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Determining the Susceptibility Pattern of Different Candida Species, Isolated From Hospitalized Immunocompromised Patients in Urmia Hospitals, to Antifungal Drugs

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ABSTRACT

Resistance to fungal infections is increasing throughout the world, and this is especially important in immunocompromised patients. Infection with candida fungi species is one of the most important causes of fungal infections in these patients, able to cause complications and mortality. The purpose of this study was to determine the susceptibility of isolated candida species to systemic antifungal drugs in immunocompromised patients in Urmia. Two hundred patients with immune deficiencies were examined for Candida fungi infection in Urmia hospitals. After isolation of Candida species causing the infection, their susceptibility to amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole and ketoconazole was investigated. Data were analyzed using SPSS21. Chi-square, Fisher's exact and Monte Carlo tests were used to compare the data. Out of the 200 patients with immune deficiencies, 45 (23%) of the patients showed infections due to Candida fungi species. The isolated species were albicans (68.9%), glabrata (13.3%), tropicalis, parapsilosis, krusei and kefyr (each 4.4%). Overall susceptibility to amphotericin B was 77.8%, fluconazole 55.6%, itraconazole 46.7%, voriconazole 91.1%, posaconazole 77.8%, and ketoconazole 40%. According to the results, Voriconazole is the best medicine for preventing or treating candidal infections in patients with immune deficiencies.

Key words: Fungus, Candida, Immune Deficiencies, Drug Susceptibility, Antifungal Drugs

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INTRODUCTION

Fungi are cosmopolitan existing in different places, such as water, solid surfaces, human skin and the digestive system. Taxonomists have estimated that there is about 1.5 to more than 5 million species of fungi in the world, but only some fungi (less than 300 species) cause diseases in human [1]. Many candida species are harmless human symbiosis, but when mucus barrier are disrupted or immune system weakens, they can be pathogenic [2]. Systemic blood and major organs infections (Candidaemia or invasive Candidiasis) engages mostly those with immune deficiencies. In addition to immune deficiencies, long-term use of antibiotics, female sex (in urogenital system infections) and diabetes are among the risk factors for candidal infections [3]. Albicans are known as the most common cause of candidal infections all over the world. Among the most important non-albicans in this area are tropicalis, parapsilosis, glabrata and krusei. Other less common strains are kefyr, auris, and lusitaniae. Candida albicans is a dimorphic fungus (with both yeast and sperm form) that exist in a part of the normal microbiome of the mouth, the digestive system, genital system, and human skin, and is the most common cause of fungal infections in the world [4].

The effect of candidiasis on human health has drastically increased in recent decades, among the causes of which the significant increase in the risk of AIDS, organ transplantation and chemotherapy that affects human microbial can be cited [5].

Studies show that candida has been recognized as the most important human fungal pathogenesis over the past three decades, which, in case of suitable conditions, can produce significant morbidity and mortality [6]. More than 150 types of candida have been known to date, of which approximately 17 types can cause invasive infections in humans [7]. The most common type of candidate with invasive power and pathogenicity is candida albicans. In a person with a healthy immune system, candida species are found mainly in the gastrointestinal tract and genitourinary tract, and although favorable conditions are needed for the pathogenesis of fungal infections, fungal infections, especially candida, can even be pathogenic in people with normal immunity [8]. The prevalence of Invasive Fungal Infections (IFIs) has greatly increased over recent years, and the studies have shown a direct correlation of that with the increase in mortality in patients with hematological malignancies [9]. Therapeutic options for coping IFIs are limited and include only four chemical classes of polyenes, triazoles, echinocandins and flucytosine. The spread of the use of antifungal drugs over the past two decades has unexpectedly contributed to the development of antifungal resistance [10, 11]. As there are not still sufficient studies in determining the exact prevalence of fungal infections and identifying the most common types of IFIs, and neutropenic patients who refer with fever with no adequate response to antimicrobial therapy, these patients need appropriate antifungal therapeutic regimen empirically to be added to antibiotics treatment to provides appropriate coverage and minimize mortality and morbidity associated with fungal infection [12]. Several studies have been conducted that have mainly evaluated the susceptibility of fungal species to antifungal drugs in both healthy children and immunocompromised patients, but no specific treatment pattern has been suggested so far [13, 14]. Thus, the purpose of this study is to present a unique therapeutic regimen for the correct treatment of fungal infections, which provides appropriate coverage according to the type of common infection.

MATERIALS AND METHODS

The study examined the susceptibility of candidate species in 200 cases of immunocompromised patients. For all the people hospitalized as patients with safety deficiencies in Urmia hospitals or diagnosed as a patient with safety deficiencies during hospitalization (200 people), a questionnaire was completed by trained personnel. The questionnaire included demographic information, type of immunodeficiency, age, gender, the history of receiving antimicrobial, antibacterial and antifungal drugs, and the patients who had received antifungal prophylaxis or antifungal prophylaxis within three months prior to the study were not included in the study.

Sampling from the hospitalized patients was done under sterile conditions using oral swabs. At first, oral samples were tested using potassium hydroxide (KOH) 10% by microscopic method for the presence of false heifers and yeast cells. Samples of oral swabs were directly cultured in Dextrose Agar (Merk) medium. Sabouraud Dextrose Agar (SDA) plates were incubated at 37°C for 7 days in aerobic conditions for all possible contaminants grow in this environment. Moreover, these samples were also cultured directly in CHROMagar Candida medium (Paris France Company). Cultured CHROMagar plates were incubated in darkness for the detection of yeast species, colony's shape and color for 72 hours at 35°C. After the incubation time, yeast species were identified by various methods including the formation of chlamydospore in Corn Meal Agar medium containing polysorbate 80 and carbohydrate adsorption.

Disk diffusion method was used to test the susceptibility to antifungal drugs. In this method, the suggested method was done for five antifungal drugs based on the Clinical and Laboratory Standard (CLS) method called M44-A. For this purpose, antibiotic discs fluconazole (25mg), ketoconazole (15mg), amphotericin B (10mg), nystatin (50µg), and colutrimazole (10µg) (Mast group LTD, UK) were prepared and used. Two standard strains - Candida Albicans (ATCC1023) and candida dubliniensis (CD36) - were used as control besides other candidate strains. Muller-Hinton Agar (MMA) plates containing 2% glucose and methylene blue (GMB) with a diameter of 4 were prepared according to the manufacturer's instructions.

The yeast cell suspension was prepared in sterile physiologic serum according to the half-MacFarland turbidity (containing 5-1×10^6 cells) and was inoculated with sterilized swabs on Muller-Hinton plates. The plates were incubated for 24 and 48 hours at 35°C. The diameter of non-

growth halo zone was calculated from the point where the yeast growth was reduced by 80%. Then, the MIC90 of derived yeast species with a non-growth halo diameter were calculated based on the CLSI and antibiotic manufacturer's recommended standards. Identification of the isolates was through direct microscopic experiments and cultivation on CHROMagar. Identification of candida species was by culture experiments in a corn-agar culture medium containing polysorbate 80 and production of germinal tubes in serum and culture media in CHROMagar and sugar-adsorption tests using AUX Kit (Biomerieux, France) API20. Colony color on CHROMagar medium, the formation of the tube mass, and the production of chlamydosporum on Corn Mill Agar with 1% of polysorbate 80 shows the presence of different types of candida. Data were analyzed using SPSS21. Chi-square, Fisher's exact and Monte Carlo test were used to compare the data. The level of significance in this study was considered less than 0.005.

RESULTS

Out of the total of 200 patients, 23 (45%) were infected with fungal infections. According to Monte Carlo test, there was a significant correlation between the age group and the presence or absence of fungus, so that the age of the patients in the group of patients with fungal infection was higher than the negative fungi group (P = 0.029) (Table 1).

In the group of patients with fungal infection (25n =), 55.6% of the patients; and in negative fungus group (n = 81) 52.3% were men with no significant statistical relationship reported between gender and fungal infection in Chi-square test. (p =0.696) (Table 2).

Various types of fungi that were found in patients with fungal infection were examined, with the greatest frequency belonging to Candida fungus (Table 3).

Table 1: Age distribution of patients with and without fungal infection

Age group	Cultivation (percent) frequency		
	Positive	Negative	Total
0-6	15 (33.3 %)	68 (43.9 %)	83 (41.5 %)
7-12	15 (33.3 %)	59 (38.1 %)	74 (37 %)
13-19	6 (13.3 %)	20 (12.9 %)	26 (13 %)
20-26	0	0	0
27-33	0	0	0
34>	9 (20 %)	89 (5.2 %)	17 (8.5 %)
کل	45 (100 %)	155 (100 %)	200 (100 %)

Table 2: Absolute and relative frequency of positive and negative culture

Gender	Culture	
	Positive	Negative
Men	25 (55.6 %)	81 (52.3 %)
Women	20 (44.4 %)	74 (47.7 %)
Total	45	155

Table 3: Variety of fungi in patients with fungal infection and their absolute and relative frequencies

Fungus type	Frequency	Percent
Candida	31	68.9
Glabrata	6	13.3
Tropicalis	2	4.4
Parapsilosis	2	4.4
Krusei	2	4.4
Kefyr	2	4.4
Total	45	100

Kruskal Wallis test showed that from among the effect of six antifungal species only candida albicans was significant (p<0.001) and in other types of fungi, these antifungal effects were not statistically significant (p>0.05). The antifungal effects of candida albicans were examined and reported to be significant for amphotericin B and ketoconazole (p = 0.001), fluconazole and voriconazole (p=0.001), itraconazole and voriconazole (p<0.001), itraconazole and posaconazole (p< 0.001) and finally voriconazole and ketoconazole (p<0.001) antifungals (Table 4).

Table 4: Absolute and relative frequency of resistance to antifungal studied according to the type of fungus

Fungus type	Resistance to antifungals					
	Amphotericin B	Fluconazole	Itraconazole	Voriconazole	Posaconazole	Ketoconazole
Albicans candida	3 (9.7 %)	2 (29 %)	14 (45.2 %)	1 (1.2 %)	2 (6.5 %)	14 (45.2 %)
Glabrata candida	2 (33.3 %)	2 (33.3 %)	2 (33.3 %)	1 (16.7 %)	1 (16.7 %)	3 (50 %)
Tropicalis candida	0	2 (100 %)	1 (50 %)	0	0	0
parapsilosis candida	0	1 (50 %)	1 (50 %)	0	1 (50 %)	1 (50 %)
krusei candida	0	1 (50 %)	0	0	1 (50 %)	1 (50 %)
Kefyr candida	0	0	1 (50 %)	1 (50 %)	0	0

Antifungal susceptibility was studied for Candida albicans. The results of the studies show that amphotericin B was more susceptible than itraconazole and ketoconazole than veraconazole, was more susceptible and voriconazole more than fluconazole and posaconazole than more itraconazole and poconazole more than ketoconazole. According to the results of this study, voriconazole antifungal fungus is superior to the other antifungals (Table 5).

Table 5: Relative and absolute frequencies of response to six antifungal types in *Candida albicans*

Antifungal	Susceptible	Medium	Resistant
Amphotericin	26 (83.9 %)	2 (6.5 %)	3 (9.7 %)
Fluconazole	20 (64.5 %)	2 (6.5 %)	9 (29 %)
Itraconazole	14 (45.2 %)	3 (9.7 %)	14 (45.2 %)
Voriconazole	30 (96.8 %)	0	1 (3.2 %)
Posaconazole	28 (90.3 %)	1 (3.2 %)	2 (6.5 %)
Ketoconazole	13 (41.9 %)	4 (12.9 %)	14 (45.2 %)

DISCUSSION

The spread of the use of antifungal drugs - which, unlike antimicrobial drugs do not form a broad spectrum and has limitations in choice - over the past two decades has unexpectedly contributed to the development of antifungal resistance [11,15]. Thus, knowing the state of drug susceptibility in preventive and therapeutic planning is critical in this regard [16]. This is especially important for immunocompromised patients, as the risk of developing fungal infections, especially infections caused by Candida species, is much higher in these groups compared to other groups. Accordingly, this study examined the susceptibility of different species of candida, isolated from immunocompromised patients, to systemic anti-fungal drugs for the first time in Urmia. According to the results of this study, candida albicans was the most common isolated strain from patients (68.9%) and candida glabrata (13.3%) was the second highest with other cases including candida tropicalis, parapsilosis, krusei and kefyr (4.4% each). In similar studies conducted by researchers in different geographic regions of the world, the prevalence of albicans species is more prevalent than other candida species [17-19]. Regarding the frequency of non-albicans strains, there are differences between different studies that might be due to geographical differences and the type of underlying illness may play a role in justifying this heterogeneity [20]. The important point in this study is the relatively high prevalence of glabrata infection, which is in line with the previous report. In recent years candida glabrata has proven an important factor in fungal infections [21].

In studying the susceptibility of isolated samples to systemic antifungal drugs in this study, we concluded that the best drug in these patients (except for candida kefyr) is voriconazole. The results of different studies in this field are very variable and heterogeneous. In the study by Kulku et al. in Russia, the resistance of the candida, isolated from hospitalized patients, to itraconazole and fluconazole was reported 10% [22], where our studied showed higher overall resistance rate (42.2 and 33.3, respectively). In the study by Nawrot et al. in Poland, the highest susceptibility was observed to amphotericin B. The results of this study are consistent with our results in terms of susceptibility to amphotericin B, although contradiction exists in other cases [23]. In the study by Passos et al. in Brazil, the least resistance, consistent with the results of our study, was for Voriconazole . Fluconazole, itraconazole and Amphotericin B were in the next ranks [24]. In the study by Ricciardi et al in Italy, voriconazole was suggested as a selective drug in patients with candida infection resistant to other treatments. This study confirms ours in terms of high susceptibility of candida species to voriconazole [25]. The results of this study and previous ones show high susceptibility to voriconazole and increase in resistance to fluconazole [17, 18, 26]. Although fluconazole has a broad treatment spectrum and low toxicity, long-term or repeated administration of this drug with low doses significantly increases the resistance of candida species, including albicans [27], which is confirmed in this study.

As using triazoles as anti-infectious agents of fungal infections is common in the hospitals in Urmia, the expected high resistance of candida species to itraconazole, fluconazole and amphotericin B was not unexpected. In Badiee et al., resistance to these three drugs was 38.4%, 35.5%, and 9.6%, respectively [28]. Amphotericin B used to be considered as the standard treatment for invasive fungal infections and our study showed that the susceptibility of this drug is still high. However, unfortunately due to some major complications of this drug, such as nephrotoxicity, it is used with a tint of caution today [29].

Like the study by Badiee et al. [28], our study, despite being expensive, suggests voriconazole as the first drug in this regard. However, it should be

emphasized that due to the small sample in these types of candida, more studies are still required.

CONCLUSION

According to the results of this study, candida albicans was the most common type of candida isolated from immunocompromised patients and candida glabrata was in the next rank. In all cases (except for Kefyr), Voriconazole was the best antifungal agent. There were differences between the other antifungal drugs based on the species of the fungus. The best anti-fungus drug considering susceptibility to candida albicans after voriconazole (96.8% susceptibility) were posaconazole (90.3) and amphotericin B (83.9). Concerning candida glabrata, voriconazole and posaconazole had susceptibility of 83.3; in the case of candida tropicalis, voriconazole, amphotericin B, posaconazole, and Ketoconazole had susceptibility of 100%. For candida parapsilosis, voriconazole and amphotericin B, the susceptibility was 100%; concerning candida krusei, voriconazole, amphotericin B and itraconazole, the susceptibility was 100% and for candida amphotericin B, fluconazole, posaconazole, Itraconazole, and Ketoconazole, it was 100%.

According to the results, the best antifungal drugs proposed in immunocompromised patients at risk of infection or already infected are as follows.

Suggestions

For prophylaxis or treatment of candida infection in immunocompromised patients, voriconazole has the priority.

For reaching definite conclusions about the susceptibility of non-albicans species to systemic anti-fungus drugs, further studies with higher sample sizes are suggested.

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