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Original Article

Fluconazole and Ibuprofen Combination: A Potential Treatment for Mucosal Candidiasis

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ABSTRACT

Background and objectives: The incidence of drug-resistant candidiasis has increased dramatically. This study aimed to evaluate antifungal effects of fluconazole alone and in combination with ibuprofen on isolates from patients with mucosal candidiasis.

Methods: *Candida* species isolates from 142 patients with suspected mucosal (oral and vaginal) candidiasis were identified by culture on CHROMagar *Candida* medium and carbohydrate assimilation test using the API 20CAUX kit. Minimum inhibitory concentration (MIC) of fluconazole alone and in combination with ibuprofen was determined by the broth microdilution method.

Results: Among isolates, 43.7% were identified as *Candida* species (*Candida albicans*, *Candida glabrata*, *Candidia parapsilosis*, *Candida tropicalis*, *Candida guilliermondii* and *Candida kefyr*). The highest rate of fluconazole resistance was observed among *C. albicans* (50%) isolates. MIC_{90} of ibuprofen-fluconazole combination against *C. albicans* isolates was 32 µg/mL, which was 8-fold less than that of fluconazole alone (MIC_{90}=256 µg/mL) ($P<0.01$). Moreover, the MIC_{90} of fluconazole-ibuprofen combination against *C. parapsilosis* isolates was 4-fold less than that of fluconazole alone.

Conclusion: Our results revealed partial fluconazole resistance among *Candida* isolates from patients with mucosal candidiasis. However, the resistance rate decreased 2.5 fold following treatment with the ibuprofen-fluconazole combination. Therefore, it is recommended to further investigate the therapeutic potential of this drug combination for treatment of fungal infections, such as candidiasis.

Keywords: Candida, Fluconazole, Ibuprofen, Antifungal effect

INTRODUCTION

Candidiasis refers to opportunistic fungal infections ranging from simple superficial infections to systemic infections. The infections can appear as acute, sub-acute or chronic in the skin, nails, oral mucosa, vagina, lungs and gastrointestinal tract that may spread to other organs such as kidneys, liver, heart, etc. (1).

Spread of fungal infections and the increasing use of antifungal drugs have dramatically increased the prevalence of resistance to antifungals, such as triazoles. Repeated exposure to antifungal drugs acts as environmental stress that stimulates cellular response to the harmful effects of the drugs, thus allowing sustainable growth of the microorganism. These drug-induced stresses are mediated through signaling pathways (2). Azoles disrupt ergosterol biosynthesis within the membrane by inhibiting fungal cytochrome P_{450}-dependent enzymes, such as lanosterol 14-alpha-demethylase, which leads to senescence or apoptosis (3, 4).

Ibuprofen [alpha-methyl-4-(2-methyl propyl) benzene acetic acid], also known by the brand names Brufen and Nurofen, is a non-steroidal anti-inflammatory drug (NSAID) used as an antipyretic and analgesic to relieve symptoms of arthritis, dysmenorrhea, gout attacks, sport injuries and inflammatory pain. Similar to other NSAIDs, it inhibits the cyclooxygenase enzyme and counteracts inflammatory precursors by reducing prostaglandin production. The World Health Organization has listed this drug as an essential medicine (5,6).

This study aimed to determine and compare the minimum inhibitory concentration (MIC) of fluconazole alone and combined with ibuprofen against *Candida* isolates from patients with oral or vaginal candidiasis.

MATERIALS AND METHODS

In the present cross-sectional study, we collected 142 clinical samples from patients (113 female, age range: 21-75 years) with suspected mucosal candidiasis who were referred to clinical laboratories in Gorgan and Bandar-e Gaz, northern Iran.

In order to identify the isolated microorganisms, swab samples were cultured on Sabouraud dextrose agar (SDA, Merck, Germany) and incubated at 35 °C for 48 hours. After direct microscopic examination using 10% KOH and subculture on SDA medium, isolates were identified with Gram staining, culture on chromogenic medium (CHROMagar, Hi-Media, India) and carbohydrate assimilation test using the API 20CAUX kit (BioMerieux, France).

Susceptibility to antifungal agents was evaluated using the broth microdilution method according to the CLSI-M27-A3 guidelines (7). Fluconazole stock solution (Gibco, Germany) was diluted with water to obtain a concentration range of 2-1024 µg/mL. The prepared fluconazole dilutions were poured into wells of a 96-well microplate containing RPMI 1640 medium (with glutamine, without bicarbonate, and with a pH indicator) and MOPS buffer (Sigma, USA). Next, a yeast suspension (1×10^3 CFU/mL) was inoculated into the wells and the plate was incubated at 35 °C for 48 hours. Finally, MIC values were recorded by reading absorbance at 530 nm using an ELISA microplate reader (Biotec, Germany). According to the CLSI-M27-A3 instructions, *Candida* strains with MIC values of ≤8 µg/mL, 16-32 µg/mL and ≥64 µg/mL were identified as susceptible, susceptible-dose dependent and resistant to fluconazole. In the present study, *Candida albicans* ATCC90028 was used as the control strain.

In order to prepare combination stock, sufficient amounts of fluconazole and ibuprofen powder were dissolved in water to obtain a concentration of 1024 µg/mL. To prepare serial dilutions, 50 µL of combination solution were added to the first well of a 96-well microplate containing 50 µL of RPMI medium. After adding 50 µL of yeast suspension (10^3 CFU/mL) and 48 hours of incubation at 35 °C, MIC values were determined and interpreted.

Data were analyzed using ANOVA and Duncan's multiple range test at a confidence level of 5%. All statistical analyses were performed using SPSS software and charts were drawn with Excel software.

RESULTS

Of 142 isolates, 62 (43.7%) were identified as *Candida* species, 66.1% of which were isolated from patients with vaginal candidiasis. The majority (79%) of *Candida* isolates were *C. albicans* (Figure 1).

The results also showed that 50% of *C. albicans* and 4.8% of *C. parapsilosis* isolates were resistant to fluconazole. The concentration of ibuprofen combined with fluconazole that inhibited 90% of *C. albicans* isolates (MIC_{90}) was 32 µg/mL, which is 8-fold lower than that of fluconazole (MIC_{90}= 256µg/mL) (P<0.01). This rate was also 4-fold for *C. parapsilosis* and *C. glabrata* isolates (Table1).

As expected, the combination of ibuprofen and fluconazole reduced the number of resistant *Candida* isolates and increased the rate of absolute susceptibility. The frequency of fluconazole-resistant *C. albicans* isolates reduced from 63.27% to 24.49% when treated with the same dose of ibuprofen-fluconazole combination (Table 2).

Figure 1. Relative frequency of *Candida* species isolates from patients with mucosal candidiasis

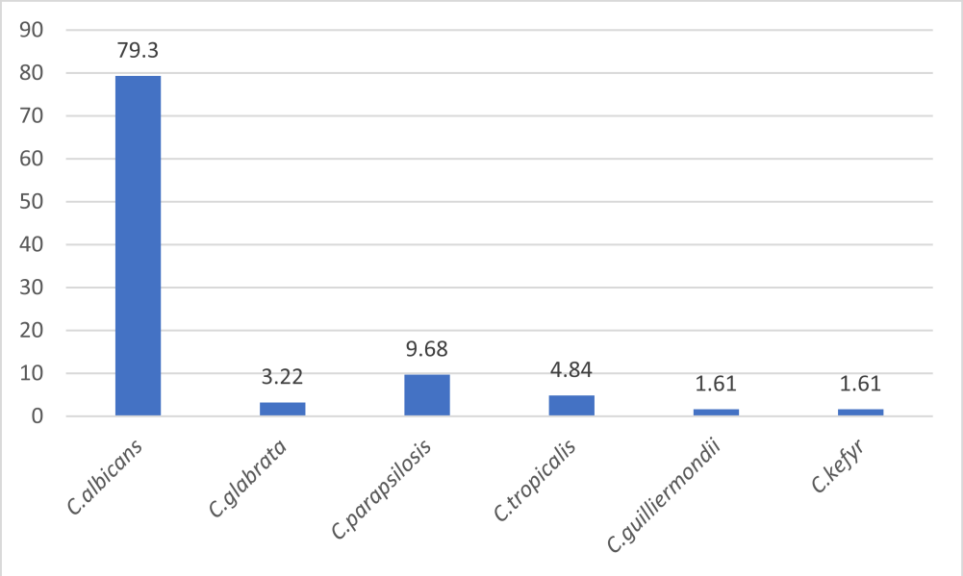

Table1. MICs of ibuprofen and fluconazole combination against *Candida* species

Strains (1×10^3cfu/ml)	MIC_{90}	MIC_{50}	Comparison
C. albicans	32 µg/ml	8 µg/ml	X^2=S
C. glabraata	128 µg/ml	32 µg/ml	X^2=S
C. parapsilosis	64 µg/ml	32 µg/ml	X^2=NS

S: Significant
NS: Not Significant

Table 2. Frequency of resistance to fluconazole and ibuprofen-fluconazole combination among *Candida* isolates

Candida species		Fluconazole	Fluconazole + ibuprofen
C. albicans	Resistant	31(66.3%)	12(24.5%)
	Susceptible-dose dependent	8(16.3%)	0(0)
	Susceptible	10(20.4%)	37(75.5%)
C. glabrata	Resistant	1(50%)	0(0)
	Susceptible-dose dependent	1(50%)	0(0)
	Susceptible	0(0)	2(100%)
C. parapsilosis	Resistant	3(50%)	1(16.7%)
	Susceptible-dose dependent	2(33.3%)	0(0)
	Susceptible	1(16.7%)	5(83.3%)
C. tropicalis	Resistant	1(33.3%)	1(33.3%)
	Susceptible-dose dependent	1(33.3%)	0(0)
	Susceptible	1(33.3%)	2(66.7%)
C. quilliermondii	Resistant	0(0)	0(0)
	Susceptible-dose dependent	1(100%)	0(0)
	Susceptible	0(0)	1(100%)
C. kefyr	Resistant	0(0)	0(0)
	Susceptible-dose dependent	0(0)	0(0)
	Susceptible	1(100%)	1(100%)

DISCUSSION

Prolonged or repeated exposure to antifungal agents is associated with increased emergence of resistant *Candida* strains (8,9). In this study, *C. albicans* (79%), *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. guilliermondii* and *C. kefyr* were the most common *Candida* species isolated from patients with suspected mucosal candidiasis. A previous study in Iran with a larger study population also reported *C. albicans* (66.5%), *C. parapsilosis* (8.6%), *C. tropicalis* (8.2%) and *C. glabrata* (6.1%) as the most common *Candida* species isolated from clinical samples (10). However, the mentioned study did not detect *C. kefyr* among the clinical isolates. In 2007, another study in Iran reported *C. albicans* as the primary cause of vaginal candidiasis (11). In 2011, *C. albicans* was found as the main cause of vaginal candidiasis in women who had been admitted to a hospital in Tonekabon, Iran (12). *C. albicans*, *C. dubliniensis* and *C. glabrata* were the most frequent causes of vulvovaginitis in women referred to a medical center in Arak, Iran (13). In our study, *C. albicans* was also the most common species isolated from vaginal specimens.

The prevalence of azole resistance has risen dramatically since 1990 (2). In the present study, 50% of *C. albicans* and 4.8% of *C. parapsilosis* isolates were resistant to fluconazole. A study in 2004 found no evidence of fluconazole resistance in *C. albicans* or isolates from superinfection with *C. glabrata* (14).

Our results revealed that a large percentage of *Candida* isolates exhibit high resistance against fluconazole. Therefore, we evaluated the antifungal efficacy of fluconazole combined with an NSAID, ibuprofen, against these isolates. According to the results, the antibiotic resistance rate decreased to 22.58% because of the synergistic effect of fluconazole and ibuprofen. In a study on the effectiveness of fluconazole-oral protexin combination for treatment of vaginal candidiasis, there was no synergistic activity observed and both drugs exhibited similar therapeutic efficacy. However, the fluconazole-protexin combination was more effective in relieving symptoms, such as dysuria compared to fluconazole alone (15). Nowrozi and Kazemi reported that ultraviolet radiation could reduce MIC of fluconazole against *Candida* strains (16).

In another study, combination of fluconazole with silver nanoparticles showed significantly higher antifungal activity compared to fluconazole alone against isolates from patients with chronic and recurrent candidal vulvovaginitis (17).

CONCLUSION

Treatment of the isolates with the fluconazole-ibuprofen combination results in a 2.5–fold decrease in drug resistance rate. Given the synergistic effect of these drugs, it is recommended to further investigate the therapeutic potential of this combination for treatment of fungal infections, such as candidiasis.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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