Rev. Inst. Med. trop. S. Paulo
48(5):257-261, September-October, 2006

ASYMPTOMATIC ORAL CARRIAGE OF *Candida* SPECIES IN HIV-INFECTED PATIENTS IN THE HIGHLY ACTIVE ANTIRETROVIRAL THERAPY ERA

Carolina Rodrigues COSTA(1), Ana Joaquina COHEN(2), Orionalda Fátima Lisboa FERNANDES(1), Karla Carvalho MIRANDA(1), Xisto Sena PASSOS(1), Lúcia Kioko Hasimoto SOUZA(1) & Maria do Rosário Rodrigues SILVA(1)

SUMMARY

Oropharyngeal candidiasis is the most common opportunistic fungal infection in individuals infected with human immunodeficiency virus. CD4+ lymphocytes count and the quantification of viral RNA in blood plasma have been found to be the main markers of HIV disease progression. The present study was conducted to evaluate *Candida* sp. diversity in the oral cavity of HIV-infected patients and to determine whether there was association of CD4+ cell count and viral load with asymptomatic oral *Candida* carriage. Out of 99 HIV-positive patients studied, 62 (62.6%) had positive culture for *Candida* (oral carriage) and 37 patients (37.4%) had *Candida* negative culture (no oral carriage). The etiologic agents most common were *C. albicans* and *C. tropicalis*. The range of CD4+ was 6-2305 cells/mm^3 in colonized patients and 3-839 cells/mm^3 for non-colonized patients, while the viral load was 60-90016 copies/mL for colonized patients and 75-110488 copies/mL for non colonized patients. The viral load was undetectable in 15 colonized patients and in 12 non colonized patients. Our results showed that there was no significant difference of the variables CD4+ cell count and viral load between oral candida carriage and no oral candida carriage patients.

KEYWORDS: Oral *Candida*; CD4+ cells; Viral load; HIV.

INTRODUCTION

Candida species that colonize the oral cavity are the source of the yeasts that cause oral candidiasis (OC)[5]. Oropharyngeal candidiasis (OPC) is the most common opportunistic fungal infection in individuals infected with human immunodeficiency virus[2,8,12].

A vast majority of HIV-positive patients develop clinical lesions of oropharyngeal or esophageal candidosis that can increase in frequency and severity with HIV disease progression[14]. Marked decrease in incidence of oral lesion has been reported in patients receiving highly active antiretroviral therapy (HAART) related to the improvement in immunological function or to inhibition of the fungal secretory aspartyl proteinases (SAPs) that play a pathogenic role in mucosal invasion[1]. Other mechanisms seem to play a fundamental role for the declining rates of OPC[9,22]. It has been suggested that low CD4+ lymphocytes count and high plasma HIV RNA levels significantly correlate with oral *Candida* carriage, thereby increasing the risk of developing symptomatic infections[21]. There are controversies about association of CD4+ cell count and viral load with asymptomatic oral yeast carriage. FONG *et al.*[11] found a correlation between asymptomatic yeast carriage, development of thrush, and low CD4+ cell count in HIV+ patients. CAMPISI *et al.*[3] verified that in AIDS patients there was more oral *Candida* colonization than control-subjects, but this characteristic was not associated with CD4+ cell count or viral load.

So, in an attempt to answer this question, we conducted the present study to evaluate *Candida* spp. diversity in the oral cavity of HIV-infected patients and to determine whether the oral *Candida* colonization in these patients was associated with CD4+ cell count or plasma HIV-1 RNA levels.

MATERIALS AND METHODS

Patients, sampling and culture of clinical isolates: This prospective study was conduced from May 2004 to May 2005, in which oral mucosa samples were collected from 99 HIV-infected patients (confirmed by Elisa and Western blot). The *Hospital de Doenças Tropicais* (HDT) in Goiás, Brazil, was requested to send samples of these patients. All the participants provided informed consent before enrollment and the Bioethics Committee of HDT approved the study.

The patients who had received treatment with antibiotics or antifungal within the last three months for therapeutic or prophylactic purposes were excluded from this study. It was established a protocol that contained data as sex, age and if they were undergoing to highly active antiretroviral therapy that included HIV protease inhibitors (Indinavir, Saquinavir) and nucleoside reverse transcriptase inhibitors

(1) Laboratório de Micologia, Instituto de Patologia Tropical e Saúde Pública da Universidade Federal de Goiás, Goiânia, GO, Brasil.
(2) Hospital de Doenças Tropicais de Goiás, Goiânia, GO, Brasil.
Correspondence to: Maria do Rosário Rodrigues Silva. Rua 15 nº. 108, apto. 700, Setor Oeste, 74140-090 Goiânia, GO, Brasil, Fax: 55-62-5211839. E-mail:rosario@iptsp.ufg.br

(Zidovudine and Lamivudine). These patients had no clinical signals of oral lesions HIV-related at clinical examination and the time of HIV diagnosis ranged from six months to 15 years (mean = seven years).

In all patients included in this study, microbiological samples were obtained by swabbing the palatine mucosa and dorsal tongue with sterile cotton swabs. These materials were then cultured aerobically on Sabouraud glucose agar at room temperature for seven days. The cultures were inspected on a daily basis for yeast growth and the pure cultures were stored in glycerine at -70 ºC until the use of them.

Identification of isolates: All isolates were identified by germ tube test, chlamydoconidia production on cornmeal agar supplemented with 1% tween 80 and by sugar and nitrogen assimilation tests[17]. Identification was confirmed by API 20C AUX identification System for yeasts (BioMerieux, Marcy L'Etoile, France). *C. albicans* ATCC 10231 and *C. parapsilosis* 22019 were included as control. Additional tests as cultivation on CHROMagar Candida medium (CHROMagar, Paris, France), growth at 45 ºC on agar Sabouraud dextrose and in hypertonic Sabouraud broth, and assimilation tests by using xylose and α-metil-D-glucoside[7,10] were used to help us to discriminate between *C. albicans* and *Candida dubliniensis*.

CD4⁺ lymphocytes and viral load measurements: Plasma HIV-1 RNA levels and CD4⁺ cell counts were measured within 48 hours of collect of oral mucosa samples. CD4⁺ cell counts were obtained from peripheral blood samples collected in ethylenediamine tetraacetic acid as anticoagulant, prepared by a whole blood lysis technique and analyzed on a FACScan flow cytometer (Becton Dickinson, San Jose, California, USA), using a two-colour monoclonal antibody panel[1].

Plasma viral load was measured using the Versant HIV-1 RNA 3.0 branched DNA assay (Bayer PLC, Newbury, UK) with a lower limit of detection of 50 HIV-1 RNA copies/mL[15].

Data analysis: The statistical analysis was performed using Statistical Programme for Social Sciences (SPSS) 11.0 for Windows. A Fisher exact test was used to determine differences in proportion of categorized variables. Continuous variables with an approximately normal distribution were tested with the Student's test. The Mann-Whitney test was used to determine significant associations of carrier rates with CD4⁺ cell count and HIV-1 viral load. A p value of < 0.05 was considered statistically significant.

RESULTS

Out of 99 patients investigated, 62 (62.6%) were found to be colonized with yeasts (oral carriage) and 37 patients (37.4%) had *Candida* negative culture (no oral carriage). Among the 62 isolates, *C. albicans* was the most frequently isolated species (50%), followed by *C. tropicalis* (20.9%). *Candida* non-albicans species were recovered in 31 isolates (50%) (Table 1).

Table 1
Species of *Candida* isolated from oral mucosa samples of 62 HIV-positive patients

Yeasts	Isolates	
	n	%
C. albicans	31	50
C. tropicalis	13	20.9
C. parapsilosis	12	19.3
C. guilliermondii	03	4.8
C. lusitaniae	01	1.61
C. kefyr	01	1.61
C. krusei	01	1.61
Total	62	99.9

The mean age of oral *Candida* carriage patients was 37.1 years (53.2% were male), while for no oral carriage the mean age was 37.9 years (56.7% were male). There was no significant difference between oral carriage and no oral carriage patients of C*andida* sp. with the respect to gender, age or antiretroviral therapy. Demographic data, and the use of antiretroviral therapy of the 99 patients are summarized in Table 2.

A broad range of CD4⁺ and viral load was observed. The range of CD4⁺ was 6-2305 cells/mm^3 in colonized patients and 3-839 cells/mm^3 for non-colonized patients, while the viral load was 60-90016 copies/mL for colonized patients and 75-110488 copies/mL for non colonized patients. The viral load was undetectable in 15 colonized patients and in 12 non colonized patients (lower limit of detection of 50 HIV RNA copies/mL). Oral yeast carriage was not associated with the number of CD4⁺ cell (mean CD4⁺ cell count was 402.2 cells/mm^3 in colonized patients and 341.1 cells/mm^3 in non colonized patients) or with detectable viral load (mean was 18543.6 copies/mL in colonized

Table 2
Profile of 99 HIV⁺ patients included in this study

Parameters	Oral *Candida* carriage n = 62	No oral *Candida* carriage n = 37	p
Mean age (years)	37.1 (range 8-63)	37.9 (range 2-55)	0.758
Sex			0.156
Male	33 (53.2%)	21 (56.7%)	
Female	29 (46.8%)	16 (43.2%)	
Antiretroviral therapy			0.181
Yes	49 (79.1%)	28 (75.7%)	
No	13 (20.9%)	09 (24.3%)	

Table 3
Immune ($CD4^+$ cell count) and viremic (HIV-1 viral load) status in oral *Candida* carriage and no oral C*andida* carriage patients

	Oral carriage		No oral carriage		*p*
	n (%)	Mean	n (%)	Mean	
CD4 (cells/mm^3)					
0 – 200	10 (16.2)	101.1	11 (29.7)	127.6	0.29
201 – 500	38 (61.2)	336.7	19 (51.4)	339.5	0.972
> 500	14 (22.6)	795.1	7 (18.9)	680.4	0.822
Total	62	402.2	37	341.1	0.291
Viral load (copies/mL)					
Undetectable	15 (24.2)	*	12 (32.4)	*	*
51 – 500	9 (14.5)	161.7	7 (18.9)	205.7	0.266
501 – 20000	21 (33.8)	5415.7	11 (29.7)	6534.9	0.463
> 20000	17 (27.4)	44492.2	7 (18.9)	42064.1	0.924
Total	62	18543.6	37	14710.9	0.317

* : The viral load was undetectable (lower limit of detection of 50 HIV RNA copies/mL)

patients and 14710.9 copies/mL in non-colonized patients) (Mann-Whitney test, $p = 0.291$ and $p = 0.317$ respectively). Similarly, there was also no significant difference when HIV^+ patients were stratified by $CD4^+$ cell count (≤ 200/mm^3, between 201-500, and > 500 cells/mm^3) and by viral load measurement (51-500, between 501-20000 and > 20000 copies/mL) (Table 3).

DISCUSSION

Asymptomatical carriage of *Candida* species is a common finding in HIV positive patients. In our study we demonstrated a high rate of oropharyngeal carriage of *Candida* (62.6%) in HIV-infected subjects. This prevalence is similar with that reported by GUGNANI *et al*.[14], who found an oral yeast carriage rate of 65.3% in HIV-seropositive patients, by CAMPISI *et al*.[3] who discovered a proportion of 61.9% in these patients and by SANCHEZ-VARGAS *et al*.[23] that observed in 66.7% of HIV/AIDS patients colonization or infection by yeasts. There are few studies about oral carriage of *Candida* species and oral infection, but FONG *et al*.[11] have demonstrated that persistent asymptomatic carriage of *Candida* species is a possible risk factor for subsequent oral infection.

Among *Candida* spp. recovered from HIV-infected patients in our study, *C. albicans* was the most common species (50%) but *Candida* non albicans species responded for 50% of all cases. *C. tropicalis* was the second species most frequently isolated (20.9%). The emergence of *C. tropicalis* in HIV-infected individuals has been reported by other authors[24,25]. This organism appears to be prevalent in tropical climates[25].

Although *C. dubliniensis* described by SULLIVAN *et al*.[27] has been recovered principally associated with oral cavities of HIV-infected individuals, this species was not isolated in our study. The prevalence of *C. dubliniensis* isolates in determined populations remains uncommon in HIV-infected patients[19,23,25]. The absence of this species above related, of *C. glabrata* and only one *C. krusei* isolate in our group may be explained by the subjects' exposure lack to antifungal agents during the previous three months. The incidence of these species has been attributed to the widespread use of fluconazole as prophylactic therapy[4].

Highly active antiretroviral therapy has been associated with a dramatic decrease in the rates of HIV related opportunistic infections[16]**.** Antiretroviral therapy including inhibitors protease (PIs) can exert a direct effect on *Candida* virulence by inhibiting the fungal secretory aspartyl proteinases (SAPs)[21]**.** In this study we did not find significant correlation between the status of *Candida* carrier and the antiretroviral therapy. This could be explained by characteristics of isolates studied. All the isolates were considered commensal yeasts and not infecting strains, so probably producing low levels of SAPs and thereby reducing the potential target for the antiretroviral agents. This finding supports the premise that strains of *C. albicans* isolated from patients with candidiasis have higher SAPs levels than strains isolated from asymptomatic carriers[6].

Although a substantial set of epidemiological data exists regarding the prevalence of *Candida* species in HIV positive individuals and its increase with disease progression, few studies have been performed about asymptomatic oral yeast carriage and its relationship to markers of immunodeficiency and HIV viral replication.

In the present study we did not find a significant correlation between oral carriage and $CD4^+$ cell count or HIV-1 RNA in plasma (mean values and stratified trends). These results were similar to the studies of CAMPISI *et al*.[3], but contrast with those of SCHOOFS *et al*.[26] that reported a significant relationship between carriage rate and CD4+ cell counts less than 200 cells/mm^3. Our data only partially agree with those of GOTTFREDSSON *et al*.[13] that found a positive association between HIV-1 RNA levels and yeast colonization in HIV patients but no correlation with $CD4^+$ cell counts.

Despite the fact that 14.5% (9/62) of the oral carriage had their $CD4^+$ cell count low of 200 cells/mm^3 and 27.4% (17/62) had their viral load above 20000 copies/mL no patient had oral lesions by *Candida*. Oropharyngeal candidiasis has been demonstrated to be

associated with oral yeast colonization and decline in CD4+ cell count[22]. The precise defense mechanisms that limit *Candida* proliferation are still undefined. Other factors such as blood group secretor status, salivary low rates, antimicrobial constituents of saliva, lysozyme and lactoferrin release, presence of normal bacteria flora and local mucosal immune system could have an impact on the development of oral infection by *Candida*[18,28].

In conclusion, oral colonization of *Candida* spp. especially *C. albicans* was detected with high frequency in HIV-infected patients. Early detection of oral carriage of *Candida* spp. is seen to be important for identification of patients with the propensity for rapid progression of HIV infection since oral carriage may influence the development of clinically significant candidiasis in these immunocompromised patients. We have also shown that the status of oral *Candida* carrier is not associated with the number of CD4 cells or the viral load.

RESUMO

Carreadores assintomáticos de espécies de *Candida* na mucosa bucal de pacientes infectados pelo HIV na era da terapia antiretroviral

Candidíase de orofaringe é a infecção fúngica oportunística mais comum em indivíduos infectados com o vírus da imunodeficiência humana. Contagem de linfócitos CD4+ e quantificação de RNA viral no plasma sanguíneo são os principais marcadores da progressão da doença pelo HIV. O presente estudo foi conduzido para avaliar a diversidade de espécies de *Candida* presentes na cavidade bucal de pacientes infectados pelo HIV e para determinar se havia associação de contagem de células CD4+ e de carga viral com carreadores assintomáticos de *Candida*, na mucosa bucal. Dos 99 pacientes HIV positivo estudados, 62 (62,6%) apresentaram cultura positiva para *Candida* sp. sendo denominados carreadores de *Candida* e os 37 pacientes (37,4%) que não possuíam leveduras do gênero *Candida* na mucosa bucal foram denominados não carreadores. Os agentes etiológicos mais comuns foram *C. albicans* e *C. tropicalis*. A variação de CD4+ foi de 6-2305 cels/mm^3 em pacientes colonizados e de 3-839 cels/mm^3 para pacientes não colonizados, enquanto a carga viral variou de 60-90016 cópias/mL para pacientes colonizados e de 75-110488 cópias/mL para não colonizados. Não foi possível a detecção de carga viral em 15 pacientes colonizados e em 12 não colonizados, porque o limite mínimo de detecção era de 50 cópias/mL. Nossos resultados mostraram que não houve diferença significante na contagem de células CD4+ e de carga viral entre os pacientes carreadores e não carreadores de *Candida* na mucosa bucal de pacientes com AIDS.

REFERENCES

1. ARRIBAS, J.R.; HERNANDEZ-ALBUJAR, S.; GONZALEZ-GARCIA, J.J. *et al*. - Impact of protease inhibitor therapy on HIV-related oropharyngeal candidiasis. **AIDS, 14:** 979-985, 2000.

2. BRAWNER, D.L. & HOVAN, A.J. - Oral candidiasis in HIV-infected patients. **Curr. Top. med. Mycol., 6:** 113-125, 1995.

3. CAMPISI, G.; PIZZO, G.; MILICI, M.E.; MANCUSO, S. & MARGIOTTA, V. - Candidal carriage in the oral cavity of human immunodeficiency virus-infected subjects. **Oral Surg. oral Med. oral Path. oral Radiol. oral Endod., 93:** 281-286, 2002.

4. COLEMAN, D.; RINALDI, M.G.; HAYNES, K.A. *et al*. - Importance of *Candida* species other than *Candida albicans* as opportunistic pathogens. **Med. Mycol., 36** (suppl. 1): 156-165, 1998.

5. DAVIES, A.N.; BRAILSFORD, S.; BROADLEY, K. & BEIGHTON, D. - Oral yeast carriage in patients with advanced cancer. **Oral Microbiol. Immunol., 17:** 79-84, 2002.

6. DE BERNARDIS, F.; CHIANI, P.; CICCOZI, M. *et al*. - Elevated aspartic proteinase secretion and experimental pathogenicity of *Candida albicans* isolates from oral cavities of subjects infected with human immunodeficiency virus. **Infect. Immun., 64:** 466-471, 1996.

7. DOLAPCI, I.; TEKELI, A.; GOCMEN, J.S.; AYSEV, D. & GURIZ, H. - Investigation of *Candida dubliniensis* in *Candida* spp.-positive hemocultures. **Acta path. microbiol. scand., 110:** 391-395, 2002.

8. DUPONT, B.; DENNING, D.W.; MARRIOT, D. *et al*. - Mycosis and AIDS patients. **J. med. vet. Mycol., 32:** 65-77, 1994.

9. EYESON, J.D.; TENANT-FLOWERS, M.; COOPER, D.J. *et al.* - Oral manifestations of an HIV positive cohort in the era of highly active anti-retroviral therapy (HAART) in South London. **J. oral Path. Med., 31:** 169-74, 2002.

10. FAGGI, E.; PINI, G.; CAMPISI, E.; MARTINELLI, C. & DIFONZO, E. - Detection of *Candida dubliniensis* in oropharyngeal samples from human immunodeficiency virus infected and non-infected patients and in a yeast culture collection. **Mycoses, 48:** 211-215, 2005.

11. FONG, I.W.; LAUREL, M. & BURFORD-MASON, A. - Asymptomatic oral carriage of *Candida albicans* in patients with HIV infection. **Clin. investigat. Med., 20:** 85-93, 1997.

12. GLICK, M.; MUZYKA, B.C.; LURIE, D. & SALKIN, L.M. - Oral manifestations associated with HIV-related disease as markers for immunesuppression and AIDS. **Oral Surg. oral Med. oral Path., 77:** 344-349, 1994.

13. GOTTFREDSSON, M.; COX, G.M.; INDRIDASON, O.S. *et al*. - Association of plasma levels of human immunodeficiency virus type 1 RNA and oropharyngeal *Candida* colonization. **J. infect. Dis., 180:** 534-537, 1999.

14. GUGNANI, H.C.; BECKER, K.; FEGELER, W. *et al*. - Oropharyngeal carriage of *Candida* species in HIV-infected patients in India. **Mycoses, 46:** 299-306, 2003.

15. HARDY, G.A.D.; IMAMI, N.; SULLIVAN, A.K. *et al.* - Reconstitution of CD4+ T cell responses in HIV-1 infected individuals initiating highly active antiretroviral therapy (HAART) is associated with renewed interleukin-2 production and responsiveness. **Clin. exp. Immunol., 134:** 98-106, 2003.

16. HUNG, C.C. & CHANG, S.C. - Impact of highly active antiretroviral therapy on incidence and management of human immunodeficiency virus-related opportunistic infections. **J. Antimicrob. Chemother., 54:** 849-853, 2004.

17. KURTZMAN, C.P. & FELL, J.W. - **The yeasts: a taxonomic study.** 4. ed. Amsterdam, Elsevier, 1998. p. 77-100.

18. LEIGH, J.E.; BAROUSSE, M.; SWOBODA, R.K. *et al*. - *Candida*-specific systemic cell-mediated immune reactivities in human immunodeficiency virus-positive persons with mucosal candidiasis. **J. infect. Dis., 183:** 277-285, 2001.

19. MARIANO, P.L.S.; MILAN, E.P.; DA MATTA, D.A. & COLOMBO, A.L. - *Candida dubliniensis* identification in Brazilian yeast stock collection. **Mem. Inst. Oswaldo Cruz, 98:** 533-538, 2003.

20. MELLORS, J.W.; RINALDO Jr., C.R.; GRUPTA, P. *et al.* - Prognosis in HIV infection predicted by the quantity of virus in plasma. **Science, 272:** 1167-1170, 1996.

21. NICOLATOU-GALITIS, O.; VELEGRAKI, A.; PAIKOS, S. *et al*. - Effect of PI-HAART on the prevalence of oral lesions in HIV-I infected patients. A Greek study. **Oral Dis., 10:** 145-150, 2004.

22. PONGSIRIWET, S.; IAMAROON, A.; SRIBUREE, P.; PATTANAPORN, K. & KRISANAPRAKORNKIT, S. - Oral colonization of *Candida* species in perinatally HIV-infected children in Northen Thailand. **J. oral Sci., 46:** 101-105, 2004.

23. SANCHEZ-VARGAS, L.O.; ORTIZ-LÓPEZ, N.G.; VILLAR, M. *et.al.* - Oral *Candida* isolates colonizing or infecting human immunodeficiency virus-infected and healthy persons in Mexico**. J. clin. Microbiol**., **43:** 4159-4162**,** 2005.

24. SANT'ANA, P.L.; MILAN, E.P.; MARTINEZ, R. *et al*. - Multicenter Brazilian study of oral *Candida* species isolated from Aids patients. **Mem. Inst. Oswaldo Cruz, 97:** 253-257, 2002.

25. SCHIMIDT-WESTHAUSEN, A.M.; BENDICK, C.; REICHART, P.A. & SAMARANAYAKE, L.P. - Oral candidosis and associated *Candida* species in HIV-infected Cambodians exposed to antimycotics. **Mycoses, 47:** 435-441, 2004.

26. SCHOOFS, A.G.; ODDS, F.C.; COLEBUNDERS, R.; IEVEN, M. & GOOSSENS, H. - Cross-sectional study of oral *Candida* carriage in a human immunodeficiency virus (HIV)-seropositive population: predisposing factors, epidemiology and antifungal susceptibility. **Mycoses, 41:** 203-211, 1998.

27. SULLIVAN, D.J.; WESTERNENG, T.J.; HAYNES, K.A.; BENNETT, D.E. & COLEMAN, D.C. - *Candida dubliniensis* sp. nov.: phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. **Microbiology, 141:** 1507-1521, 1995.

28. YEH, C.K.; FOX, P.C.; SHIP, J.A. *et al*. - Oral defense mechanisms are impaired early in HIV-1 infected patients. **J. AIDS, 1:** 361-366, 1988.

Received: 28 September 2005
Accepted: 17 May 2006