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Colonization by *Candida* Species of the Oral and Vaginal Mucosa in HIV-Infected and Noninfected Women

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Abstract

Candidiasis in HIV/AIDS patients continues to be a public health problem. Effective antifungal therapies are few in number and have inherent problems such as selecting for drug-resistant strains of *Candida* species. To evaluate the state of *Candida* colonization of the oral and vaginal mucosa, we recruited 80 women, both HIV-infected and HIV-uninfected, from the Women's Interagency HIV Study (WIHS). Diet diaries were collected by participants to examine the role of diet on fungal growth. Baseline studies were initially done in participants that followed the colonization of both mucosal sites over 0–90 days. The most common *Candida* species from both groups of patients were *C. albicans* and *C. glabrata*. Among the HIV-infected cohort, the percentage of participants who were positive for *Candida* spp. was higher than in the HIV-uninfected control group. Furthermore, the frequency of colonization (1 episode versus >1 episode) was also increased in the HIV-infected cohort. These data indicate that *Candida* species remain an important component of the microbial community in both populations.

Introduction

Vaginal candidiasis is very common in both immunocompetent and immunocompromised individuals while oral candidiasis is much more common in immunocompromised individuals.[1] Oral candidiasis is an important health problem that disproportionately affects HIV-infected and AIDS patients, with 90% of AIDS patients being afflicted with oral candidiasis.[2] Unfortunately, candidiasis has limited treatment options, many of which are refractory to traditional treatments.[3]

Since the initiation of highly active antiretroviral therapy (HAART) there has been a decrease in the incidence of oropharyngeal candidiasis.[4] In a study of opportunistic illnesses in 8,070 patients from 1994 to 2007, esophageal candidiasis, like other oral infections, decreased significantly but has stabilized since 2003–2007.[5] However, a study that followed 744 HIV patients on HAART found oral lesions were identified in 35% of patients. Oral pharyngeal candidiasis was the most frequent disease reported (74.9%).[6]

The purpose of this study was to compare the proportions of which oral and vaginal mucosa of HIV-infected and HIV-uninfected cohorts are colonized by *Candida* species.

Materials and Methods

Design

A prospective noninterventional cohort was followed for 90 days. The cohort was composed of 84 women, 59 HIV-infected and 25 HIV-uninfected, from which oral and vaginal samples and self-reported diaries were collected.

Study population

Participants were recruited from the Women's Interagency HIV Study (WIHS), an ongoing longitudinal study evaluating HIV prognosis and related conditions in women, previously described in the literature.[7,8] The WIHS currently has 2,895 HIV-infected women and 972 HIV-uninfected women recruited from six national metropolitan locations. HIV-uninfected women are matched for socioeconomic status, age, race, and relative risk for developing HIV. Participants were recruited from the WIHS study population in Washington, DC. The women are interviewed and undergo a physical and gynecologic examination and laboratory testing every 6 months. The laboratory testing consists of typical HIV tests, such as viral load and CD4 count, and other tests, such as a

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complete metabolic panel and thyroid testing. Women were excluded from this candidiasis study if they had a hysterectomy, were trying to get pregnant, or were being treated with any antifungals during screening. The local institutional review board at each site approved the study protocol and all participants gave written informed consent.

Data collection

The participants collected self-administered oral and vaginal culture swabs [BBL CultureSwab (Becton, Dickinson, Sparks, MD)] four separate times on days 0, 30, 60, and 90, between October 2010 and May 2011. The swabs were transferred to the laboratory on ice and frozen at −80°C before batch analysis. A standard follow-up questionnaire was conducted on days 30, 60, and 90 inquiring about general health including vaginal infections, diarrhea, menstruation, sexual activity, doctor visits, medication use, etc. The data gathered were compared to CD4 and viral load data collected 1–6 months prior. The following covariates were examined: constipation, diarrhea, headache, nasal congestion, prescription and nonprescription drugs, menstruation, vaginal infection, and vaginal cleansing.

Fungal identification

Direct microscopy (10% KOH) of swabs is effective to detect fungal morphological forms (budding yeast or hyphae) in smears when the organism presents in high numbers and is therefore most probably causing disease. However, species identification requires other selective media or biochemical assays. Each swab was therefore inoculated on both Sabouraud's dextrose agar with chloramphenicol (SABC) as well as CHROMagar. Since some *Candida* species do not grow on SABC medium, Sabouraud's dextrose agar (SAB) without chloramphenicol was used. CHROMagar-Candida is useful in differentiating among species of *Candida* since they produce different colored colonies, especially *C. albicans* and *C. glabrata* (and these two species alone account for the majority of all *Candida* isolates).[9] Germ tube and chlamydospore assays were done for *C. albicans* only. In the germ tube assays, yeast was inoculated in 0.5 ml of 10% sera and incubated aerobically at 37°C for 2 h. A drop of the yeast–serum mixture was examined microscopically and the appearance of small filaments projecting from the yeast cell surface indicates that the germ tube was positive. In the chlamydospore assays, yeast cells were plated onto cornmeal agar under a glass coverslip to maintain a semianaerobic condition and grown in the dark for 7 days at 25°C. Plates were examined over the following 21 days for chlamydospores. The remaining undetermined strains from the assays mentioned above were tested on the API 20C system (BioMerieux) to assign a species. All isolates were stored at −80°C for future fluconazole susceptibility tests.[10,11]

Outcome variables

Each oral and vaginal swab was tested for vaginal or oral fungal growth. The outcome was dichotomized as present for growth or not present during the four tested periods.

Covariates

Possible factors that may affect fungal growth were also selected, such as diarrhea, constipation, currently menstruating, and diagnosis as vaginal infection. CD4 (cells/mm^3) value was defined in analysis in three groups: <350, 350–500, and >500. Log viral load was used in the analysis. Both CD4 and viral load data were collected from participants during WIHS core visits every 6 months.

Statistical analysis

Logistic regression with the generalized estimating equation (GEE) model for repeated measures (0, 30, 60, and 90 days) was used to assess the association between oral or vaginal fungal growth, as reflected by odds ratio and their 95% confidence intervals. Covariates in multivariate models were chosen based on prior knowledge of factors that may affect fungal growth and statistical significance in univariate analysis. To avoid multicollinearity, two different multivariate models were fitted.

Results

Originally, 84 women were enrolled in our substudy from the Washington, DC metropolitan area; 59 women were HIV-infected and 25 women were HIV-uninfected. There were no statistically significant differences between the two groups (Table 1). Four participants subsequently dropped out of the study. Table 2 shows the characteristics of the study participants, compared with the entire WIHS cohort. In our study, there were more African-American women and more women who had a baseline education level above high school. Also, women in our study had higher viral load values than that of the WIHS cohort (log mean value: 6.12 versus 5.42).

Vaginal candidiasis

Among the study women, 15 (18.8%) reported a vaginal infection during the study period; two women reported more than one vaginal infection. Among these 15 women, 13 were HIV-infected.

Among the HIV-infected women, 54% had vaginal fungal growth at least one time during the study period. Of this group with vaginal fungal growth, 38% were positive during more than one period. Of the HIV-uninfected women, 41% had vaginal fungal growth during the study period and 18% of those participants were positive for the presence of vaginal

Table 1. Characteristics of HIV-Infected Participants and HIV-Uninfected Participants

Characteristics	*HIV-infected participants (N = 56)*	*HIV-uninfected participants (N = 24)*
Race (%)		
Other	3.6	4.2
Hispanic	3.6	8.3
Black (non-Hispanic)	82.1	83.3
White (non-Hispanic)	10.7	4.2
Income (>$12,000/year %)	52.7	65.2
Depression (%)	26.8	12.5
Education (%)		
Above high school	41.4	29.2
High school	37.5	45.8
Less than high school	21.4	25.0

Table 2. Characteristics of Study Participants and All Participants in the Women's Interagency HIV Study

Characteristics	*All study participants (N = 80)*	*All WIHS participants (N = 1901)*
Race (%)[b]		
Other	3.75	3.68
Hispanic	5.00	27.51
Black (non-Hispanic)	82.50	56.18
White (non-Hispanic)	8.75	12.62
Income (≥$12,000/year %)	56.41	53.15
Depression (%)	22.50	30.02
Education (%)[b]		
Above high school	37.50	33.19
High school	40.00	29.97
Less than high school	22.50	36.83
CD4 (cells/mm^3) (%)		
<350	25.00	25.25
≥350 and ≥500	12.50	13.73
>500	62.50	61.02
HIV-infected (%)	70.00	71.60
HAART use[a] (%)	82.14	84.13
Log viral load[a] (mean)[b]	6.12	5.42

[a]HIV-infected only.
[b]The differences are statistically significant.
WIHS, Women's Interagency HIV Study; HAART, highly active antiretroviral therapy.

fungal growth during more than one period (Table 3). Interestingly, bowel movements appeared to correlate with fungal growth, as diarrhea was associated with an increase in vaginal fungal growth. Women who had diarrhea in the past 30 days were approximately two times more likely to have fungal growth than those who did not have diarrhea, which reached statistical significance in both models (model 1: OR = 2.08, CI = 1.01–4.3; model 2: OR = 2.65, CI = 1.13–6.2).

The dominant species of yeast from all vaginal isolates in the HIV-infected group were *C. albicans* (73%) followed by *C. glabrata* (18%). Other species accounted for 9% of all isolates.

Oral candidiasis

Among the HIV-infected women, 67% had oral fungal growth at least one time during the study period. Of that population, 62% were culture positive during more than one period. Of the HIV-uninfected women 53% had positive cultures during the study period and 41% of those participants were culture positive during more than one period. Women with higher HIV viral loads tended to have a higher risk for oral fungal growth (OR = 1.19, 95% CI = 1.03–1.38) (Table 4). Using over-the-counter medications resulted in a lower presence of oral fungal growth, while illness resulted in a very significant increase in oral fungal growth (Table 4).

The frequency of oral *Candida* species among all isolates was also determined. In HIV-infected patients, 68% of isolates were *C. albicans*, followed by *C. glabrata* (21%), *C. tropicalis*, *C. parapsilosis*, and *C. lusitaniae*, collectively (~9%).

Discussion

Candida commonly colonizes the vaginal and oral mucosa in healthy women of all ages; at least 75% of women will experience at least one vaginal infection during their lifetime.[12] Carrier rates of *Candida* ranging from 17% to 75% have been reported, depending on the population studied.[13] However, we found substantial rates of both in our cohorts. As expected, the rates were higher in the HIV-infected group compared to the HIV-uninfected group. One surprising and potentially very important finding is the high rate of oral growth in both groups, 67% of HIV-infected women and 53% of HIV-uninfected women. The potential for these organisms to become pathogenic is significant and important, as treatment options are limited.

The fungal microbiome of humans is not nearly as well-studied as the bacterial microbiome. However, recently Ghannoum *et al.* studied 20 healthy volunteers using pan-fungal internal transcribed spacer (ITS) primers to identify the oral fungal population in healthy adults.[14] They found that

Table 3. Odds Ratios for Vaginal Fungal Growth Dependent on Studied Variables

	Univariate model		*Multivariate model*			
			Model I		*Model II*[a]	
Variable	*Odds ratio*	*95% CI*	*Odds ratio*	*95% CI*	*Odds ratio*	*95% CI*
CD4 group (cells/mm^3)						
<350	1.64	(0.72, 3.72)	1.50	(0.57, 3.94)		
≥350 and ≤500	0.94	(0.32, 2.74)	1.09	(0.32, 3.71)		
>500	1.00		1.00			
HIV-infected (HIV-uninfected is reference)	1.89	(0.85, 4.20)	1.86	(0.68, 5.07)		
Log RNA[a]	1.04	(0.90, 1.20)			1.05	(0.88, 1.26)
Used over-the-counter medication in past 30 days	1.96	(1.15, 3.32)	1.65	(0.92, 2.97)	2.04	(0.95, 4.37)
Had diarrhea in past 30 days	**2.22**	**(1.06, 4.64)**	**2.08**	**(1.01, 4.30)**	**2.65**	**(1.13, 6.20)**
Had vaginal infection in past 30 days	3.36	(1.09, 10.42)	3.14	(0.93, 10.63)	2.22	(0.44, 11.21)
Consuming yogurt or probiotic products	1.13	(0.65, 1.96)	1.00	(0.50, 2.02)	0.78	(0.34, 1.79)

[a]HIV-infected only.
Model I: CD4 + HIV status + used over-the-counter medication + had diarrhea + had vaginal infection + eating yogurt or not.
Model II: log viral load + used over-the-counter medication + had diarrhea + had vaginal infection + eating yogurt or not.
Note: Variables in bold indicated a statistically significant result.

Table 4. Odds Ratios for Oral Fungal Growth Dependent on Studied Variables

	Univariate model		*Multivariate model*			
			Model I		*Model II*[a]	
Variable	*Odds ratio*	*95% CI*	*Odds ratio*	*95% CI*	*Odds ratio*	*95% CI*
CD4 group (cells/mm^3)						
<350	1.76	(0.72, 4.27)	1.48	(0.56, 3.96)		
≥350 and ≤500	1.59	(0.46, 5.49)	1.20	(0.31, 4.58)		
>500	1.00		1.00			
HIV-infected (HIV-uninfected is reference)	1.99	(0.88, 4.54)	2.12	(0.82, 5.49)		
Log RNA[a]	**1.17**	**(1.01, 1.36)**			**1.19**	**(1.03, 1.38)**
Used over-the-counter medication in past 30 days	**0.51**	**(0.33, 0.80)**	**0.43**	**(0.26, 0.69)**	**0.39**	**(0.23, 0.68)**
Had illness that resulted in change of activities in past 30 days	**2.18**	**(1.32, 3.59)**	**2.92**	**(1.73, 4.95)**	**3.17**	**(1.75, 5.74)**
Eating yogurt or not	0.83	(0.62, 1.10)	0.80	(0.48, 1.33)	0.75	(0.39, 1.43)

[a]HIV-infected only.

Model I: CD4 + HIV status + used over-the-counter medicine in past 30 days + had illness that resulted in change of activities in past 30 days + eating yogurt or not.

Model II: log viral load + used over-the-counter medicine in past 30 days + had illness that resulted in change of activities in past 30 days + eating yogurt or not.

Note: Variables in bold indicated a statistically significant result.

Candida species were the most common inhabitants, found in 75% of participants. The frequency of *C. albicans* vaginal colonization was reduced in healthy woman by lactobacilli, although the authors suggest additional studies are required to establish treatment efficacy.[15] Symptomatic cure of vulvovaginal candidiasis (VVC) was also noted in another study of healthy women in which *Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14 were used in combination therapeutically.[16]

Quantitation of *Candida* species from body sites is imprecise, since these organisms grow in tissues as both yeast and filamentous forms.[17] Additionally, filamentous growth is linear, not exponential and, consequently, the colony-forming units measurement is an approximation.[18] However, the presence of fungal growth was greatly influenced by HIV status and for both groups was higher than we anticipated.

Our study is limited by self-report of vaginal infections. We also had only limited clinical data and were not able to correlate fungal growth with clinical symptoms. Our data would be more robust if our sample size was larger. Our cohort is rather compliant with 82% on HAART and 75% with CD4 counts greater than 350. A larger cohort would have allowed us to examine fungal growth in subgroups that were less compliant.

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Author Disclosure Statement

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