9 (34.62)	
Age	$\mu \pm SD$	40.93 \pm 9.65	38.46 \pm 9.05	33.42 \pm 7.39	ANOVA p = 0.00093
	Median	40.00	37.00	33.00	
	Min - Max	20 - 67	19 - 62	18 - 46	

N = number of patients.

Table 2 - Distribution of *C. albicans* carriers per medication group.

<i>C. albicans</i>	Group A N = 111	Group B N = 56	Group C N = 26	Test
Negative	62 (55.85)	25 (44.64)	14 (53.85)	Chi-square $\chi^2_2 = 4.034$ p = 0.133
Positive	49 (44.15)	31 (55.36)	12 (46.15)	

N = number of patients.

Table 3 - Clinical features of candidiasis in each group.

Clinical diagnosis	Group A N = 111	Group B N = 56	Group C N = 26	Test
Without oral candidiasis	93 (83.78)	46 (82.14)	19 (73.08)	Chi-square $\chi^2_2 = 6.616$ p = 0.158
Erythematous candidiasis	12 (10.81)	7 (12.50)	2 (7.69)	
Pseudomembranous candidiasis	6 (5.41)	3 (5.36)	5 (19.23)	

N = number of patients.

Table 4 - Cd4 cell counts of the medication groups.

Cd4	Group A N = 111	Group B N = 56	Group C N = 26	Test
$\mu \pm SD$	412.96 \pm 291.14	431.61 \pm 249.38	515.96 \pm 384.18	Anova p = 0.28
Median	357.00	399.50	511.00	
Min - Max	18 - 1,678	33 - 1,208	16 - 1,924	

N = number of patients.

The Cd4 cell counts showed no statistically significant difference (ANOVA) among the studied groups (Table 4).

The clinical forms showed statistical differences related to Cd4 cell counts (p = 0.036, Table 5). The Pseudomembranous form presented smaller cell counts than the erythematous form (p = 0.037) and without lesions (P = 0.0167). The correlation between cd4 cell count and the isolation of *C. albicans* was statistically significant (p = 0.046 - t-test).

The relationship between the levels of cd4 cells

and the clinical forms of candidiasis (Table 6) showed statistically significant differences (p = 0.029).

Discussion

Earlier studies on AIDS emphasized the prevalence of opportunistic infections, mainly oral candidiasis, in HIV-infected individuals, and observed that the clinical signs of oral candidiasis increased with the progression of the disease.³ Today, the related literature remains controversial about the reasons for the high incidence of candidiasis in HIV+

Table 5 - Relationship between clinical forms of candidiasis and Cd4 cell counts.

Cd4	Without oral candidiasis N = 158	Erythematous candidiasis N = 21	Pseudomembranous candidiasis N = 14	Test
$\mu \pm SD$	451.32 \pm 306.21	416.33 \pm 214.58	240.93 \pm 184.14	ANOVA p = 0.036
Median	399.00	368.00	174.00	
Min - Max	16 - 1,924	131 - 818	20 - 542	

N = number of patients.

Table 6 - Levels of Cd4 cells and the clinical forms of candidiasis.

Cd4	Without oral candidiasis N = 158	Erythematous candidiasis N = 21	Pseudomembranous candidiasis N = 14	Test
1	56 (35.44)	8 (38.10)	2 (14.29)	Chi-square $\chi^2_4 = 10.819$ p = 0.029
2	71 (44.94)	9 (42.86)	4 (28.57)	
3	31 (19.62)	4 (19.05)	8 (57.14)	

N = number of patients.

patients. Many believed that an immune system deficiency triggered by AIDS was responsible for the severity of oral candidiasis, and not the high virulence of the yeast, since most studies had found no differences between the isolates from HIV-infected and non-infected individuals.¹⁷

The importance of candidiasis in AIDS patients led to the introduction of preventive protocols with antifungal drugs, mainly fluconazol. This may have been responsible for the selection of strains resistant to azolic derivatives.¹⁸ Another important fact is that the resistance to azolics presented by *C. albicans* is not evidenced in the same manner as in non-HIV patients.¹⁹

There are indications that the selection of more pathogenic strains is possible, and this possibility must not be neglected while caring for HIV+ patients infected with *C. albicans*. Certain strains of *C. albicans* have a specific Ca3 repetitive sequence of DNA which is more frequent in individuals with AIDS when compared with samples isolated from individuals that are non-carriers of the HIV virus.²

Characterization of the differences among strains isolated from HIV+ individuals and control groups began to increase in the 1990s. Virulence factors from *C. albicans* were shown to be more expressive in HIV-carrier individuals.^{20,21}

The frequency of oral candidiasis in HIV+ patients varies among the different reports, and can af-

fect up to 94% of infected individuals. However, the prevalence of candidiasis in HIV+ individuals has diminished.^{22,23} The data of the present study corroborate this affirmation, as the incidence of candidiasis in the present study was 18.13%, which can be considered low when compared with the values of 80.1% found in the related literature.²⁴ Many studies attribute this reduction due to the use of protease inhibitors for the treatment of patients with AIDS.^{9,13,25,26}

The present study was unable to make this correlation, since the incidence of 18.13% of oral candidiasis, although low, did not present statistically significant differences between the medication groups (p = 0.158). It was expected that the patients in Group A (n = 49/111), who used PI drugs, would present a lower incidence in comparison with that of the other groups. PI also had no influence on the frequency of *C. albicans* isolates. When the cd4 cell count was correlated with the isolation of *C. albicans*, a statistically significant difference was observed between the group of colonized patients, who had a lower cd4 cell count, and the group without isolation (p = 0.046).

The relationship between PI drugs and a decreased frequency of oral candidiasis is credited by many authors to a direct action of the PI drugs on the yeast.^{3,16,27}

The antifungal effect of the PI indinavir and saquinavir was demonstrated, *in vitro* and *in vivo*, at

a minimum inhibitory concentration much higher than that attained during the conventional treatments using these drugs.¹⁰

Therapy with PI drugs has influenced not only the prevalence of oral candidiasis, but the indexes of other pathosis have also been diminished,^{1,12,28} in which the pathogen was not directly inhibited by the PI. This led the authors to believe that restoration of the HIV+ patient's immune system provided by the PI is the most relevant fact to be considered, during the course of treatment.

The cd4+ T lymphocyte counts showed no statistically significant differences among the medication groups. However, when the presence of candidiasis and its different clinical forms was assessed (Table 5), it was found that patients with pseudomembranous candidiasis presented a lower cd4 count in comparison with patients that had the erythematous form, and patients without candidiasis ($p = 0.036$), evidencing the importance of the immune response with regard to this illness.

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Conclusions

1. The frequencies of *C. albicans* isolates in the oral mucosa of the evaluated patients were statistically similar for the 3 medication groups.
2. Cd4 values were significantly lower in patients who were *C. albicans* carriers.
3. The frequencies of oral lesions compatible with candidiasis did not differ statistically among the medication groups.
4. The cd4 cell counts were similar for the different medication groups.
5. The cd4 cell values of the patients with pseudomembranous candidiasis were lower when compared with those of patients without lesions and with erythematous lesions.

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