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Susceptibility pattern of anti-candida drugs in the pediatric patients with acute leukemia

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Abstract

Background: Pediatric patients on chemotherapy are vulnerable to invasive fungal infection especially Candida spices. Resistance to antifungal agents has increased in Candida spp., especially in non-albicans species. This study aims to assess the susceptibility of Candida spp. strains isolated from children with acute leukemia less than 18 years.

Materials and Methods: This prospective cross-sectional study was conducted during March 2011 to March 2012. Participants were 188 children aging from 1 month to 18 years, who had acute leukemia, were admitted in Amir Oncology Hospital affiliated to Shiraz University of Medical Science, Shiraz, Iran.

Identification of Candida strains was performed using germ tube and chlamydospore production tests on an Application Programming Interface (API) 20 C AUX yeast identification system. Susceptibility testing for 7 antifungal agents was performed by the agar-based E-test method. Fungal cultures were carried out from nose, oropharynx, stool, and urine specimens.

Results: A total of 229 yeasts were isolated. C. albicans was the most common species found, followed by C. krusei, C. parapsilosis, C. glabrata, and other Candida species. Candida glabrata was the most highly resistant of the yeasts isolated, being 100% resistant to fluconazole and itraconazole, 88% to posaconazole, and 75% to amphotericin B and ketoconazole.

Conclusion: In this study, caspofungin was the most effective antifungal agent against the colonized Candida spp. found, followed by conventional amphotericin B. Knowledge about susceptibility patterns of colonized Candida spp. can be of help to clinicians managing pediatric patients on chemotherapy.

Key Words: Acute Leukemia, Antifungal Drugs, Cancer, Neutropenia, Pediatric

Introduction

Despite progress in supportive care and treatment strategy of children with malignant disorders, invasive fungal infections associated with significant morbidity and mortality in these patients, especially those with hematological malignancies, increases in related healthcare costs. Choosing the appropriate treatment is not always simple because of the possibility of drug interactions and side effects (1). These infections are influenced by various factors including, the use of chemoprophylaxis, central venous catheters and local epidemiology (2). Candida has been observed as a primary fungal pathogen, particularly in immunosuppressed patients (3). Candida albicans has been the most common causal agent in these infections, affecting 48% of patients (4). Recent studies have showed a reduction in the rates of C. albicans infection and a relative shift toward non-albicans Candida spp (5-8). Early recognition, prompt diagnosis and treatment are also serving as a diagnostic tool to manage patients and avert Candida transmission to others (9, 10). Nonetheless, several studies revealed increasing resistance of Candida spp. to common used antifungal agents (6, 11,

12). Distribution and susceptibility patterns of predominant Candida spp. information could be of help to clinicians involved in the management of these patients. The aim of this study was to determine the antifungal susceptibility pattern of the isolated fungi in children with acute leukemia on chemotherapy.

Materials and Methods

A prospective study was conducted between March 2011 and March 2012 to investigate drug susceptibility patterns in isolated fungi from patients treated in a large tertiary-care referral pediatric hematology/oncology center in Southern Iran. The study included 188 pediatric patients (101 boys and 87 girls). All of patients had acute leukemia including acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL), and had undergone courses of chemotherapy and/or hospitalizations. All the eligible participants were included after taking consents from their parents or their legal guardians. The study was reviewed and approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran. Neutropenia was considered as less than 1500 cells/mL and severe Neutropenia as absolute neutrophil count (ANC) less than 500 cells/mL or an ANC that is expected to reduce to <500 cells/mL during the next 48 hours. About 90% of patients had a prior history of antifungal prophylaxis consumption. On admission, specimens were obtained from nose, oropharynx, stool, and urine for fungal surveillance cultures. Samples were placed on Sabouraud dextrose agar (Merck, Germany) and incubated at 24°C for 10 days. The purity of the isolate was evaluated by culturing the isolate on potato dextrose agar (OXOID LTD, Basingstoke, Hampshire, England) twice for 48h at 35°C. Identification of Candida strains was performed using germ tube, chlamydospore production tests, and carbohydrate assimilation reactions on the API 20 C AUX system (bioMeriéux, France) according to the manufacturer's instructions. Two Candida spp., including Candida parapsilosis (ATCC 22019) and Candida krusei (ATCC 6258) were used as quality controls.

Susceptibility testing for ketoconazole, fluconazole, itraconazole, voriconazole, amphotericin B, caspofungin, and posaconazole was performed using an agar-based E-test method (bioMeriéux, Sweden). RPMI 1640, supplemented with 1.5% agars and 2% glucose and buffered to pH 7.0 with 0.165 M morpholine propane sulfonic acid (MOPS), was used to prepare the plates. These were inoculated by dipping a sterile swab into an inoculum suspension adjusted to the turbidity of a 0.5 McFarland standard (106 cells/ mL) and streaking the agar in three directions. The minimum inhibitory concentration (MIC) endpoints were determined after 24h and 48h of incubation at 35°C. Concentrations of each drug ranged from 0.002-32 µg/mL, with the exception of fluconazole, with 0.016-256 µg/mL concentrations.

The MIC for ketoconazole, itraconazole, fluconazole, voriconazole, posaconazole, and caspofungin, was based on significant inhibition of 80% of growth. For amphotericin B, the MIC was determined as the point of complete inhibition (100%). The resistance breakpoints for the antifungal were as follows: ketoconazole ≥4.0, fluconazole ≥64, itraconazole ≥1.0, voriconazole ≥8.0, amphotericin B >1.0 and caspofungin >2.0 micrograms per milliliter (13-18). The resistant breakpoint for posaconazole has not been established as per Clinical & Laboratory Standards Institute (CLSI) document M27-A3 (12). The MIC50 and MIC90 (i.e., the MIC at which 50% and 90% of isolates are inhibited) were also calculated. Statistical analysis was performed using SPSS software (version 17, Chicago, Illinois, USA) and were analyzed using descriptive statistics and cross tabulation.

Results

During the study period, overall, 229 yeasts were isolated and 88 of 188 children were found to be colonized with Candida spp., yielding an overall colonization rate of 46.8%. Colonization of the oral cavity was significantly more frequent than in the other body sites investigated. The highest percentage of Candida colonization was found in those with acute lymphoblastic leukemia.

Candida spp. were isolated from more than one body site in 26 (29.5%) colonized patients, and in some cases, more than one species was isolated from each site. C. albicans was the most common species found, being detected in 117 patients (51.2%), followed by C. krusei in 18 patients (7.9%), C. glabrata in 14 (6.3%), C. tropicalis in 11 (4.7%), C. famata in 11 (4.7%), C. parapsilosis in 8 (3.5%), C. dubliniensis in 6 (2.4%), C. kefyr in 4 (1.8%), Cryptococcus (Cr) in 16 (7%) including C.terreus and Cr. laurentiiand other Candida species, including C. guilliermondii, C. rugosa, C. lusitaniae, C. lambica, as well as Rhodotorula spp. in 24 patients(10.5%).

Candida albicans, the species most frequently isolated, was sensitive to amphotericin B, fluconazole, voriconazole, itraconazole, ketoconazole, posaconazole, and caspofungin at rates of 97%, 58.5%, 57%, 72%, 31%, 48%, and 97%, respectively. Candida glabrata was the most resistant species among the isolated yeasts, with resistance rates of 100% to fluconazole and itraconazole, 88% to posaconazole, and 75% to amphotericin B and ketoconazole. All the fungal species exhibited highest sensitivity to caspofungin. The detected 16 Cryptococcus isolates were divided into two subspecies; Cr. terreus and Cr. laurentii. The results of antifungal susceptibility testing by E-test in Candida isolates are shown in Table I. The lowest MIC90 was observed for caspofungin.

Table I: Antifungal Susceptibility of Candida spp isolated from pediatric patients with acute leukemia

Antifungal agent	Species (no. isolates)	No isolates	MIC50 (µg/mL)	MIC90 (µg/mL)	Range	Resistance No. (%)
Amphotericin	*C. albicans*	117	0.5	0.75	0.047-1.5	4 (3%)
	C. krusei	*18*	4	6	0.38-6	16 (80%)
	C. glabrata	*14*	3	6	0.25-6	12 (75%)
	Cryptococcus spp	*16*	0.38	1	0.016-2	2 (11%)
Fluconazole	*C. albicans*	117	1.5	256	0.094-256	54 (41.5%)
	C. krusei	18	96	256	24-256	16 (80%)
	C. glabrata	14	96	256	32-256	16 (100%)
	Cryptococcus spp	16	2	16	2-256	6 (33%)
Voriconazole	*C. albicans*	117	0.125	32	0.003-32	56 (43%)
	C. krusei	18	0.5	2	0.125-32	2 (10%)
	C. glabrata	14	1.5	2	0.75-32	4 (25%)
	Cryptococcus spp	16	0.125	3	0.016-3	0
Itraconazole	*C. albicans*	117	0.032	32	0.016-32	36 (28%)
	C. krusei	18	0.75	4	0.19-32	6 (30%)
	C. glabrata	14	32	32	4-32	16 (100%)
	Cryptococcus spp	16	0.75	32	0.023-32	8 (44%)
Ketoconazole	*C. albicans*	117	0.125	32	0.016-32	90 (69%)
	C. krusei	18	6	8	1-32	14 (70%)
	C. glabrata	14	6	32	2-32	12 (75%)
	Cryptococcus spp	16	1.5	12	0.032-32	6 (33%)
Posaconazole	*C. albicans*	117	0.047	32	0.008-32	*
	C. krusei	18	0.75	8	0.008-32	
	C. glabrata	14	32	32	1-32	
	Cryptococcus spp	16	0.032	4	0.032-32	
Caspofungin	*C. albicans*	117	0.094	0.125	0.032-8	4 (3%)
	C. krusei	18	0.125	0.094	0.032-4	4 (20%)
	C. glabrata	14	0.094	0.19	0.064-0.19	0
	Cryptococcus spp	16	0.094	1.5	0.032-4	1 (5%)

Species present in small numbers are not mentioned in the table.

Discussion

In the present study, 46.8% of pediatric patients were colonized with Candida spp. Patients on chemotherapy and documented prior Candida colonization are at considerable risk of developing systemic candidiasis (19). There is evidence that Candida colonization is a risk factor for developing invasive Candida infection (20) and candidemia (5) in hospitalized patients. In one study, the reported rate of nosocomial candidemia increased more than 2-fold over the 9-year study period (3), while in another research, nosocomial candidemia was diagnosed in 6.9% of colonized neonates, compared with 0.76% of non-colonized neonates (21). It is Reported colonization rates are 12.1% in neonates (21), 12.4% in infants (22), and 55.2% in adults with hematological malignancies (8). In current study, the most commonly colonized sites belonged to the oral cavity and rectum, in keeping with the findings of other studies (8, 22).

Among the Candida species isolated in the present report, 51.2% were identified as C. albicans. In other studies, C. albicans detection rates have been reported at 50% (23), 64.2% (22), 48.6% (24), 42% (21), and 55% (12). In blood cultures, C. albicans isolates accounted for 37.2% 2 and 39.2% (25) of Candida spp. found. The isolation rate of non-albicans Candida in this study was 48.8%, whereas rates of 35.8% (21) and 78.2% (26) have been reported in the literature. In our study, Cryptococcus spp. accounted for 7% of the isolates. Colonization with this yeast has been less extensively described. The increased use of antifungal in patients on chemotherapy, mainly for prophylaxis, is considered the strongest contributory factor to the changes in species distribution, which have subsequently affected mortality and the choice of empirical treatment (27).

Resistance to antifungal agents is associated with high mortality rate in at-risk hospitalized patients especially on chemotherapy. It can be categorized into primary, acquired, and clinical resistance. Primary resistance to antifungal agents is known as intrinsic and occurs when the organism is naturally resistant to the antifungal agent without exposure to anti-fungal, such as C. krusei, which is known to be universally resistant to fluconazole (28). Acquired resistance develops during treatment, and often occurs as a result of genetic mutations (29). Clinical resistance, i.e., failure of anti-fungal therapy, depends on a variety of factors, like the host immune system condition, pharmacokinetics of the antifungal agent, and the species involving in fungal infection. Intrinsic resistance to amphotericin B is rare and acquired resistance during therapy is even less common (30, 31). C. glabrata and C. krusei tend to have higher MICs than C. albicans, and a small proportion of them have been found to be resistant to amphotericin B with MIC $\geq$ 2 μg/mL (32). Candida glabrata with amphotericin B MIC $\geq$ 2 μg/ mL was reported in less than 1% of USA and in 4.4% of European isolates 33. Furthermore, difficulty in the treatment of infection with C. glabrata, that is often resistant to many azole antifungal agents, especially fluconazole, is also reported 34. Recent studies have revealed that the MICs of triazoles, voriconazole, itraconazole and fluconazole, for C. glabrata were higher than those seen for most other Candida species (12, 23, 24).

Amphotericin B is recommended as first-line therapy for invasive mycoses, and is most commonly used in pediatrics (26). The use of this drug is limited by the toxicity of the conventional formulation and the high cost of the lipid emulsions. In the present study, susceptibility testing revealed that all isolates of C. albicans were more sensitive to amphotericin B and caspofungin than to the other antifungal studied. However, resistance to amphotericin B was seen in 80% of C. krusei and 75% of C. glabrata isolates. In one study, amphotericin B resistance was

found in nearly 20% of C. parapsilosis isolates (35).

Triazole antifungal drugs alter the fungal cell membrane by inhibiting ergosterol synthesis through an interaction with 14-demethylase, which leads to alterations in cellular permeability and a loss of membrane fluidity and integrity (36). The currently available triazole antifungals include fluconazole, voriconazole, itraconazole, ketoconazole, and posaconazole. Resistance to azoles was found in all Candida spp., with species-specific trends. Gene mutations related to ATP dependent pumps CDR genes in Candida spp. appears to confer resistance to multiple azoles and have been associated with fluconazole treatment, and cross-resistance with other azoles may also be possible (36,37) which was well documented by molecular methods (38). According to previous studies, resistance to older azoles is most commonly demonstrated in C. krusei and C. glabrata (23, 24). In the present study, fluconazole resistance was observed in 41.5%, 80%, and 100% of C. albicans, C. krusei, and C. glabrata isolates and resistance to itraconazole was found in nearly 28%, 30% and100% of C. albicans, C. krusei, and C. glabrata strains, respectively percentages that are consistent with the findings in previous reports (4,23). Cross-resistance between the newer and older azoles has been a concern, particularly for pairs of congeners, such as fluconazole - voriconazole and itraconazole-posaconazole (39, 40). In the present study, voriconazole and fluconazole resistance was documented in 10% of C. krusei and 25% of C. glabrata isolates. Posaconazole is the newest orally administered triazole antifungal with an extended spectrum of activity. Due to cross activity between this antifungal agent and other azoles, in this study, the MICs for many Candida spp. were more than 2 µg/mL.

Our susceptibility data showed that the newer antifungal agents are effective for the treatment of yeast infections. Echinocandins such as caspofungin are active against many species of fungi and have been approved by the U.S.A Food and Drug Administration (FDA) for the treatment of candidemia and invasive candidiasis. In our study, caspofungin was the most effective agent, with lower MIC50 and MIC90 values against all the Candida spp. studied.

Conclusion

Knowledge about the susceptibility patterns of colonized Candida spp. can be of help to clinicians who must decide which is the best option for the management of their high-risk patients. In pediatric patients with hematologic malignancies and especially neutropenia, we found that caspofungin was the best antifungal agent against Candida colonization, followed by conventional amphotericin B.

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Conflict of interest

The authors declare that there is no conflict of interest.

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