Short Communication

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Molecular Identification of Candida Species in Bronchoalveolar Lavage Specimens of Hospitalized Children with Pulmonary Disorders

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ABSTRACT

BACKGROUND AND OBJECTIVE: Fungal agents, especially Candida species, can cause fungal and life-threatening lung infections in children. The present study was performed to identify Candida isolates obtained from bronchoalveolar lavage (BAL) specimens of children admitted to Akbar Children's Hospital in Mashhad.

METHODS: In this cross-sectional study, 210 BAL specimens of hospitalized children with pulmonary disorders during 27 months (April 2018 to June 2020) were examined. Routine mycology tests including direct examination with 20% potassium hydroxide (KOH) and culture on Sabouraud dextrose agar containing chloramphenicol were performed for these specimens. The genomic DNA of Candida colonies were amplified by Multiplex PCR, and Candida species were identified based on the electrophoresis pattern.

FINDINGS: The patients included 90 females (42.9%) and 120 females (57.1%) and their age range was between 2 months to 16 years and their mean age was 4.6±3.16 years. Among clinical specimens, 20 specimens (9.5%) had direct microscopic results and positive culture for Candida. Identified Candida isolates included C. albicans in 15 cases (75%), C. dubliniensis in 2 cases (10%), C. parapsilosis in 2 cases (10%) and C. tropicalis in 1 case (5%). Among the underlying factors, corticosteroid receptors and patients with neutropenia had the highest frequency (73 patients, 34.8%).

CONCLUSION: The results of the study showed that C. albicans was the most common Candida isolate in the BAL specimen of hospitalized children with pulmonary disorders. Children under 4 years of age were the most affected group and pneumonia was the most common clinical form in patients with Candida infection.

KEY WORDS: *Candida, Children, BAL, Pulmonary, Infection*.

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Introduction

Fungal infections of the lungs are relatively common and potentially life-threatening in immunocompromised children (1, 2). Patients undergoing chemotherapy for cancer and receiving broad-spectrum antibiotics and transplant recipients are most susceptible to fungal pneumonia (1). The most common fungal agents that cause this type of pulmonary disorder are Candida and Aspergillus (3, 4). In fungal infection caused by Candida, C. albicans is the most common pathogen, but C. dubliniensis, C. tropicalis, C. glabrata, C. krusei, C. parapsilosis, C.kefyr and C. guilliermondii can also be mentioned in causing this infection (3).

The importance of non-albicans species has increased in recent years due to their resistance to some antifungal drugs (5, 6). However, the susceptibility of children during different ages to Candida infection has been reported to be very different. In a study on children, infants 1-2 months old hospitalized in the ICU were the most affected (7). Clinical signs and symptoms in fungal infections are not specific and can even be observed simultaneously with other infectious agents (1).

Therefore, clinical symptoms alone cannot help differentiate these types of infections and require paraclinical examinations. Differentiating fungal infections from other microbial infections and accurately identifying the causative agents can be helpful in their targeted treatment. The use of various molecular techniques is very helpful in accurately identifying and differentiating microbial agents (8). Therefore, the aim of this study was to identify Candida species obtained from bronchoalveolar lavage (BAL) specimens of hospitalized children with pulmonary disorders referred to Akbar Children's Hospital in Mashhad.

Methods

This cross-sectional study was approved by the ethics committee of Mashhad University of Medical Sciences with ethics code IR.MUMS.MEDICAL.REC.1397.773. It was conducted on 210 BAL specimens of hospitalized children with pulmonary disorders by a pediatric pulmonologist with the help of a bronchoscope. Sampling was performed from April 2018 to June 2020 (in a period of approximately 2 years). The BAL specimens of hospitalized children with clinical pulmonary symptoms were included in the study, and specimens with severe bacterial contamination that interfered with the growth of Candida were excluded. After transfer to the laboratory, the specimens were centrifuged at 3000 rpm for 15 minutes and a portion of the precipitated liquid was microscopically examined by direct examination using 20% potassium hydroxide (KOH). The other part of the specimen was inoculated and cultured in Sabouraud dextrose agar containing chloramphenicol. The culture media were incubated at 35 °C for 3 days and after the growth of yeast colonies, a part of the colonies was cultured on CHROMagar (CHROMagar company, Paris, France). The plates were re-incubated at 35°C for 24-48 hours and examined and purified for the presence of more than one Candida species (3).

Purified yeast colonies were identified using molecular multiplex PCR method according to primers and PCR program presented by Arastehfar et al. (9). In this molecular method, all primers (7 primer pairs) were mixed together in a microtube for each specimen. The volume of each primer was 0.5 µl for each specimen (final volume of primers was 7 µl), 20 µl of MasterMix (MasterMix RED Taq DNA Polymerase), 20 µl of distilled water and 3 µl of DNA in a final volume of 50 µl. After preparing and dividing the reaction materials, PCR tubes were transferred to a thermocycler and the genome was replicated under thermal cycler: initial denaturation at 95°C for 5 minutes (one cycle), denaturation at 95°C for 30 seconds, annealing at 66 °C for 30 seconds, extension at 72°C for 30 seconds (35 cycles in total) and final extension at 72°C for 5 minutes. After PCR reaction, the product was electrophoresed on 2% agarose gel for observation. The data were analyzed using Chi-square, t-test and Fisher's exact test and using SPSS software version 22 and $p<0.05$ was considered significant.

Results

In this study, of the 210 hospitalized children with pulmonary disorders, 120 patients were male (57%) and 90 were female (43%). The age range of the studied children varied from 2 months to 16 years and their mean age was 4.6±3.16 years (Figure 1). In terms of the type of underlying factors, corticosteroid receptors with 208 patients (99%) and neutropenia with 73 patients (34.8%) and also in terms of symptoms of respiratory disorders, patients with pneumonia had the highest frequency (Table 1). In addition, concurrent cases have been reported in several patients.

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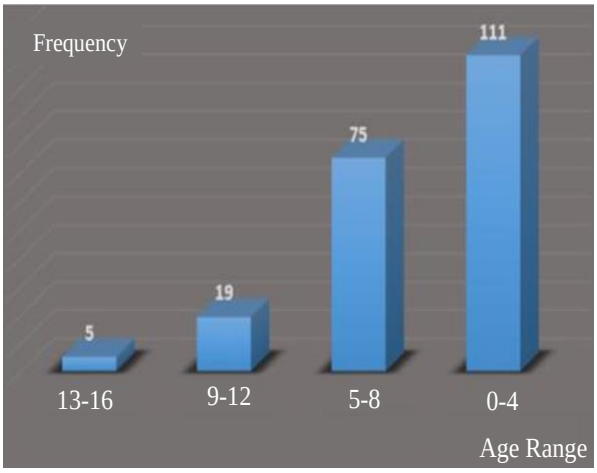

Figure 1. Frequency of susceptible children with pulmonary disorders admitted to Akbar Hospital in Mashhad based on age group

Table 1. Symptoms and pulmonary disorders and predisposing factors in susceptible children admitted to Akbar Hospital in Mashhad

	Number(%)
Symptoms of respiratory disorders	
Pneumonia	54(26.6)
Asthma and allergies	29(13.8)
Collapsed lung	12(7.5)
Hemangioma	5(2.4)
Underlying genetic diseases	
Cystic fibrosis	8(8.3)
Favism	3(5.1)
Multiple Sclerosis	2(1)
Down syndrome	2(1)
Congenital immunodeficiency	2(1)
Other underlying factors	
Corticosteroid receptors	208(99)
Neutropenia	73(34.8)
hydatid cyst	6(8.2)
Tuberculosis	4(2)
Systemic lupus erythematosus	2(1)

Out of 210 studied specimens, 20 specimens (9.5%) had positive direct test results (budding cells and pseudomycelium, or mycelium) and positive yeast culture. Identified yeast species included C. albicans, C. dubliniensis, C. parapsilosis, and C. tropicalis (Figure 2). Out of 20 children with pulmonary candidiasis, 12 patients (60%) were male and 8 patients (40%) were female. In terms of age in children with lung infection, 12 patients (60%) were up to 4 years and 6 patients (30%) were in the age range of 4-8 years and 2 patients (10%) were in the age range of 8-12 years. There was no statistically significant difference between the factor of age and patients with pulmonary candidiasis. There was no statistically significant relationship between the factor of gender and patients with pulmonary candidiasis. In addition, the result of Fisher exact test showed that there was no significant difference between the obtained Candida species and the gender of the patients. Of all patients, 9 patients (45%) had neutropenia, and based on Fisher's exact test, no relationship was found between neutropenia and different Candida species.

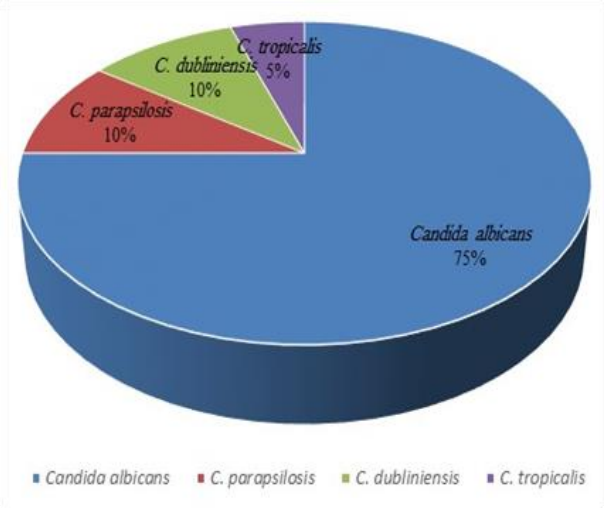

Figure 2. Frequency of different Candida species in susceptible children with pulmonary disorders admitted to Akbar Hospital in Mashhad

Discussion

According to the findings of this study, out of 210 BAL specimens of susceptible children, 20 specimens (9.5%) were positive for Candida. Pulmonary fungal infections with opportunistic fungal infections are particularly prevalent in immunocompromised patients. These infections are often transmitted to patients through inhalation, direct contact with hospital staff, and artificial ventilation (3, 10, 11). These infections can be life threatening in immunocompromised children. Intravascular catheters can cause pulmonary candidiasis as the secondary site of infection (12). Initiation of appropriate treatment for these fungal pulmonary infections depends on accurate and rapid diagnosis of the causative agent. In the present study, 20 specimens (9.5%) were positive for Candida. This frequency percentage was similar to the research of

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Anoshirvani et al. in 2018 with 9.5% frequency and Wood et al. in 2005 with 7.9% frequency (13, 14), which could indicate similar condition of the studied patients, because each of the underlying diseases will provide special conditions for opportunistic infections. In this study, the prevalence of lung infections was 60% in males and 40% in females, which was relatively higher in males. However, there was no statistically significant difference between the gender and fungal infections, and this issue has been evaluated in other studies. In the study of Zarrinfar et al. in 2015, 61% of men and 39% of women had pulmonary candidiasis (3). This increase in prevalence in males compared to females is probably due to the greater exposure of males to pathogens, and physiological and hormonal conditions.

In this study, the highest age range for pulmonary fungal infection was less than 4 years (60%), which was not consistent with the study by Burgos et al. in 2000-2005 with an average age of 9.9 years and also the study by Tschiedel et al. in 2011-2017 with an average age of 6.5 years (15, 16). These differences can be due to differences in the immune status of patients and the number of cases examined, because in most studies, the immune status factor (intrinsic or acquired) has been introduced as the most important factor.

In this study, the most common Candida species isolated from children were C. albicans (75%), followed by C. parapsilosis (10%), C. dubliniensis (10%) and C. tropicalis (5%). In the study of Stjärne Aspelund et al. on BAL specimens of lung transplant recipients, fungal agents were: C. albicans (33%), non-albicans Candida (13%), A. fumigatus (17%), Aspergillus non-fumigatus (12%), Penicillium spp. (9%), and Fusarium spp. (2%) (17). In the study of Zarrinfar et al. on BAL specimens of patients with respiratory disorders, C. albicans (52%), C. tropicalis (24%), C. glabrata (14.7%), C. krusei (5.3%) C. parapsilosis (1.3%), C. kefyr (1.3%), C. guilliermondii (1.3%) were found (3). As can be seen, C. albicans as a common species is still isolated in patients prone to lung infection, which is consistent with the results obtained in the present study. This may be due to the lack of experimental treatments in these patients and the reduction of non-albicans in these patients. This is due to the increase in non-albicans occurs following the overuse of antifungal drugs and the development of drug resistance. In the study of Kianipour et al. on BAL specimens of patients with respiratory disorders of fungal species, C. albicans (42.9%), C. glabrata (28.7%), C. krusei (11.4%), C. tropicalis (5.6%), C. dubliniensis (5.6%), C. kefyr (2.9%), C. guilliermondii (2.9%) were observed (18). Differences in the presence of species that cause fungal infections in different studies can have other reasons, including laboratory methods and techniques used in differentiating fungal species, because each has a different sensitivity and specificity. However, definitive identification of fungal isolates at the species level requires molecular techniques (9).

Identification of isolates at the species level can also be helpful in epidemiological studies and can raise awareness for treatment and prognosis decisions. Among molecular techniques, the multiplex PCR method has a good sensitivity and specificity that can save time and cost by simultaneously identifying several primers in a microtube and simultaneously identifying different species (9). Therefore, this technique was used in the present study and all Candida isolates were identified based on this technique. Therefore, performing and using this technique to investigate and identify yeast agents in other laboratories is also recommended.

According to the results of the present study, susceptible children with clinical pulmonary symptoms under the age of 4 years showed higher frequency than other age groups. Among different Candida species isolated from the lungs of these patients, C. albicans had the highest prevalence. Among the underlying factors, corticosteroid receptors and patients with neutropenia were the most common.

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