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Azole Resistance in *Candida albicans* Isolates from Oropharyngeal Candidiasis is Associated with *ERG11* Mutation and Efflux Overexpression

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Abstract

Background: Azole resistance rates are rising in *Candida* species. Fluconazole is one of the most important antifungal drugs used in candidiasis treatment.
Objectives: We identified the molecular mechanisms of fluconazole resistance of *Candida albicans* oropharyngeal candidiasis (OPC) isolates obtained from head and neck cancer patients, a study carried out between 2018 and 2020.
Methods: One hundred and twenty-five *C. albicans* clinical isolates were collected. Antifungal susceptibilities were determined by the CLSI- M27-A3 method. The *ERG11* gene was amplified and sequenced to discover SNP mutation. Moreover, real-time PCR was carried out to measure the mRNA levels of *ERG11*, *CDR1*, *CDR2*, and *MDR1*.
Results: Resistance to fluconazole was found in 15 *C. albicans* isolates. Amino acid substitutions E266D and D116E were observed in resistant, sensitive dose-dependent (SDD), and susceptible *C. albicans* isolates. K128T, G465S, A114S, Y257H and V488I were in relation to fluconazole resistance. D504A, P375A, W520C, G59S, and V51L were novel substitutions detected in the isolates; except for D504A, other mutations were observed only in resistance isolates. The expression levels of *CDR2*, *CDR1*, *MDR1*, and *ERG11* were increased compared to susceptible isolates, respectively.
Conclusions: *ERG11* mutation was the principal mechanism for fluconazole resistance in *C. albicans* isolated from oropharyngeal candidiasis patients, and caspofungin can be used as the effective antifungal substance in fluconazole resistance situation for *C. albicans* infection.

Keywords: *Candida albicans*, Oropharyngeal Candidiasis, Azole-resistance, Gene

1. Background

Among human fungal diseases, *Candida albicans* is considered the most common opportunistic pathogen, which causes various illnesses ranging from superficial to life-threatening conditions (1-3). Although *C. albicans* may be harmless due to the balance kept with other existing microbiota in healthy individuals, it can immediately turn into a pathogen under certain circumstances (4, 5). In other words, alterations in the immune system and variations in host health can result in enabling these pathogens to cause infection (6, 7). It has been revealed that candidiasis is a fungal infection in which *C. albicans* is considered the most prevalent causative agent (8). Radiation therapy on head and neck cancer patients enhances the risk of developing candidiasis. This condition leads to oropharyngeal candidiasis (OPC) (9, 10), which is a common oral complication causing systemic infection in vulnerable hosts (11).

Significant research has been done on antifungal drugs to provide treatment for systemic yeast infections. These compounds with therapeutic properties include four main classes: azoles (fluconazole, itraconazole, voriconazole, posaconazole), polyenes (amphotericin B), echinocandins (caspofungin, micafungin, anidulafungin, aminocandin), and antimetabolites (5-flourocytosine) (12, 13). Fluconazole, a bis-triazole antifungal agent, is thought to as the first-line cure for systemic infection of *Candida*

(14, 15). Fluconazole's function is to hamper lanosterol C-14-a-demethylase enzyme, which is required for converting lanosterol to ergosterol. Subsequently, aggregation of 14-a-methyl sterol and drop in ergosterol in yeast cell wall leads to prevention of cell proliferation (16).

As aforementioned, the extensive long-term administration of agents with fungicidal activity, especially azoles, has led to the emergence of a variety of species resistant to these drugs (17). Several classical mechanisms developing antifungal resistance include mutations in the target enzyme, alterations in permeability of efflux pump proteins (ATP transport system superfamily (ABC) like *CDR1*p and *CDR2p*, and major facilitator (MFs), including *MDR1* gene), and changes in modification or degradation of drugs inside the cell. Mutations in targeted enzymes and shifts in efflux pumps are shown to be of greater importance among various species of *Candida* (18-20). *Candida albicans* species can become resistant to antifungal drugs through mutations in which the gene of the target enzyme alters (21). For instance, a point mutation in *ERG11* gene, which encodes the major target of azoles, 14a-demethylase, results in amino acid alteration leading to decreased enzyme affinity to azole drugs. Hence, the resistance against these agents is strengthened (22, 23). However, azole resistance can be caused by overexpression of Ca*ERG11* as well (19, 22).

2. Objectives

This study aimed to determine antifungal susceptibility of clinical *C. albicans* isolates and *ERG11* gene mutation. Moreover, the changes in the expression of genes responsible for azole resistance were measured using RT-PCR.

3. Methods

3.1. Patient and Fungal Isolates

Here 116 head and neck cancer patients with OPC were selected during the period of two years (Jul 2018 to Oct 2020) at Cancer Institute of Imam Khomeini Hospital in Tehran. No treatment of antifungals and prophylaxis was done on the patients, just radiotherapy. The study was informed and patient's agreement was prepared. The data of patients, including age, sex, type of malignancy, and stage of cancer (The rate of spread of cancer in the patient's body), were obtained in patients' sheets. Oral samples were collected with sterile swabs. Oropharyngeal candidiasis was confirmed by the existence of white plaques and certified by funding of yeasts and pseudohyphae on KOH 10% examinations and positive culture. Initial differentiation was performed by following phenotypic methods: culturing on CHROMagar *Candida* (CHROMagar Company, Paris, France) medium, chlamydoconidia, and germ tube production and carbohydrate assimilation. Finally, it was confirmed by multiplex PCR method (24). All *Candida* isolates were kept in 1.5 ml sterile 20% glycerol solution at -80°C for future use.

3.2. Antifungal Susceptibility Testing

Specific antifungal drugs containing fluconazole (Sigma-Aldrich, St. Louis, MO, USA), itraconazole (Sigma-Aldrich, St. Louis, MO, USA), voriconazole (Sigma-Aldrich, St. Louis, MO, USA), caspofungin (CFG, Merck Sharp & Dohme, Haarlem), and amphotericin B (Sigma-Aldrich, St. Louis, MO, USA) were used for this experiment according to the Clinical and Laboratory Standards Institute M27-A3 standard method (25). Various concentrations of antifungals were prepared in RPMI medium (Sigma-Aldrich, St. Louis, MO, USA) and added to the 96-well microtiter plates. Fungal suspension was prepared from 48 h cultured colonies, and inoculums were prepared in RPMI 1640 (0.5 - 2.5 $\times 10^3$ cell/mL). Then, 100 μl of cell suspensions were added in each well containing 100 μl of various amounts of antifungals. The plate was kept at 37°C, and minimum inhibitory concentrations (MICs) were determined visually after 24 h (26). *Candida albicans* ATCC10231 was considered control. All the experiments were done in triplicate. According to the CLSI description, all isolates with a MIC of $\geq$ 8 μg/mL were defined as having resistance; $\geq$ 2 μg/mL as susceptible and 4 μg/mL as sensitive dose-dependent (SDD) to fluconazole (27).

3.3. DNA Extraction, PCR Amplification, and Sequencing

Candida albicans cell disruption was done using glass beads, and extraction of total genomic DNA was carried out using phenol-chloroform-isoamyl alcohol method (28). *ERG11* was amplified by PCR using specific primers (Table 1) (29). In a reaction volume of 50 μl, amplifications were done using Taq PCR Master Mix, Ampliqon (Ampliqon, Denmark). PCRs were implemented with an initial incubation at 95°C, 5 min; 45 cycles of 95°C, 10 secs; 58°C, 150 secs; 72°C, 90 sec; followed by 72°C for 5 min (29). The *ERG11* sequences were analyzed by MEGA6 software, and SNP were detected by comparing the whole *ERG11* open reading frame of fluconazole susceptible strain previously submitted (XM-711668.2) by *ERG11* sequence (30, 31).

3.4. Gene Expression by Real-time PCR

The *ERG11*, *CDR1*, *CDR2*, and *MDR1* gene expressions were evaluated in 42 fluconazole-resistant, susceptible-dose-dependent (SDD), and susceptible *C. albicans* isolates from OPC head and neck cancer patients as follows. Whole RNA extraction was done from the homogenized fungal cells by GITC (Guanidinium Isothiocyanate) reagent and

Table 1. The Primers Used for PCR and Real-time PCR Experiments

Genes	Primer Sequence (5'-3')	Size (bp)
	PCR	
ERG11		1587
F	5'-GTTGAAACTGTCATTGATGG-3'	
R	5'-TCAGAACACTGAATCGAAAG-3'	
	Real-time PCR	
ERG11		91
F	5'- AACTACTTTTGTTTATAATTTAAGATGGACTATTGA-3'	
R	5'- AATGATTTCTGCTGGTTCAGTAGGT-3'	
CDR1		96
F	5'- TTTAGCCAGAACTTTCACTCATGATT-3'	
R	5'- TATTTATTTCTTCATGTTCATATGGATTGA-3'	
CDR2		80
F	5'- GGTATTGGCTGGTCCTAATGTGA-3'	
R	5'- GCTTGAATCAAATAAGTGAATGGATTAC-3'	
MDR1		83
F	5'- TTACCTGAAACTTTTGGCAAAACA-3'	
R	5'- ACTTGTGATTCTGTCGTTACCG-3'	
ACT1		85
F	5'- TTGGTGATGAAGCCCAATCC-3'	
R	5'- CATATCGTCCCAGTTGGAAACA-3'	

Abbreviations: *ERG11*, ergosterol gene 11; *CDR1*, cerebellar degeneration related protein 1; *CDR2*, cerebellar degeneration related protein 2; *MDR1*, multidrug resistance protein 1; ACT1, actin 1.

glass beads, then treatment with RNase-free DNase was done (Thermo Fisher Scientific, USA) (32). From a total of 1,000 ng RNA, single-stranded cDNA was prepared using Revert Aid M-MuLV and random hexamer primers in cDNA synthesis kit (Yekta Tajhiz, Iran). Real-time PCR was performed (33) by SYBR green master mix (Sina Clone, Iran). The final volume of each reaction was 25 μL performed by a Rotor gene 6,000 (Corbett System). The specific primer sets were showed in Table 1 (34). Real-time PCR was performed by the following program: 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min (35). All tests were done in triplicate. The results were determined by relative quantification, using *ACT1* expression as the reference gene. Gene folding change levels were measured by $2^{-\Delta CT}$ method. Determination of fold increases (FI) was done by the relative threshold method ($2^{-\Delta\Delta CT}$) (33).

3.5. Statistical Analysis

The data of gene expression were considered for the analysis of variance (One-way ANOVA) in Tukey range. The differences with a P-value < 0.05 were considered significant. For P-value evaluation, one-way ANOVA test using GraphPad Prism 6 (San Diego, CA, USA) was used.

4. Results

4.1. Clinical Data

In this study, 217 *Candida* isolates were obtained from 116 OPC patients with head and neck cancer in Imam Khomeini Hospital, institute of cancer in Tehran, for about two years (Jul 2018 to Oct 2020). Of the total isolates, 125 isolates were *C. albicans*, and 92 isolates were *Candida* non-*albicans*. Only *C. albicans* isolates were included in this study. Mixed isolates were observed in 5 patients. The fungal yield of each sample was 97 - 15 ± 5 CFU/plate after culturing on Sabouraud dextrose agar (SDA) medium. The results of initial differentiation showed that seven (5.6%) and nine (7.2%) *C. albicans* isolates did not produce any germ tube and chlamydoconidium, respectively. Also, four (3.2%) *C. albicans* isolates represented white color on CHROMagar *Candida* medium. All of these isolates were detected as *C. albicans* by multiplex PCR (the total number of *C. albicans* isolates was 125 isolates). Our data showed that 73 (58.4%) *C. albicans* isolates were obtained from males, and 52 (41.6%) *C. albicans* isolates were obtained from females. More patients were in stage 2 (57.6%), followed by stage 1 (32%) and stage 3 (10.4%). The patients' ages ranged from 12 to 93 years, and the average age was 52. Most of the patients had face basal cell carcinoma (BCC, 45%), maxillary squamous cell carcinoma (SCC, 28%), tongue SCC (19%), and the rest of the patients (8%) had other head and neck malignancies.

4.2. Antifungal Susceptibility Profiles

The results of antifungal susceptibility of *C. albicans* isolates showed that 15 isolates (12%) from 125 *C. albicans* isolates represented a reduction in susceptibility to fluconazole. Moreover, 12 (9.6%) isolates were considered to be

SDD, and 98 (78.4%) isolates were susceptible to fluconazole. Moreover, 14 isolates (11.2%) displayed susceptibility reduction to voriconazole, and 16 isolates (12.8%) represented reduced susceptibility to itraconazole. In addition, reduced sensitivity to amphotericin B was observed in 23 (18.4%) isolates and in relation to caspofungin in 13 (10.4%) isolates. The demographic data and details of all *C. albicans* isolates with antifungal susceptibility are shown in Tables 2 and 3.

4.3. ERG11 Sequencing Results

The *ERG11* gene sequence of 42 *C. albicans* isolates, 15 fluconazole-resistant, 12 SDD, and 15 susceptible isolates were done and deposited in GenBank database under accession number OM774349-OM774390. *ERG11* coding region was amplified by PCR in the size of 1,640 bp. The results of *ERG11* sequencing from 42 *C. albicans* isolates are shown in Table 3. We found 34 mutations that 20 of which were silent mutations and did not change amino acid. Fourteen missense mutations were detected in 42 R/SDD/S *C. albicans* isolates. Among the 14 missense mutations identified, nine mutations had been identified previously, including D116E, K128T, G465S, E266D, V488I, A114S, Y257H, K344E, and R523G. Five mutations were reported newly, including D504A, P375A, W520C, G59S, and V51L (Table 3). D116E and E266D are the most common mutations among 42 *C. albicans* isolates.

4.4. Efflux Transporters Gene Expression Levels

The expression levels of *CDR1*, *CDR2*, *ERG11*, and *MDR1* for 42 clinical *Candida* isolates were measured. The expression of the genes in *C. albicans* strains was compared with the mean expression level of 15 fluconazole-susceptible isolates by quantitative PCR. At least 2-fold increased overexpression was observed (36). The results showed that five (33.3%) resistant isolates with MIC $\geq$ 32 μg/mL represented increased expression levels of *CDR1* and *CDR2*, five isolates with MIC 8 μg/mL showed *CDR1* and six isolates represented *CDR2* expression levels upper than 2-fold of the mean of susceptible isolates. *MDR1* and *ERG11* expression levels were increased in two (13.3 %) resistant isolates with MIC $\geq$ 32 μg/mL (Table 3, Figure 1). Statistical analysis represented a significant difference in the mean of gene expression of resistance isolates in comparison to the mean of gene expression in susceptible isolates, $P < 0.0001$ (Figure 1).

5. Discussion

Oropharyngeal candidiasis is considered to be the most frequent oral clinical *Candida spp*. manifestation in people with head and neck malignancies (37). For oropharyngeal candidiasis treatment, Azoles have been the choice (34). The high frequency of azole resistance and the speed at which *C. albicans* resistance is acquired is a crucial concern for clinicians, especially in the case of immunocompromised patients (38). Azole resistance in *C. albicans* might be caused by overexpression of genes, which encode efflux pumps or might result from mutations in or overexpression of *ERG11* (34). Since there are a few studies about antifungal resistance of species of *C. albicans* that are relevant to OPC in Iranian patients with head and neck cancer, the current study was conducted to evaluate antifungal susceptibility patterns and molecular mechanisms of these isolates in Institute of Cancer in Imam Khomeini Hospital located in Tehran. Among 125 *C. albicans* isolates, 27 isolates were R, and SDD to fluconazole, and 98 isolates were susceptible. Therefore overall, 42 isolates (27 R and SDD beside 15 susceptible isolates) were considered to be done for *ERG11* sequence and real-time PCR analysis.

Our results illustrated 14 missense mutations in *ERG11* gene that substituted amino acid sequence. Among them, D116E and E266D were expressed in all of the resistant isolates, including SDD and some susceptible *C. albicans* isolates, which is consistent with other studies, showing that these mutations probably have no effects in reduction of azole susceptibility (31, 39, 40). It has been reported (30) E266D substitution in resistant isolates of *C. albicans*, while other studies demonstrated this amino acid substitution in both azole resistance and azole susceptible isolates. It has been demonstrated that the amino acid substitution D116E was not associated with the azole-resistant phenotype (39). Furthermore, previous studies showed that A114S, Y257H, K128T, and V488I mutations were responsible for fluconazole susceptibility reduction in *C. albicans* isolates (38, 41-43).

In our study, *C. albicans* resistant isolates represented A114S, Y257H, and K128T amino acid substitutions (K128T substitution was found in 14 isolates, A114S was found in four isolates and Y257H was found in one isolate of *C. albicans*), which strongly suggests that these are associated with the azole-resistant phenotype. Combined substitutions of Y257H and A114S have been informed in fluconazole-resistant isolates. These substitutions were demonstrated to increase fluconazole resistance as well. Since the location of A114S is next to the *ERG11*p substrate channel, the interference with active site binding or inhibitor may occur due to its mutations; however, Y257H does not appear to affect the *ERG11*p for azoles since it is located in the G helix, which is far away from substrate channel of the protein. Therefore, further verification of Y257H mutation association with azole resistance must be carried out (30). Here we found new amino acid substi-

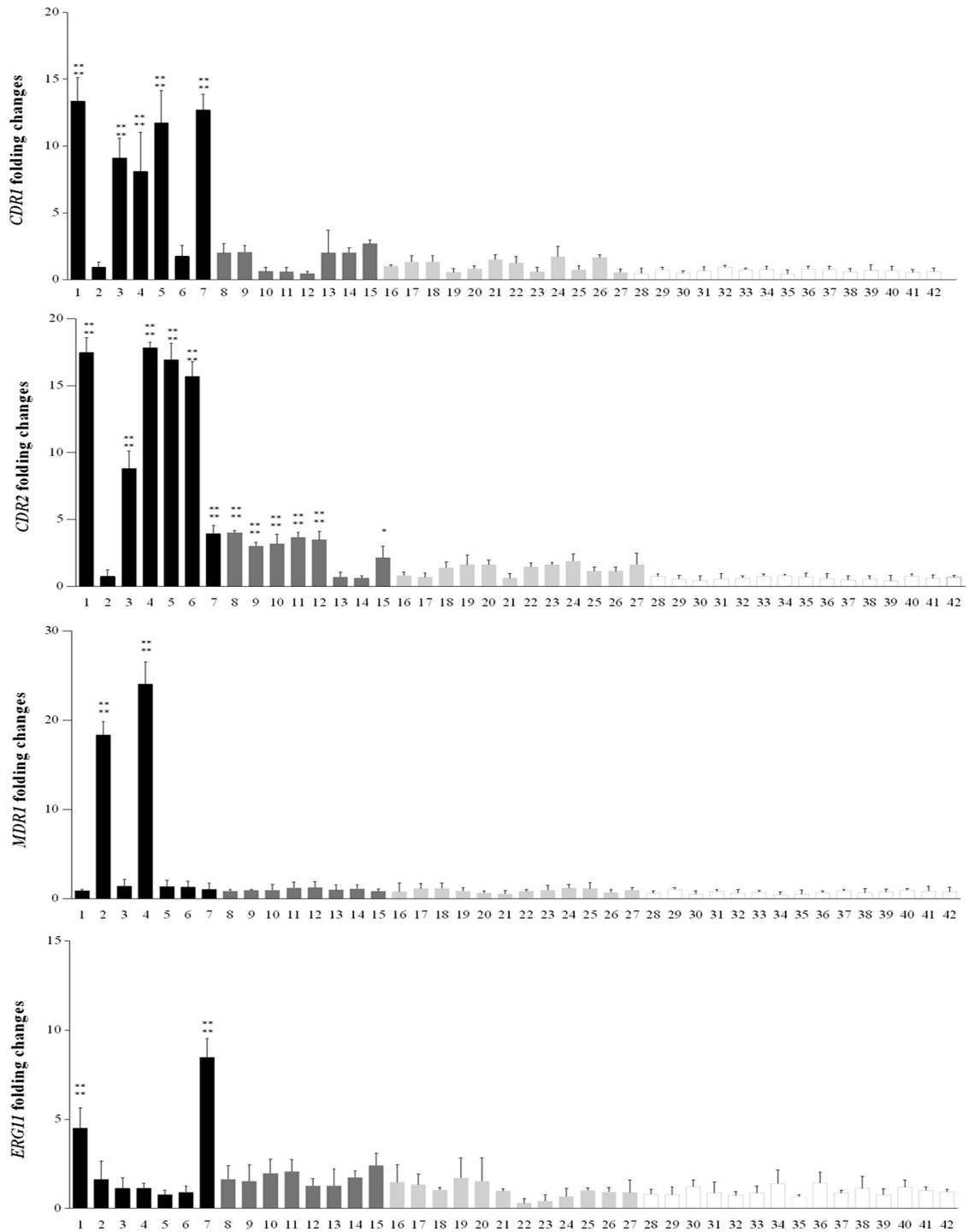

Figure 1. *CDR1*, *CDR2*, *MDR1*, and *ERG11* fold expression levels in azole-resistance with MIC $\geq$ 32 μg/mL (n = 7; black bars) and MIC = 8 μg/mL (n = 8 dark grey bars), SDD with MIC = 4 μg/mL (n = 12; light grey bars) and susceptible with MIC < 2 (n = 15; white bars) groups of *Candida albicans*. Each of the target gene expression levels was measured by the $2^{-\Delta\Delta Ct}$ method using *ACT1* housekeeping gene as the internal control. The process of each sample was done in triplicate. Error bars show the standard deviations; *Statistically significant difference with the mean of gene expression in susceptible isolates, P < 0.0001.

Table 2. Antifungal Susceptibility Results of 125 Clinical *Candida albicans* Isolates Recovered from Oropharyngeal Candidiasis in Patients Suffering from Head and Neck Cancer [a]

Antifungal Agents	Category, No. (%)				CBPs (μg/mL)				ECV (μg/mL)
	S/WT	SDD/I	R/NWT	Gmean (μg/mL)	S	SDD	I	R	
Fluconazole	98 (78.4)	12 (9.6)	15 (12)	4.018	$\leq$ 2	4	-	$\geq$ 8	-
Itraconazole	68 (54.4)	41 (32.8)	16 (12.8)	0.371	$\geq$ 0.12	0.25 - 0.5	-	$\geq$ 1	-
Voriconazole	74 (59.2)	37 (29.6)	14 (11.2)	0.314	$\geq$ 0.12	-	0.25 - 0.5	$\geq$ 1	-
Caspofungin	87 (69.6)	25 (20.0)	13 (10.4)	0.373	$\geq$ 0.25	-	0.5	$\geq$ 1	-
Amphotericin B	102 (81.6)	-	23 (18.4)	0.739	-	-	-	-	2

Abbreviations: WT, wild type; NWT, non-wild type; CBPs, clinical breakpoints.
[a] The MIC of *Candida albicans* ATCC 10231 (control) for Azoles was 0.5 to 0.0625 μg/mL, and for Caspofungin and Amphotericin B was 0.25 μg/mL.

tutions, including D504A, P375A, W520C, G59S, and V51L. Interestingly, D504A was observed in susceptible isolates, but others were found in R and SDD isolates (MIC $\geq$ 8 μg/mL and MIC = 4 μg/mL), suggesting that they contributed to reduced susceptibility isolates.

Real-time PCR was carried out to discover the expression levels of *CDR1*, *CDR2*, *ERG11*, and *MDR1* genes for all resistant, SDD, and susceptible isolates. Our results indicated that *CDR2* gene showed increased expression in more resistant isolates compared with other tested genes, followed by the *CDR1* gene. Studies have demonstrated that expression of *CDR1* and *CDR2* were elevated in the azole-resistant isolates, in comparison with isolates susceptible to azole, since *CDR2* expression was at higher levels compared to *CDR1* (38). Interestingly, our results showed that the expression level of *CDR2* gene was higher than *CDR1*.

In this study, gene expression increased by two folds compared with the mean of susceptible isolates that was considered the target gene overexpression. Five isolates with MIC $\geq$ 32 represented overexpression in *CDR1* with the range of 13.36 to 8.09, and the expression level of *CDR1* in five isolates with MIC = 8 μg/mL was 2 to 2.7. Moreover, in the eight SDD isolates (MIC = 4 μg/mL), the expression level of *CDR1* was 1 to 1.6 (The mean expression level in susceptible isolates was 0.666) (Figure 1). Also, five *C. albicans* isolates showed overexpression range of 17.8 to 8.8 in *CDR2* gene (The mean *CDR2* expression level was 0.615 in susceptible isolates). In some isolates with MIC = 8 μg/mL and 4 μg/mL, the expression levels more than twice as high as the average of susceptible isolates were seen as the expression level was between three to four in six isolates with MIC = 8 μg/mL (Table 3, Figure 1).

The overexpression of *ERG11*, encoding lanosterol demethylase, a key enzyme in the ergosterol biosynthesis pathway, is a significant reason for fluconazole resistance in *C. albicans*. Flowers et al. demonstrated that *ERG11* overexpression was observed in most of the fluconazole-resistant isolates. They suggested that *ERG11* overexpression is a common contributor to resistance in *C. albicans* (36). Furthermore, Liu et al. reported that *ERG11* was not overexpressed in fluconazole-resistant *C. albicans* isolates. They suggested that *ERG11* overexpression is not crucial for azole resistance induction in *C. albicans* (38). However, here we demonstrated that *ERG11* overexpression was observed just in two fluconazole-resistant isolates (expression level ranges from 8 to 4.5) but not in all of them. It has been demonstrated that resistance to fluconazole results from excessive *MDR1* expression.

It shed light on a principal mechanism of clinical resistant isolates (44). Our study also indicated that the expression of *MDR1* increased more than *ERG11* in isolates that showed reduced sensitivity to fluconazole (Table 3, Figure 1). It has been shown that fluconazole-resistant isolates represented high levels of gene expression in *MDR1* (44). *MDR1* gene overexpression was seen in two resistant isolates in the range of 24.06 to 18.34. This work has some limitations, such as our inability to analyze the molecular epidemiology of *C. albicans* isolates to determine the relationship between fluconazole resistance and genetic affinity of the clinical *C. albicans* isolates.

Taken together, as there is little information about antifungal resistance pattern of *C. albicans* clinical isolates from OPC in Iranian head and neck cancer patients, our study aimed to evaluate fluconazole resistance mechanism of these clinical isolates and to find a correlation between sex and age of these patients and the drug resistance for the first time. Moreover, in this study, there was no relationship between drug resistance, cancer type, and sex and age of patients, suggesting that a large sample size might be needed to find the relationship between them.

5.1. Conclusions

This study demonstrated that mutation in *ERG11* gene was the most causative mechanism for fluconazole resistance in *C. albicans* isolates that were obtained from patients with head and neck cancer suffering from oropharyngeal candidiasis. Additionally, we found that caspofungin was the effective antifungal substance in fluconazole resistance situations for *C. albicans* infection in these isolates. Identification of drug resistance mechanisms and

Table 3. *In vitro* Azole Susceptibility, *ERG11* Sequence, and Gene Expression Levels for the Clinical Isolates of *Candida albicans*[a]

No.	Isolate	Accession No.	MIC (μg/mL)					Amino Acid Substitution(s) in *ERG11*	Gene Overexpression
			FLZ	VRC	ITZ	AMB	CAS		
1	2pr-55	OM774349	**> 64**	**4.00**	**8.00**	**4.00**	**2.00**	**D116E, K128T, G464S**	***CDR1, CDR2, ERG11***
2	2pr-22	OM774363	**> 64**	0.50	0.50	0.50	0.125	**A114S, Y257H, K344E, R523G**	***MDR1***
3	2pr-73	OM774368	**> 64**	0.125	0.50	0.50	**1.00**	**D116E, E266D, G464S**	***CDR1, CDR2***
4	2pr-125	OM774374	**> 64**	**1.00**	**2.00**	0.50	0.125	**A114S, K128T, K344E, R522G**	***CDR1, CDR2, MDR1***
5	2pr-114	OM774373	**> 64**	0.50	**1.00**	0.50	0.50	**D116E, K128T, E266D, G464S**	***CDR1, CDR2***
6	2pr-142	OM774378	**64**	0.50	0.125	1:00	0.0312	**D116E, K128T E266D,**	***CDR2***
7	2pr-145	OM774379	**32**	0.50	0.25	**2.00**	0.25	**A114S, E266D**	***CDR1, ERG11***
8	2pr-195	OM774384	**8.00**	**1.00**	0.125	0.0312	0.50	**V488I, W520C**	*CDR1, CDR2*
9	2pr-354	OM774390	**8.00**	0.50	0.125	1.00	1.00	**E266D, V488I, W520C**	*CDR1, CDR2*
10	2pr-9	OM774357	**8.00**	**1.00**	0.125	**4.00**	0.25	**D116E, E266D, P375A**	*CDR2*
11	2pr-135	OM774376	**8.00**	0.0625	0.125	1:00	0.25	**D116E, E266D, V488I**	*CDR2*
12	2pr-15	OM774360	**8.00**	0.50	**1.00**	0.125	**1.00**	**E266D, V488I, W520C**	*CDR2*
13	2pr-56	OM774361	**8.00**	0.125	0.50	0.50	0.125	**G59S, D116E, E266D,**	*CDR1*
14	2pr-42	OM774364	**8:00**	0.50	**1.00**	0.125	0.125	**E266D, W520C**	*CDR1*
15	2pr-278	OM774386	**8.00**	0.50	0.50	**2.00**	0.125	**G59S, E266D**	*CDR1, CDR2*
16	2pr-58	OM774362	4.00	0.125	0.125	1.00	0.0312	**V51L**	*CDR1*
17	2pr-65	OM774354	4.00	0.50	0.50	1.00	0.25	**D116E, E266D**	*CDR1*
18	2pr-47	OM774366	4.00	**1.00**	**2.00**	0.50	0.50	**D116E, E266D**	*CDR1, CDR2*
19	2pr-87	OM774367	4.00	**1.00**	0.50	0.125	0.25	**D116E, E266D**	*CDR2*
20	2pr-77	OM774369	4:00	0.50	**2.00**	0.125	**1.00**	**D116E, E266D**	*CDR2*
21	2pr-57	OM774350	4.00	0.25	0.50	**2.00**	0.50	**D116E, E266D,**	*CDR1,*
22	2pr-59	OM774351	4.00	0.0312	**2.00**	0.0625	**8.00**	**E266D**	*CDR1, CDR2*
23	2pr-61	OM774352	4.00	0.125	0.125	0.50	0.25	**D116E, E266D**	*CDR2*
24	2pr-63	OM774353	4.00	0.50	**2.00**	0.125	0.25	**D116E, E266D**	*CDR1, CDR2*
25	2pr-7	OM774356	4.00	0.25	0.125	0.50	0.25	**E266D**	*CDR2*
26	2pr-36	OM774365	4.00	**1.00**	0.50	1.00	0.25	**D116E, E266D**	*CDR1, CDR2,*
27	2pr-83	OM774371	4.00	0.50	0.125	0.125	0.0625	**D116E, E266D**	*CDR2,*
28	2pr-329	OM774387	0.0312	0.125	0.50	1.00	0.125	**D116E, E266D**	-
29	2pr-332	OM774388	0.125	0.50	0.125	0.0312	0.50	**D116E, E266D**	-
30	2pr-339	OM774389	1.00	0.125	0.125	0.50	0.25	**D116E**	-
31	2pr-11	OM774358	2.00	0.50	0.50	1.00	**1.00**	**E266D, D504A**	-
32	2pr-13	OM774359	0.50	0.125	0.50	1.00	0.50	**D116E**	-
33	2pr-108	OM774372	1.00	0.50	0.50	1.00	**2.00**	**E266D**	-
34	2pr-149	OM774381	2.00	0.125	0.50	0.125	0.0312	**D116E, E266D**	-
35	2pr-168	OM774383	2.00	0.0312	0.50	0.0625	0.25	**D116E**	-
36	2pr-201	OM774385	1.00	0.50	0.125	1:00	0.125	**E266D**	-
37	2pr-129	OM774375	0.125	0.0312	0.125	1.00	0.25	-	-
38	2pr-93	OM774370	0.0312	**1.00**	0.125	1:00	0.125	-	-
39	2pr-136	OM774377	0.50	0.50	0.50	0.125	0.0312	-	-
40	2pr-163	OM774382	0.50	0.0312	0.50	1.00	0.25	-	-
41	2pr-67	OM774355	**< 0.0312**	**1.00**	0.125	**< 0.0312**	**> 16**	-	-
42	2pr-146	OM774380	2.00	0.125	0.0312	0.125	0.25	-	-

Abbreviations: FLZ, fluconazole; IRZ, itraconazole; VRC, voriconazole; AMB, amphotericin B; CAS, caspofungin; S, susceptible; R, resistant; SDD, susceptible-dose-dependent; I, intermediate.

[a] The genes with overexpression levels are shown in bold font. The genes with expression more than twice the mean of susceptible are shown in normal font. The amounts of MICs in resistance concentrations are shown in bold font.

antifungal susceptibility patterns are considered helpful in using appropriate antifungal drugs and preventing antifungal resistance.

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Footnotes

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