Dental Research Journal

Original Article

Antifungal effect of atorvastatin in comparison with fluconazole on *Candida* species isolated from patients undergoing head-and-neck radiotherapy

Zahra Golestannejad[1], Parvin Dehghan[2], Nadia Najafizade[3], Mahnaz Kheirkhah[4], Maryam Emami Bafrani[5], Adel Tabesh[1], Farshad Nadian[6], Faezeh Khozeimeh[1]

[1]Department of Oral and Maxillofacial Medicine, Dental Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Sciences, [2]Department of Mycology and Parasitology, Faculty of Medicine, Isfahan University of Medical Sciences, [3]Department of Radiation Oncology, Isfahan University of Medical Sciences, [4]Department of Mycology and Parasitology, School of Medicine, Isfahan University of Medical Sciences, [5]Dental Students' Research Committee, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, [6]Department of Prosthodontics, School of Dentistry, Shahre Kord University of Medical Sciences, Shahre Kord, Iran

Received: 27-Aug-2023
Revised: 29-Oct-2023
Accepted: 31-Oct-2023
Published: 20-Dec-2024

Address for correspondence:
Dr. Faezeh Khozeimeh, Department of Oral and Maxillofacial Medicine, Dental Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: khozeimeh@dnt.mui.ac.ir

ABSTRACT

Background: Head-and-neck radiotherapy can change oral *Candida* species and lead to the development of refractory oral candidiasis resistant to the commonly prescribed antifungal medications such as fluconazole. Atorvastatin exerts an antifungal effect by inhibiting the synthesis of fungal wall ergosterol and impairing mitochondrial function. This study aimed to compare the antifungal effects of fluconazole and atorvastatin on *Candida* species isolated from patients undergoing head-and-neck radiotherapy.

Materials and Methods: In this clinical *in vitro* study, swab samples were collected from 33 patients admitted to Isfahan Seyed-O-Shohada Hospital before the onset and 2 weeks after the initiation of radiotherapy. The antifungal effects of fluconazole and atorvastatin were evaluated by the microdilution test according to the Clinical and Laboratory Standards Institute standards, and measuring their minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Data were analyzed by the Mann–Whitney *U*-test and the statistical significance level was considered $P < 0.05$.

Results: The results showed that the MIC24, MIC48, and MFC of fluconazole were significantly lower than those of atorvastatin for *Candida albicans*, *Candida tropicalis, and Candida glabrata* both before ($P < 0.001$ for all) and during ($P < 0.001$ to $P = 0.003$) radiotherapy.

Conclusion: According to the results, fluconazole has antifungal effects comparable to those of atorvastatin, but in much lower doses. Atorvastatin showed optimal antifungal effects but in doses beyond the clinically applicable threshold.

Key Words: Atorvastatin, candidiasis, fluconazole, head-and-neck neoplasm, oral, radiotherapy

INTRODUCTION

Patients with head-and-neck malignancies receive a high dose of radiation in the oral cavity during radiotherapy, which can change their oral environment and commonly cause early complications such as mucositis and xerostomia, and subsequent colonization

Access this article online

Website: www.drj.ir
www.drjjournal.net
www.ncbi.nlm.nih.gov/pmc/journals/1480
DOI: 10.4103/drj.drj_550_23

How to cite this article: Golestannejad Z, Dehghan P, Najafizade N, Kheirkhah M, Bafrani ME, Tabesh A, *et al*. Antifungal effect of atorvastatin in comparison with fluconazole on *Candida* species isolated from patients undergoing head-and-neck radiotherapy. Dent Res J 2024;21:68.

of the oral cavity and infection with different *Candida* species.[1-3] Candidiasis is the most common infection of the oral cavity in patients under radiotherapy for head-and-neck malignancies. Although the majority of *Candida* infections in such patients are caused by *Candida albicans*, infection with non-*albicans* species has also been reported in such patients since 1990.[2,4-6] *Candida* species are present in the normal oral flora of approximately 80% of the population. However, they can cause clinical candidiasis following alteration of the oral environment and systemic conditions after radiotherapy.[7] The main changes caused in the oral environment by radiotherapy include quantitative and qualitative changes in the saliva due to degeneration and decreased function of the salivary glands.[1-3] Considering the high prevalence of oral candidiasis and its potential morbidity and mortality, prompt diagnosis and management of such cases is highly important.[8]

Currently, several groups of topical and systemic antifungal medications with different formulations are available for the treatment of candidiasis. Polyenes such as nystatin and amphotericin B, and azoles such as fluconazole, ketoconazole, and itraconazole are among the most important groups of antifungal medications. Among antifungal agents, fluconazole is more extensively used for the treatment of local and systemic cases of candidiasis due to its optimal dissemination in almost all body tissues. Due to the need for long-term intake of antifungal medications, such patients often suffer from the medication side effects, and are at risk of developing resistance to routine antifungals such as fluconazole.[9] Some recent studies evaluated the antifungal activity of statins. This hypothesis was developed after noticing a reduction in the number of reported cases of zygomycosis in diabetic patients since 1990 following the widespread use of statins.[10]

Statins are among the serum cholesterol-lowering medications. They are successfully used by patients with hypercholesterolemia and have significantly decreased the incidence of cardiovascular diseases. Statins prevent cholesterol synthesis by inhibition of 3-hydroxy-3-methyl-glutaryl-CoA reductase and lower the serum cholesterol level as such. Yeasts use the same enzymatic pathway to produce ergosterol, instead of cholesterol, as a final product. Furthermore, statins impair the production of isoprenoid and subsequently the mitochondrial function and cell respiration in *Candida*. They also change the lipid structure and metabolism and dynamically alter the cell membrane of *C. albicans*.[11-13]

Considering the shift in microorganisms causing *Candida* infection in patients with malignancy from *C. albicans* to non-*albicans* species, increased risk of *Candida* infection with species resistant to routine antifungal therapy in such patients, and the inhibitory effects of atorvastatin on the enzymatic pathway of yeasts, this study aimed to compare the antifungal effects of fluconazole and atorvastatin on *Candida* species isolated from patients undergoing head-and-neck radiotherapy.

MATERIALS AND METHODS

This clinical *in vitro* study was conducted from February 2019 to March 2019 (ethical code: IR.MUI.RESEARCH.REC.1398.152). The methodology of this study has been described in detail in the previous study.[14] *Candida* species used in this study had been isolated from patients and stored in the Parasitology and Mycology Department of Isfahan University of Medical Sciences. These species had been clinically isolated from 33 patients hospitalized in Isfahan Seyed-O-Shohada Hospital before and after 2 weeks of head-and-neck radiotherapy. The strain type had been identified by polymerase chain reaction-restriction fragment length polymerization. The study population included 18 females and 15 males between 38 and 74 years. Twenty-one out of 33 patients were positive for *Candida* before radiotherapy. At 2 weeks after the onset of radiotherapy, six patients were excluded (4 males and 2 females); of the remaining 27 patients, 19 were positive for *Candida.* To prepare the fungal suspension, *Candida* species were first cultured on Sabouraud dextrose agar, and incubated at 35°C for 24 h.

Laboratory phase

A 0.5 McFarland standard suspension of the isolated species was prepared and confirmed spectrophotometrically (Wet Process Analyzer, Biowave II, Biochrom UK) at 530 nm wavelength. According to the Clinical and Laboratory Standards Institute (CLSI) standards, 11.2 mg fluconazole powder was added to 1 mL of methanol, and 10.24 mg atorvastatin was dissolved in 1 mL of dimethyl sulfoxide and stored at room temperature for 30 min to obtain homogeneous stock solutions of the two medications.[15]

Susceptibility testing

To assess the antifungal efficacy of fluconazole and atorvastatin, their minimum inhibitory

concentration (MIC) and minimum fungicidal concentration (MFC) for each *Candida* species were separately calculated. The MIC of both medications was determined by the broth microdilution method. For this purpose, 96-well ELISA microplates were used. Ten wells were considered for 0.12–64 μg/mL concentrations of fluconazole, and 10 wells were considered for 8–1024 μg/mL concentrations of atorvastatin in Roswell Park Memorial Institute (RPMI) medium. Two wells were assigned to the positive and negative controls. Next, 100 μL of the fungal suspension was added to each well. Finally, 100 μL of the fungal suspension containing 1×10^3 fungi plus 100 μL of RPMI were added to the positive control well (no medication), while the negative control well only contained 100 μL of pure RPMI with no medication or microorganism according to CLSI-M27.[14] After incubation at 35°C for 24 and 48 h, the turbidity of the wells was evaluated, and the first well with no turbidity after 24 and 48 h was recorded as the MIC24 and MIC48, respectively.

To determine the MFC, 20 μL of the suspension in the MIC well and the next wells were added to Sabouraud dextrose agar plates and after swab culture, incubated at 35°C for 24–48 h. To determine the MFC, the first plate with <5 grown colonies was selected.

Statistical analysis

Data were analyzed by SPSS version 22 (IBM, Chicago, IL, USA). To assess the antifungal efficacy of fluconazole and atorvastatin, the MIC24, MIC48, and MFC of each medication were separately calculated for *C. albicans, Candida glabrata,* and *Candida tropicalis* clinical isolates, and their median, range, and mode were calculated. The Mann–Whitney *U*-test was applied to compare the antifungal activity of the two medications (MIC24, MIC48, and MFC) once before the onset of treatment and once during the radiotherapy separately for each *Candida* species. The statistical significance level was considered $P < 0.05$.

RESULTS

The antifungal efficacy of fluconazole and atorvastatin is reported in Tables 1 and 2. The frequency value could not be calculated for *Candida parapsilosis*, and *Candida krusei* since they were isolated from only one patient each. Furthermore, since these two species were not isolated from any patient before initiation of radiotherapy, the antifungal efficacy of fluconazole and atorvastatin before and during radiotherapy could not be compared for these two species, and only the following values could be calculated for them:

Antifungal efficacy of fluconazole against *C. parapsilosis*: MIC24 = 1 μg/mL, MIC48 >64 μg/mL, and MFC = 64 μg/mL. Antifungal efficacy of fluconazole against *C. krusei*: MIC24 = 32 μg/mL, MIC48 >64 μg/mL, and MFC = 64 μg/mL. Antifungal efficacy of atorvastatin against *C. parapsilosis*: MIC24 = 32 μg/mL, MIC48 = 256 μg/mL, and MFC = 512 μg/mL. Antifungal efficacy of atorvastatin against *C. krusei*: MIC24 = 256 μg/mL, MIC48 = 512 μg/mL, and MFC = 768 μg/mL.

A comparison of the antifungal efficacy of fluconazole and atorvastatin is presented in Tables 3 and 4. The Mann–Whitney *U*-test revealed that both before and during radiotherapy, the MIC24, MIC48, and MFC of fluconazole were significantly lower than the MIC24 of atorvastatin for all three *Candida* species ($P < 0.001$ to $P = 0.003$).

Since 7 out of 33 patients were positive for *C. albicans* both before and during radiotherapy, the difference in all three parameters of MIC24, MIC48, and MFC of fluconazole and atorvastatin against *C. albicans* was calculated before and during radiotherapy and analyzed by the Mann–Whitney *U*-test [Table 5]. The Mann–Whitney *U*-test revealed that the MIC24 of fluconazole and atorvastatin increased significantly ($P = 0.007$) following treatment, while MIC48 and MFC had no significant alteration ($P > 0.05$).

DISCUSSION

The present results showed that fluconazole had higher antifungal effects on all *Candida* species isolated from patients under head-and-neck radiotherapy than atorvastatin both before and during radiotherapy, such that the MIC24, MIC48, and MFC of fluconazole were lower than those of atorvastatin.

In the present study, in addition to the assessment of MIC24, MIC48, and MFC, susceptibility to fluconazole was also evaluated such that the breakpoint of 8 μg/mL was defined for fluconazole MIC.[16] In other words, species with MIC values equal or higher than 8 μg/mL were considered resistant, while those below this threshold were considered sensitive to fluconazole. Since atorvastatin is not an antifungal medication, a breakpoint is not defined for it.

Table 1: Median and range of minimum inhibitory concentration 24, minimum inhibitory concentration 48, and minimum fungicidal concentration of fluconazole for *Candida* species isolated from patients before and during radiotherapy

Parameter	Species (μg/mL), median (range)					
	Candida glabrata		*Candida tropicalis*		*Candida albicans*	
	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 of atorvastatin for *Candida* species isolated from patients before and during radiotherapy

Parameter	Species (μg/mL), median (range)					
	Candida glabrata		*Candida tropicalis*		*Candida albicans*	
	Before radiotherapy	During radiotherapy	Before radiotherapy	During radiotherapy	Before radiotherapy	During radiotherapy
MIC24	128 (64–256)	128 (128–256)	128 (32–512)	128 (64–512)	128 (64–128)	384 (256–512)
MIC48	256 (128–>1024)	256 (256–>1024)	256 (64–>1024)	384 (256–>1024)	256 (256–>1024)	384 (256–512)
MFC	512 (128–>1024)	768 (128–>1024)	1024 (128–>1024)	1024 (512–>1024)	1025 (1024–>1024)	768 (512–1024)

MIC: Minimum inhibitory concentration; MFC: Minimum fungicidal concentration

Table 3: Comparison of minimum inhibitory concentration 24, minimum inhibitory concentration 48, and minimum fungicidal concentration of fluconazole and atorvastatin for different *Candida* species isolated from patients before radiotherapy

Parameter	Species					
	Candida glabrata		*Candida tropicalis*		*Candida albicans*	
	Fluconazole	Atorvastatin	Fluconazole	Atorvastatin	Fluconazole	Atorvastatin
MIC24						
Median (range) (μg/mL)	3 (1–>64)	128 (64–256)	2 (0.5–8)	128 (32–512)	1 (0.5–1)	128 (64–128)
P	0<0.001		0<0.001		0<0.001	
MIC48						
Median (range) (μg/mL)	>64 (1–65)	256 (128–>1024)	>64 (4–>64)	256 (64–>1024)	2 (2–8)	256 (256–>1024)
P	0<0.001		0<0.001		0<0.001	
MFC						
Median (range) (μg/mL)	64 (2–>64)	512 (128–>1024)	64 (4–>64)	1024 (128–>1024)	4 (4–16)	1025 (1024–>1024)
P	0<0.001		0<0.001		0<0.001	

MIC: Minimum inhibitory concentration; MFC: Minimum fungicidal concentration

To date, no previous study has compared the antifungal effects of fluconazole and atorvastatin on patients under radiotherapy. The results of the present study are different from Esfahani *et al*.[17] study. For *C. albicans* both MIC and MFC of fluconazole were lower than Esfahani *et al*. study.[17] On the other hand, both MIC and MFC of atorvastatin were higher than in that study. Of note, in the present study, MIC and MFC of fluconazole were lower than that of atorvastatin, while in Esfahani *et al*.'s study, it was vice versa. For *C. glabrata* comparing the results of these two studies comes into the same trend.[17]

The possible reasons for the difference in the results of the present study and those of Esfahani *et al*.[17] can be the difference in treatment protocols of the study populations which included radiotherapy and chemotherapy in their study, and the role of radiotherapy in xerostomia and its impact on oral microorganisms. The effects of radiotherapy on the oral environment include xerostomia, changes in the quality of the saliva, changes in oral pH, mucositis,

Table 4: Comparison of minimum inhibitory concentration 24, minimum inhibitory concentration 48, and minimum fungicidal concentration of fluconazole and atorvastatin for different *Candida* species isolated from patients during radiotherapy

Parameter	Species					
	Candida glabrata		*Candida tropicalis*		*Candida albicans*	
	Fluconazole	Atorvastatin	Fluconazole	Atorvastatin	Fluconazole	Atorvastatin
MIC24						
Median (range) (μg/mL)	2 (0.5–>64)	128 (128–256)	1 (1–4)	128 (64–512)	0.25 (0.25–0.25)	384 (256–512)
P	0<0.001		0<0.001		0.002	
MIC48						
Median (range) (μg/mL)	>64 (2–65)	256 (256–>1024)	2 (2–>64)	384 (256–>1024)	2.25 (0.5–4)	384 (256–512)
P	0<0.001		0<0.001		0.003	
MFC						
Median (range) (μg/mL)	>64 (4–>64)	768 (128–>1024)	3 (2–>64)	1024 (512–>1024)	34 (4–64)	768 (512–1024)
P	0<0.001		0<0.001		0.003	

MIC: Minimum inhibitory concentration; MFC: Minimum fungicidal concentration

Table 5: Mean difference of minimum inhibitory concentration 24, minimum inhibitory concentration 48, and minimum fungicidal concentration of fluconazole and atorvastatin for Candida species isolated from patients before and during radiotherapy

Mean difference before and during radiotherapy (μg/mL)	Medication		
	Fluconazole	Atorvastatin	*P*
MIC24	+16.5	+9.1	0.007
MIC48	0	+16.8	0.776
MFC	−10.33	−146	0.228

MIC: Minimum inhibitory concentration; MFC: Minimum fungicidal concentration

changes in the physiological behavior and morphology of *Candida* species, and changes in pathogenesis and virulence of yeasts.[7,18-20]

Szenzenstein *et al*.[21] evaluated the effect of atorvastatin alone (without comparing it to fluconazole) on *Candida* species isolated from the blood of hospitalized patients. They evaluated *C. parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis*. Since in the present study, *C. parapsilosis* was isolated from only one patient 2 weeks after the initiation of radiotherapy, the results of their study and the present investigation can only be compared regarding *C. parapsilosis*. In the present study, the MIC of atorvastatin for *C. parapsilosis* was found to be 32 μg/mL which was close to the value of the study by Szenzenstein *et al*. which was 25 μg/mL.[21]

Brilhante *et al*.[22] showed that atorvastatin had widely variable effects on different *Candida* species such that its mean MIC was 52.6 μg/mL for *C. albicans*, 165.34 μg/mL for *C. tropicalis,* 755.06 μg/mL for *C. krusei*, and 1491.37 μg/mL for *C. parapsilosis*. In contrast to their findings, the present results revealed that the MIC of atorvastatin for *C. parapsilosis* and *C. krusei* was lower than that for other species, and much lower than the value reported by Brilhante *et al*.[22] However, the present results were close to the findings of Brilhante *et al*.[22] regarding *C. albicans* and *C. tropicalis*.

Possible reasons for the difference between their results and the present findings include different sources of isolates, methodological differences in the measurement of MIC, and the atorvastatin solvent. A systematic review by Ting *et al*.[23] showed that the MIC of statins may change depending on the type of solvent, form of statin used, methodology, culture media, and exposure time to statin. Atorvastatin has been extensively used as a blood cholesterol-lowering medication since 1990.[10] Recently, atorvastatin was evaluated as a possible new antifungal agent that can inhibit the biosynthesis in the fungal cell wall,[23] with the assumption that it might be a good alternative antifungal medication to control oral candidiasis in cases with drug resistance potential.

Brilhante *et al*.[22] and Kurnatowski *et al*.[24] demonstrated that *C. albicans* and *C. tropicalis* isolated from the oral cavity of patients with head-and-neck cancer before and during radiotherapy were resistant to fluconazole. In contrast to their studies, Bulacio *et al*.[25] revealed that *C. albicans* and *C. tropicalis* species isolated during radiotherapy were sensitive to fluconazole. Consistent with the results of Bulacio *et al*.[25] and in contrast to the findings of Brilhante *et al*.[22] and Kurnatowski *et al*.,[24] the present study indicated that *C. albicans*, *C. tropicalis*, and *C. glabrata* were sensitive to fluconazole both before and during radiotherapy.

Differences between studies may be due to the different origins of *Candida* species (e.g., isolated from animals), different sampling methods (swabbing vs. mouthwash), and different inclusion criteria.

Radiotherapy changes the oral environment and causes xerostomia, changes the saliva quality, alters the oral pH, causes mucositis, changes the physiological and morphological behavior of *Candida* species, and eventually changes the pathogenesis and virulence of yeasts.[7,18,20] Karbach *et al*.[26] in 2012 demonstrated that reduction in saliva following head-and-neck radiotherapy is correlated with the occurrence of treatment-resistant *Candida* species. Evidence shows that environmental changes cause fast alterations in gene expression in *C. albicans* and its adaptation to environmental conditions.[27] Moreover, the majority of infection-dependent changes in gene expression of *C. albicans* are a reflection of its environmental adaptation.[28] Paula *et al*.[29] and Zida *et al*.[30] reported that *Candida* species showed resistance to fluconazole in 13% of HIV-positive patients with no clinical evidence of candidiasis and 66.5% of symptomatic hospitalized patients. In contrast to the abovementioned theory, the present results regarding the MIC24 of fluconazole showed that all three species of *C. albicans*, *C. tropicalis*, and *C. glabrata* isolated from patients before and during radiotherapy were sensitive to fluconazole (MIC <8 μg/mL).

No resistance development to fluconazole following radiotherapy in the present study may be due to the fact that a minimum radiation dose of 2000 cGy is required for the parotid gland to cause a significant reduction in saliva flow.[31] No previous study has precisely calculated the radiation dose of the parotid gland, and only the radiation dose based on the field and direction of irradiation has been estimated.[32] The present study was the first to precisely calculate the radiation dose of the parotid gland using professional software (TiGRT, LinaTech LLC, USA) separately for each patient. The results showed that in only 39% of patients, the radiation dose of the parotid gland was over 2000 cGy in the 2 weeks. Thus, only 39% of patients received the minimum required dose to decrease the saliva flow and quality, which may also lead to the emergence of drug-resistant species.

Atorvastatin has gastrointestinal absorption and a systemic effect and may be used in the abovementioned cases at risk of candidemia. The serum level of atorvastatin in the Iranian population with a daily dose of 40 mg is reported to be 50 ± 30 ng/mL.[33] The present results showed that the minimum concentration of atorvastatin that can inhibit *Candida* was 32 μg/mL. Since this concentration is almost 1000 times the serum level of atorvastatin, despite the oral bioavailability of atorvastatin, it cannot be recommended to control possible candidemia following orofacial *Candida* infection in patients under radiotherapy.

Another finding of the present study was that radiotherapy had a significant effect on the MIC24 of both medications and increased their efficacy since the values before radiotherapy were larger than the values during radiotherapy. Ramla *et al*.[7] demonstrated an increase in the production of hydrolytic enzymes including phospholipase and proteinase by the fungi following radiotherapy, which may change the efficacy of antifungal medications following radiotherapy. However, since these enzymes are correlated with colonization of the host tissue by the fungi and tissue invasion, they do not affect the *in vitro* susceptibility of fungi. Future studies with a higher sample size might elucidate the explanation for such facts.

CONCLUSION

The present results indicated that considering no resistance development by the *Candida* species to fluconazole following radiotherapy and also high MIC of atorvastatin (1000 times its serum level), fluconazole is still recommended for treatment of oral candidiasis in patients under radiotherapy, and atorvastatin is not suitable for this purpose.

Financial support and sponsorship
Nil.

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.

REFERENCES

1. Ainapur MM, Mahesh P, Kumar KV, Deepak A, Mrinal U, Sharada P. An *in vitro* study of isolation of candidal strains in oral squamous cell carcinoma patients undergoing radiation therapy and quantitative analysis of the various strains using CHROMagar. J Oral Maxillofac Pathol 2017;21:11-7.
2. de Freitas EM, Nobre SA, Pires MB, Faria RV, Batista AU, Bonan PR. Oral *Candida* species in head and neck cancer patients treated by radiotherapy. Auris Nasus Larynx 2013;40:400-4.

3. Miceli MH, Díaz JA, Lee SA. Emerging opportunistic yeast infections. Lancet Infect Dis 2011;11:142-51.
4. Hachem R, Hanna H, Kontoyiannis D, Jiang Y, Raad I. The changing epidemiology of invasive candidiasis: *Candida glabrata* and *Candida krusei* as the leading causes of candidemia in hematologic malignancy. Cancer 2008;112:2493-9.
5. Raj S, Sharma D, Mate P, Capoor MR, Bhowmik KT. A study of changes in the oral fungal flora of patients on radiotherapy for head and neck malignancies and their correlation with funguria and fungemia. Indian J Cancer 2017;54:39-42.
6. Sipsas NV, Lewis RE, Tarrand J, Hachem R, Rolston KV, Raad II, *et al.* Candidemia in patients with hematologic malignancies in the era of new antifungal agents (2001-2007): Stable incidence but changing epidemiology of a still frequently lethal infection. Cancer 2009;115:4745-52.
7. Ramla S, Sharma V, Patel M. Influence of cancer treatment on the *Candida albicans* isolated from the oral cavities of cancer patients. Support Care Cancer 2016;24:2429-36.
8. Suryawanshi H, Ganvir SM, Hazarey VK, Wanjare VS. Oropharyngeal candidosis relative frequency in radiotherapy patient for head and neck cancer. J Oral Maxillofac Pathol 2012;16:31-7.
9. Farmakiotis D, Tarrand JJ, Kontoyiannis DP. Drug-resistant *Candida glabrata* infection in cancer patients. Emerg Infect Dis 2014;20:1833-40.
10. Kontoyiannis DP. Decrease in the number of reported cases of zygomycosis among patients with diabetes mellitus: A hypothesis. Clin Infect Dis 2007;44:1089-90.
11. Westermeyer C, Macreadie IG. Simvastatin reduces ergosterol levels, inhibits growth and causes loss of mtDNA in *Candida glabrata*. FEMS Yeast Res 2007;7:436-41.
12. Forrest GN, Kopack AM, Perencevich EN. Statins in candidemia: Clinical outcomes from a matched cohort study. BMC Infect Dis 2010;10:152.
13. Macreadie IG, Johnson G, Schlosser T, Macreadie PI. Growth inhibition of *Candida* species and *Aspergillus fumigatus* by statins. FEMS Microbiol Lett 2006;262:9-13.
14. Golestannejad Z, Khozeimeh F, Najafizade N, Tabesh A, Faghihian E, Maheronnaghsh M, *et al.* Comparison of oral candidiasis characteristics in head-and-neck cancer patients before and during radiotherapy. Dent Res J (Isfahan) 2023;20:63.
15. Wayne P. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard. Wayne, Pennsylvania, USA: CLSI; 2002.
16. Katiraee F, Teifoori F, Soltani M. Emergence of azole-resistant *Candida* species in AIDS patients with oropharyngeal candidiasis in Iran. Curr Med Mycol 2015;1:11-6.
17. Esfahani AN, Golestannejad Z, Khozeimeh F, Dehghan P, Maheronnaghsh M, Zarei Z. Antifungal effect of atorvastatin against *Candida* species in comparison to fluconazole and nystatin. Med Pharm Rep 2019;92:368-73.
18. Davis D. Adaptation to environmental pH in *Candida albicans* and its relation to pathogenesis. Curr Genet 2003;44:1-7.
19. Sun Y, Cao C, Jia W, Tao L, Guan G, Huang G. pH regulates white-opaque switching and sexual mating in *Candida albicans*. Eukaryot Cell 2015;14:1127-34.
20. Bensadoun RJ, Patton LL, Lalla RV, Epstein JB. Oropharyngeal candidiasis in head and neck cancer patients treated with radiation: Update 2011. Support Care Cancer 2011;19:737-44.
21. Szenzenstein J, Gácser A, Grózer Z, Farkas Z, Nagy K, Vágvölgyi C, *et al.* Differential sensitivity of the species of *Candida parapsilosis* sensu lato complex against statins. Mycopathologia 2013;176:211-7.
22. Brilhante RS, Caetano EP, Oliveira JS, Castelo-Branco Dde S, Souza ER, Alencar LP, *et al*. Simvastatin inhibits planktonic cells and biofilms of *Candida* and *Cryptococcus* species. Braz J Infect Dis 2015;19:459-65.
23. Ting M, Whitaker EJ, Albandar JM. Systematic review of the *in vitro* effects of statins on oral and perioral microorganisms. Eur J Oral Sci 2016;124:4-10.
24. Kurnatowski P, Moqbil S, Kaczmarczyk D. Signs, symptoms and the prevalence of fungi detected from the oral cavity and pharynx of radiotherapy subjects with head and neck tumors, and their susceptibility to chemotherapeutics. Ann Parasitol 2014;60:207-13.
25. Bulacio L, Paz M, Ramadán S, Ramos L, Pairoba C, Sortino M, *et al.* Oral infections caused by yeasts in patients with head and neck cancer undergoing radiotherapy. Identification of the yeasts and evaluation of their antifungal susceptibility. J Mycol Med 2012;22:348-53.
26. Karbach J, Walter C, Al-Nawas B. Evaluation of saliva flow rates, *Candida* colonization and susceptibility of *Candida* strains after head and neck radiation. Clin Oral Investig 2012;16:1305-12.
27. Wilson D, Thewes S, Zakikhany K, Fradin C, Albrecht A, Almeida R, *et al.* Identifying infection-associated genes of *Candida albicans* in the postgenomic era. FEMS Yeast Res 2009;9:688-700.
28. Brown AJ, Odds FC, Gow NA. Infection-related gene expression in *Candida albicans*. Curr Opin Microbiol 2007;10:307-13.
29. Paula SB, Morey AT, Santos JP, Santos PM, Gameiro DG, Kerbauy G, *et al.* Oral *Candida* colonization in HIV-infected patients in Londrina-PR, Brazil: Antifungal susceptibility and virulence factors. J Infect Dev Ctries 2015;9:1350-9.
30. Zida A, Yacouba A, Bamba S, Sangare I, Sawadogo M, Guiguemde T, *et al*. *In vitro* susceptibility of *Candida albicans* clinical isolates to eight antifungal agents in Ouagadougou (Burkina Faso). J Mycol Med 2017;27:469-75.
31. Burlage FR, Coppes RP, Meertens H, Stokman MA, Vissink A. Parotid and submandibular/sublingual salivary flow during high dose radiotherapy. Radiother Oncol 2001;61:271-4.
32. Dambroso D, Svidzinski TI, Svidzinski AE, Dalalio M, Machado DO, Moliterno RA. Radiotherapy effect on frequency of *Candida* spp. and on virulence of *C. albicans* isolated from the oral cavity of head and neck cancer patients. J Basic Appl Pharm Sci 2009;30:153-9.
33. Chou YC, Wang YK, Charng MJ, Ueng YF. Determination of serum atorvastatin concentrations in lipid-controlling patients with and without myalgia syndrome. J Food Drug Anal 2013;21:147-53.