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ORIGINAL ARTICLE

Predisposing factors for oropharyngeal colonization of yeasts in human immunodeficiency virus-infected patients: A prospective cross-sectional study

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Background: Oropharyngeal candidiasis continues to be a major opportunistic infection in human immunodeficiency virus (HIV)-infected patients. The objectives of this study were to investigate the prevalence, associated factors, and microbiologic features for oropharyngeal yeast colonization in HIV-infected patients.
Methods: From October to December 2009, consecutive HIV-infected patients older than 18 years were recruited in this study. Demographic information, underlying conditions, and clinical histories were collected. Oropharyngeal swab cultures for yeasts and antifungal drug susceptibilities of the isolates were performed.
Results: Of the 105 HIV-infected patients, 54 (51.4%) were colonized with yeasts, including 11 patients (20.4%) with more than one species. Among the 68 isolates, *Candida albicans* accounted for 73.5%, followed by *Candida tropicalis* (5.9%), *Candida glabrata* (5.9%), and *Candida dubliniensis* (4.4%). There were 7.5% and 6% *Candida* isolates resistant to fluconazole and voriconazole, respectively. All of the *Candida* isolates were susceptible to amphotericin B. A higher prevalence of yeast colonization was noted in patients with a CD4 cell count

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≤200 cells/μL ($p = 0.032$). Multivariate regression analysis showed that intravenous drug use was an independent associated factor for oropharyngeal yeast colonization (odds ratio, 5.35; 95% confidence interval, 1.39—20.6; $p = 0.015$), as well as protease inhibitor-containing antiretroviral therapy (odds ratio, 3.59; 95% confidence interval, 1.41—9.12; $p = 0.007$).
Conclusion: Despite previous studies showing that protease inhibitors decreased *Candida* adhesion to epithelial cells *in vitro*, the current study found protease inhibitor-containing antiretroviral therapy predisposed to oropharyngeal yeast colonization in HIV-infected patients.

Introduction

Human immunodeficiency virus (HIV)-infected patients are prone to opportunistic infections with the progression of cell-mediated immunodeficiency. Mucosal fungal infection, especially oropharyngeal and esophageal candidiasis, is one of the most common manifestations of HIV infections.[1,2] More than 80% of patients had oropharyngeal colonization by *Candida* species during the course of HIV infection.[3,4] Despite the introduction of highly active antiretroviral therapy since 1996, *Candida* species continue to be a major pathogen for HIV-infected patients.[5,6]

Several virulent factors are involved in the pathogenesis of *Candida*. In particular, secreted aspartic proteinases (which are encoded by the *SAP* gene family and are responsible for adherence, tissue damage, assimilating nitrogen from proteinaceous sources, and evasion of host immune responses) play a key role in the pathogenesis of *Candida*.[7] Interestingly, the secreted aspartic proteinase of *Candida* and the HIV protease both belong to the aspartyl proteinase family.[8,9] The resolution of oral candidiasis in HIV-infected patients was observed in clinical studies after the introduction of protease inhibitors (PIs).[10,11] However, there are limited clinical studies comparing the effect of PIs with other antiretroviral agents on oropharyngeal carriage of *Candida*.

Although oral colonization of yeasts in HIV-infected patients has been reported, the comprehensive associated factors, such as underlying illnesses, modes of HIV transmission, the past antimicrobial agents history, and current antiretroviral regimens, have not been extensively investigated. These factors are likely to influence the manifestation of oropharyngeal yeast colonization. To address these issues and to better understand the epidemiology, we conducted a prospective cross-sectional study to survey the prevalence of oropharyngeal yeast colonization in HIV-infected patients from industrial metropolitan areas in Taiwan. We also determined the *in vitro* susceptibilities of these isolates to fluconazole, voriconazole, and amphotericin B.

Methods

Study setting

This study was carried out at E-Da Hospital, which serves more than one million residents in the industrial metropolitan areas and is a major referral hospital for HIV care in southern Taiwan. This study was approved by the Institutional Review Board of E-Da Hospital (EMRP-098-027).

Study protocol

From October to December 2009, consecutive HIV-infected patients older than 18 years in the outpatient unit of E-Da Hospital were evaluated. If oropharyngeal candidiasis was noted, these patients were excluded from our study. Demographic information about age and sex, underlying illnesses, clinical conditions, the history of antibiotics and antifungal therapy in the past 6 months, status of hospitalization in the past 12 months, and the use of antiretroviral agents was collected from medical records in a standardized data form. Plasma HIV viral load and CD4 lymphocyte count were also determined at the time of data collection.

Sample collection and culture

Oropharyngeal samples were obtained by swabbing the oropharyngeal mucosa of patients with a dry cotton swab (EZ Culturette; Becton Dickinson, Sparks, MD, USA). All swabs were streaked onto Chromagar *Candida* medium (CHROMagar Microbiology, Paris, France) and incubated at 30°C for 72 hours. Three (if there were) independent colonies from each positive culture were collected. Additional colonies were selected when the cultures had more than one morphotype present. To distinguish *Candida albicans* from *Candida dubliniensis*, all isolates were subjected to VITEK Yeast Biochemical Card (YBC, bioMérieux, Marcy l'Etoile, France). When the YBC identification probability was less than 90% or when uncommon species were reported, the sequences of the internal transcribed spacer (ITS) region and/or the D1/D2 region of ribosomal DNA were used for species identification. The ITS regions were amplified by the primers ITS1, 5′-TCCGTAGGT GAACCTGCGG-3, and ITS4 5′-TCCTCCGCTTATTGATATGC-3′, and the D1/D2 regions were amplified by the primers NL1 5′-GCATATCAATAAGCGGAGGAAAAG-3′ and NL4 5′-GGTCCGTGTTTCAAGACGG-3′.[12]

Antifungal susceptibility tests

Minimum inhibitory concentrations (MICs) of fluconazole, voriconazole, and amphotericin B were determined by the broth microdilution method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[13] Briefly,

in-house prepared microplates containing fluconazole (range, 0.125—64 μg/mL), voriconazole (range, 0.0156—8 μg/mL), and amphotericin B (range, 0.0313—16 μg/mL) in RPMI medium 1640 (31800-022, Gibco BRL) were used. After incubation at 35°C for 48 hours, the growth of each isolate was measured by Biotrak II plate spectrophotometric reader (Amersham Biosciences, Biochrom Ltd., Cambridge, England). Following the guidelines of CLSI document M27-A3,[13] isolates were considered resistant to fluconazole, voriconazole, and amphotericin B if their MICs were ≥64 μg/mL, ≥ 4 μg/mL, and ≥2 μg/mL, respectively. Quality control strains included *C. albicans* ATCC 90028, *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC 750. The MICs at which 50% and 90% of the total population were inhibited were defined as MIC_{50} and MIC_{90}, respectively.

Data analysis

SPSS version 14.0 (SPSS Inc., Chicago, IL, USA) was used for the analysis of results. Categorical variables were analyzed using the χ^2 test or Fisher exact tests, as appropriate. Continuous variables were analyzed using the Student *t*-test. To identify the associated factors, underlying conditions that could contribute to oropharyngeal colonization of yeasts and were associated with a level of significance of <0.20 in univariate analyses were included in a logistic regression model for multivariate analysis. Odds ratio (OR), 95% confidence interval (CI), and *p* value were calculated for each factor. Hosmer-Lemeshow goodness-of-fit test was used to assess the fitness of the model. All *p* values were two-tailed and $p < 0.05$ was considered statistically significant.

Results

During the study period, 105 consecutive HIV-infected patients were enrolled in this study. There were 99 males (94.3%) and 6 females (5.7%), with a mean ± standard deviation (SD) age of 39.5 ± 10.1 years (range, 20—70 years). Only three patients had a history of antibiotics or antifungal use (duration, 3—6 months and 7—130 days, respectively). The duration of HIV infection was 5.9 ± 4.3 years (mean ± SD). Use of intravenous drugs was found in 17 patients (16.2%) (Table 1).

Among these 105 HIV-infected patients, 54 (51.4%) were colonized with yeasts. There were no significant differences in age, sex, underlying illness, antibiotics or antifungal therapy within the previous 6 months, duration of HIV infection, or a high HIV viral load (> 50 copies/mL) between yeast culture-positive and -negative patient groups. However, a history of hospitalization within the previous 12 months ($p = 0.027$) and a CD4 cell count ≤200 cells/μL ($p = 0.032$) were significantly associated with positive yeast cultures (Table 1). The prevalence of positive oropharyngeal cultures also differed significantly according to the modes of HIV transmission ($p = 0.031$) and regimens of antiretroviral therapy ($p = 0.022$).

Multivariate regression analysis demonstrated that intravenous drug use was an independent associated factor for oropharyngeal yeast colonization when compared with male homosexuality (OR, 5.35; 95% CI, 1.39—20.6; $p = 0.015$; Table 2) in regard to the mode of HIV transmission. Patients who received PI/nucleoside reverse transcriptase inhibitors (NRTIs) were more likely to be associated with oropharyngeal yeast colonization than those who received nonnucleoside reverse transcriptase inhibitor (NNRTI)/NRTIs (OR, 3.59; 95% CI, 1.41—9.12; $p = 0.007$). Further analysis demonstrated that the difference of colonization rate between PI- and NNRTI-containing regimens was independent of the CD4 cell count and plasma HIV viral load ($p = 0.804$ and 0.228, respectively).

Of the 68 yeasts isolated from the 54 patients, 67 were *Candida* species (Table 3). Multiple yeast species were identified in 11 patients (20.4%), including eight patients with two species and three with three species. *C. albicans* accounted for 73.5% of the isolates, followed by *C. tropicalis* (5.9%), *Candida glabrata* (5.9%), and *C. dubliniensis* (4.4%). Neither mode of HIV transmission ($p = 0.999$) nor antiretroviral regimens ($p = 0.611$) was associated with the species of isolates.

The results of susceptibility tests are shown in Table 3. Of the 67 *Candida* isolates, 7.5% were resistant to fluconazole and 6% were resistant to voriconazole. All *Candida* isolates were susceptible to amphotericin B. The five fluconazole-resistant isolates included two *C. tropicalis* and one each of *C. albicans*, *C. glabrata*, and *Candida krusei*. Among the four voriconazole-resistant isolates, two were *C. albicans* and the others were *C. tropicalis*. One *C. albicans* and two *C. tropicalis* were cross-resistant to both fluconazole and voriconazole. There was no statistically significant difference between the CD4 counts and the susceptibility to fluconazole (susceptible, 452 ± 271; nonsusceptible, 339 ± 108; $p = 0.362$) and voriconazole (susceptible, 452 ± 269; nonsusceptible, 310 ± 182; $p = 0.301$).

Among five patients receiving antimicrobial drugs 6 months before the survey, one had antibiotic and antifungal drug, two had antibiotic, and two had antifungal drug. The duration for antibiotics ranged from 90 to 180 days and for antifungal drugs ranged from 7 to 130 days. Four of the five patients were colonized by yeasts. *Candida* isolates were susceptible to fluconazole despite the fact that they were recovered from patients receiving fluconazole therapy before the survey.

Discussion

As the disease progresses, HIV-infected patients become more vulnerable to a variety of opportunistic infections, including candidiasis. The prevalence of oropharyngeal yeast colonization in HIV-positive patients varies from 44% to 62% in the antiretroviral therapy era.[5,14—16] In the current study, 51.4% of HIV-infected patients had yeast colonization in the oropharyngeal cavity. We also found a higher rate of oropharyngeal yeast colonization in HIV-positive patients if their CD4 lymphocyte counts were ≤200 cells/μL. This finding is similar to that of previous studies.[3,14,16] In addition to the low CD4 lymphocyte count, several other factors could influence the occurrence and distribution of oropharyngeal yeast colonization.

Table 1 Demographic characteristics, underlying illnesses, laboratory findings, and medication histories of HIV-infected patients

Characteristics	All cases	Yeast culture		*p*
		Positive ($n = 54$), *n* (%)	Negative ($n = 51$), *n* (%)	
Age, mean ± SD (y)	39.5 ± 10.1	40.8 ± 11	38.1 ± 8.8	0.171
Sex, male	99 (94.3)	50 (92.6)	49 (96.1)	0.679
Underlying illness				
Diabetes mellitus	7 (6.7)	4 (7.4)	3 (5.9)	0.999
Hypertension	3 (2.9)	2 (3.7)	1 (2)	0.999
Modes of HIV transmission				0.031
Homosexual	60 (57.1)	25 (46.3)	35 (68.6)	
Heterosexual	28 (26.7)	16 (29.6)	12 (23.5)	
Intravenous drug use	17 (16.2)	13 (24.1)	4 (7.8)	
Hospitalization within the previous 12 mo	6 (5.7)	6 (11.1)	0	0.027
Antibiotics therapy within the previous 6 mo	3 (2.9)	2 (3.7)	1 (2)	0.999
Antifungal therapy within the previous 6 mo	3 (2.9)	3 (5.6)	0	0.243
HIV infection				
Duration of HIV infection (y)	5.9 ± 4.3	6.1 ± 4.3	5.7 ± 4.3	0.627
HIV viral load > 50 copies/mL	26 (24.8)	16 (29.6)	10 (19.6)	0.234
CD4 count (cells/μL)	480 ± 258	455 ± 265	508 ± 250	0.294
CD4 count ≤ 200 cells/μL	9 (8.6)	8 (14.8)	1 (2)	0.032
Antiretroviral therapy				0.022
NNRTI + 2 NRTIs	46 (43.8)	16 (29.6)	30 (58.8)	
Duration, mean ± SD (mo)	33.9 ± 21.7	26.6 ± 21	37.8 ± 21.3	0.094
Protease inhibitor + 2 NRTIs	46 (43.8)	30 (55.6)	16 (31.4)	
Duration, mean ± SD (mo)	41 ± 21.9	38.4 ± 23.6	45.9 ± 18	0.238
Integrase inhibitor + 2 NRTIs	4 (3.8)	2 (3.7)	2 (3.9)	
Duration (mo)	3, 3, 9, 38	3, 38	3, 9	
Three NRTIs	1 (1)	0	1 (2)	
Duration (mo)	61	—	61	
None	8 (7.6)	6 (11.1)	2 (3.9)	

CI = confidence interval; HIV = human immunodeficiency virus; NNRTI = nonnucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor; OR = odds ratio; SD = standard deviation.

Phelan et al[17] and Lamster et al[18] reported a much higher prevalence rate of oral *Candida* infection in intravenous drug users than in homosexual men. Even in seronegative patients, a higher percentage of oral candidiasis was observed among intravenous drug users.[18,19] Opioids, particularly morphine and its derivatives, have been shown to depress the immune system by influencing the function of T lymphocytes.[20] Therefore, in HIV-infected patients who also receive injections of opioids, their immunity could be further depressed by the dual effect of opioids and HIV. In the current study, we found the prevalence of oropharyngeal yeast colonization was significantly higher in HIV seropositive intravenous drug users than in homosexual men. This observation lends further support to the immunosuppressive effect of opioids in HIV-infected patients. Nevertheless, whether abuse of opoids is detrimental to immunity needs further investigation.

PI, an important component of highly active antiretroviral therapy, blocks the action of the HIV protease, which is required for protein processing in the viral replication cycle.[21] *In vitro* studies demonstrated that PIs could inhibit secreted aspartic proteases of *C. albicans* and consequently attenuate their adherence to epithelial cells, interrupt the integrity of yeast cell membrane, and decrease the viability of *Candida*.[9,22–24] These results suggested that the reduction of oral *Candida* colonization in HIV-infected patients who received antiretroviral therapy might not only be due to reconstitution of the immune system, it could also result from the effect of direct inhibition to secreted aspartic proteases by PIs.[9,22,23]

Despite these *in vitro* studies, there is limited literature comparing the clinical differences of antiretroviral regimens on the prevalence of oropharyngeal yeast colonization in HIV-infected patients.[16,25,26] Cassone et al[25] found that PI-containing antiretroviral therapy exerted an early, immune reconstitution-independent effect on attenuation of oral candidiasis compared with NNRTI-containing regimens. Pomarico et al[26] investigated the effect of antiretroviral therapy on oral candidiasis and found that PI-containing regimens reduced the prevalence rate of oral candidiasis in HIV-positive children. However, Delgado et al[16] reported that PI-containing regimens exerted no different effect on the oral carriage of *Candida* when compared with NNRTI-containing regimens. Interestingly,

Table 2 Multivariate logistic regression model of associated factors for oropharyngeal yeast colonization in HIV-infected patients[a]

Associated factors	OR (95% CI)	*p*
Modes of HIV transmission		
Homosexual	1 (Reference)	—
Heterosexual	2.01 (0.76–5.35)	0.162
Intravenous drug use	5.35 (1.39–20.6)	0.015
Hospitalization within the previous 12 months		
No	1 (Reference)	—
Yes	—	0.999
Antiretroviral therapy		
NNRTI + 2 NRTIs	1 (Reference)	—
Protease inhibitor + 2 NRTIs	3.59 (1.41–9.12)	0.007
Integrase inhibitor + 2 NRTIs	2.33 (0.28–19.39)	0.434
Three NRTIs	—	0.999
None	5.23 (0.84–32.69)	0.077

CI = confidence interval; HIV = human immunodeficiency virus; NNRTI = nonnucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor; OR = odds ratio.

[a] All variables were entered into the model in one single step.

the current study demonstrated that the prevalence of yeast colonization in oropharyngeal cavity was significantly higher in patients receiving PI-containing antiretroviral therapy than in those with NNRTI-containing regimens. Moreover, this difference was independent of the patients' CD4 cell count and plasma HIV viral load. However, all of these clinical studies, including the current report, were not randomized, double-blind, parallel-group investigations. To better understand the influence of different antiretroviral regimens on the distribution of oropharyngeal *Candida* colonization in HIV-infected patients, further well-designed head-to-head comparison studies are warranted.

Although *C. albicans* is still the most common species, the prevalence of *Candida* species other than *C. albicans* isolated from HIV-infected patients is increasing.[3,4,14,27] *C. dubliniensis*, *C. glabrata*, and *C. tropicalis* are considered as emerging pathogens.[14,27,28] Compared with the current study with a proportion of 73.5%, the percentage of *C. albicans* decreased from 86.7% in 2002[14] to 68.5% in 2005[27] in Taiwan. The widespread use of antiretroviral agents, antibiotics, and antifungal agents were assumed to have an effect on the distribution of oropharyngeal yeast colonization.[5,29] Since the introduction of highly active antiretroviral therapy, there was a significant decline of fluconazole- and itraconazole-resistant *Candida* colonizing the oral cavity of HIV-infected patients.[30] In the current study, the susceptibility of *Candida* to antifungal agents was similar to previous reports in Taiwan.[14,27] Because *Candida* species other than *C. albicans* are known to have higher resistant rates to antifungal agents,[30] these

Table 3 The minimum inhibitory concentrations of antifungal agents for isolated yeasts in HIV-infected patients

Isolates	Minimum inhibitory concentration (μg/mL)		
	Fluconazole	Voriconazole	Amphotericin B
Candida albicans (*n* = 50)			
Range	0.125–64	0.016–8	0.5–1
MIC_{50}	0.25	0.016	0.5
MIC_{90}	1	0.06	1
Candida tropicalis (*n* = 4)			
Range	0.5–64	0.016–8	0.5–1
MIC_{50}	1	0.03	0.5
MIC_{90}	64	8	1
Candida glabrata (*n* = 4)			
Range	8–64	0.25–0.5	0.5–1
MIC_{50}	8	0.5	1
MIC_{90}	64	0.5	1
Candida dubliniensis (*n* = 3)			
Range	0.125–0.25	0.016	0.25
MIC_{50}	0.125	0.016	0.25
MIC_{90}	0.25	0.016	0.25
Candida parapsilosis (*n* = 2)	0.5–2	0.016–0.06	0.5–0.5
Candida krusei (*n* = 1)	64	0.25	1
Candida utilis (*n* = 1)	2	0.25	0.5
Candida guilliermondii (*n* = 1)	1	0.03	0.5
Candida (*Stephanoascus*) *ciferrii* (*n* = 1)	0.125	0.016	0.25
Geotrichum klebahnii (*n* = 1)	—	—	—

HIV = human immunodeficiency virus; MIC = minimum inhibitory concentration.

gradually increasing species may constitute an important issue in the treatment of opportunistic infections among HIV-infected patients.

There are several limitations to our study. First, this study was conducted only at a single hospital in southern Taiwan, limiting its generalizability. Second, the duration of our study was only 3 months and the number of enrolled cases was small. Therefore, the strength of our study could be influenced. Third, the underlying illnesses were collected from medical records. There may be inconsistencies with the completeness of these data. Some associated factors may not have been detected or explored in our study.

In conclusion, the current study found a higher prevalence of oropharyngeal yeast colonization in HIV-infected patients with a low CD4 cell count. Intravenous drug use and PI-containing antiretroviral therapy were significantly associated with oropharyngeal yeast colonization independent of the CD4 cell count and HIV viral load. Although *C. albicans* was still the prevalent species colonizing the oropharyngeal cavity of HIV-infected patients, the increasing multidrug-resistant *Candida* species other than *C. albicans* present an emerging challenge in fungal infection management.

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