Clinical Evaluation and Microbiology of Oropharyngeal Infection Due to Fluconazole-Resistant *Candida* in Human Immunodeficiency Virus–Infected Patients


Signs and symptoms of oropharyngeal candidiasis (OPC) were correlated with microbiology and clinical response to fluconazole in a cohort of patients with advanced human immunodeficiency virus (HIV) infection and recurrent OPC. Sixty-four HIV-infected patients with a median CD4 cell count of <50/mm³ (range, 3–318/mm³) who presented with OPC were enrolled in a longitudinal study. Specimens for cultures were taken weekly until clinical resolution. Therapy with fluconazole was increased weekly as required to a maximum daily dose of 800 mg until resolution of symptoms and oral lesions. Resistant or dose-dependent susceptible yeasts, defined as a minimum inhibitory concentration of ≥16 μg/mL, were detected in 48 (31%) of 155 episodes. Clinical resolution with fluconazole therapy occurred in 107 (100%) of 107 episodes with susceptible yeasts vs. 44 (92%) of 48 episodes with resistant or dose-dependent susceptible strains (P = .008). Patients from whom fluconazole-resistant yeasts were isolated required longer courses of therapy and higher doses of fluconazole for response, but overall, excellent responses to fluconazole were seen in patients with advanced HIV infection.

Oropharyngeal candidiasis (OPC) is the most common fungal infection in patients infected with HIV. Currently, there are many effective therapeutic options for thrush. The newer azole antifungal compounds are commonly used because of the high rate of efficacy, ease of administration, and low levels of toxicity [1]. Fluconazole, the most commonly used newer azole, is an extremely effective, very well-tolerated, and simple treatment [1–3]. As a result, fluconazole has been widely used to treat OPC, both for acute episodes (intermittent therapy) and for prophylaxis (continuous therapy) [2, 3].

The most common organism isolated is *Candida albicans*, which is usually exquisitely susceptible to fluconazole, with MIC₉₀ values for isolates of ≤1 μg/mL [4]. Some non-*albicans* yeasts have been noted to have much higher rates of resistance, including *Candida glabrata* and *Candida krusei*, which are being increasingly isolated [5]. With the widespread use of fluconazole in treating and preventing yeast infections, clinical resistance is becoming a serious problem [6, 7]. In addition, recently there have been several reports of *C. albicans* with decreased susceptibility to fluconazole [8–10].

Correlation of in vitro and in vivo resistance continues to be evaluated. In this study, we used a novel chromogenic agar dilution method to prospectively screen for yeasts with decreased fluconazole susceptibility and to establish the microbiology of OPC in a cohort of patients with advanced HIV infection longitudinally followed up for recurrent OPC. Signs, symptoms, and response to fluconazole in HIV-positive patients with episodes of OPC with or without resistant isolates were evaluated.

**Patients and Methods**

HIV-infected patients receiving health care at the University of Texas Health Science Center at San Antonio and the South Texas Veterans Health Care System, Audie L. Murphy Division, San Antonio, Texas, were evaluated in a longitudinal study of OPC. Enrollment criteria were evidence of active thrush by examination of a potassium hydroxide preparation and culture, a CD4 cell count of <350/mm³, and no current therapy with any azole compound for a preexisting fungal infection. Symptoms were assessed as absent (none present), mild (altered taste, dry mouth, and/or minimal pain), moderate (extensive pain), or severe (unable to eat or swallow). The extent of lesions was recorded as absent (none), mild (single lesion), moderate (multiple lesions), or severe (confluent lesions or generalized erythema).

Clinical samples were obtained weekly during therapy and quarterly for surveillance cultures by swabbing individual le-
sions and by patients swishing 10 mL of normal saline and spitting. One hundred microliters of swish solution was plated on media with or without fluconazole and incubated at 30°C for 48 hours before growth was assessed. Three to five colonies from each sample were submitted for determination of MICs by a macrobroth method according to a standard of the National Committee for Clinical Laboratory Standards (NCCLS) [11] to correlate with appearance on fluconazole-containing media as either susceptible or resistant. CHROMagar Candida medium (CHROMagar Company, Paris) with fluconazole was used to improve detection of non- _C. albicans_ species and resistant isolates [10, 12]. Resistance was defined as an MIC of $\geq 16 \mu g/mL$ by the NCCLS macrobroth method.

Clinical evaluation was performed by the same health provider. Therapy consisted of fluconazole tablets (200 mg on the first day and 100 mg for 6 days) and was continued until complete resolution of symptoms and oral lesions (clinical response). The fluconazole dose was increased weekly as required to a maximum daily dose of 800 mg. Patients with recurrent episodes began treatment with the dose of fluconazole that was effective for the previous episode of OPC. A microbiological response was defined as sterile cultures at the end of treatment.

The $\chi^2$ test (Epi-Info Version 6, Centers for Disease Control and Prevention, Atlanta) and Student’s $t$-test (Microsoft Excel 5.0, Microsoft, Redmond, WA) were used where needed.

**Results**

Sixty-four HIV-infected patients presenting with OPC were enrolled in a longitudinal study. There was not complete documentation of at least one episode of OPC in six patients, and these patients were excluded from analysis. One hundred fifty-five episodes of OPC were identified in 58 patients. The median CD4 cell count for the 58 patients evaluated was 30/mm$^3$ (range, 4–318/mm$^3$); 56 of 58 patients had a CD4 cell count of $<$200/mm$^3$.

At presentation, symptoms were assessed as none in 7 episodes, mild in 23, moderate in 73, and severe in 52. At presentation, the extent of disease was recorded as absent in 4 episodes, mild in 10, moderate in 103, and severe in 38. OPC resolved in 1 week in 72 episodes, 2 weeks in 50, 3 weeks in 21, and $\geq4$ weeks in 12. Clinical response to fluconazole occurred in 151 (97%) of 155 episodes. In the 151 episodes responding to fluconazole, 94 (62%) required 100 mg/d, 35 (23%) required 200 mg/d, 9 (6%) required 300–400 mg/d, 2 (1%) required 500–600 mg/d, and 11 (7%) required 800 mg/d. Clinical response to fluconazole occurred in 107 (100%) of 107 episodes with fluconazole-susceptible yeasts (MIC, $\leq 8 \mu g/mL$) vs. 44 (92%) of 48 episodes with yeasts for which the MIC was $\geq 16 \mu g/mL$ ($P = .008$).

Outcomes according to the maximal MIC for each episode are presented in figure 1. The only clinical failures occurred in episodes with MICs of fluconazole for yeast isolates of $\geq 64 \mu g/mL$. In addition, microbiological responses were seen almost exclusively in episodes where the maximal MIC was $< 8 \mu g/mL$, even though in episodes with MICs of $\leq 1 \mu g/mL$, the rate of microbiological response was only 50%.

The issue of whether a microbiological response affects the time to relapse was assessed in 90 episodes with documented relapses. The mean time to relapse for episodes with only a clinical response was 52 days (range, 17–155 days; median, 45 days) vs. 63 days (range, 19–191 days; median, 49 days) for episodes with both clinical and microbiological responses ($P = .21$).

Resistant yeasts, defined as an MIC of $\geq 16 \mu g/mL$, were detected in 48 (31%) of 155 episodes: 22 (46%) with resistant _C. albicans_, 16 (33%) with resistant non-_C. albicans_ species, and 10 (21%) with mixed resistant yeasts. Symptoms were moderate to severe in 84 (79%) of 107 episodes with susceptible yeasts vs. 41 (85%) of 48 episodes with resistant strains ($P = .43$). Moderate to severe infection occurred in 94 (88%) of 107 episodes with susceptible strains and 47 (98%) of 48 episodes with resistant strains ($P = .06$). Clinical resolution occurred with 100 mg of fluconazole/d in 79 (74%) of 107 episodes with only susceptible isolates vs. 15 (31%) of 48 episodes with resistant isolates ($P < .001$); clinical resolution occurred within 2 weeks in 93 (87%) of 107 episodes with susceptible yeasts vs. 26 (54%) of 48 episodes with resistant strains ($P < .001$). The mean daily dose of fluconazole used in episodes with only susceptible yeasts was 134 mg (median, 100 mg) vs. 350 mg (median, 200 mg) for episodes with resistant yeasts ($P < .001$). When comparing episodes with resistant _C. albicans_ vs. those with resistant non-_C. albicans_ species (with
susceptible *C. albicans*), there was no difference in the dose (*P* = .48) or duration (*P* = .79) of fluconazole required for therapy.

**Discussion**

Therapy for OPC in HIV-infected patients has become simple and highly effective since the introduction of fluconazole. As a result, fluconazole has been widely used for both intermittent therapy and prophylaxis for OPC [2, 3]. Recently, yeast isolates with decreased susceptibility to fluconazole have been an increasing problem in the treatment of OPC in HIV-infected patients, especially that due to *C. albicans*, although non–*albicans* species are also frequently isolated [5, 7, 10, 13, 14]. Clinical resistance due to these isolates has been the subject of several reports [9, 10, 15–17]. We analyzed our experience with 155 well-documented episodes of OPC in HIV-infected patients in a prospective study.

Antifungal susceptibility testing is an important issue in considering fluconazole resistance in *Candida* species and has been difficult to standardize, although NCCLS guidelines have been approved for standard yeast susceptibility testing [11]. Correlation between in vitro susceptibility and clinical response has been the focus of much investigation, which suggests that in cases of OPC dose-dependent clinical responses occur [18]. Recently, the NCCLS has reported a study on interpreting MICs of fluconazole associated with clinical resistance [18]. Recommended susceptibility breakpoints are an MIC of ≤8 μg/mL as susceptible, an MIC of 16–32 μg/mL as susceptible but dose-dependent, and an MIC of ≥64 μg/mL as resistant [18]. In this study, an MIC of 16 μg/mL was used because clinical resistance may occur with 100 mg/d, a dosage commonly employed for treatment of OPC.

Risk factors for the development of fluconazole-resistant isolates causing OPC in HIV-infected patients include advanced immunosuppression, indicated by low CD4 cell counts, and prior fluconazole use [6, 9, 10, 13]. In addition, total use of >10 g of fluconazole has been significantly associated with the development of resistance [10, 13].

In our experience with late-stage HIV infection, we noted a high prevalence of resistant yeasts by using chromogenic media with fluconazole for screening cultures; this finding was confirmed with NCCLS macrobroth testing. Despite the frequent isolation of yeasts for which MICs were >16 μg/mL, almost all patients responded clinically to fluconazole at daily doses of up to 800 mg. The presence of resistant isolates did not significantly affect the severity of symptoms or the extent of lesions at initial presentation. However, there was a trend toward more severe disease with the presence of resistant isolates.

Clinical resistance leading to failure of 800 mg of fluconazole/d was rare, seen in only four (2.6%) of 155 episodes. In all four of these episodes, isolates for which MICs of fluconazole were ≥64 μg/mL were recovered. Although several reports have commented on the frequent isolation of resistant yeast isolates in this setting, there are relatively fewer reports of failure of high doses of fluconazole. Episodes requiring ≥200 mg of fluconazole/d for resolution were quite common, seen in 57 (37%) of 155 episodes. Since fluconazole is very well tolerated, use of high doses appears reasonable and is more efficacious in the treatment of episodes with resistant isolates. However, the use of an itraconazole solution is another option for patients for whom high doses of fluconazole have failed, as not all isolates are cross-resistant [19].

Patients from whom resistant isolates (*C. albicans* or non–*albicans* species) were recovered were more likely to require higher doses of fluconazole and require longer courses of treatment than were patients from whom only susceptible isolates were recovered. However, clinical response to 100 mg of fluconazole/d was seen in 22% of episodes with yeast isolates for which MICs were >64 μg/mL. In these episodes, the predominant isolates were susceptible and more likely responsible for the clinical response. It is interesting that there was no significant difference in the dose or duration of therapy required when comparing episodes with either resistant *C. albicans* or resistant non–*albicans* species (with susceptible *C. albicans*) alone in culture, although the number of episodes with resistant non–*albicans* species and susceptible *C. albicans* was small. However, persistence of infection for >2 weeks suggests the presence of resistant yeasts, with clinical implications of increasing the fluconazole dose for clinical response. It may also be reasonable to consider persistence of infection as an indication that susceptibility testing may be warranted.

Another finding of our study suggests that the goal of sterile cultures during therapy for OPC in HIV-infected patients may not be clinically useful in preventing or delaying relapses during intermittent therapy with fluconazole. It should also be emphasized that sterile cultures were achieved only in one-half of the episodes, even those with very susceptible isolates. In addition, a sterile culture was not a consistent outcome of treatment, even for the same patient for whom MICs were stable. Sterile cultures at the end of therapy were unlikely in episodes with yeast isolates for which cultures revealed MICs of ≥8 μg/mL.

In summary, fluconazole at daily doses of up to 800 mg is highly effective therapy for OPC in HIV-infected patients, even in cases where yeast isolates with decreased susceptibility to fluconazole are present. There is a strong correlation with the presence of resistant isolates as determined with NCCLS methodology and the requirement of higher doses of fluconazole for clinical resolution of OPC. Clinical symptoms do not appear to be affected by the presence of resistant isolates, although there may be a trend toward moderate to severe disease being more common in cases where resistant isolates are present. Finally, microbiological response may not be important in determining the time to relapse after successful treatment with fluconazole.
References


