Original Article

Identification of *Candida* species in patients with oral lesion undergoing chemotherapy along with minimum inhibitory concentration to fluconazole

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Abstract

Background: Various species of *Candida*, especially *Candida albicans* was known as the most important etiological agent of fungal infections. Oral candidiasis is the most common fungal infection in patients undergoing chemotherapy. The purpose of this study was to identify *Candida* species from oral lesions of these patients and antifungal susceptibility of the clinical isolates.

Materials and Methods: Among 385 patients with cancer, 55 (14.3%) showed oral lesions. Oral swabs were performed to identify the yeasts using direct smear and CHROMagar medium. Micro dilution method was prepared in different concentrations of fluconazole and minimum inhibitory concentration and minimum fungicidal concentration of each species were compared.

Results: Oral candidiasis confirmed in 36 cases by direct examination and culture. *C. albicans* and non-*albicans* represented in 26 (72.2%) and 10 (27.8%) of the isolates, respectively. 76.5% of *C. albicans* and 23.5% non-*albicans* isolates were resistant to fluconazole. Data were shown that 62% and 30.7% of resistant strains of *C. albicans* were found in patient with gastrointestinal cancer and lymphoma respectively.

Conclusion: Data were shown that *C. albicans* is the most commonly identified species in oral candidiasis and majority of fluconazole resistant *C. albicans* were found in patients with gastrointestinal cancer and lymphoma. Therefore, we recommend an alternative drug instead of fluconazole as a first line of treatment for these type of cancers and administration of fluconazole in patients undergoing chemotherapy should be prescribed in accordance with the type of cancer.

Key Words: *Candida*, chemotherapy, fluconazole, microbial sensitivity test, oral candidiasis

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INTRODUCTION

The most essential fungal opportunistic pathogen is *Candida albicans*. It generally resides as a commensal in the mouth, digestive and genitourinary tracts.[1-4] These yeasts can cause infection when the host becomes weak or immunocompromised. The infections may be superficial and affect the mucous membrane or may attack the bloodstream and spread into internal organs.[5] The main risk factors for invasive candidiasis

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include; long-term remain in an intense care unit, previous administration of wide-spectrum antibiotics or immunosuppressive agents.[6-9] By these reasons, the people may be more sensitive to fungal infection diseases compared with healthy population.

Candida species are opportunistic pathogens which could associate with the virulence attributes of the organism and also the host factors.[10] There are various types of oropharyngeal candidiasis including acute pseudomembranous (thrush), acute atrophic, and angular cheilitis.[11]

Oral candidiasis is the most common fungal infection among children <1-month-old, the elderly, and also in cancer patients undergoing chemotherapy. These patients should be monitored in clinical trials of oral candidiasis.

In healthy population, carriage rates have been presented to confine from 20% to 75% with no sign.[12] In denture carriers, it has been reported 50–65%, people with long-term care facilities 65–88%, patients with leukemia and undergoing chemotherapy 90%, and patients with AIDS 95%.[13-17]

In immunocompromised patients, the infection may expand through the bloodstream or upper gastrointestinal tract leading to intense infection with morbidity and mortality. In patients with systemic candidiasis, mortality rate is from 71% to 79%.[14]

Fluconazole is an antifungal agent that is administered orally or intravenously. It is used to treat various fungal infections, especially vaginal, oral candidiasis. It is also used to prevent infections in people with weak immune systems, including neutropenic patients due to cancer chemotherapy, transplant patients, and premature babies. The mechanism of action involves interfering with synthesis of the fungal cell membrane. Fluconazole is an inhibitor of the human cytochrome P450 system.[18]

Most studies on oral candidiasis with cancer, come from USA, Europe, and other developed countries, and the subject of cancer rarely studied in developing countries or Iran. This is the first study in patients with various types of cancer who were under chemotherapy hospitalized in Seyed Al-Shohada, Isfahan, Iran. *In-vitro* susceptibility test of an antifungal agent; fluconazole was also evaluated on isolated *Candida* species.

MATERIALS AND METHODS

This study was conducted on patients undergoing chemotherapy with an average of 5 days staying in the hospital (3–7 days). The immunocompromised, diabetic patients, denture wearers, and the people with mental retardation were excluded from the study.

A questionnaire form was developed to record the medical history of patients, type of cancers, and demographic data. This research has been approved by Isfahan University of Medical Sciences/Ethics Committee).

Among 385 cancer patients undergoing chemotherapy, who were examined for lesions with creamy, whitish, curd-like plaques or pseudomembranes in oropharyngeal mucosa and the tongue, 55 cases showed oral lesions. Sampling was carried out by two wet swabs transferring in tubes containing 0.5 ml of saline solution. The swabs were used for direct examination and culture on CHROMagar *Candida* medium (CHROMagar Company, France).

Culture media were incubated in 35°C for 48 h, and if ≥10 CFU yeasts grown from each swab emerged on the plate, the sample was considered as positive. Stock cultures were grown on Sabouraud dextrose agar (SDA), (Merck, Germany) and were incubated at 35°C for 24 h to determine the minimum inhibitory concentration (MIC) of the respective antifungal agent.

The following methods were used for detection of *C. albicans*:

1. Detection of colored colony morphology on CHROMagar *Candida*
2. Discrimination of *C. albicans* by forming chlamydoconidium on Corn Meal Agar-Tween 80 (Merck, Germany), and incubation at 30°C for 3 days.

The MIC of fluconazole was performed using the broth microdilution technique proposed by the National Committee for Clinical Laboratory Standards (NCCLS).

All isolates of *Candida* species were sub-cultured at 30°C for 24 h on SDA plates. At least five colonies of yeasts were suspended in 5 ml of sterile saline (0.85%). The resulting suspension was vortexed for 15 s, and the turbidity of each suspension was adjusted at 0.5 McFarland standard (corresponding to 1×10^6–5×10^6 cells per ml) at 530 nm wavelength by the method of the (NCCLS). A working suspension was made by a 1:100 dilution followed by a 1:20 dilution of the stock suspension with RPMI 1640 broth medium, which resulted in 5.0×10^2–2.5×10^3 cells per ml.

The antifungal drug was purchased as stock powder (Sigma-Germany). In order to prepare 1280 μg/ml concentration, 13.061 mg/ml of stock solution was dissolved

in dimethyl sulfoxide (DMSO) (Merck-Germany). Small volumes of the sterile stock solution were dispensed into the sterile vials. Then they were kept at –20°C. For microdilution procedure, the drugs were diluted 1:5 with RPMI to achieve the 2 times strength needed for the broth microdilution test. The azole stock solutions were serially diluted in DMSO in accordance with the NCCLS M27-A guidelines. The range of concentrations tested was 0.25–128 μg/ml for fluconazole. A constant volume (100 μl) of the inoculum was added to each microdilution well-containing 100 μl of the serial dilution of antifungal agents to reach final concentrations. Two wells were used as positive and negative controls.[19]

The microplates were incubated at 35°C for 48 h. The MIC values for fluconazole were collated to the Clinical and Laboratory Standards Institute interpretative guideline on antifungal susceptibility testing. When ≥64 μg/ml of fluconazole is used and the fungal growth is continued, the yeast is susceptible, and it is considered as susceptible dose-dependent when 16–32 μg/ml of fluconazole is used and considered as susceptible when ≤8 μg/ml used. The minimum fungicidal concentration (MFC) was the lowest drug concentration that showed either no growth or fewer than three colonies to obtain approximately 99–99.5% killing activity.

RESULTS

Among 55 (14.3%) patients with oral lesions and various types of cancers, oral candidiasis was confirmed in 36 (65.4%) cases by direct examination and culture methods. The causative agents of oral candidiasis in cancer patients undergoing chemotherapy were *C. albicans* 26 (72.2%) and non-*albicans*10 (27.8%).

As it is shown in Table 1, among 8 types of cancer in patients undergoing chemotherapy; oral candidiasis was more frequent in patients with gastrointestinal cancer, leukemia, and lymphoma, respectively.

The MIC and MFC of each (range 0.25–128 μg/ml) were performed. Seventeen (47.2%) of the isolates were susceptible to fluconazole (MIC ≤ 8 μg/ml), 17 (47.2%) were fluconazole resistant (MIC ≥ 64 μg/ml), and 2 (5.6%) were susceptible dose dependent [Table 2].

Among 36 isolates, 26 (76.7%) were *C. albicans* and 10 (23.5%) were non-*albicans* isolates.

Table 1: Demographic characterization of cancer patients with candidiasis

Patient characteristic	*n* (%)
Sex	
Male	19 (52.8)
Female	17 (47.2)
Age (years)	
Range	25-81
Mean	53
Days admitted in hospital (days)	
Range	3-7
Mean	5
Cancer type	
Gastrointestinal	11 (30.5)
Leukemia	7 (19.5)
Liver	3 (8.3)
Lymphoma	6 (16.7)
Breast	3 (8.3)
Bladder	3 (8.3)
Lung	2 (5.6)
Bone	1 (2.8)
Total	36 (100)

DISCUSSION

C. albicans is the most important fungal pathogen that exists as a commensal in human gastrointestinal, urinary tract, and mouth.[15,16] This yeast can invade the cutaneous mucocutaneous membranes or disseminates to internal organs of immunocompromised hosts.[5] Candidal infections are the major problem in all patients around the world, particularly in those undergoing chemotherapy.[17] In addition, the transplant recipients, the patients using central venous catheters are high risk to accrue invasive forms.[20] Non-*C. albicans* species such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis,* and *C. krusie* cause 90% of invasive infections, and the relative prevalence of these species depends on the geographic area of the patients population.[21,22]

The frequency of *C. albicans* and other yeasts in patients are quite different according to the kind of cancer. At the present study of 385 patients undergoing chemotherapy 36 oral candidiasis in 8 types of cancer were confirmed. The most frequency of oral candidiasis was in patients with gastrointestinal cancer, leukemia, and lymphoma, respectively, and *C. albicans* was the most common agent in the mouth of patients with different type of cancers (72.2%). These results were consistent with the findings of other researchers.[23]

Due to ineffective diagnostic methods and inapplicable initial antifungal therapies, mortality rates are estimated to be as high as 45%.[24,25]

Fluconazole with favorable oral bioavailability and safety profiles has been used largely for chemoprophylaxis and treatment of systemic fungal infections in the past decade.[26,27] There are

Table 2: MIC, MFC, and sensitivity of fluconazole on 36 clinical isolates

Species code	Species	MIC	MFC	Sensitivity
1	*Candida* sp.	0.5	4	Sensitive
2	*Candida albicans*	2	4	Sensitive
3	*Candida albicans*	2	8	Sensitive
4	*Candida albicans*	2	8	Sensitive
5	*Candida albicans*	>128		Resistant
6	*Candida* sp.	0.25	1	Sensitive
8	*Candida* sp.	0.5	2	Sensitive
9	*Candida albicans*	2	8	Sensitive
11	*Candida albicans*	4	16	Sensitive
13	*Candida albicans*	2	4	Sensitive
14	*Candida albicans*	>128		Resistant
18	*Candida albicans*	>128		Resistant
19	*Candida albicans*	4	8	Sensitive
21	*Candida albicans*	>128		Resistant
22	*Candida albicans*	2	8	Sensitive
23	*Candida albicans*	>128		Resistant
24	*Candida albicans*	2	8	Sensitive
25	*Candida* sp.	16	32	Susceptible dose-dependent
27	*Candida albicans*	>128		Resistant
28	*Candida* sp.	2	8	Sensitive
30	*Candida* sp.	8	16	Sensitive
35	*Candida albicans*	2	8	Sensitive
36	*Candida albicans*	>128		Resistant
37	*Candida albicans*	>128		Resistant
38	*Candida albicans*	4	16	Sensitive
40	*Candida albicans*	>128		Resistant
41	*Candida* sp.	>128		Resistant
42	*Candida albicans*	>128		Resistant
44	*Candida albicans*	0.5	4	Sensitive
46	*Candida albicans*	>128		Resistant
55	*Candida albicans*	16	32	Susceptible dose-dependent
47	*Candida* sp.	>128		Resistant
48	*Candida* sp.	>128		Resistant
58	*Candida* sp.	>128		Resistant
49	*Candida albicans*	>128		Resistant
50	*Candida albicans*	>128		Resistant

Sensitive=47.2%, Resistant=47.2%, Susceptible dose-dependent=5.6%.
MIC: Minimum inhibitory concentration, MFC: Minimum fungicidal concentration

three pathways by which a patient might acquire a resistant organism: (a) Colonizing or infecting organisms initially susceptible but mutates and becomes resistant, (b) the patient is colonized or infected with more than one strain or species and an inherently resistant strain or species is selected or, (c) the patient is initially colonized or infected with an inherently resistant species.[28] The least satisfactory definition of resistance is one based solely on MICs, but unfortunately, isolates are sometimes described as resistant on the basis of promptly chosen breakpoints without reference to the clinical outcome. The presence or development of elevated MICs is unnecessary if the patient improves clinically. This is true even if the MICs increase the achievable focus of the given antifungal agent in serum.[29]

The treatment fail to eliminate the fungus is not simply clarified because sometimes the clinical status of many patients with resistant yeasts improved.[28] Various studies have been conducted using wide-spectrum of antifungal drugs resistance on *C. albicans* and non-*albicans* species such as *C. glabrata* and *C. krusei*.

In this study, the majority of resistant strains in patients with gastric cancer and lymphoma have demonstrated high incidence (84%) of oral colonization with *C. albicans* yeasts. Seven of 11 isolated *Candida* from gastrointestinal cancer (87.5%), and 4 (66.6%) with the agent of *C. albicans* isolated from lymphoma patients were resistant to fluconazole. Hence, according to present data, it will be suggested fluconazole should be prescribed in accordance with the type of cancer. Clarkson *et al*. found the same results about the oral colonization of *Candida* in gastrointestinal cancer and they proved that ketoconazole and clotrimazole were more sensitive than fluconazole in the treatment of oral candidiasis.[30]

In general, topical agents are considered superior to systemic agents due to lower risk of side effects and drug interactions. The Infectious Diseases Society of America (IDSA) guidelines recommend that clotrimazole troches or nystatin suspension/pastilles can use for the treatment of moderate oropharyngeal candidiasis as first line antifungal drugs. Advantages of nystatin rinse include its availability and ease of use.[31]

For fluconazole-refractory disease, the IDSA guidelines recommend itraconazole or posaconazole, with voriconazole and amphotericin B reserved for refractory cases.[32]

CONCLUSION

Altogether our data showed that *C. albicans* is the most commonly identified species in oral candidiasis and majority of fluconazole-resistant *C. albicans* were found in patients with gastrointestinal cancer and lymphoma. Therefore, we recommend to use alternative drug instead of fluconazole as a first line of treatment for these type of cancers and administration of fluconazole in patients undergoing chemotherapy should be prescribed in accordance with the type of cancer and the severity of the infection. Initially, according to IDSA guidelines recommendations, the use of clotrimazole troches or nystatin suspension/

pastilles as first-line therapy for the management of mild oropharyngeal candidiasis and other systemic drugs are recommended to determine the MIC on samples isolated from the patients.

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Conflicts of interest

There are no conflicts of interest.

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