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Frequency of *Candida* and Candidiasis in Tehran, Iran

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ABSTRACT

Aims: In imbalanced conditions, *Candida* species colonization as a normal microflora of human skin and some mucosal surfaces is replaced by invasive forms (budding yeast cells, pseudohyphae, and true hyphae). This study aimed to investigate the frequency of *Candida* species and candidiasis with emphasis on the presence and propensity of different *Candida* species for pseudohyphae and true hyphae formation in clinical samples taken from various clinical forms of candidiasis.

Materials & Methods: In this cross-sectional study (2018 to 2019), sampling was done from 492 patients suspected to candidiasis, referred to the Medical Mycology Laboratory. Employing direct microscopy and culturing methods, the *Candida* species were identified using morphological and biochemical characteristics and also PCR-RFLP and DNA sequencing.

Findings: From a total of 96 candidiasis patients, 44.9% were identified with superficial-cutaneous and 55.1% with visceral candidiasis. The most clinical strains were isolated from fingernail scrapings (33.2%), followed by bronchoalveolar lavage samples (17%). The mycelium was found in 55.2% of the cases, and the highest frequency was related to the nail specimens (34%, p <.05). *C. albicans* was the predominant species forming mycelium (69.8%), followed by *C. tropicalis,* but no mycelium was found in *C. guilliermondii* cases. Mycelium formation was observed more in patients with an underlying disease such as AIDS and organ transplantation (p <.05).

Conclusion: Non-*albicans Candida* species have also the propensity to induce an invasive form of mycelial in the skin and to increase internal organs temperature, exacerbating clinical symptoms. This finding is important for choosing proper antifungal treatments and should be taken into account by clinicians.

Keywords: *Candida,* Invasion, Mycelium, Non-*albicans,* Pseudohyphae, PCR.

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Introduction

Candidiasis is a primary or secondary fungal infection. More than 17 different *Candida* species are known to be as the etiological agents of candidiasis; however, the most common of which is *C. albicans*. It may appears as acute, subacute, or chronic clinical forms. *Candida* species could cause infections in the human skin, finger, toenails, mouth tissue, throat, trachea, lungs, genitalia, and gastrointestinal tract. They may also lead to a variety of systemic infections such as meningitis, endocarditis, and septicemia. Oral candidiasis, also known as oral thrush, is one of the most commonly reported clinical forms of *Candidia* infection [1-2]. *Candida* species are present in the gastrointestinal tract of about 30-60% of healthy people and up to 92% of patients with diabetes mellitus [3-5]. The rate of morbidity caused by candidiasis among the hospitalized patients is approximately 5-10% so that *Candida* species are the 11th common cause of morbidity and mortality among this population [6-7]. Urinary tract candidiasis is known as the most frequent nosocomial fungal infection worldwide and accounts for 17.1% of all urinary tract infections [7-8]. Also, the rate of *Candida* infection incidence in infants admitted to the NICU is 20%, and the mortality rate in this group is 30% [9]. Superficial-cutaneous candidiasis has the second rank among the cutaneous infections caused by fungal microorganisms, and onychomycosis is the most common form of cutaneous candidiasis [10]. *Candida* species are opportunistic fungal pathogens found as a part of the normal microflora in human skin and mucosal surfaces. Since they are well-balanced in the human body, the infection occurs when some factors change this normal balance. For example, long-term use of broad-spectrum antibiotics to treat non-fungal infections may affect this balance. Other factors include the immune system status, age, physiological changes, physical and mental disabilities, and debilitating diseases, diabetes, clinical use of immunosuppressive drugs, occupation, obesity, vascular disease, alcoholism, and avitaminosis [1-3]. Also, *Candida* species express several virulence factors contributing to pathogenicity [1-3, 11]. *Candida* species could grow in different morphological forms (yeast, blastoconidia, pseudohyphae, and true hyphae). These morphological forms play various roles during communal growth and infection. For example, blastoconidia form of this organism readily undergoes the morphological change from yeast to hyphal or pseudohyphal form. Ovoid yeast cells are the predominant form during the communal conditions, while pseudohyphae and filamentous hyphae are crucial for tissue invasion [1-2, 12-13]. However, in several studies, *Candida* species able to produce mycelium in host tissue and laboratory conditions have been identified [1-2, 11, 13], but there is no complete information about the ability of different species to form mycelium in various types of clinical specimens (superficial-cutaneous and visceral samples). Also, there is no comprehensive information about the effect of host underlying and predisposing factors on the ability to invade and form mycelium in any *Candida* species, especially non-*albicans* species.

Objectives: Therefore, the aim of this study was to determine the presence and propensity of different *Candida* species for pseudohyphae and true hyphae formation in clinical samples taken from various clinical forms of candidiasis.

Materials and methods

Sampling: This study was conducted on suspected patients to superficial-cutaneous and visceral candidiasis, referred to the Medical Mycology Laboratory of Tehran University of Medical Sciences. During January 2018 to January 2019, 492 specimens were collected from bronchoalveolar lavage, sputum, blood, stool, urine, wound swabs, mouth tissue, peritoneal fluid, abdominal fluid, lung cavity lesion, maxillary sinus specimens, fingernail

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scraping, the skin in the groin, toenail scraping, and the axillary skin. Samples were prepared by KOH 10% and cultured on Sabouraud's dextrose agar (Merck, Germany) and brain heart infusion agar and kept at 25 and 37°C for at least 2 weeks. Isolates were identified based on germ tube test, chlamydoconidia production in cornmeal agar (Becton, France), colony color on chromogenic CHROMagar *Candida* medium (CHROMagar, Paris, France), and API 20 C AUX kits (bioMérieux, Marcy l'Etoile, France). Also, unidentifiable isolates were subjected to PCR-RFLP and sequencing techniques to confirm species identification.

DNA extraction: Fungal DNA was extracted using the glass bead disruption method from harvested Candida colonies [14-15]. Briefly, some of fresh yeast was suspended in 300 µL of lysis buffer (10 mM Tris, 1% SDS, 100 mM NaCl, 1 mM EDTA PH 8, 2% Triton X-100). Then, 300 µL of phenol-chloroform (1 : 1) and 300 mg of glass beads (0.5 mm in diameter), were added and vortexed for 5 min for disrupting of the cells completely. By centrifugation at 10,000 RPM for 5 min, the debris was separated and the aqueous layer was extracted tow time with an equal volume of chloroform. Total DNA in the supernatant was precipitated with isopropanol, washed with 70% ethanol. Finally it was airdried and re-suspended in 100 µL of TE buffer (1 mM EDTA, 10 mM Tris), and preserved at -20 °C until future use.

PCR conditions and sequencing: By using the universal primers (ITS1 and ITS4), the ITS1-5.8S-ITS2 regions of yeast genomic rDNA were amplified [15]. With a final volume of 100 µL, PCR amplification was carried out. Each reaction mixture contained 0.2 µM of each forward (ITS1, 5'-TCC GTA GGT GAA CCT GCG G-3') and reverse (ITS4, 5'-TCC TCC GCT TAT TGA TAT GC-3') primers, 1 µL of template DNA, 0.1 mM of each deoxynu-cleoside triphosphate dNTPat, 2.5 U of TaqDNA polymerase and 10 µL of 10×PCR buffer. The steps of thermocycler program were accordance with an initial denaturation at 94°C for 5 min and 25 cycles of denaturation at 94°C for 30 s, followed by annealing at 56°C for 45 s, extension at 72°C for 1 min and a final extension step at 72°C for 7 min. The PCR amplified products were visualized by agarose gel electrophoresis (1.5% w/v) in TBE buffer (0.09 M Tris, 20 mM EDTA and 0.09 M boric acid with pH 8.3) and stained with ethidium bromide(0.5 µg/mL). Negative and positive controls were used in PCR procedure. For sequencing, the positive PCR products were sent to Bioneer Advanced Nucleic Acids core facility. By using the BLAST algorithm available at NCBI website (http: www.ncbi.nlm.nih.gov.BLAST.), the ITS sequences were then analyzed from the coating and separately used to perform individual nucleotide–nucleotide searches. Identification of the Candida species was done based on the maximum identities ≥ 99% and query coverage ≥ 98%.

Statistical tests: The data of the present study, were analyzed using SPSS software Version 22.0. The methods of one tail chi-square test and Fisher's exact test were used for categorical data. Statistically, the p-value<.05 was considered as significant.

Findings

Among the 492 clinical cases suspected to superficial-cutaneous and visceral candidiasis, 96 patients (19.5 %) were identified with candidiasis. The study population comprised 46 (47.9%) females and 53 (55.1%) males with the age ranges from 1 to 86 years in both sexes. Most infected patients were over 50 years old (53.1%). The highest isolation rate of *Candida* species was related to fingernails (32 cases, 33.2%), followed by Bronchoalveolar lavage samples (16 cases, 17%), and the lowest isolation rate was related to urine, peritoneal fluid, and maxillary sinus specimens (each with 1 positive case, 1%).

The most commonly isolated *Candida* species in both superficial-cutaneous and

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visceral specimens were as follows: *C. albicans* (50 cases, 52.1%), *C. glabrata* and *C. tropicalis* (each with 16 cases, 16.7%), *C. parapsilosis* (10 cases, 10.4%), *C. krusei* (2 cases, 2.1%), *C. guilliermondii* and *C. kefyr* (each with 1 case, 1%) (Table 1).

Mycelium formation was observed in 53 clinical specimens (55.2%), and the propensity of various *Candida* species for mycelium formation was significantly different according to the type of clinical specimen. The highest rate of mycelium formation was related to nail specimens (34%, p <.05), and in the urine and peritoneal specimens, mycelium formation was not observed. Also, mycelium formation was more common in patients aged over 50 years and in females. *C. albicans* was the predominant species with regard to the mycelium formation (69.8%), while this ability was not observed in *C. guilliermondii* (Table 2).

In this study, 11 cases (11.5%) suffered from underlying diseases and special conditions, including HIV infection and organ transplantation. Visceral candidiasis was reported in all of whom. *C. albicans* was the predominant species isolated from these patients, and the highest isolation rate was related to the mouth tissue, regarding to the type of clinical specimens. It should be noted that the mycelium formation was observed in most patients of this group (p< .05) (Figure 1). Also, the results of this study showed that only 1 year old infants had *Candida* septicemia, and the etiologic agent in these cases was *C. glabrata*.

Discussion

Imbalances between normal fungal flora, normal bacterial flora, and immune defense mechanisms could lead to fungal overgrowth, formation of invasive *Candida* morphology, and candidiasis. Some of the host factors affecting normal balance conditions are age, physiological changes, long-term use of antibiotics, immunodeficiency disease such as AIDS and transplantation, chemotherapy, diabetes, and the use of immunosuppressive drugs [1-3]. Also, *Candida* species express several virulence factors contributing to pathogenicity. These factors include surface adhesion molecules, secreted aspartyl proteases and phospholipases and morphogenesis (the reverse transition between unicellular yeast cells and filamentous, growth forms), and secreted aspartyl proteases and phospholipases. Additionally, 'phenotypic switching' in *C. albicans* and several other *Candida* species is accompanied by changes in antigen expression, colony morphology, and tissue affinities and leads to the deep penetration of keratinized epithelia, assisted by hypha formation. Switching might provide cells with a flexibility, resulting in the adaptation of the organism to the hostile conditions and prevention from the defense of host immune system [1-3, 11].

Furthermore, environmental factors such as acidity (PH), temperature, glucose state, and tissue type could be effective on the ability of *Candida* flora to be converted into invasive forms [1-2].

In *Candida* species, germ tube formation followed by hyphal growth is a critical property in the pathogenesis of symptomatic visceral infections and superficial-cutaneous candidiasis. In these infections, the infected tissues often contain invasive forms, including budding yeasts, pseudohyphae, or true hyphae [1-2].

In this study, the ability of different *Candida* species to form pseudohyphae and true hyphae was investigated, and these invasive forms were observed in 55.2% of cases. The invasion was observed more in females and in their superficial-cutaneous specimens. Also, in this gender, fingernails were the most frequent anatomical site of *Candida* infection; therefore, there was a statistically significant relationship between the gender

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Table 1) The frequency of *Candida* species in different types of clinical specimens

Candida Species	*Candida. albicans*		*Candida. tropicalis*		*Candida. glabrata*		*Candida. parapilosis*		*Candida. krusei*		*Candida. lliermondii*		*Candida. kefyr*		Total	
Specimen	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Broncho alveolar lavage	10	10.4	3	3.1	3	3.1	0	0	0	0	0	0	0	0	16	16.6
Sputum	6	6.3	1	1	1	1	0	0	0	0	0	0	0	0	8	8.3
Blood	0	0	0	0	1	1	2	2.1	0	0	0	0	0	0	3	3.1
Stool	2	2.1	0	0	0	0	0	0	0	0	0	0	0	0	2	2.1
Urine	1	1.1	0	0	0	0	0	0	0	0	0	0	0	0	1	1.1
Wound swabs	6	6.2	2	2.1	2	2.1	0	0	0	0	0	0	0	0	10	10.4
Mouth tissue	4	4.2	1	1	1	1	0	0	0	0	0	0	0	0	6	6.2
Peritoneal fluid	0	0	1	1.1	0	0	0	0	0	0	0	0	0	0	1	1.1
Abdominal fluid	0	0	0	0	2	2.1	1	1.1	0	0	0	0	0	0	3	3.3
Lung cavity lesion	1	1.1	0	0	1	1.1	0	0	0	0	0	0	0	0	2	2.1
Maxillary sinus specimens	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Finger nail clipping	10	10.4	8	8.3	5	5.2	5	5.2	2	2.1	1	1	1	1	32	33.3
The groin	6	6.2	0	0	0	0	2	2.1	0	0	0	0	0	0	8	8.3
Toenail clipping	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
The axillary skin	2	2.1	0	0	0	0	0	0	0	0	0	0	0	0	2	2.1
Total	50	52.1	16	16.6	16	16.6	10	10.4	2	2.1	1	1.1	1	1.1	96	100

No.: Number, %: percentage

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Table 2) The frequency of mycelium formation (creation invasive forms) by different *Candida* species in various types of clinical specimens

Candida Species	*Candida. albicans*		*Candida. tropicalis*		*Candida. glabrata*		*Candida. parapilosis*		*Candida. krusei*		*Candida. guilliermondii*		*Candida. kefyr*		Total	
Specimen	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Broncho alveolar lavage	3	5.7	3	5.7	0	0	0	0	0	0	0	0	0	0	6	11.3
Sputum	5	9.4	1	1.9	0	0	0	0	0	0	0	0	0	0	6	11.3
Blood	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stool	2	3.8	0	0	0	0	0	0	0	0	0	0	0	0	2	3.8
Urine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wound swabs	6	11.3	2	3.8	0	0	0	0	0	0	0	0	0	0	8	15.1
Mouth tissue	3	5.7	0	0	0	0	0	0	0	0	0	0	0	0	3	5.7
Peritoneal fluid	0	0	1	1.9	0	0	0	0	0	0	0	0	0	0	1	1.9
Abdominal fluid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lung cavity lesion	1	1.9	0	0	0	0	0	0	0	0	0	0	0	0	1	1.9
Maxillary sinus specimens	1	1.9	0	0	0	0	0	0	0	0	0	0	0	0	1	1.9
Finger nail clipping	9	17	3	5.7	0	0	4	7.5	1	1.9	0	0	1	1.9	18	34
The groin	5	9.4	0	0	0	0	0	0	0	0	0	0	0	0	5	9.4
Toenail clipping	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
The axillary skin	2	3.8	0	0	0	0	0	0	0	0	0	0	0	0	2	3.8
Total	37	69.9	10	18.8	0	0	4	7.5	1	1.9	0	0	1	1.9	53	100

No.: Number, %: percentage

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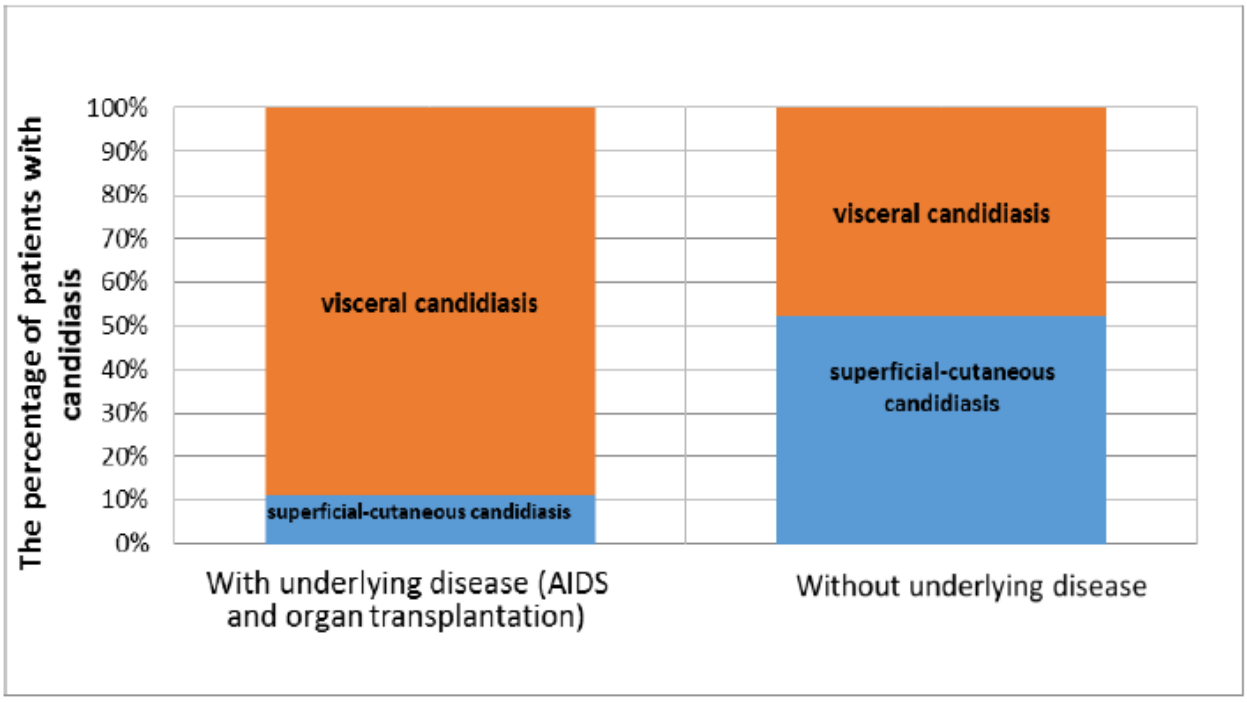

Figure 1) The percentage of mycelium formation (creation of invasive form) in different types of candidiasis (superficial, cutaneous and invasive) with regard to underlying diseases

and anatomical site of infection (p<.05). These findings are consistent with other research results in Iran [10, 14]. Given the greater chance of hands for contact with pollution sources, and due to the fact that prolonged contact of hands with water creates suitable conditions for their easy colonization, the increased chance of *Candida* infection in this anatomical site is explainable [1,7,15]. Also, the effect of female sex hormones on reducing the immune response in this gender might be another reason for this finding [1-2, 16]. Although the optimum temperature for hyphae formation is 37°C, the results of this study highlight the ability of *Candida* species to adapt with skin temperatures (lower than 37 °C) and to form mycelium in this temperature [1-2,17].

The highest rate of invasion was observed in patients over 50 years. This result may be due to the development of dry skin and reduced shedding along with general body weakness and probable immune response deficiency in this age group. Also, increased age is a risk factor for fungal colonization and infection due to reduced T-cell proliferation and cellular immune deficiency (CMI) [1-2, 16, 18]. In this study, *C. albicans* was the most prevalent species involved in invasive forms (69.8%). Frequent isolation of this species from clinical infections and its virulence factors such as hyphae formation could be the reasons for this observed result [1-2, 11].

In 11.5% of patients with an underlying disease (including AIDS and transplantation), only visceral candidiasis was observed, with the mouth as the most commonly infected anatomical site in them. Also, *C. albicans* was the most frequent isolated species in this specific group of patients. It should be noted that the ability to form mycelium was observed in most of these patients (Figure 1, p<.05). This finding emphasizes the association between the immunodeficiency and the use of immunosuppressive drug with increased risk of *Candida* infections [2, 16, 18]. Although most clinical manifestations were found in people over 50 years, and *C. albicans* was the predominant isolated agent in this age group, this study showed that *Candida* septicemia was observed only in

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infants, and its etiologic agent in all cases was *C. glabrata*. This species had the second rank among the causative agents of candidiasis in this study. Given the resistance of *C. glabrata* to antifungal drugs and due to the relatively under-developing immune system in infants and life threatening clinical manifestations of septicemia, special attention should be paid to this species by laboratory experts and clinicians.

Conclusion

According to this study results, the highest isolation rate of *Candida* species was related to fingernails, followed by Bronchoalveolar lavage samples; which is necessary to be taken into account by both patients and physicians. Patients with underlying diseases were the group most at risk of visceral candidiasis complication. It should be noted that mycelium formation was observed in most patients in this group. In addition to *C. albicans* high frequency and its predominant mycelium formation, other non-*albicans* species such as *C. tropicalis* (first rank of non-albicans *Candida*) could have an invasive form and mycelium formation at 37°C or below, exacerbating clinical symptoms. Due to the species-dependent resistance of *Candida* species to antifungal drugs, this finding is important for treating *Candida* infections and choosing appropriate antifungal agents, which should be considered by both laboratory technicians and clinicians.

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Ethical Permissions: This study was approved by the ethical committee of Tehran University of Medical Sciences (the number of Ethics Committee protocol: IR.TUMS.SPH.REC.1397.167).

Conflict of interest: The authors have no conflicts of interest to declare for this study.

Authors' Contribution: All authors contributed to this study.

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