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Original Article

Comparison of the antifungal effect of voriconazole and fluconazole on oral candidiasis before and during radiotherapy

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ABSTRACT

Background: Head-and-neck radiotherapy can change oral *Candida* species and cause candidiasis resistance to common antifungals by making the changes to the oral cavity environment. Voriconazole is a synthetic azole with extensive antifungal activity. The current study aimed at comparing the antifungal activity of fluconazole and voriconazole on *Candida* species isolated from the oral cavity of patients undergoing head-and-neck radiotherapy.

Materials and Methods: The present *in vitro* study was performed on samples isolated from patients undergoing head-and-neck radiotherapy, before and during radiotherapy. After the identification of the species, the antifungal effect of fluconazole and voriconazole was determined by the microdilution method, and the minimum inhibitory concentration (MIC), the minimum fungicidal concentration, and the antifungal susceptibility of the isolated strains were also measured. The data were analyzed by the Chi-squared and then two-sided Fisher's exact tests. $P < 0.05$ was considered statistically significant.

Results: The study findings showed no significant difference in the susceptibility of *Candida albicans* to voriconazole and fluconazole before and during radiotherapy. Before radiotherapy, both voriconazole and fluconazole had similar effects on *Candida tropicalis*, but after radiotherapy, voriconazole was less effective. However, both before and during radiotherapy, fluconazole had a greater antifungal effect than voriconazole on *Candida glabrata* strains. The MICs of voriconazole and fluconazole for both *Candida parapsilosis* and *Candida krusei* isolates were within the susceptible or dose-dependent range.

Conclusion: The current study results showed that voriconazole was not more effective than fluconazole in the treatment of oral candidiasis in patients undergoing head-and-neck radiotherapy.

Key Words: Antifungal agents, candidiasis oral, fluconazole, head-and-neck neoplasms, radiotherapy, voriconazole

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INTRODUCTION

Radiotherapy is one of the main therapies for head-and-neck malignancies with adverse effects on the irradiated field.[1] Radiation to the head-and-neck region increases the pathogenicity of fungal species and causes resistance to routine antifungal agents, in addition to changing and increasing the number of *Candida* species in the oral cavity.[2-7]

Fluconazole, a member of the azole class, is one of the most common antifungal agents used to treat local and systemic oral candidiasis.[8,9] Its mechanism of action is the inhibition of ergosterol production in the fungal cell membrane by inhibiting 14α-demethylase and prohibiting lanosterols demethylation, which disrupt ionic homeostasis of the cell and vacuolar activity, and ultimately, fungal cell death.[10,11] *Candida albicans* and nonalbicans *Candida* species showing resistance to common antifungals, especially fluconazole, are more frequently isolated from patients receiving head-and-neck radiotherapy; the fact that emphasizes the need for further investigations.[6,7,12,13] Singh *et al*., in a study on fungal strains isolated from patients undergoing head-and-neck chemotherapy reported that only 66% of the isolates were susceptible to fluconazole and 33% were resistant, whereas all the isolated strains of *C. albicans* were susceptible to amphotericin B and voriconazole.[14]

Voriconazole is a triazole and synthetic derivative of fluconazole and a slight change in its chemical structure has elevated its antifungal activity compared to fluconazole. Its mechanism of action is similar to those of the other azoles. It is effective against all *Candida* species, including *Candida krusei* and *Candida glabrata* which are intrinsically resistant to antifungals and *C. albicans* strains that acquired resistance to fluconazole.[15,16]

Considering the incidence of oral candidiasis and antifungal resistance during radiotherapy and also the potential inhibitory effect of voriconazole on fluconazole-resistant fungal species, the current study aimed to compare the susceptibility of *Candida* species to fluconazole and voriconazole before and during radiotherapy.

MATERIALS AND METHODS

This *in vitro* study was done between March 2020 and April 2021.

The study samples

Candida species studied in the current study were previously collected and stored at the Department of Medical Parasitology and Mycology, Isfahan University of Medical Sciences (under publishment). These species were isolated from 33 patients in Sayed-al-Shohada Hospital in Isfahan, Iran, before and during head-and-neck radiotherapy (3D conformal method). The sample group consisted of 18 women and 15 men at the beginning of the study (aged between 38 and 74 years). Twenty-one out of 33 patients were *Candida*-positive before treatment. In the 2nd week of radiotherapy, six patients expired (four men and two women), and 19 out of 27 patients were *Candida*-positive. Some patients had more than one *Candida* strain, for example, *C. albicans* and *Candida tropicalis* simultaneously.

The isolated strains were identified by polymerase chain reaction-restriction fragment length polymorphism [Figure 1]. To prepare the fungal suspension, the *Candida* strains were first cultured on Sabouraud Dextrose Agar (SDA) and incubated at 35°C for 24 h.

Laboratory process

A suspension adjusted to match the turbidity standard of 0.5 MacFarland was prepared for each isolated strain, and the light absorption of the prepared suspensions was then adjusted to 530 nm using a spectrophotometer WPA(Wet Process Analyzer) Biowave II wavelength (Biochrom UK).

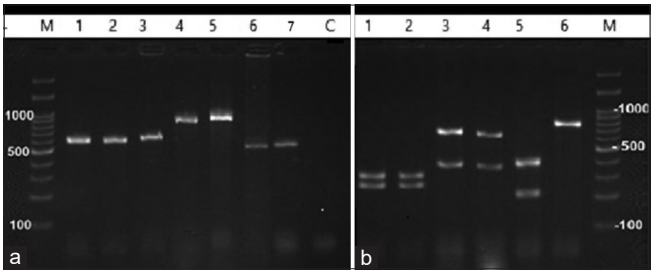

Figure 1: The PCR-RFLP. (a) PCR products of *Candida* isolates on agarose gel electrophoresis. No. 1-3 *C. albicans*, Nos. 4 and 5 *C. glabrata*, No. 6 *C. tropicalis*, No. 7 *C. parapsilosis.* No. 9 Negative Control and M: Marker is 100 pairs; (b) PCR products of *Candida* isolates on agarose gel electrophoresis after endonuclease digestion with the *MSP* I enzyme. No. 1-2 *C. albicans*, Nos. 3–4 *C. glabrata*, No. 5 *C. tropicalis*, No 6 *C. parapsilosis,* M: Marker is 100 pairs. PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism; C. *parapsilosis: Candida parapsilosis; C. albicans: Candida albicans; C. glabrata: Candida glabrata; C. tropicalis: Candida tropicalis.*

The Clinical and Laboratory Standards Institute (CLSI) guidelines were used to prepare the primary stocks of the antifungal agents as 1.280 mg for fluconazole (Sigma-Aldrich; Germany), and 2.3 mg for voriconazole (Merck-Germany) were separately dissolved in 1 ml dimethyl sulfoxide (DMSO; Merck-Germany) and incubated for 30 min at the room temperature to obtain a homogenized stock.[17]

Susceptibility assessment

To evaluate the antifungal activity of fluconazole and voriconazole, their MIC24, MIC48, and minimum fungicidal concentration (MFC) were separately measured for *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei,* and *Candida parapsilosis*.

MICs (minimal inhibitory concentration) of both antifungal agents were determined by the microdilution inhibitory method. For this purpose, the enzyme-linked immunoassay 96-well microplates were utilized; 10 wells were used for 0.5–128 µg/mL of fluconazole and 10 wells for 0.06–16 µg/mL of voriconazole in Roswell Park Memorial Institute (RPMI); two wells were also assigned to positive and negative controls. Then, 100 µL of the fungal suspension provided from each isolated strain was added to each well. Finally, 100 µL of the 1×10^3 fungal suspension plus 100 µL of RPMI was added to the positive control well, and the negative control well was only filled with 100 µL of pure RPMI without drugs and microorganisms according to CLSI-M27.[17] After incubation at 35°C for 24 and 48 h, the turbidity in the wells was evaluated, and the first wells without turbidity after 24 and 48 h were considered the MIC24 and MIC48, respectively.

To determine MFC, 20 µL of the suspension in the MIC well and the following wells were added to SDA plates, and after swab culturing, the plates were incubated for 24–48 h at 35°C. Plates with <5 grown colonies were used to determine MFC.

To determine the breakpoint of fluconazole, species with MIC ≤8 were considered sensitive and the ones with MIC ≥64 resistant; species with 16 < MIC <32 were considered susceptible-dose dependent (SDD). To determine the breakpoint of voriconazole, species with MIC ≤1 were sensitive and the ones with MIC ≥4 resistant; in addition, species with MIC = 2 were considered SDD.

Statistical analysis

Data were analyzed using the SPSS software version 22 (Chicago, IL, USA). To investigate the antifungal effects of fluconazole and voriconazole, their MIC24, MIC48, and MFC were separately measured, and the median, range, and mode were also determined. To compare the antifungal effect of fluconazole and voriconazole, the resistance and susceptibility of the isolates to them were determined. To compare susceptible and resistant species, statistical analysis was performed using the Chi-squared and two-sided Fisher's exact tests. $P < 0.05$ was considered statistically significant.

RESULTS

The studied *Candida* samples from the mycological collection of the Department of Medical Parasitology and Mycology, Isfahan University of Medical Sciences, included 14 *C. albicans*, five *C. tropicalis,* and two *C. glabrata* before radiotherapy, and 12 *C. albicans*, four *C. tropicalis,* two *C. glabrata*, one *C. parapsilosis,* and one *C. krusei* in the 2nd week of radiotherapy.

Table 1 and 2 show the medians and ranges of MIC24, MIC48, and MFC for fluconazole and voriconazole against *C. albicans*, *C. glabrata*, and *C. tropicalis*.

The frequency was not determined for two species of *C. krusei and C. parapsilosis* since only one strain of each was isolated from the patients during radiotherapy. Furthermore, MIC24, MIC48, and MFC of fluconazole and voriconazole before and during radiotherapy could not be compared since neither of the species was isolated before radiotherapy from the patients. According to the findings, MIC24, MIC48, and MFC of voriconazole were 0.03, 0.03, and < 0.03 µg/mL against *C. parapsilosis and* 0.03, 0.03, and 1 µg/mL against *C. krusei,* respectively. MIC24, MIC48, and MFC of fluconazole were 1, >64, and 64 against *C. parapsilosis* and 32, >64, and 64 against *C. krusei,* respectively.

According to the results, 78.5% of the isolated *C. albicans* strains were sensitive or SDD to fluconazole, whereas 100% of such strains were sensitive or SDD to voriconazole. Analysis of the data using the Chi-square and two-sided Fisher's exact tests showed that the susceptibility of *C. albicans* strains to fluconazole had no significant difference from that of voriconazole before radiotherapy ($P = 1$); the same result was obtained in the 2nd week of radiotherapy ($P = 0.7$). In other words, *C. albicans* strains that had similar sensitivity to both agents

before radiotherapy was still the same during the 2nd week of radiotherapy.

Since 100% of the isolated strains of nonalbicans *Candida* species – i.e., *C. glabrata* and *C. tropicalis* – were susceptible to fluconazole before and during the radiotherapy, statistical analyses were not performed in the current study. The frequency of sensitive, resistant, and SDD species is shown in Table 3.

Regarding the two species of *C. krusei and C. parapsilosis* with only one isolate during radiotherapy and no isolates before radiotherapy, the results showed that *C. krusei* was SDD to fluconazole (MIC = 32) and sensitive to voriconazole (MIC = 0.03 μg/mL). *C. parapsilosis* was 100% sensitive to both voriconazole (MIC = 0.03 μg/mL) and fluconazole (MIC = 1 μg/mL).

DISCUSSION

The results of the present study showed no significant difference in the sensitivity of the isolated *C. albicans* strains to fluconazole and voriconazole before and during radiotherapy. Both studied agents had similar antifungal activity against *C. tropicalis* before radiotherapy, but the effectiveness of voriconazole was reduced after radiotherapy. Similarly, the antifungal activity of fluconazole was greater than that of voriconazole against *C. glabrata* strains isolated from patients before and during radiotherapy.

Table 1: Median and range of minimum inhibitory concentration 24, minimum inhibitory concentration 48, and minimum fungicidal concentration (μg/mL) of fluconazole against fungal species isolated from patients before and during radiotherapy

Antifungal activity	Strain, median (range)					
	Candida albicans		*Candida tropicalis*		*Candida glabrata*	
	Before radiotherapy	During radiotherapy	Before radiotherapy	During radiotherapy	Before radiotherapy	During radiotherapy
MIC24	3 (1->64)	2 (0.5->64)	2 (0.5-8)	1 (1-4)	1 (0.5-1)	0.25 (0.25-0.25)
MIC48	>64 (1-65)	>64 (2-65)	>64 (4->64)	2 (2->64)	2 (2-8)	2.25 (0.5-4)
MFC	64 (2->64)	>64 (4->64)	64 (4->64)	3 (2->64)	4 (4-16)	34 (4-64)

MIC: Minimum inhibitory concentration; MFC: Minimum fungicidal concentration

Table 2: Median and range of minimum inhibitory concentration 24, minimum inhibitory concentration 48, and minimum fungicidal concentration (μg/mL) of voriconazole against fungal species isolated from patients before and during radiotherapy

Antifungal activity	Strain, median (range)					
	Candida albicans		*Candida tropicalis*		*Candida glabrata*	
	Before radiotherapy	During radiotherapy	Before radiotherapy	During radiotherapy	Before radiotherapy	During radiotherapy
MIC24	0.3125 (0.03->16)	0.25 (0.03-16)	0.06 (0.03->16)	16 (16-16)	0.155 (0.25-0.6)	1.0150 (0.03-2)
MIC48	1.0625 (0.03->16)	8 (0.03->16)	0.06 (0.03->16)	>16 (16->16)	0.53 (0.06-1)	0.515 (0.03-1)
MFC	12 (0.03->16)	6 (0.03->16)	1 (<0.03->16)	>16 (>16->16)	12 (8-16)	0.515 (0.03-1)

MIC: Minimum inhibitory concentration; MFC: Minimum fungicidal concentration

Table 3: The percent of sensitive, resistant, and susceptible dose-dependent *Candida* spp. to fluconazole and voriconazole before and during the 2nd week of radiotherapy

Percent of susceptibility	Strain											
	Candida albicans				*Candida glabrata*				*Candida tropicalis*			
	Voriconazole		Fluconazole		Voriconazole		Fluconazole		Voriconazole		Fluconazole	
	Before (%)	During (%)	Before (%)	During (%)	Before (%)	During (%)	Before (%)	During (%)	Before (%)	During (%)	Before (%)	During (%)
Sensitive	57.1	58.3	71.4	75	100	50	100	100	80	-	100	100
SDD	42.9	41.7	7.1	16.7	-	50	-	-	-	-	-	-
Resistant	-	-	21.4	8.3	-	-	-	-	20	100	-	-

SDD: Susceptible dose-dependent

In 2005, Bagg showed that the MIC of *C. albicans* strains ranged 0.5–64 μg/mL against fluconazole and 0.016–8 μg/mL against voriconazole;[18] the present study findings were in line with those of Bagg since MIC of isolated *C. albicans* was 0.5−>64 μg/mL against fluconazole and 0.03–16 μg/mL against voriconazole.

Inconsistent with the current study findings, Bulacio *et al*., Bashir and Ahmad, and Belazi *et al*. showed that 100% of the isolated C. *albicans* were sensitive to fluconazole and voriconazole.[19-21] A possible reason for this discrepancy can be the method used to evaluate the antifungal effect of the studied drugs. Bashir and Ahmad and Bulacio *et al*. used the zone of inhibition method[1,2], whereas Balazi utilized E-test to determine the antifungal effect of the drugs[3], which is less sensitive compared to the microdilution method used in the present study.[22]

In the present study, 100% of *C. glabrata* strains were sensitive to both of the studied agents before radiotherapy, whereas 50% were sensitive and 50% SDD to voriconazole after radiotherapy; in terms of fluconazole, the results of both before and during radiotherapy were similar, and 100% of *C. glabrata* strains were sensitive to this drug, and no resistance was observed. Inconsistent with the findings of the present study, Belazi *et al*. and Bashir and Ahmad, in their studies on patients undergoing head-and-neck radiotherapy, showed that 66% and 57.4% of *C. glabrata* strains were resistant to fluconazole, respectively, whereas 100% were sensitive to voriconazole[1,3]. Unlike the present study, Bashir and Ahmad and Belazi *et al*. only evaluated the postradiotherapy isolated strains and did not examine the sensitivity of the preradiotherapy isolated strains[1,3]; similarly, the intrinsic resistance of *C. glabrata* to fluconazole might be the reason for the results of the two mentioned studies, and the resistance of the isolated strains might not correlate with radiotherapy. According to previous studies, *C. glabrata* might be intrinsically resistant to fluconazole or acquire resistance due to prophylactic administration.[23-26]

In the present study, 100% of the *C. tropicalis* strains isolated before and after radiotherapy were sensitive to fluconazole. The results showed that 80% of the isolated strains of this species were sensitive to voriconazole, before radiotherapy and only 20% were resistant; however, 100% of the strains were resistant to voriconazole during radiotherapy. The changes in the resistance pattern of *C. tropicalis* strains to voriconazole during radiotherapy may be due to the impact of environmental changes on the strain's resistance. The radiation therapy could result in azole resistance by morphological and physiological changes in *Candida* species, such as accelerated growth, increased cell adhesion, and increased pseudohyphae and blastopore production.[4]

In a study, Zuza-Alves *et al*. reported that a saline environment may increase the expression of the efflux pump gene in *C. tropicalis* and as a result, increase the resistance of fungi strains to antifungal agents.[27] Consistent with the current study findings, Jahanshiri *et al*. in a study on patients undergoing radiotherapy reported fluconazole as the most effective agent against nonalbicans *Candida* species, including *C. tropicalis*.[28] Furthermore, in a study with *Candida* samples of different parts of the body, 7.7% of tropicalis strains were resistant to voriconazole (R), whereas 100% of these strains were sensitive (S) to fluconazole.[29] Fluconazole inhibits ergosterol biosynthesis in fungal cells by binding to their cytochrome P450 sterol 14α-demethylase.[10,11] Voriconazole has the same mechanism of action but the increased affinity of voriconazole for 14-alpha sterol demethylase makes it useful against some fluconazole-resistant organisms.[15,16] Rubio showed that fungistatic or fungicidal effect of voriconazole against *Candida* spp. is species dependent, the voriconazole has the fungistatic activity against *C. tropicalis*;[30] therefore, according to the results of the present study showing that 100% of *C. tropicalis* strains isolated during radiotherapy were sensitive to fluconazole and resistant to voriconazole, and considering the fungistatic activity of voriconazole, its administration to critically ill immunocompromised patients should be performed cautiously.

One of the limitations of the present study was the small sample size, especially for *C. parapsilosis* and *C. krusei,* which could not be statistically analyzed, and the results were expressed descriptively. The *in vitro* model of the study is another limitation.

It is suggested that further studies be performed to compare the antifungal effect of these two drugs in both *in vitro* and *in vivo* conditions in patients with clinical manifestations of oropharyngeal candidiasis (OPC) and to evaluate the clinical improvement indices.

CONCLUSION

According to the obtained results showing that there were no significant differences in the sensitivity *of C. albicans* to fluconazole and voriconazole, both fluconazole and voriconazole are suggested in the treatment of oral candidiasis caused by *C. albicans* following radiotherapy.

On the other hand, the nonalbicans *Candida* strains evaluated in the current study showed different susceptibility patterns to fluconazole and voriconazole. According to the current study, it is recommended to prescribe fluconazole for oral candidiasis caused by *C. tropicalis, C. glabrata*, and *C. parapsilosis* following radiotherapy, whereas in OPC caused by *C. krusei*, voriconazole is recommended.

Furthermore, it is recommended to prescribe both fluconazole and voriconazole for oral candidiasis caused by *C. albicans* and *C. glabrata* and fluconazole for oral infections caused by *C. tropicalis*.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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