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Original Article

Evaluation and comparison of antifungal effect of voriconazole with nystatin on *candida* species derived from neoplastic patients undergoing maxillofacial radiotherapy

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ABSTRACT

Background: Radiotherapy is a common treatment for head-and-neck malignancies and causes complications such as oral candidiasis and the change of oral *Candida* species from *albicans* to *nonalbicans*. Voriconazole has acceptable antifungal effect. The aim of this study was to determine and compare the antifungal effect of nystatin with voriconazole on these species.

Materials and Methods: The samples used in this *in vitro* study were identified by polymerase chain reaction-restriction fragment length polymorphism from patients before and 2 weeks after head-and-neck radiotherapy in Seyed Al-Shohada Hospital. The antifungal effect of nystatin and voriconazole was determined by microdilution method and measurement of the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration, and the results were analyzed by Mann–Whitney analysis.

Results: The results showed that all species before and after radiotherapy showed 100% sensitivity to nystatin. Prior to radiotherapy, 57.1% of albicans species isolated were in the sensitive range (MIC ≤1) and 42.9% were in the dose-dependent range (MIC = 2) to voriconazole. After radiotherapy, 58.3% of albicans species were in the sensitive range and 41.7% of these species were in the dose-dependent range to voriconazole.

Conclusion: The results of the present study showed that before radiotherapy, all species were sensitive to nystatin, while a percentage of albicans and nonalbicans were resistant to voriconazole. In the 2nd week of radiotherapy similar to prior to radiotherapy, all species isolated from patients were sensitive to nystatin, while a percentage of albicans and nonalbicans were resistant to voriconazole.

Key Words: Neoplastic patients, nystatin, oral candidiasis, radiotherapy, voriconazole

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INTRODUCTION

Radiation therapy may change the quantity and quality of saliva, cause mucositis, and increase

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candidiasis incidence through damaging the salivary glands.[1-5] Various studies on patients receiving head-and-neck radiation therapy since 1990 have shown that both albicans and nonalbicans *Candida* species are involved in oral colonization and candidiasis in radiation-treated patients.[1,4,6] The main causative species of oral candidiasis is *Candida albicans*. However, nonalbicans species also occur as important pathogens in these infections, and concomitant infection with albicans and nonalbicans *Candida* species is more severe and more resistant to treatment.[3,4,7]

Nystatin belongs to the group of polyenes that are antifungal medicines used topically to control oral candidiasis. Polyenes impose antifungal effects by forming pores in the membrane through direct binding to fungal membrane ergosterol, leading to intracellular ionic imbalance, membrane changes, and cell death.[8] Ergosterol is a membrane bio-regulator involved in the integrity of the membrane of fungal cells. Triazole medications, including fluconazole and ketoconazole, dissociate ergosterol by inhibiting 14α-demethylase and cause the sterol precursors to accumulate, resulting in the formation of a membrane with altered structure and function. The antifungal activity of new triazole derivatives, such as voriconazole, is attributed to cytochrome P450 and 14α-sterol demethylase inhibition. The epidemiology of fungal infections is shifting to species naturally resistant to most of the used antifungal therapies.[9] Although some studies have recently reported the resistance of *Candida* species to nystatin, this resistance is generally rare.[10-12]

Resistance to polyenes occurs through three mechanisms, including reducing the total ergosterol content of cells, rearranging or masking existing ergosterol, and exchanging all or part of the sterols bound to polyenes.[13,14] Mechanisms of *Candida* resistance to azoles, such as voriconazole, include changes in the quality or quantity of the target enzyme, restricted access to the target, or a combination of these two routes.[15] Studies have demonstrated that treatment with voriconazole or voriconazole plus fluconazole is effective against recurrent candidiasis infections.[16]

Some alterations might occur in oral *Candida* species following head-and-neck radiation therapy, and species resistant to common antifungal medicines, including nystatin, may emerge. On the other hand, voriconazole has an inhibitory effect on resistant *Candida* species. Therefore, this study aimed to compare the antifungal impacts of nystatin and voriconazole on *Candida* species isolated from patients before and during head-and-neck radiation therapy.

MATERIALS AND METHODS

Type of study: *In vitro* study

Ethical code: IR.MUI.RESEARCH.REC.1398.151.

Candida spp. in the present study were isolated from 33 patients in sayed Al-Shohada Hospital in Isfahan, Iran, before and during head-and-neck radiotherapy. The isolated strains were identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). *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 publish). To prepare the fungal suspension, the *Candida* strains were first cultured on Sabouraud dextrose agar (SDA) and incubated at 35°C for 24 h.

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

According to the Clinical and Laboratory Standards Institute (CLSI) for making the initial stock of the Nystatin and Voriconazole studied, respectively: 12.5 mg nystatin powder (Sigma-Aldrich, Germany) in 1 ml methanoland 3.2 mg voriconazole powder (Merck, Germany)was dissolved in 1 ml of dimethyl sulfoxide (Merck,Germany) and placed at laboratory temperature for 30 min to homogenize the resulting stock.[17]

The MIC (minimum inhibitory concentration) of both drugs were determined by serial dilution method (broth microdilution method). In this method, in 96-micron plates of enzyme-linked immunosorbent assay from 12 wells, ten wells were prepared for concentrations of 0.58–128 μg/ml of nystatin and 0.03–16 μg/ml of voriconazole and two positive and negative control wells. 100 μl of fungal suspension of one species was then added to each well. Of the two remaining wells, one is considered positive control and the other is negative control. In the positive control well, 100 μl of the organism suspension with

a density of 1 × 103 cells per ml and 100 µl Roswell Park Memorial Institute (RPMI), and in the negative control well, 100 µl RPMI and 100 µl of RPMI were added with the drug according to the CLSI-M27 protocol.[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 minimum fungicidal concentration (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.

Data analysis

Data were analyzed by SPSS version 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). 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 nystatin and voriconazole, the resistance and susceptibility of the isolates to them were determined. To compare susceptible and resistant species, statistical analysis was performed using Chi-squared and two-sided fissure exact tests.

RESULTS

This candida samples were from the collection of the Mycology Department of Isfahan University of Medical Science which was identified by PCR-RFLP method(the article is under published) candida samples before radiotherapy are as follows: 14 C. albicans, 5 *Candida tropicalis,* 2 *Candida glabrata* and after radiotherapy changed as follows: 12 *C. albicans,* 4 *C. tropicalis,* 2 *C. glabrata,* 1 *Candida parapsilosis,* 1 *Candida krusei*.

To measure the antifungal effect of two drugs, voriconazole and nystatin, three indicators (MIC24, MIC48, and MFC) for each drug and separately for isolated species before and after radiotherapy was used. three isolated species before radiotherapy including: *C. albicans, C. glabrata,* and *C. tropicalis* and five isolated species after radiotherapy including:

C. albicans, C. glabrata and *C. tropicalis, C. krusei,* and *C. parapsilosis* after radiotherapy. and the middle and range are shown in Tables 1 and 2.

Antifungal indices of two species of *c krusei and c parapsilosis were isolated only in one sample after radiotherapy an the indices of this two are as follow*:

Antifungal indices of nystatin on *C. parapsilosis* species: MIC24 = 0.5 µg/ml, MIC48 = 0.5 µg/ml and MFC = 1 µg/ml. Nystatin antifungal indices on *C. krusei* species: MIC24 = 1 µg/ml, MIC48 = 1 µg/ml and MFC = 1 µg/ml. Antifungal markers of voriconazole on *C. krusei* species: MIC24 = 0.03 µg/ml, MIC48 = 0.03 µg/ml and MFC = 1 µg/ml. Indicators of the antifungal drug voriconazole on *C. parapsilosis* were reported: MIC24 = 0.03 µg/ml, MIC48 = 0.03 µg/ml and MFC = 0.03 µg/ml.

In this study, to compare the antifungal effect of nystatin and voriconazole, drug sensitivity and resistance for each of the two drugs were determined. The break point of voriconazole was defined as MIC ≤1 µg/ml values as sensitive, MIC = 2 µg/ml values as dose-dependent, and MIC ≥4 µg/ml values as resistant. Nystatin clearance point was also considered MIC ≤2 µg/ml as sensitive and MIC >2 µg/ml as resistant.

The percentage of resistant, dose-dependent, and susceptible species is given in Tables 3 and 4, respectively.

The results showed that before radiotherapy, 57.1% of albicans species isolated were in the sensitive range (MIC ≤1 µg/ml) and 42.9% were in the dose-dependent range (MIC = 2 µg/ml) to voriconazole. Prior to radiotherapy, 100% of glabrata species were in the sensitive range (MIC ≤1 µg/ml) to voriconazole, but 80% of the tropicalis species were in the susceptible range and 20% of these species were in the resistant range (MIC ≥4 µg/ml). While 100% of all species, including albicans, tropicalis, and glabrata, were in the sensitive range (MIC ≤2 µg/ml) to nystatin before radiotherapy.

After radiotherapy, 58.3% of albicans species were in the sensitive range and 41.7% of these species were in the dose-dependent range of voriconazole. After radiotherapy, 50% of glabrata species were in the sensitive range and 50% were in the dose-dependent range of voriconazole. One hundred percent of *tropicalis* species were in the voriconazole-resistant range after radiotherapy, while 100% of all species after radiotherapy, including albicans and tropicalis, and glabrata, were in the nystatin-sensitive range. Therefore, nystatin was more effective than voriconazole before and after radiotherapy.

Table 1: Median and range of minimum inhibitory concentration 24, minimum inhibitory concentration 48, and minimum fungicidal concentration of nystatin on species isolated from radiotherapy 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	1 (<0.5-2)	0.75 (0.5-1)	1 (0.5-1)	1 (0.5-1)	1 (1-1)	0.5 (0.5-0.5)
MIC48	1 (0.5-4)	1.5 (0.5-2)	2 (0.5-2)	1.5 (1-2)	2 (1-2)	0.5 (0.5-0.5)
MFC	1 (0.5-32)	1 (<0.05-1)	1 (0.5-1)	1 (0.5-8)	1 (0.5-2)	0.5 (0.5-0.5)

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 of voriconazole on species isolated from patients undergoing radiotherapy before and during radiotherapy treatment

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: Percentage of sensitive, resistant, and dose-dependent Candida strains to voriconazole and nystatin by strain type before radiotherapy

Percent of susceptibility	Strain					
	Candida albicans		*Candida tropicalis*		*Candida glabrata*	
	Voriconazole (%)	Nystatin (%)	Voriconazole (%)	Nystatin (%)	Voriconazole (%)	Nystatin (%)
Sensitive	57.1	100	80	100	100	100
SDD	42.9	0	0	0	0	0
Resistant	0	0	20	0	0	0

SDD: Susceptible dose dependent

Table 4: Percentage of sensitive, resistant, and dose-dependent Candida strains to voriconazole and nystatin by strain type in the 2nd week of radiotherapy

Percent of susceptibility	Strain					
	Candida albicans		*Candida tropicalis*		*Candida albicans*	
	Voriconazole (%)	Nystatin (%)	Voriconazole (%)	Nystatin (%)	Voriconazole (%)	Nystatin (%)
Sensitive	58.3	100	0	100	50	100
SDD	41.7	0	0	0	50	0
Resistant	0	0	100	0	0	0

SDD: Susceptible dose dependent

MIC24, MIC48, and MFC nystatin on parapsilosis were 0.5 μg/ml, 0.5 μg/ml, and 1 μg/ml, respectively, and on the krusei species were 1 μg/ml, 1 μg/ml, and 1 μg/ml, respectively. The three mentioned indices of voriconazole reported on parapsilosis were 0.03 μg/ml, 0.03 μg/ml, and 1 μg/ml, respectively, and on the krusei species 0.03 μg/ml, 0.03 μg/ml, and 0.03 μg/ml, respectively.

DISCUSSION

The results of the present study showed that before radiotherapy, all species isolated from patients, including *C. albicans*, *C. tropicalis,* and *C. glabrata*, were sensitive to nystatin, while a percentage of albicans and tropicalis were resistant to voriconazole. In the 2nd week of radiotherapy similar to prior to

radiotherapy, all species isolated from patients were sensitive to nystatin, while a percentage of albicans and glabrata and all species of tropicalis were resistant to voriconazole.

In general, the present study showed that before and after radiotherapy, the antifungal effect of nystatin was greater than voriconazole.

However, topical formulations of nystatin in radiation therapy patients with mucositis, pain, nausea, and swallowing difficulty are less acceptable than the systemic formulations of some agents, such as voriconazole.[18] Voriconazole exerts more fungistatic effects than fungicide influences. Moreover, it may cause various side effects, such as reversible visual impairment, skin lesions, photosensitivity reactions, elevated liver enzymes, headache, nausea, diarrhea, abdominal pain, and visual hallucinations.[19]

In this study, the antifungal effect of voriconazole on *C. albicans* isolated from patients before radiotherapy and in the 2nd week of radiation therapy revealed that the susceptibility of this strain did not change significantly following radiotherapy. It was observed that 57.1% of patients had an allergic reaction to voriconazole before radiotherapy, and 58.3% had reactions in the 2nd week of radiotherapy. This difference might result from the ecological adaptation of *C. albicans* species.

Studies have shown that environmental changes lead to rapid changes in the gene expression and adaptation of *C. albicans* to environmental conditions.[20] Most infection-related changes in *C. albicans* gene expression reflect the environmental adaptation of this species.[21] Paula and Zida demonstrated that *Candida* is resistant to nystatin in 3% of HIV-positive patients with no clinical evidence of candidiasis and 4.8% of inhospital patients.[11,12] Contrary to the mentioned theory, we observed that all species isolated from radiation therapy patients were susceptible to nystatin both before and after radiotherapy (2 μg/ml < MIC), which was similar to the findings of Bulacio *et al*.[18] and Kurnatowski *et al*.[22]

The meta-analysis by Clarkson *et al*. in 2007 showed a significant benefit in using the absorbed drugs (fluconazole) in gastrointestinal (GI) tract rather than those not absorbed (nystatin) to prevent oral candidiasis for patients with cancer receiving treatment. There were no significant differences between patients receiving either absorbed or drugs not absorbed from the GI tract for the following outcomes: systemic fungal infection, death, empirical antifungal treatment, toxicity, and compliance.[23]

Regarding the isolated nonalbicans strains, the results showed that 100% of the *C. tropicalis* strains were susceptible to nystatin on the 2nd week of radiation therapy, while 100% were resistant to voriconazole. The mechanism of resistance to voriconazole is changing the permeability of the fungal cell membrane following the overexpression of the *ERG11* gene and the altered function of the ergosterol produced by this gene.[24] da Silva *et al*. in 2017 showed that the virulence of tropicalis strain elevated after radiotherapy because its adhesion to surfaces, biofilm formation, and phagocytosis index augmented.[25]

Contrary to the study of Schelenz *et al.* (2010)[5] and Bulacio *et al.*[18] all *tropicalis* strains isolated from the surveyed patients were shown to be 100% susceptible to both nystatin and voriconazole. These variable results could be attributed to the difference in the studied patients. In the current study, *Candida* samples were taken from the saliva of patients receiving head-and-neck radiotherapy. On the other hand, Karimi F and Gamaletsou MN. sampled the patients with malignancies and radiotherapy in the head and neck, as well as cases with other malignancies, hematological malignancies, and patients undergoing different treatments for malignancies, including chemotherapy, radiation therapy, and surgery. Chemotherapy and hematological malignancies that predispose patients to neutropenia may be associated with the development of *Candida* species resistant to antifungal agents.[26,27]

In this study, 100% of *C. glabrata* strains were susceptible to nystatin and voriconazole before radiotherapy. In comparison, 100% of the strains were susceptible to nystatin postradiation, 50% were susceptible to voriconazole, and 50% were dose-dependent. One reason for this is the increased resistance of *C. glabrata* to antifungal medicines, such as voriconazole, after radiation therapy. Dahiya *et al*. in 2003 introduced *C. glabrata* as the causative agent for oral *Candida* in patients with head-and-neck cancer who received radiation therapy. They found that the postradiotherapy required dose of azoles, including fluconazole, to treat candidiasis due to *C. glabrata* was twice the preradiotherapy dose. It shows the increased resistance of *C. glabrata* to antifungal therapy after radiation therapy.[28]

The only *C. parapsilosis* species in the current study was isolated from a patient after radiation therapy, while it was not isolated from this patient before radiotherapy. *C. parapsilosis* is stable in the hospital environment and spreads through contaminated hands.[29] The isolate found in this study was susceptible to nystatin and voriconazole with no difference in resistance to the two agents (MIC nystatin: 0.5 μg/ml and MIC voriconazole: 0.03 μg/ml). In 2015, Chakrabalti demonstrated that *C. parapsilosis* was the second most common cause of candidemia in patients admitted to the intensive care unit.[29] The systemic control of the latter species is essential because of its potential for candidemia after malignancy treatment and the importance of *C. parapsilosis* species. Therefore, despite the low MIC for this species, nystatin may not be a suitable medication for treating or preventing *C. parapsilosis* in people receiving radiation therapy due to the lack of GI absorption.

Only two studies by Bulacio *et al*.[18] and Schelenz *et al*.[5] performed an *in vitro* investigation on the antifungal effects of voriconazole and nystatin in patients with head-and-neck malignancy who received radiation therapy. The results of Bulacio *et al.* were in line with the present study that showed 100% of all *Candida* strains were susceptible to nystatin after radiation therapy. In contrast to the current study, all *C. albicans* strains were 100% susceptible to voriconazole after radiotherapy. In the present study, 58.3% and 41.7% of *C. albicans* strains were susceptible to voriconazole after radiotherapy and in the dose-dependent range for voriconazole after radiotherapy, respectively. Bulacio *et al*. noted that 100% of *C. tropicalis* strains were susceptible to voriconazole after radiotherapy, which is not consistent with our findings indicating that 100% of these strains were resistant to voriconazole.

In the present study, in contrast to previous studies, preradiation therapy samples were also isolated, and their resistance and susceptibility to both medications were investigated. The results showed that 57.1% of the albicans strains were susceptible to voriconazole, 42.9% were dose dependently susceptible to voriconazole, and 100% were susceptible to nystatin before radiation therapy. Therefore, the possible reason for the difference between the results of this study and the mentioned studies is the initial resistance of the albicans strain to these two medicines (before radiation therapy). Note that the first sampling of this study (before radiation therapy) was from patients with head-and-neck malignancies. The malignancies themselves contribute to antifungal drug resistance.[30] Consequently, the resistance of albicans strain to voriconazole may be due to the potential malignancies of the patients examined in this study. In the research by Schelenz *et al*.[5] on patients with head-and-neck malignancies, in line with the results of the present study, almost 100% of all the strains of *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis* were 100% susceptible to nystatin and voriconazole with very low resistance.

Belazi *et al*.[31] investigated the antifungal effect of voriconazole in patients who received radiation therapy. Consistent with the results of this study, 100% of *C. albicans* strains were susceptible or within the dose-dependent range. Furthermore, all *C. albicans* strains had a very low MIC for voriconazole and were susceptible to the medicine. These authors reported all strains of *C. tropicalis* to be susceptible to voriconazole, whereas, in this study, 100% of these strains were resistant to voriconazole. In the study by Bansal R, which is in line with the findings of the present study, all the strains of *C. glabrata*, *C. parapsilosis*, and *C. krusei* were susceptible to voriconazole. In contrast to previous studies, preradiation samples were isolated, and their resistance and susceptibility to voriconazole were investigated. As a result, the initial resistance of albicans strain to nystatin and voriconazole can be due to underlying malignancy in patients studied in the present study.[30] The other contributing factor might be the difference in the method of assessing the antifungal effect of this medication.

Owotade *et al*. investigated that 100% of *C. albicans* species derived from patients with cancer were susceptible to voriconazole. The possible reason for the difference between the results of this study and the mentioned studies is that patients do not receive radiotherapy that may change the susceptibility of fungal species.[32]

In the study of Vazquez in 2010 about management of oropharyngeal and esophageal candidiasis in patients living with HIV infection, it was shown that in patients with fluconazole-refractory mucosal candidiasis, treatment options now include itraconazole solution, voriconazole, posaconazole, and the newer echinocandins.[33]

In general, the results of this study showed that before radiation therapy, all species isolated from patients,

including *C. albicans*, *C. tropicalis*, and *C. glabrata*, were susceptible to nystatin. On the other hand, a percentage of *C. albicans* and *C. tropicalis* were resistant to voriconazole. In the 2nd week of radiation therapy, all isolates were susceptible to nystatin, similar to before radiotherapy. However, some *C. albicans* and *C. glabrata*, as well as all *C. tropicalis* strains, were resistant to voriconazole.

CONCLUSION

According to the results of the present investigation, all oral *Candida* isolated from patients before and during radiotherapy were susceptible to nystatin, while some strains showed resistance to voriconazole both before and during radiotherapy. Therefore, nystatin therapy is recommended to prevent and treat fungal infections in people receiving head-and-neck radiation therapy. On the other hand, treatment with voriconazole is not recommended for resistant strains.

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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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