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Original Article

Effectiveness of Fluconazole for Suppressive Maintenance Therapy in Patients with RVVC: a Randomized Placebo-Controlled Study

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Abstract

Recurrent vulvovaginal candidiasis (RVVC) is seen in 5% of women with *Candida* vaginitis. Use of fluconazole as prophylactic treatment has been suggested for RVVC. The aim of the present study was to evaluate the efficacy of fluconazole suppressive therapy in RVVC patients, as a randomized, placebo-controlled, double-blind prophylactic study. Among the 330 women with acute symptomatic vulvovaginal candidiasis referred to Mirza-kouchak Khan gynecology hospital, 64 eligible subjects with RVVC were enrolled. Then, all of them were treated with fluconazole (150 mg orally every 3 days, for three doses). This was followed by microscopical and clinical examination. Next, patients were randomly divided into two groups. In the fluconazole group (n = 32), patients received fluconazole 150 mg, per week for 6 months and in the control group (n = 32) they received placebo. All the patients were revisited on a monthly basis and at the end of treatment, as well as 3 and 6 months after treatment. At the end of treatment, the frequency of positive culture in fluconazole group was significantly lower than the placebo group (25% vs. 62.5%, P = 0.05). Furthermore, the rate of clinical recurrence was significantly lower in the fluconazole group,with respect to the placebo group (18.8% vs. 50%, P = 0.017). However, following band 6 months after treatment, patients who received fluconazole maintenance therapy had a non-significant differences, compared to the placebo group, based on the rate of clinical recurrence or the frequency of positive cultures. Resistance to fluconazole (MIC ≥ 64) was comparable between groups. Despite the fact that fluconazole is well tolerated by the patients, suppressive treatment with fluconazole in RVVC patients had an insufficient effect on prevention from recurrence of clinical signs and the improvement of vaginal mycological status in long term (3-6 months after treatment).

Keywords: Fluconazole; Suppressive therapy; Recurrent vulvovaginal candidiasis (RVVC); Randomized clinical study.

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Introduction

It has been estimated that 75% of all adult women experience at least one episode of vulvovaginal candidiasis (VVC) in their lifetime. Recurrent vulvovaginal candidiasis (RVVC) occurs in approximately 5% of women with *Candida* vaginitis, which is defined usually as ≥4 episodes of VVC that occur within a 12-month period and may disrupt seriously a woman's social and sexual life and frequently leads to frustration (1-4). Known predisposing host factors, which include uncontrolled diabetes mellitus, contraceptive use, compromise of the immune system, pregnancy, and hormone replacement therapy, only partially explain RVVC. It has been also suggested that the administration of broad-spectrum antibiotics is a risk factor for both acute and recurrent VVC (5-7).

Knowledge about pharmacological properties of drugs used in the treatment of vulvovaginal candidiasis allows for individual therapy to each patient. Antifungal agents are available for oral and intra-vaginal treatment of uncomplicated vulvovaginal candidiasis. Patients are more likely to prefer oral versus intra-vaginal anti-fungals (8, 9). The triazoles family is the most widely used antifungal agents today. The drugs in this class offer activity against many systemic fungal infections without the serious nephrotoxic effects observed with amphotericin B administration. There are currently four members of triazole class licensed for use in the United States (Fluconazole, itraconazole, voriconazole and posaconazole). In addition, there are three compounds in various phases of clinical development (ravuconazole, albaconazole, and isavuconazole or BAL8557) (10-12).

Fluconazole is a modern and up to date option for treatment of VVC and RVVC. Based on previous studies, after the ingestion of a single 150 mg tablet of fluconazole, concentrations of fluconazole above the minimal inhibitory concentration (MIC) that inhibits the growth of 90 percent of *Candida* species isolates (MIC_{90}) are achieved for 72 to 96 h in vaginal tissue and secretions, an efficacy that allows for weekly administration (13-15). The treatment of patients with RVVC consists of fluconazole (150 mg every 72 h for three doses). Patients should then be maintained on a suppressive dose of fluconazole (150 mg weekly) for six months (16). After on interruption treatment regimen of 6 months with weekly 150 mg oral fluconazole, the relapse rate increases again and 4-5 out of every 10 women could experience recurrence a year after the start of therapy (2).

Studies comparing fluconazole with ketoconazole and topical antifungal agents, such as clotrimazole and miconazole, have documented that fluconazole could be equally efficacious with minimal adverse effects (17). Several high reliability clinical trials have confirmed short- and long-term therapeutic efficacy of fluconazole. Most of these trials have used a single-dose of fluconazole, which would theoretically lead to a high degree of medication compliance. A wide range of single therapeutic dose, good tolerance and a high degree of patient acceptance gives the specialist a powerful and efficient tool for management of VVC/RVVC (8, 17-21). The objective of this prospective and randomized clinical study was to evaluate fluconazole efficacy in the treatment of recurrent VVC versus placebo.

Experimental

This study was performed between March 2006 and October 2008 at the Mirza-kouchak Khan gynecology hospital, Tehran University of Medical Sciences and included 330 out-patients with active and acute *Candida* vaginitis.

Selection of patients

Inclusion criteria

Female outpatients aged 18-45 years who had an acute, symptomatic, culture-confirmed episode of VVC and a history of at least four documented episodes of *Candida* vaginitis in the previous 12 months (including the current episode) were enrolled. All patients provided a written informed consent. Among patients who had received antifungal maintenance therapy within the previous 12 months, a history of at least 4 episodes of vaginal candidiasis within a year before the start of maintenance therapy was necessary for inclusion. Patients who previously received prophylactic treatment were allowed

to participate, provided they had not been using local or systemic antimycotic medication during the previous four weeks or had not used any other vaginal medication or vaginal douching during the last 48 h.

Exclusion criteria

Patients with negative *Candida* cultures or with diabetes mellitus, either by history or by testing at entry, pregnant women and women who intended to become pregnant during the study were not allowed to participate. Other exclusion criteria were mixed infections, known seropositivity for the human immunodeficiency virus (HIV), and receipt of antifungal agents in the previous four weeks. Patients were prohibited to use any antibiotics or antimycotics, besides the study medication, during the study period.

Data collection

Of the 330 women with acute symptomatic vulvovaginal candidiasis, 97 patients with entry criteria were enrolled, and 33 patients were excluded (20 patients during the treatment, 12 women during the follow-up period and 1 participant because of pregnancy).

At enrollment, a detailed medical history was collected, a pelvic examination was performed, two microscopic examination of vaginal secretions with the use of 10% potassium hydroxide and normal saline solution and two vaginal fungal cultures were obtained. Swab sample from the vaginal fluid was placed on two microscope slide. One slide was used for confirmatory wet mount reading after rehydration with saline solution.

Wet mount should be examined for evidence of coexisting trichomoniasis or bacterial vaginitis. A single drop of 10% potassium hydroxide (KOH) solution was then added to the specimen on the other slide. The KOH dissolves the skin cells, but leaves the *Candida* cells intact, permitting visualization of hyphae and yeast cells which are typical of many *Candida* species (22-24). Samples of patients with a positive microscopic finding were sent for culturing. Final confirmation of the diagnosis depended on positive *Candida* cultures, which were investigated for the formation of chlamydospores and germ tubes (1). All patients who were eligible for inclusion, received three sequential 150 mg doses of fluconazole, orally, as an induction dose at 72 h intervals (induction phase) and were asked to return for evaluation two weeks after enrollment. At the first visit, symptoms had to be normalized, and microscopic studies as well as *Candida* cultures had to be negative to proceed to the maintenance period. Eligible subjects were randomized at a ratio of 1:1 to receive either a single oral, 150 mg dose of fluconazole (n = 32) or an oral placebo (n = 32) tablet weekly for six months (suppression or maintenance phase). Patients were prohibited from using other antifungal, broad-spectrum antibiotics or topical steroid therapies at any time during the study. During the maintenance period, patients were seen monthly so that symptoms and possible side effects of the medication could be registered, a pelvic examination was performed and microscopic studies as well as cultures were also conducted. Patients who were still free of symptoms and without signs of *Candida* on microscopic study and culture, remained in the study until the next visit. At the end of maintenance therapy, patients had a visit and were followed without treatment for six months (observation phase). Patients were seen at months 9 and 12, during the observation phase and were checked for symptoms, as well as microscopic or cultural evidence of *Candida* and fluconazole resistance in isolates of *Candida albicans*. In vitro susceptibilities of *Candida albicans* isolates to fluconazole were determined by the broth microdilution method (M27-A2) based on to the Clinical Laboratory Standards Institute (CLSI) guidelines. The Minimal Inhibitory Concentration (MIC) (the lowest concentration of antifungal that completely inhibits 50 percent growth of the organism) was determined. Isolates were considered susceptible, when the MIC value was $\leq$ 8 μg/mL for fluconazole, whereas isolates with MIC $\geq$64 μg/mL were considered to be resistant (25).

Statistical analysis

Descriptive statistics were described as mean ± SE or as frequencies and associated percentages.

The 95% confidence interval (95% CI) was calculated. A p-value <0.05 was used to denote statistical significance. Patients were well

Table 1. Microbiology of baseline vaginal fungal isolates in patient.

Organism	Frequency (n)	Percent (%)
Candida albicans	50	78.13
Candida glabrata	10	15.63
Candida parapsilosis	2	3.12
Candida guikrmordi	1	1.56
Candida krusei	1	1.56
Total	64	100

matched in both treatment groups. Quantitative variables were compared with the student t-test. For the comparison of independent and qualitative variables between two groups, the $\chi 2$ test or Fisher's exact test were applied. Statistical calculations were carried out using the SPSS for Windows Version 15.0 software.

Results

Based on the results, the average age of participants was 31.9 ± 6.9 years (range, 18 to 45 years). The results of microbiologic analysis of the baseline vaginal isolates from the 64 patients are summarized in Table 1. The majority of baseline vaginal yeast isolates were identified as *Candida. albicans* (78.13%), and the next most common species were *Candida glabrata* (15.63%). Mean ± SE of age and parity of fluconazole and placebo groups who participated in this study are compared in Table 2. The difference was not statistically significant ($P > 0.05$). For comparative study, patients who were randomly assigned to receive fluconazole and those randomly assigned to receive placebo were similar, regarding age and the number of live births. At the observation period, immediately after the cessation of therapy, frequency of patients with clinical recurrence and positive culture in the fluconazole group was significantly lower than the placebo group ($P = 0.017$ and $P = 0.05$, respectively) (Table 3). Resistance of isolates to fluconazole between two groups was not meaningful ($P > 0.05$).

Discussion

According to the results of this study, the average age of participants was 31.9 ± 6.9 years (range, 18 to 45 years). Vulvovaginal candidiasis occurs with the highest prevalence in women aged 20-30 years (26-28). It seems that the mean age in this study was high. The mean age of patients in Sobel et al study (14) was 33.8 years. Previous epidemiologic studies have indicated that women who are aged less than 45 years are at increased risk of VVC (29).

Table 2. Comparison of mean ± SE of age and parity between fluconazole and placebo groups (n=32).

Variable	Fluconazole	Placebo	P-value
Age (years)	31.9 ± 7.5	31.8 ± 6.4	0.93
Parity	2.34 ± 1.42	1.88 ± 1.04	0.14

No significant inter-group differences in the mean of age and parity of the two groups were observed ($P > 0.05$). *Candida albicans* was the most common species among the isolates, followed by *Candida glabrata.* Other *Candida* species isolated were *Candida parapsilosis, Candida guikrmordi* and *Candida krusei,* respectively.

Based on our data, immediately after the cessation of therapy, 6 out of every 32 patients receiving fluconazole (18.8%) were without a clinical recurrence, as compared with the 16 out of every 32 patients receiving placebo (50%) (Table 3). Eight out of every 32 patients in the fluconazole group (25%) had a positive culture versus the 20 out of every 32 of those who received placebo (62.5%) (Table 3). However, the differences in frequency of patients with clinical recurrence and positive culture in the fluconazole and placebo groups in months 3 and 6 after weaning of the medication were not statistically significant. Although at these months, more symptomatic episodes of VVC were observed in patients who had previously been protected by fluconazole than in those who had received placebo. Hence, clinical recurrence occurred 43.8% and 71.9% at 3 and 6 months after ending of maintenance phase, respectively, versus 62.5% and 81.3% in the placebo group. The rate of a positive vaginal culture in the observation phase was significantly higher in the group that had previously been treated by fluconazole (62.5% and 78.1% in months 9 and 12 of study, respectively, versus 78.1% and 87.5% in the placebo group). The results

Table 3. Comparison of treatment outcome between fluconazole and placebo groups immediately, three-months and six-months after the cessation of therapy.

Observation period	Variable	Number of patients		P-value*
		Fluconazole group (n = 32)	Placebo group (n = 32)	
Immediately after the cessation of therapy	Positive culture	8 (25%)	20 (62.5%)	0.05
	Clinical recurrence	6 (18.8%)	16 (50%)	0.017
3 months after the cessation of therapy	Positive culture	20 (62.5%)	25 (78.1%)	0.247
	Clinical recurrence	14 (43.8%)	20 (62.5%)	0.210
6 months after the cessation of therapy	Positive culture	25 (78.1%)	28 (87.5%)	0.509
	Clinical recurrence	23 (71.9%)	26 (81.3%)	0.556

*P-values of <0.05 were considered statistically significant.

of Sobel et al study (14) showed that, with the use of fluconazole in a weekly regimen, 90% of women were free of clinical signs after 6 months; however, after cessation of therapy, the recurrence rate steeply rose to 57% at 1 year, although still less than in the placebo group (78%). In their study, mycologic recurrence occurred in 30% and 70% after 6 and 12 months of treatment, respectively, versus 75% and 85% of patients who received placebo.

In a clinical trial, 50 women with chronic vulvovaginal candidiasis caused by *Candida albicans* with three or more recurrences per year were enrolled in an open-trial prospective study and fluconazole with dose of 150 mg (3 caps. x 50 mg) once weekly for a period of 6 months was prescribed. Clinical improvement was observed in 81% of the patients after treatment and microbiological cure was observed in 86% of the patients (30). In another study, fluconazole (150 mg per month), has been found to decrease the recurrence rate by 50% (31).

Most studies recommend antifungal therapy for six months. Many women have recurrences after cessation of medication. Thus, these patients may need to stay on medication for a longer period of time (22). Randomized controlled trials, for treatment of recurrent VVC, have shown the effectiveness of oral fluconazole and itraconazole maintenance therapy taken for 6 months after an initial regimen (32-33). Results of this study and findings of Sobel et al have shown the effectiveness of oral fluconazole administration in reducing the recurrence rate of vulvovaginal candidiasis immediately after cessation of therapy.

Based on the findings of this study, antifungal susceptibility testing revealed that 70% of the *Candida* isolates tested were resistant to fluconazole, but resistance of isolates to fluconazole between the two groups was not statistically significant ($P > 0.05$).

Treatmeant of vulvovaginal candidiasis frequently fails due to the emergence of azole resistance. In a study, fluconazole resistance has been reported in 14 out of 393 (3.6%) *Candida albicans* isolates, whilst resistance to itraconazole was considerably higher (16.2%) (34-38). The pharmacokinetic characteristics of fluconazole and safety of weekly administration are major factors contributing to its overall clinical success (14). Since fluconazole is administered orally, treatment compliance is better than those medications, administered intravaginally (22). Patients treated with fluconazole report headache, abdominal pain and nausea (39). In our study, side effects during the treatment were not significant and might not be directly correlated with the administration of fluconazole. There was no cessation of therapy

due to side effects of fluconazole. Fluconazole is easily taken, well tolerated and is suitable for the long-term treatment of chronic vulvovaginal candididasis. A concern about long-term therapy has been the probability of the emergence of higher frequency of azole resistance in isolates of *Candida albicans* or more frequent isolation of species other than *Candida albicans* (14).

Conclusion

In this randomized clinical study, we evaluated the effectiveness of fluconazole for suppressive maintenance therapy in patients with RVVC versus placebo. Based on our results, by administration of fluconazole 150 mg per week as maintenance therapy for 6 months, therapeutic cures (clinically and microbiologically) were achieved in fluconazole group at the end of treatment. However, three-months and six-months after treatment, patients who received fluconazole maintenance therapy had a non-significant difference, compared to the placebo group. Recurrence of clinical sings, as well as a positive vaginal *Candida* culture were seen in 60%-70% of the participants. Although several clinical trials have confirmed short- and long-term therapeutic efficacy of fluconazole, nevertheless development of new antifungal agents with minimal adverse effects is recommended to overcome *Candida* infections (40).

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