ORIGINAL ARTICLE

Asymptomatic oral yeast carriage in HIV-infected patients: frequency and fluconazole susceptibility profile

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Objectives: Fluconazole-resistant oropharyngeal candidiasis (OPC) is a rapidly growing problem in HIV-infected patients. To better understand the pathogenesis of fluconazole resistance in this setting, asymptomatic candidal carriage was determined by means of oral swabs regularly performed in all patients without clinical signs of OPC seen at our HIV outpatient clinic. Controls were 204 asymptomatic healthcare workers without previous exposure to fluconazole.

Methods: Swabs were plated on three solid media and put in a Sabouraud broth. Phenotypically different colonies were identified to the species level. Susceptibility to fluconazole was determined using a disk diffusion test with 50 μg fluconazole disks on yeast nitrogen agar, with a cut-off value of 25 mm.

Results: Swabs were performed in 538 consecutive HIV-positive patients, of whom 216 (40%) had had prior episode(s) of OPC and/or were previously exposed to fluconazole. Yeasts were grown in 418/538 HIV-positive patients (78%), compared to 57/204 controls (28%) ($p < 0.05$). In HIV-positive patients, yeasts were grown in 189/216 (88%) of those with past fluconazole exposure, and in 229/322 (71%) without exposure ($p < 0.05$). A total of 589 isolates were grown in the 538 HIV-positive patients (451 *C. albicans,* 88 *C. glabrata,* 22 *C. tropicalis,* 11 *C. krusei,* and 17 isolates from 12 other species). Resistance to fluconazole was present in 121/589 (21%) *Candida* species isolates in HIV-positive patients and in 2/59 (3%) in controls. Among *C. albicans* isolates, there were 18 fluconazole-resistant strains in HIV-positive patients (4%) and none in controls.

Conclusions: Using sensitive culture methods, oral yeast colonization was detected significantly more frequently in HIV-infected patients (78%) than in a control group of HIV-negative persons (28%). In addition, yeast colonization was quantitatively more important in patients with lower $CD4^+$ lymphocyte counts and for those who had been exposed to fluconazole for episode(s) of OPC. Fluconazole-resistant *C. albicans* isolates were observed only in HIV-positive patients, and all patients (17/18) for whom this information could be ascertained had had prior exposure to fluconazole.

Key words: Oropharyngeal candidiasis, fluconazole, resistance, *Candida albicans, Candida* spp., yeast carriage

Oropharyngeal candidiasis (OPC) is the most frequent HIV-related infection, and develops at some time during the course of HIV illness in up to 90% of the patients [1]. In this context, the efficacy and the safety of fluconazole have been shown to be superior to those of ketoconazole [2], and fluconazole has become the regimen of choice for therapy of OPC in many HIV outpatient clinics. However, we [3,4] and others [5–7] recently observed an increasing number of OPC episodes clinically not responding to fluconazole therapy. *Candida albicans* strains isolated in patients with clinical failure have been shown to have a decreased susceptibility to fluconazole [3,6,7]. Patients with fluconazole-resistant OPC have been previously exposed significantly more often to fluconazole than patients whose episodes of OPC responded to the drug [4,6]. Moreover, using genotyping of consecutive *C. albicans* isolates cultured in the same patients, we showed that resistance was probably acquired under fluconazole exposure by the initial susceptible *C. albicans* isolate [4], a mechanism often – but not always – observed (for review see Rex et al [8]). To confirm and extend these observations, we decided to regularly

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perform oral swabs in all symptomatic or asymptomatic patients followed at our HIV outpatient clinic. Data obtained in patients without signs of OPC at the time of swab collection are presented here.

METHODS

Patients

In our institution, patients are followed by the HIV outpatient clinic at least twice a year, irrespective of the stage of their HIV infection. The following data were prospectively collected at the time of visit and oral swabbing: $CD4^+$ lymphocyte count, previous number of OPC episodes and antifungal therapy, and clinical evidence of active candidiasis.

Controls

There were 204 asymptomatic healthcare workers without previous exposure to fluconazole. There were 66 males and 138 females, and the median age was 28 (range: 16 to 54).

Microbiology

Swabs were performed on anterior and posterior palatal mucosa in each patient, using a standardized procedure. All swabs were plated onto three solid media (Sabouraud dextrose agar, chocolate blood agar, 5% sheep blood agar) and put into a Sabouraud broth. Broths were secondarily plated onto Albicans ID selective and differential medium (bioMerieux, Marcy-L'Etoile, France). Yeasts were identified to genus and species level using conventional procedures (germ tube test and biotyping). Fluconazole susceptibility was tested with an agar disk diffusion method using yeast nitrogen agar with a 50-μg disk of fluconazole. In vitro resistance to fluconazole was defined by diameters of inhibition < 25 mm, which has been shown to correlate with MIC > 25 μg/ml [3]. Our method has shown an excellent correlation with the disk diffusion method developed by Troke [9].

RESULTS

From February 1993 to June 1994, oral swabs were systematically performed in 538 consecutive HIV-positive outpatients without clinical evidence of OPC. There were 443 males and 95 females. HIV infection was acquired through intravenous drug use in 226 patients, homosexual relations in 167, heterosexual relations in 102, and the administration of blood products in 27. The route of HIV infection could not be determined in 16 cases. The median $CD4^+$ cell count was 245/mm^3 (range: 1 to 1500). Of the 538 patients, 216 (40%) had been previously exposed to fluconazole, because of past episode(s) of OPC (214 patients) or cryptococcal infection (2 patients).

Oral yeast colonization was detected in 78% of HIV-infected patients and in 28% of HIV-negative controls (Table 1). Moreover, isolates were retrieved from broth culture only in the majority of HIV-negative persons (74%) with positive cultures, and grew both on primary plates and in broth in most of the

Table 1 Oral yeast carriage in HIV-infected patients without OPC and in HIV-negative controls: quantitative yeast growth characteristics

	HIV-negative		HIV-positive		*P*
Number of subjects	204		538		
Oral yeast carriage	57/204	(28%)	418/538	(78%)	< 0.05
Growth on primary plates	15	(26%)	309	(74%)	< 0.05
Growth in broth only	42		109		
Mixed culture (≥ 2 yeast spp.)	2	(4%)	124	(30%)	< 0.05
Total number of isolates	59		589		
C. albicans	51	(86%)	451	(77%)	= 0.05
Other yeasts	8		138		
C. glabrata	1		88		
C. tropicalis	–		22		
C. krusei	–		11		
Others	7		17		
Fluconazole-resistant isolates	2	(3%)	121	(21%)	< 0.05
C. albicans	0		18		
Other yeasts	2		103		
C. glabrata	1		85		
C. tropicalis	–		1		
C. krusei	–		11		
Others	1		6		

HIV-positive patients (74%) with positive cultures (Table 1). Rates of oral colonization were 88% in HIV-positive patients with past fluconazole exposure and 71% in HIV-positive patients without exposure. Oral yeast colonization was quantitatively more important in patients previously exposed to fluconazole, and with lower $CD4^+$ lymphocyte counts (Table 2). Fifty-nine isolates were recovered in the 57 HIV-negative persons with oral yeast colonization: there were 51 *C. albicans* and 8 other yeasts (3 *Geotrichum* spp, and *C. glabrata, C. norvegiensis, C. valida, Saccharomyces cerevisiae, Trichosporon capitatum*) (1 each). A total of 589 isolates were recovered in the 418 colonized HIV-positive patients [451 *C. albicans*, 88 *C. glabrata*, 22 *C. tropicalis*, 11 *C. krusei*, 4 *Saccharomyces cerevisiae*, 2 *C. parapsilosis*, 2 *Geotrichum* spp., and one isolate each of *C. colliculosa, C. intermedia, C. kefir, C. lusitaniae, C. norvegiensis, Kloeckera apiculata, K. apis, Rhodotorula glutinis, R. rubra* (Table 1)]. Resistance to fluconazole was present in two of the 59 isolates in controls, both belonging to species with known reduced susceptibility to fluconazole (*C. glabrata* and *C. norvegiensis*) (Table 1). In the 418 HIV-positive patients with oral yeast colonization, there were 121 fluconazole-resistant isolates: 103 were of species intrinsically less susceptible to the drug (85 *C. glabrata*, 11 *C. krusei*, 3 *S. cerevisiae*, and 1 isolate each of *C. tropicalis, C. norvegiensis, Rhodotorula glutinis* and *R. rubra*). In addition, there were 18 isolates of *C. albicans* that were resistant to fluconazole: prior exposure to the compound was documented in 17 of them and was not assessable in the last patient (Table 2).

Table 2 Oral yeast carriage in HIV-infected patients: quantitative growth and isolate characteristics according to previous exposure to fluconazole

	Total HIV-positive patients	Previous fluconazole exposure		*P*
		No	Yes	
Number of subjects	538	322	216	
Mean $CD4^+$ lymphocytes	261±10	324±15	180±13	<0.05
Oral yeast carriage	418/538 (78%)	229/322 (71%)	189/216 (88%)	<0.05
Growth on primary plates	309	155	154	<0.05
Growth in broth only	109	74	35	
Mixed culture (≥2 yeast spp.)	124	69	55	NS
Total number of isolates	589	325	264	
Fluconazole-resistant	121 (21%)	55 (17%)	66 (25%)	<0.05
C. albicans	451	258	193	
Fluconazole-resistant	18[a]	0	17 (8.8%)[a]	<0.05

[a] Prior exposition to fluconazole not assessable in one patient.

Of the 189 HIV-infected patients with previous exposure to fluconazole who were found to be colonized with yeasts, all carried at least one *C. albicans* isolate (two different *C. albicans* in four patients), with or without additional non-*C. albicans* yeasts. All patients with a *C. albicans* fluconazole-resistant strain carried it in pure culture, whereas the 49 other non-*C. albicans* yeasts resistant to fluconazole (mostly *C. glabrata*) were found in mixed culture with a fluconazole-susceptible *C. albicans* strain.

DISCUSSION

Candida organisms are normal inhabitants of the oropharyngeal cavity, and isolation rates of yeasts from the mouths of healthy individuals have been reported to range between 2% and 37%, mainly depending on the culture method used [10]. Oral yeast colonization is higher in hospitalized patients, with rates ranging from 13% to 76% [11], but data in HIV-infected patients are limited so far. Sangeorzan et al found 84% of 92 HIV-infected patients to be colonized, mostly (81%) by *C. albicans* [12].

Using sensitive culture methods, we detected oral yeast colonization in the mouths of 57 of 204 healthy HIV-negative persons (28%), as compared to 418 of 538 patients at various stages of their HIV infection (78%). In addition, yeast colonies were significantly more numerous in the latter patients. This observation can be explained by several factors, among which are the elimination of bacterial competitive flora in HIV-positive patients by concurrent antibiotic therapy or prophylaxis for opportunistic infections, the frequency of infection- or drug-related lesions of oral mucosa, and the underlying HIV-related immunodeficiency. Oral yeast colonization was quantitatively and qualitatively more important in HIV-positive patients who had presented prior episode(s) of OPC, and who had lower $CD4^+$ lymphocyte counts. As there is a clear correlation between $CD4^+$ lymphocyte count and the number of OPC episodes [3,6,13], it was not possible to determine the relative importance of these two factors. In addition, yeasts usually cannot be eradicated under treatment for OPC because of the fungistatic effect of azole antifungal drugs and because of the persistence of the underlying HIV-associated immunosuppression. Consequently, recurrences are reported in 50–100% of the patients [14,15]. Due to its efficacy and safety, fluconazole usually represents the first-line therapy for OPC and has been used in over 15×10^6 patients (data on file, Pfizer Inc.). However, chronic exposure to fluconazole has been statistically related to the development of clinical and microbiological resistance to the drug [4,6]. Our work indirectly

confirms these observations, since isolates of *C. albicans* resistant to fluconazole were cultured only in HIV-positive patients, all but one of them with known prior exposure to fluconazole.

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