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Distribution of invasive fungal infections: Molecular epidemiology, etiology, clinical conditions, diagnosis and risk factors: A 3-year experience with 490 patients under intensive care

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ABSTRACT

Recently, the prevalence of invasive fungal infections (IFIs) is rising. The global mortality rate of IFIs is 10–49%. This study aimed to determine the prevalence, the causative agents, and the risk factors associated with the invasive fungal infections in a tertiary health center to provide valid decision-grounds for healthcare professionals to effectively prevent, control, and treat fungal infections. The current study was conducted on 1477 patients suspected to have systemic fungal infections from different units of the hospital. After screening using routine mycological examination, the patients were confirmed with complementary mycological and molecular methods. Patients were included based on the confirmed diagnosis of IFI and excluded based on lack of a microbiologically and histologically proven diagnosis of IFI. Of the 1477 patients recruited in this study, confirmed cases of fungal infection were 490 (169 proven; 321 cases probable). Among the fungi recovered, *Candida* species had the highest frequency 337 (68.8%) followed by *Aspergillus* species 108 (22.1%), *Zygomycetes* species 21 (4.3%), non-*Candida* yeast 9 (1.8%). Others were black fungi 5 (1%), mycetoma agents 5 (1%), *Fusarium* 4 (0.8%), and *Trichoderma* (0.2%). Hematologic malignancies and diabetes mellitus were the most common underlying diseases among IFI-confirmed patients. This study observed an increased frequency of invasive candidiasis with non-*albicans Candida* and other invasive saprophytic fungal infections. The increased rate of invasive candidiasis with non-*albicans* agents highlights a new perspective in the epidemiology and treatment of invasive fungal infections.

1. Introduction

Fungal infections represent a spectrum of mild to severe systemic and life-threatening diseases [1]. Recently, systemic fungal infections have been rising probably due to improved diagnostic methods and an increasing number of susceptible patients [2]. Systemic fungal infections

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can be categorized into the primary systemic fungal infections that are observed in people with competent immune systems, often caused by fungi, such as *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Paracoccidioides brasiliensis,* and *Sporothrix schenckii* - usually correspond with specific geographical areas - and the opportunistic systemic fungal infections that are seen in people with defective immune systems [3].

On the global estimate, more than 800 million people suffer from invasive fungal infections (IFIs) and the yearly death rates of IFI (1,660,000) are higher than that of malaria (445,000) and comparable to that of tuberculosis (1,700,000) [4]. IFI is 3–15 times more common in Asian countries than in the West [3]. Apart from the high mortality rates (10–49%), prolonged hospital stay and serious financial repercussions are major economic setbacks of IFIs [5]. Factors associated with increased risk of IFI include malignancy; AIDS; protracted hospital stay; neutropenia; contamination of in situ foreign objects such as urinary catheters, intravenous catheters, feeding or draining tubes; persistent use of corticosteroids and broad-spectrum antibiotics [6,7].

Invasive aspergillosis and candidiasis account for more than 90% of hospital fungal infections, with a combined mortality rate between 60 and 95%, especially in children and infants [8,9]. Moreover, the recent emergence of unusual species that are resistant to treatment such as *Candida auris* has compounded challenges of systemic mycoses management [2]. Patients in the extreme of ages are more susceptible to these infections due to their weak immunity [10]. For instance, in the past decade, the mortality rate of candidemia in children was reported to be 19–31% and even higher for aspergillosis (68–77%) [11]. Systemic candidiasis was reported to be the second cause of death in premature babies; about 20% of the patients succumb despite receiving antifungal drugs [12]. *Candida*-related fungemia in both adults and children has been a serious challenge in intensive care delivery and contributes to 10–15% of hospital-acquired blood infections [5].

The similarities between clinical symptoms of invasive fungal diseases and that of other infections further complicate the diagnosis of IFIs. The definitive diagnosis of such infections entails observing the incriminating fungus is the patients' samples even in autopsies after the patient's death. From the standpoint of clinical epidemiology, early detection of invasive fungal infections and their associated risk factors can improve the therapeutic outcome and decrease the mortality rates among patients.

This study aimed at determining the prevalence, causative agents, and risk factors associated with invasive fungal infections in a tertiary health center, in Tehran, to provide a valid ground that may guide healthcare professionals to effectively prevent, control, and treat fungal infections.

2. Material and methods

2.1. Study design and population

This cross-sectional study was conducted at Amir-Alam and Imam Khomeini hospitals, Tehran, the capital of Iran between November 2016 and December 2018. The hospitals are the referral centers for patients with different diseases from all parts of the country. We included 1477 patients referred to the centers with suspected FRS (Fungal Rhino Sinusitis) according to the Helsinki Declaration, after obtaining written from the patients. The inclusion criteria were: confirmed diagnosis of IFI according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria (i.e., clinical, microbiological, and histological evidence of invasive fungal infection). We excluded any patient that did not have a microbiologically and/or histologically proven diagnosis of IFI. Different specimens, such as bronchoalveolar lavage (BAL), blood, bone marrow, catheter tips, cerebrospinal fluid (CSF), ear, eye, endotracheal tubes, gastric fluid, hard palate, lumbar vertebra, nail, peritoneum, pleura, portal vein, stool, paranasal sinuses, sputum, sternum, synovial fluid, thorax, tongue, urine, vagina, and wound were collected, transported to and processed at Medical Mycology Laboratory, Faculty of Public Health, Tehran University of Medical Sciences (TUMS), Tehran, Iran. Along with the sample, we used questionnaires to collected patient's information, such as age, gender, the location of the lesion, the duration of the sickness, medical and drug history, and other essential information from the patient's records.

2.2. Mycological studies

2.2.1. Direct examination

All samples collected were examined directly using KOH wet preparation by placing a small portion of the sample on a microscopic slide containing KOH solution and observed for fungal elements, such as mycelium (with or without cell-wall), spore, yeast (without or without bud), pseudohyphae, etc.

2.2.2. Culture technique

To culture the fungal agents, we inoculated brain heart infusion agar (BHIA) and Sabouraud's dexterous agar (SDA) containing chloramphenicol with the sample under sterile conditions. We incubated the inoculated plates at 30 °C for 4 weeks and thereafter examined for the growth of fungal agents daily. At this stage, we could partially determine the genus and the species of the fungus.

2.2.2.1. Culture results interpretation.

1. Isolation of one fungus colony from sterile body fluids like blood or CSF is significant.
2. Isolation of several colonies of one fungus type at the inoculation site is significant.
3. Extracting several colonies from two or more different fungi at inoculation sites is significant (co-infection = repeating the culture to confirm results)
4. Isolation of colonies of two or more fungi other than at inoculation sites is not significant (due to contamination).

2.2.3. Indian ink test

To detect the glycopolysaccharide capsule of *Cryptococcus neoformans*, we negatively stained suspected samples with Indian ink and visualized the capsule as a transparent halo in a black background.

2.3. PCR & sequencing

We used PCR-sequencing methods to confirm the identity of some isolates that were not identifiable using conventional methods. Specific primers ITS1, ITS4, β-tubulin, and elongation factors were utilized for this analysis. The results of sequencing evaluated and compared using of NCBI BLAST searches against fungal sequences existing in DNA databases (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.4. Other confirmatory methods

Other tests used to confirm the isolates include germ tube formation, chlamydoconidia formation, culture on Chromogenic *Candida* agar, and the fungal β (1,3)-D-glucan assay.

2.4.1. Germ-tube test

The germ tube test was performed by inoculating 0.5 mL samples of pooled human sera in borosilicate tubes by touching colony surfaces with a sterile Pasteur pipette tip and then gently emulsifying the cells which adhered to the pipette into the serum. Open tubes were incubated

at 37 °C for 2–4 h. Suspect germ tubes were confirmed by microscopy at 40×. Germ-tube was indicative of *C. albicans* or *C. dubliniensis.*

2.4.2. Chlamydoconidia formation test

Chlamydoconidia formation test was performed using corn meal agar medium supplemented by 1% of Tween 80. The samples previously grown in SDA were seeded as 3 parallel streaks in a rectangular piece of corn meal agar placed between two slides, incubated in wet chamber at 30 °C for 72 h and visualized in an optical microscope (10× and 40× magnification). The formation of rounded spores with double-wall isolates was observed as chlamydoconidia, and was indicative of *C. albicans* or *C. dubliniensis*. For all phenotypic tests, negative control (*C. glabrata*) and positive controls (*C. albicans* and *C. dubliniensis*) were considered.

2.4.3. Chromogenic agar culture

Yeast strains were cultured routinely on Sabouraud dextrose agar (SDA) at 30 °C for 48 h. Then, pure colonies were transferred on CHROMagar *candida* for the isolation and presumptive identification of *Candida* species. The *Candida* isolates were identified after incubation for 48 h at 37 °C. The strains were identified according to the manufacturer's instructions, which define *C. albicans* or *C. dubliniensis* as green colonies, *C. tropicalis* as steel blue colonies, *C. krusei* colonies as showing rose color and rough aspect, and the other species as developing colonies from white to rose.

2.4.4. Fungal β (1,3)-D-glucan assay

Patients' sera recovered from arterial and/or venous blood specimens were tested for Beta-(1, 3)-D-glucan as recommended by the manufacturer (Fungitell®; Associates of Cape Cod Inc, Falmouth, MA, USA). The concentration of Beta-(1, 3)-D-glucan in each sample was automatically calculated using a calibration curve with standard solutions ranging from 31.25 to 500 pg/mL. The manufacturer's Beta-(1, 3)-D-glucan cut-off of 80 pg/mL was used. All samples were analyzed in triplicate and the mean was assigned as the final result for the specimen.

2.5. Statistical analysis

We analyzed the obtained data using SPSS software employing statistical tests such as Chi-Square, Fischer, and *t*-test. P-value <0.05 was considered significant.

3. Results

A total of 490 cases met the criteria for invasive fungal infection (169 cases/Proven; 321 cases/Probable IFIs). Out of 490 inpatients studied, 293 were men (59.8%), and 197 women (40.2%). The mean age of the patients was 47.2 ± 3.8 years; the majority (34.1%) were at least 50 years. Accordingly, the majority of sample examine were BAL (33.3%); sputum (15.3%); nasal sinus (14.5%); wound (6.9%); endobronchial (6.3%); urine (4.9%); biopsy (4.5%); blood (3.7%); ascites and hard plate each (2.7%); abscess (1.8%), CSF, ear, Vaginal swab, S/E and orbit each (0.6%); and gastric acid and pleura fluid (0.2%). In this study, 54.9% of IFIs were primarily localized to the lung, (14.5%) to sinuses, (6.9%) to wound, (4.5%), to biopsied tissue, and (3.7%) to the bloodstream.

Table 1 shows the clinical characteristics of 490 cases of proven or probable invasive fungal infection among 1477 Iranian patients.

Table 2 depicts the distribution and frequency of the samples examined in this study while the distribution and the frequency of the underlying diseases are shown in Table 3.

Among fungi recovered, 346 (70.6%) were yeast and 144 (29.4%) were saprophytes. The majority of the yeast identified belong to *Candida* species 337 (68.8%) with few non-*Candida* yeasts 9 (1.8%). Species of the genus *Aspergillus* were the most common saprophytes 108 (22.1%) followed by *Zygomycetes* species 21 (4.3%), black fungus and mycetoma agents each 5 cases (1%), *Fusarium* 4 (0.8%), and *Trichoderma* 1 (0.2%) [Table 4].

The distribution of the saprophytes (*Aspergillus* and other molds) in comparison with the yeasts isolated from systemic fungal infections is shown in Table 5.

Table 6 shows the relationship between gender and multiple fungi infections. Accordingly, systemic fungal infections were higher in men (59.8%) than in women (40.2%). Similarly, the table shows that

Table 1
Clinical characteristics of 490 cases of proven or probable invasive fungal infection among 1477 Iranian patients.

Characteristics of infections and isolates	Frequency (%)
Site of infection	
Lung	269 (54.9)
Sinuses	71 (14.5)
Blood	18 (3.7)
CNS	3 (0.6)
Orbit	3 (0.6)
Tissue/Biopsy	22 (4.5)
Urinary tract	24 (4.9)
Needle aspiration	24 (4.9)
Wound	34 (6.9)
Other	22 (4.5)
Certainty of diagnosis	
Proven	169 (34.5)
Probable	321 (65.5)
Fungal species	
Yeast/Yeast-like	**346 (70.6)**
Candida Albicans	108 (22)
Candida spp	229 (46.7)
Cryptococcus spp	3 (0.6)
Geotrichum spp	3 (0.6)
Trichosporon spp	3 (0.6)
Mold/Mold-like	**144 (29.4)**
Aspergillus spp	108 (22)
Alternaria Alternata	2 (0.4)
Actinomyces	4 (0.8)
Fusarium spp	4 (0.8)
Mycetoma agents	1 (0.2)
Nattrassia Mangiferae	2 (0.4)
Phoma spp	1 (0.2)
Trichoderma spp	1 (0.2)
Zygomycetes spp	21 (4.3)

Table 2
Distribution of samples examined in cases with invasive fungal infections.

Condition	No. of patients (%)	P-value		
	2016 (n = 155)	2017 (n = 172)	2018 (n = 163)	
BAL	59 (38.1)	55 (32)	49 (30.1)	0.3857
Sputum	26 (16.8)	25 (14.5)	24 (14.7)	0.4217
Abscess	4 (2.6)	2 (1.2)	3 (1.8)	0.2677
B/C	5 (3.2)	5 (2.9)	8 (4.9)	0.1840
Biopsy	6 (3.9)	9 (5.3)	7 (4.3)	0.5812
Gastric acid	1 (0.7)	0 (0)	0 (0)	0.3299
Endotracheal	15 (9.7)	8 (4.7)	8 (4.9)	0.7766
CSF	1 (0.7)	1 (0.6)	1 (0.7)	0.2423
Ear	2 (1.3)	1 (0.6)	0 (0)	0.1057
Ascites	2 (1.3)	7 (4.1)	4 (2.5)	0.3559
Sinonasal	7 (4.5)	34 (19.8)	30 (18.4)	0.1112
Pleura Fluid	0 (0)	1 (0.6)	0 (0)	0.4367
UA/UC	9 (5.8)	5 (2.9)	10 (6.1)	0.1857
S/E	1 (0.7)	1 (0.6)	1 (0.7)	0.4554
Wound	12 (7.8)	13 (7.6)	9 (5.5)	0.3999
Vaginal swab	2 (1.3)	1 (0.6)	0 (0)	0.1423
Hard palate	2 (1.3)	4 (2.3)	7 (4.3)	**0.0001***
Orbit	1 (0.7)	0 (0)	2 (1.3)	0.1797
	155 (100)	**172 (100)**	**163 (100)**	

Blood culture (B/C), Cerebrospinal fluid (CSF), Stool examination (S/E), Urine analysis (U/A), Urine culture (U/C), Bronchoalveolar lavage (BAL).

Table 3
Distribution of underlying diseases in cases with invasive fungal infections. Primary diseases No. of patients (%).

Primary diseases	No. of patients (%)		
	2016 (n = 155)	2017 (n = 172)	2018 (n = 163)
Haematol. Malignancies	67 (43.3)	74 (43.1)	69 (42.4)
Organ transplantation	7 (4.5)	12 (7)	13 (8)
AIDS	3 (1.9)	5 (2.9)	6 (3.7)
Aplastic syndromes	8 (5.2)	7 (4.1)	4 (2.5)
Solid tumors	6 (3.9)	9 (5.3)	10 (6.2)
DM	28 (18)	31 (18.1)	26 (15.9)
Chronic diseases	16 (10.3)	19 (11.1)	15 (9.2)
Other diseases	19 (12.3)	14 (8.2)	20 (12.2)
Non	1 (0.6)	0 (0)	0 (0)

Table 4
Species-specific distribution of the etiology of systemic fungal infections.

Fungus	No. of patients (%)			P-value
	2016 (n = 609)	2017 (n = 456)	2018 (n = 412)	
Actinomyces	3 (0.5)	1 (0.22)	0 (0)	0.089
Alternaria Alternata	1 (0.16)	1 (0.22)	0 (0)	0.7857
Aspergillus Clavatus	2 (0.33)	0 (0)	0 (0)	0.1431
Aspergillus Flavus	20 (3.3)	23 (5.1)	31 (7.52)	**0.0001***
Aspergillus Fumigatus	4 (0.65)	6 (1.3)	8 (1.94)	**0.0001***
Aspergillus Niger	1 (0.16)	3 (0.65)	4 (0.97)	**0.0001***
Aspergillus Terreus	2 (0.32)	3 (0.65)	0 (0)	0.2464
Aspergillus Tubigensis	0 (0)	1 (0.22)	0 (0)	0.1444
Candida Albicans	46 (7.6)	45 (9.8)	17 (4.1)	0.3251
Candida glabrata	18 (2.9)	25 (5.4)	35 (8.5)	**0.0001***
Candida parapsilosis	15 (2.4)	19 (4.2)	19 (4.6)	**0.0001***
Candida tropicalis	9 (1.4)	11 (2.4)	15 (3.6)	**0.0001***
Candida dubliniensis	9 (1.4)	8 (1.7)	5 (1.2)	0.3330
Candida kefyr	5 (0.8)	4 (0.87)	3 (0.72)	0.7420
Candida krusei	3 (0.49)	4 (0.87)	4 (0.97)	**0.0001***
Candida guilliermondii	4 (0.65)	5 (1.1)	2 (0.48)	0.4857
Candida lusitaniae	0 (0)	2 (0.32)	3 (0.72)	**0.0001***
Candida intermedia	2 (0.33)	0 (0)	0 (0)	0.2887
Cryptococcus spp	1 (0.16)	1 (0.22)	1 (0.24)	0.0677
Fusarium spp	0 (0)	1 (0.22)	3 (0.72)	**0.0001***
Geotrichum spp	2 (0.33)	1 (0.22)	0 (0)	0.3668
Mycetoma	0 (0)	0 (0)	1 (0.24)	0.1857
Nattrassia Mangiferae	1 (0.16)	0 (0)	1 (0.24)	0.2887
Didymella pedeiae	0 (0)	0 (0)	1 (0.24)	0.1075
Trichoderma spp	1 (0.16)	0 (0)	0 (0)	0.1887
Trichosporon spp	1 (0.16)	1 (0.22)	1 (0.24)	0.0820
Zygomycetes spp	5 (0.82)	7 (1.53)	9 (2.18)	**0.0001***
Total	**155 (10.5)**	**172 (37.7)**	**163 (39.6)**	**0.0001***

infections caused by yeasts (70.6%) are higher than those by saprophytes (29.4%) in both men and women.

Patients over the age of 50 years were afflicted more with IFIs (43%) than those in the age group of 20–30 years (10%). Moreover, infections with *Candida* species were the most common among all age groups.

4. Discussion

Diagnosis of IFI may be very complex in clinical practice (with the possible exception of candidemia). This is because only the diagnosis from a sterile site (specimen) or biopsied tissue can be confidently confirmed as a proven infection. For this reason, several definitions have been validated to standardize the diagnosis of IFI when such specimens are not available; to allow for comparability of results from different studies. The most known are the EORTC/MSG definitions of IFI for neutropenic patients and patients with hematological malignancies - that still has the limitations of being less accurate in other populations. Although in other groups of patients, different criteria exist. For example, in non-neutropenic patients in ICU, the AspICU (Aspergillus algorithm for use in critically ill patients) algorithm can be used and other initiatives to improve standardization of definitions are coming up (see the Protocol of the FUNgal infections definitions in ICU patients/ FUNDICU project). Probable invasive fungal diseases (IFDs) are defined based on the presence of a host factor, a clinical, and a mycological criterion. Cases that meet the clinical criteria and a host factor but could not attain the mycological criteria are considered possible IFDs.

In this study, out of 1477 cases suspected to have fungal infections, 490 cases met the criteria for invasive fungal infection (proven or probable). The results of the current study proved that candidiasis is the most prominent cause of fungal diseases among hospitalized patients followed by aspergillosis, which is consistent with most reports from other parts of the world. Based on our findings, non-*albicans Candida* accounts for 68% (229 cases) of the 337 *Candida* cases and *Aspergillus flavus* was the most prevalent cause of invasive fungal infections among other species of *Aspergillus*.

Falagas et al. conducted a systematic review of candidemia from 1996 to 2009 and found that *C. albicans* was the dominant species in most of the studies reviewed [13]. However, the review noted that non-*albicans* species were prominent in South America, Asia, and Southern Europe; *C. glabrata* was most isolated in the US and Northern/Central Europe; *C. parapsilosis* in South America, Southern Europe, and several parts of Asia; and *C. tropicalis* in South America and Asia.

Interestingly, invasive candidiasis is now common in many non-neutropenic patients admitted to ICUs. It was estimated that of 5000 cases suspected of candidiasis in the UK, 40% would be admitted to the intensive care unit [14]. Other studies corroborate that projection [15, 16]. Candidemia has been associated with a significant death rate. A recent study showed a death rate of 60% in 30 days, while in a series of retrospective cases in the USA, the mortality rates from septic shock among candidemia patients were approximately 90% [17]. Some studies estimated assignable mortality rates due to candidemia among patients with fungemia to be around 15% [18,19]. Arendrup et al. claimed that *C. krusei* showed the highest mortality rates (36%) in comparison to *C. parapsilosis* (25%), and other species of *Candida* (14%). Barchiesi et al. demonstrated that *C. krusei* had the highest and *C. parapsilosis* the lowest mortality rate. Although many studies suggested that *C. albicans* has the highest death rate [20], Gamelatsou et al. in their study showed that *C. glabrata* had the highest and *C. parapsilosis* had the lowest mortality rate [21]. These contradictory findings could be due to differences in study populations, variations in the study design, or geographical location.

Cryptococcosis is a hallmark disease in HIV/AIDS patients [22]. In this study, 3 cases (0.6%) of cryptococcosis were identified; all among patients with HIV/AIDS. A research carried out in Brazil, Chile, and Venezuela - on a group in which 60% of the patients were HIV positive - showed that out of 100 cryptococcosis patients, 89 cases were infected with *C. neoformans*, and 11 with *C. gattii*. Shreds of evidence suggest that *C. gattii* spreads from hot places with semi-arid climates to other places like the Northwest Pacific Ocean [23], Canada [24], and Europe [25, 26]. Findings regarding *C. laurentii* and *C. albidus* infections have also been reported.

Despite antifungal treatment, invasive aspergillosis remains a life-threatening infection especially if diagnosed lately. In our centers, invasive aspergillosis is the second most common infection accounting for 22% (108 cases) of cases. In their 10- year research in France, Bital et al. reported that the occurrence of invasive aspergillosis was 4.4% in a year with a rate of 1.1–1.8 per 100000 population [27]. Dasbach et al. observed lower indices in the US in 1996 [28]. In another study conducted on 960 patients of invasive aspergillosis, 48.3% had hematological malignancy, 29.2% were solid organ transplant recipients, 27.9% were hematopoietic stem cell transplant (HSCT) recipients, and the rest were patients with other immunosuppressive conditions [29]. In ICU, patients with underlying diseases are also susceptible to invasive fungal

Table 5
The Comparison of etiologic agents of systemic fungal infections over 3 years.

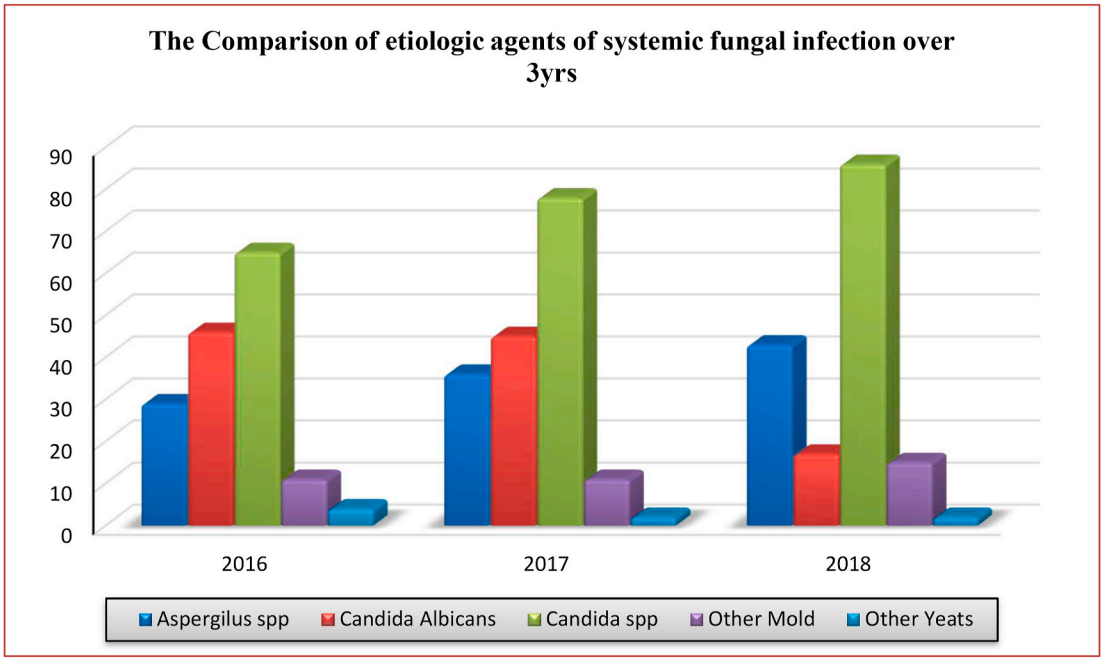

Table 6
The relationship between gender and the occurrence of multiple fungal infections.

			Mycetoma	Black fungi	Aspergillus	Candida yeast	Non-candida yeast	Trichoderma	Fusarium	Zygomycetes	Total
Sex	**F**	Count	3	3	41	133	2	1	3	11	**197**
		% of Total	0.6%	0.6%	8.4%	27.1%	0.4%	0.2%	0.6%	2.3%	40.2%
	M	Count	2	2	67	204	7	0	1	10	**293**
		% of Total	0.4%	0.4%	13.7%	41.7%	1.4%	0%	0.2%	2%	59.8%
Total		Count	5	5	108	337	9	1	4	21	**490**
		% of Total	1.0%	1.0%	22.1%	68.8%	1.8%	0.2%	0.8%	4.3%	100%

infections. In this group, the occurrence of invasive aspergillosis was reported to be 1.7–6.31 per 1000 adult admissions [30,31]. Similarly, in children, the yearly spread of invasive aspergillosis was 0.4%, among whom 75% harbored immunosuppressive conditions or malignant tumors [32]. Other researchers reported the mortality rates of invasive aspergillosis in adult and children admitted in ICU to be 33.1% and 37.5%, respectively [33]. In the current study, *Aspergillus flavus* was the most implicating species of invasive aspergillosis. However, in other studies, isolates recovered from invasive aspergillosis mainly belong to *Aspergillus fumigatus* (92%) [34], followed by *A. flavus, A. nigger, and A. terreus.* A significant proportion of invasive infections were mixed (up to 36%) [35], except in Brazil, where fusariosis was reported more often [36].

The main underlying condition among our patients was hematologic malignancies followed by organ transplants, with the majority of patients suffering from invasive lung diseases. Reports from other studies indicated that among patients with hematologic blood malignancies, the spread of invasive aspergillosis ranges from 0.3% to 2.8%; however, the rates vary depending on the underlying diseases [37,38]. In a 10-year--long study in the USA, Neofytos et al. demonstrated that the overall incidence in transplant recipients was 49% for lung, 11% for liver, 10% for heart 10%, and 2% for the kidney. Except in lung and liver transplant recipients, the diagnosis of invasive aspergillosis occurred with some delay, and thus, the death rates reported 12 weeks after the primary diagnosis were calculated as follows: 47.1% for liver, 27.8% for kidney, 16.7% for heart, and 9.5% for lung [39]. The fatality rates of invasive aspergillosis in non-neutropenic patients was estimated to be around 63–72%, which could be primarily due to the delay in the diagnosis of the disease [31,40,41].

Invasive zygomycosis parallels poor prognosis, especially when the central nervous system (CNS) is involved. In our study, *Zygomycetes* spp was the second most frequently isolated molds in 21 (4.3%) of cases. The largest epidemiologic study on invasive mucormycosis was conducted on 900 cases from 1855 to 1999 [42]. According to this research, *Rhizopus* species was the most common cause of mucormycosis (47%), followed by *Mucor* spp (18%), *Cunninghamella* spp. (7%), *Apophysomyces elegans* (5%), *Lichtheimia* spp. (5%), *Saksenaea* spp. (5%), *and Rhizomucor* spp. (4%). Based on this study, more than 80% of the cases had at least one of the following underlying conditions: diabetes (36%), malignancy (17%), solid organ transplant (7%), deferoxamine therapy (6%), using injectable drugs (5%), bone-marrow transplant (5%), kidney failure (4%), lightweight infant (3%), diarrhea and malnutrition (3%), HIV (2%), and systemic lupus erythematosus (SLE) (1%). Sinuses were reported to be the most common site of infection (39%) followed by lungs (24%), skin (19%), brain (9%), gastrointestinal tract (7%), and disseminated form (3%). Skiada et al. reported *Rhizopus* spp. (34%), *Mucor* spp. (19%), and *Lichtheimia* spp. (19%) as the most common isolated species in their study [43].

In the US, studies reported *Rhizopus* species (52%) and *Mucor* species (23%) to be the dominant isolates. Others were *Rhizomucor* spp. (7%), *Lichtheimia* spp. (3%) and 14% unknown species [44]. In most studies, the death rates reported were more than 50% [44–48]. Roden et al.

demonstrated that the difference in the death rates was proportional to the underlying diseases, the localization/type of infection (100% in a disseminated form), and infectious organisms (*Cunninghamella* species showed the highest death rate) [42]. There are increasing reports of invasive fusariosis in patients with blood malignancies and stem cell transplant recipients. *Fusarium* infections are described to be the second leading cause of invasive mycosis in these groups of patients [49].

5. Conclusion

We reported an increased frequency of non-*albicans Candida* over the *C. albicans* in the etiology of invasive fungal infections. We also noted the prominence of *Aspergillus flavus* in the saprophytes implicated with IFDs. The increased rates of invasive candidiasis with non-*albicans* agents give new perspectives on shifts in epidemiology and possibly treatment of invasive fungal infections. Details of the underlying diseases can improve the diagnosis, methods of treatment, and attendant mortality rates associated with invasive fungal infections in our study centers.

CRediT authorship contribution statement

Study concept and design: OR, SSH, ZB. Collecting samples and laboratory work: SSH, FR, MY, VR, AS, ZB, LH, MM, ASK. Analysis and interpretation of data: MZ, VM, OR, MSM. Drafting of the manuscript: AS, MG, and OR. Critical revision of the manuscript for important intellectual content: OR, MG. Statistical analysis: MSM, MZ, VM, AS.

Declaration of competing interest

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