CONCISE COMMUNICATIONS

Association of Plasma Levels of Human Immunodeficiency Virus Type 1 RNA and Oropharyngeal *Candida* Colonization

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The pathophysiology of oropharyngeal candidiasis in patients infected with human immunodeficiency virus (HIV) type 1 is poorly understood. Association between oropharyngeal yeast carriage and various clinical factors in HIV-1–infected patients was studied in 83 patients with no clinical evidence of thrush and no recent antifungal use. Of the clinical factors measured, the only correlate of yeast colonization was with plasma HIV-1 RNA levels (P = .001), whereas the correlation with CD4 cell count was poor (P = .36). By multivariable regression modeling, plasma HIV-1 RNA was the only parameter that correlated with the extent of colonization with *Candida* infection (P = .003). These data indicate that the presence and amount of asymptomatic oropharyngeal yeast carriage in persons with HIV-1 infection is more significantly correlated with plasma HIV-1 RNA levels than with CD4 cell count. Further studies on the effect of HIV-1 on oropharyngeal yeast colonization, infection, and local immunity are warranted.

Oropharyngeal candidiasis (OPC), primarily caused by *Candida albicans,* is relatively common in persons infected with human immunodeficiency virus (HIV) type 1. The point prevalence of OPC in HIV-1–infected persons is 2.2%–7%, and the lifetime risk is >90% [1]. The presence of OPC is a predictor of poor outcome in HIV-1–infected persons [2]. A statistical association exists between few CD4 cells and the development of OPC, but this association is weak [1, 3]. In fact, some HIV-1–infected persons with relatively high CD4 cell counts can have OPC, as can some persons with acute HIV-1 infection syndrome [4]. Thus, the pathophysiology of OPC remains poorly understood, and factors that probably play a role in this disease are yet unidentified.

Recent experience suggests that the prevalence of most opportunistic infections, including OPC, are declining in HIV-1–infected persons [5]. It is likely that most of the decrease in the prevalence of opportunistic infections is due to improvements in immune function in patients who receive highly active antiretroviral therapy (HAART) [6]. The improvements in immune function in patients responding to HAART are incompletely understood. They do not appear to be solely reflected by increases in numbers of CD4 lymphocytes, since the rise in CD4 cells in patients responding to HAART lags behind other qualitative and quantitative measures of immunity [5].

In order to improve our understanding of how carriage of yeast in the oropharynx is determined in HIV-1 disease, we conducted a cross-sectional study of several clinical factors and their association with yeast colonization. Our hypothesis was that the presence and amount of yeasts would be correlated with both plasma HIV-1 RNA levels (viral load) and peripheral blood CD4 lymphocyte counts. Furthermore, of these two potential predictors, we believed that plasma HIV-1 RNA would most closely correlate with yeast colonization.

Materials and Methods

Study design. This cross-sectional study was conducted in an academic medical center outpatient clinic. Baseline clinical variables and laboratory data were collected prospectively. This clinic provides care for >1000 HIV-1–infected patients and serves as the primary care provider for the vast majority of its patients.

Patient evaluations. At enrollment, the study subjects were interviewed and examined and asked to swish 20 mL of 0.9% sterile saline in their mouths for 15 s and to collect the contents into a sterile container. Subsequently, 1 mL was plated onto CHROMagar (Hardy Diagnostics, Santa Maria, CA). After the plates were

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Informed consent was obtained from all patients prior to study participation, in compliance with human experimentation guidelines of the US Department of Health and Human Services. The study was approved by the Duke Institutional Review Board Committee.

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incubated at 30°C for 48 h, growth on the plates was quantitated, and the *Candida* load was expressed as colony-forming units (cfu) per milliliter. If a dense growth of yeast was noted after 24 h of incubation, the culture was diluted 10-fold and replated. Colonies with the diagnostic green appearance were assumed to be *C. albicans;* all other organisms were speciated by use of standard growth and morphologic criteria as well as sugar assimilation profiles (API 20; BioMerieux, Hazelwood, MO). All enrolled patients had recent HIV-1 RNA and CD4 cell measurements (<30 days for plasma HIV-1 RNA and <90 days for CD4 cell count) while on a stable antiretroviral regimen. Most of the patients (64/83) had plasma HIV-1 RNA determined by reverse transcriptase–polymerase chain reaction (Roche Diagnostics, Alameda, CA) and CD4 lymphocyte counts determined by flow cytometry on the same day that the culture was obtained. The following exclusion criteria were used: topical or systemic antifungal use within 30 days, clinical evidence of OPC, poor dentition, oral prosthetic devices, including dentures, and use of antibacterial agents other than those used for *Pneumocystis carinii* pneumonia (PCP) prophylaxis within 30 days. Over an 8-month period, 83 patients were enrolled in the study. Data on patient demographics, history of prior fungal infections, antifungal treatment, and medications at the time of enrollment were collected (table 1).

Statistical methods. Prior to data analysis, 5 variables were identified as likely to be related to oropharyngeal colonization with *Candida* organisms: CD4 lymphocyte counts, plasma HIV-1 RNA levels, history of prior fungal infections, number of antiretroviral agents, and PCP prophylaxis. The association of these factors with oropharyngeal *Candida* species was calculated by Spearman's nonparametric correlation coefficient. Bivariate and multivariable logistic regression analyses were then used to assess these relationships further. Poisson regression analysis was performed by use of *Candida* load as the dependent variable in both bivariate and multivariable analyses. For the Poisson regression analysis, the *Candida* load was categorized as follows: no growth, 1–50 cfu/mL, 51–200 cfu/mL, 201–500 cfu/mL, 501–1500 cfu/mL, and >1500 cfu/mL. Goodness-of-fit was evaluated by Pearson's χ^2 test for each model, as well as by residual plots. One outlier was identified (1533 CD4 cells/μL, CD4/CD8 ratio of 1.13, *Candida* load <25); this subject was influential in the models in which CD4 cell count was used as an independent variable. Since no other data points were in this range, we chose to delete this subject from the final analysis. Statistical analysis was performed by use of SAS software (SAS Institute, Cary, NC).

Results

Patient characteristics. Characteristics of the 83 patients enrolled in the study are shown in table 1. In all, 58 (70%) were colonized with yeast. The only 2 yeast species cultured were *C. albicans* (55 isolates) and *C. glabrata* (5 isolates); 56 patients had a single species isolated.

Association between amount of oropharyngeal Candida and plasma HIV-1 RNA. The relationships between colonization with *Candida* species and previously identified clinical and laboratory parameters were analyzed by use of Spearman's cor-

Table 1. Characteristics of the 83 patients participating in the study.

Parameter	Colonized n = 58 (%)	Not colonized n = 25 (%)
Age (years), median	34 (range, 19–63)	35 (range, 27–77)
Sex		
Women	25 (43)	10 (40)
Men	33 (57)	15 (60)
Pneumocystis carinii prophylaxis		
Yes	17 (29)	8 (32)
No	41 (71)	17 (68)
Previous history of fungal infection		
Yes	21 (36)	6 (24)
No	37 (64)	19 (76)
Current use of antiretroviral agents		
Yes	42 (72)	20 (80)
No	16 (23)	5 (20)
No. of antiretroviral agents		
0	16 (28)	5 (20)
1	0 (0)	1 (4)
2	13 (22)	5 (20)
3	25 (43)	14 (56)
>3	4 (7)	0 (0)
Protease inhibitors	28 (48)	13 (52)
CD4 cells/μL, median	382 (range, 7–860)	381 (range, 44–876)
Plasma HIV-1 RNA (copies/mL), median	5378 (range, 76–553,090)	1298 (range, 90–100,385)
Log_{10} HIV-1 RNA, median	3.73	3.10
Colonization with *Candida albicans*	55/58 (95)	0
Colonization with *Candida*, non-*albicans*	5/58 (9)[a]	0
Candida load (cfu/mL), median	60 (range, 1–1832)	0

NOTE. Data are median (range) for continuous variabled and no. of patients (%) for categorical variables. Cfu, colony-forming units.

[a] Nos. >100% because 2 patients had mixed colonization.

relation coefficients. As shown in table 2, the only predictor of *Candida* load was plasma HIV-1 RNA ($r = .35$, $P = .001$). When only the 58 patients who were colonized with yeasts were analyzed, the correlation with HIV-1 RNA was even stronger, and no other potential predictors reached statistical significance (data not shown). Numbers of yeasts in the oropharynx were generally low in patients with a plasma HIV-1 RNA of <4 $\log_{10}$ copies/mL, but counts rose steeply in persons with higher HIV-1 loads (>4 $\log_{10}$ copies/mL).

By bivariate logistic regression analysis, the presence of *Candida* species in the oropharynx was associated with higher HIV-1 loads (odds ratio, 1.9 for each 1 $\log_{10}$ HIV-1 RNA increase; 95% confidence interval, 1.12–3.09; $P = .016$). No other patient factors, including CD4 cell count, use of PCP prophylaxis, history of fungal infection, and number of antiretroviral agents, were significantly associated with yeast carriage, by multivariate logistic regression (data not shown).

Statistical modeling. After categorization of the *Candida* load into 6 different categories, as described in Materials and Methods, bivariate Poisson regression analysis showed that HIV-1 RNA level was the only variable significantly associated with *Candida* load ($P = .003$). Addition of other parameters to the model did not alter the relationship between *Candida* load and HIV-1 load (data not shown). A final model using 5 variables revealed HIV-1 RNA as the single significant predictor ($P < .001$) of the presence and amount of *Candida* species in the oropharynx (table 2). This model had good statistical fit (Pearson's χ^2, 0.97).

Discussion

In this study, 70% of the patients were colonized with yeast, which is similar to findings studied for asymptomatic HIV-1–infected patients by other investigators [7]. The recent decrease in the incidence of opportunistic infections in HIV-1–infected persons receiving HAART suggests, however, that partial reconstitution of the immune system can result from retroviral suppression. We hypothesized that the yeast carriage in patients with HIV-1 infection was more closely associated with the level of retroviral replication and its effects on mucosal immunity rather than on peripheral blood CD4 lymphocyte counts. This hypothesis was based on several observations. Some patients can have OPC during acute HIV-1 infection syndrome, when virus load measurements are generally very high and CD4 cells are usually relatively preserved [4]. Furthermore, antiretroviral monotherapy is associated with a significant reduction in OPC, despite minimal effects on CD4 cell counts [8]. In addition, recent reports showed resolution of oropharyngeal thrush after initiation of HAART [9, 10], even though CD4 lymphocyte counts remained low (<140 cells/μL) in all patients at the time that infection resolved [10].

Prior to the era of HAART, the presence of yeast in HIV-1–infected patients predicted subsequent development of OPC [3]. We conducted this cross-sectional study to determine whether oropharyngeal carriage of yeast was correlated more strongly with HIV-1 load than with peripheral CD4 cells. As shown in table 2, we found that the quantity of yeasts in the oropharynx was significantly associated with the level of HIV-1 RNA levels but not with the number of CD4 cells. In fact, the HIV-1 load measurement was the only significantly associated variable among those tested. We tried to eliminate factors that may influence the oropharyngeal cultures by excluding persons with recent antifungal use and those who used dentures. We also looked for correlates between the presence of yeast and factors such as age, sex, history of fungal infections, use of PCP prophylaxis, and the number of antiretroviral agents that the patients were receiving at the time of culture. However, none of these factors had a significant association with the

Table 2. Spearman's rank correlation coefficients between yeast colonization ($\log_{10}$ cfu/mL) and demographic and laboratory results of 83 patients, and final model (Poisson regression modeling) using five variables to predict *Candida* colonization.

Parameter	Spearman's rank correlation coefficient	Parameter estimate	*P*
Spearman's rank correlation coefficient			
Age (years)	−0.05		.67
Sex	0.02		.83
PCP prophylaxis	0.11		.34
History of fungal infection	0.15		.18
Any antiretroviral therapy	−0.04		.75
No. of antiretroviral agents	−0.10		.35
CD4 cells/μL	−0.10		.36
HIV-1 RNA ($\log_{10}$ copies/mL)	0.35		.001
Poisson modeling			
HIV RNA ($\log_{10}$ copies/mL)		0.409	<.001
CD4 cells/μL		<.001	.27
History of previous fungal infection		0.142	.49
PCP prophylaxis		0.279	.24
No. of antiretroviral agents		0.029	.71

NOTE. Scaled Pearson's χ^2 for Poisson model was 0.97. PCP, *Pneumocystis carinii* pneumonia.

presence and number of yeasts, in the final statistical model (table 2). Improvements in systemic immune function that occur during HAART [5] may by mirrored at the mucosal surfaces, explaining our findings of an association between HIV-1 load and oropharyngeal yeast colonization. Conceivably, the lowering of viral replication in plasma results in stronger mucosal immunity, but there is no consensus on how to quantitate this improvement. HIV-1 envelope proteins may directly suppress phagocytosis and intracellular killing of *Candida* by neutrophils and monocytes [11, 12]. Accordingly, high degrees of viral replication may facilitate yeast proliferation on the oropharyngeal mucosal surface by suppressing host responses. Local immune function therefore may be partially restored by lowering HIV-1 load.

The absence of *Candida* organisms in the oral cavity of some of the patients demonstrates that other factors, in addition to HIV-1 replication, are important in the pathogenesis of OPC in HIV-1–infected persons. The factors that are also likely to be of importance are genetic, such as expression of certain intercellular adhesion molecules [13], specific receptors for *Candida* species in the host [14], and the balance between Th1 and Th2 responses in mucosal immunity [15].

In conclusion, we found oropharyngeal *Candida* colonization to be more strongly associated with plasma HIV-1 RNA than with peripheral CD4 cell counts or any other potential risk factors studied. We feel that, as a direct result of high levels of HIV-1 replication, the local defenses are compromised, leading to increasing yeast proliferation in the oropharynx. These data also suggest that a decrease in the oropharyngeal carriage of yeast may be accomplished without specific antifungal therapy. Further studies of patients initiating HAART will be of great interest to better define the relationship between HIV-1 load, antiretroviral activity, oropharyngeal yeast carriage, and the development and regression of thrush.

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