

Original Article

Candidosis in Children with Onco-Hematological Diseases in Chennai, South India

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SUMMARY: *Candida* spp. are recognized as a leading contributor to mortality and morbidity in patients with onco-hematological malignancies. The rates and risk factors for mycotic infections in pediatric oncology patients are undetermined, particularly for those treated at centers in developing countries. The objective of the present study was to prospectively evaluate the species stratification and antifungal susceptibility profile of *Candida* spp. associated with superficial and systemic infection in children with onco-hematological diseases. Acute lymphoblastic leukemia was the most common underlying disease (71.4%) among the 91 children under study. *Candida albicans* was the predominant species, with 17/29 isolates (58.6%); followed by *C. tropicalis*, with 10/29 isolates (34.5%). The drug susceptibility data analysis for the clinical isolates of *Candida* revealed 17.2% (5/29) resistance to fluconazole. This study reinforces the need for the systematic surveillance of candidosis for the correct management of such life-threatening infections.

INTRODUCTION

Fungal infections remain a major cause of morbidity and mortality in neutropenic patients, especially in those treated for leukemia. *Candida* spp. and *Aspergillus* spp. now rank among the 10 most prominent pathogens in leukemic patients (1-5). These pathogens account for 75% of fungal infections in general, and infection results in 25-60% mortality. However, less common fungal pathogens including zygomycetes, *Trichosporon* spp., phaeohyphomycetes, *Scedosporium* spp., *Fusarium* spp., and other rare or "exotic" fungal pathogens, which were in earlier times believed to be nonpathogenic in humans, but are now reported to cause invasive disease in granulocytopenic patients (6). Neutropenia, chemotherapy-induced mucosal damage, and the prolonged use of broad-spectrum antibacterial drugs that destroy the normal microbiota, are the major causes of opportunistic fungal infections. Mycotic infections have been estimated to constitute 20-30% of the life-threatening infections in patients with acute leukemia, 10-15% in patients with lymphoma, 5% in patients with massive tumours, 21-57% in patients with bone marrow transplants, and 5-15% in renal transplant recipients (7).

Early detection and identification of fungal pathogens is essential for targeted antifungal therapy (8). Unfortunately, with the widespread use of azole antifungals, especially fluconazole, as prophylaxis in neutropenic cancer patients, problems with resistant strains has increased. One phenomenon that has been increasingly reported among patients receiving fluconazole is a shift from highly susceptible to less susceptible species of *Candida*. Epidemiological studies

performed in patients with cancer and fungemia have shown that while the number of cases caused by *Candida albicans* has decreased, the frequency of infection due to *C. krusei* and *C. glabrata* has increased substantially (5,9).

There is a paucity of data on candidosis in children with onco-hematological diseases in Chennai, India. This study was therefore undertaken to determine the prevalence of candidosis, distribution of various *Candida* spp., and their antifungal susceptibility patterns in a pediatric population with onco-hematological diseases.

MATERIALS AND METHODS

This prospective study was carried out in the Department of Haematology, Institute of Child Health, Egmore, Chennai from September 2002 to July 2003. The study cohort consisted of 91 children with onco-hematological diseases. Thirty-one normal children from the Government High School, Adyar, Chennai formed the control group. Informed verbal consent was obtained from the parents or guardians of all the children studied.

Oral candidosis was defined as clinically apparent infection of the oropharynx with characteristic white plaques with positive cultures. An episode of candidemia was defined as the finding of one positive blood culture containing *Candida* spp., accompanied by symptoms of infection-like fever, neutropenia, non-responsiveness to antibacterial therapy, etc. Candiduria was defined as isolation of $>10^5$ yeast colonies in 1 ml mid-stream urine along with symptoms such as burning micturition, etc. An absolute neutrophil count of $<1,000$ cells/mm³ was suggestive of neutropenia.

Oral swabs, urine, etc., were collected depending on the nature of the suspected infection. Blood from suspected cases of fungal blood stream infection, i.e., those not responding to an antibacterial drug regimen, was inoculated into in-house biphasic brain-heart infusion bottles. Other specimens were inoculated onto blood agar, MacConkey agar, and Sabouraud

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Table 1. Distribution of *Candida* spp. in children with onco-hematological diseases

Specimens (n)	No. of <i>Candida</i> isolates	<i>C. albicans</i>	<i>C. tropicalis</i>	<i>C. krusei</i>
Case*				
Blood ¹⁾ (52)	3	1	2	–
Oral swabs				
OPC positive (21)	22	15	6	1
OPC negative (31)	3	1	1	1
Urine ²⁾ (37)	1	–	1	–
Total (141)	29	17	10	2
Control**				
Oral swabs (31)	10	4	4	2

*Case: Distribution of *Candida* spp. in children with onco-hematological diseases.

**Control: Distribution of *Candida* spp. in control population.

OPC, oropharyngeal candidosis.

¹⁾: Other significant isolates: coagulase negative staphylococci (5), *E. coli* (2), *S. aureus* (2).

²⁾: Other significant isolates: enterococci (2).

dextrose agar (SDA) with chloramphenicol (50 µg/ml), and the samples were incubated aerobically at 37°C. Blood cultures were maintained for up to 4 weeks before they were considered as negative. All fungal isolates were maintained on SDA slopes kept at 4°C until further use.

The yeasts were identified by a battery of tests that included germ-tube formation in pooled human serum, chlamyospore formation on corn meal agar with 1% Tween 80, characteristic colony color on HiCrom *Candida* agar (HiMedia, Mumbai, India), and sugar fermentation and assimilation tests (10).

Antifungal susceptibility testing of all *Candida* isolates was carried out by the disk diffusion method on glucose-methylene blue-supplemented Mueller-Hinton agar according to the proposed M44-P guidelines of the National Committee of Clinical Laboratory Standards (NCCLS) subcommittee on antifungal susceptibility testing. The antifungals tested included the azoles (fluconazole [25 µg], ketoconazole [10 µg], and clotrimazole [10 µg]) and polyenes (amphotericin B [100 U] and nystatin [100 U]). Quality control was performed using the NCCLS-recommended ATCC *C. albicans* 90028 (11,12).

RESULTS

A total of 91 children with onco-hematological diseases were studied. The mean (SD) patient age was 6.21 (3.06) years and the mean neutrophil count was 793.8 (424.1). There were 57 males and 34 females with a male:female ratio of 1.7:1. Acute lymphoblastic leukemia (ALL) was the most common underlying hematological malignancy (71.4%), with acute myeloblastic leukemia (AML) being present in 14.3%, non-Hodgkin's lymphoma (NHL) in 8.8%, aplastic anemia (AA) in 3.3%, and Hodgkin's lymphoma (HL) in 2.2% cases. All subjects had a peripheral IV line. None of the patients had a central IV or a urinary catheter.

Twenty-nine *Candida* strains were isolated from 141 specimens collected. *C. albicans* (58.6%) was the predominant species isolated, followed by *C. tropicalis* (34.5%) and *C. krusei* (6.9%). Among the control population of 31 children, oral colonization of *Candida* was seen in 10 subjects (Table 1). *Candida* was isolated from 48% of the oral swabs cultured. In addition, 42.3% patients had pseudo-membranous candidosis, whereas 5.7% patients had *Candida* colonization

Table 2. Distribution of candidosis in various onco-hematological diseases

	ALL n (%)	AML n (%)	NHL n (%)	AA n (%)	HL n (%)
OPC	18 (86)	1 (4.8)	1 (4.8)	–	1 (4.8)
Candidemia	2 (67)	–	–	1 (33)	–
Candiduria	1 (100)	–	–	–	–

OPC, oropharyngeal candidosis; ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; NHL, non-Hodgkin's lymphoma; AA, aplastic anemia; HL, Hodgkin's lymphoma.

without thrush. Here, 64% of the oral isolates were identified as *C. albicans*, followed by *C. tropicalis* (28%) and *C. krusei* (8%).

During the course of this study, the incidence of candidemia was 5.7%. *C. tropicalis* was the predominant species (66.7%), followed by *C. albicans* (33.3%). ALL was the most common underlying disease (in 2 cases). One patient with candidemia also had candiduria due to *C. tropicalis* (Table 2). All three children with candidemia were on intensive chemotherapy. Two of the patients had received steroids and were neutropenic.

Other pathogens isolated from the blood cultures were coagulase-negative staphylococci (5 cases), *Escherichia coli* (2 cases), and *Staphylococcus aureus* (2 cases). In general, *Candida* spp. ranked second among the bloodstream pathogens.

The drug susceptibility data analysis of the clinical isolates of *Candida* revealed 17.2% (5/29) resistance to fluconazole. The resistance among *C. albicans* isolates to fluconazole was 6.9% (2/29), whereas 10.3% of the *C. tropicalis* isolates (3/29) were resistant to fluconazole. No resistance was observed against the other antifungals tested. Among the bloodstream infection isolates, one strain of *C. albicans* was resistant to fluconazole, whereas the other *C. albicans* and *C. tropicalis* isolates were susceptible to all azole and polyene groups of antifungals tested. The three cases of candidemia responded to treatment with amphotericin B (0.5 mg/kg of body weight).

DISCUSSION

Nosocomial fungal infection adversely affects the outcome of underlying disease and significantly increases the cost of

care of hospitalized patients (13). Agranulocytosis, indwelling central venous catheters, extensive ulceration of the mucous membrane, and treatment with multiple broad-spectrum antibiotics are important contributing factors in such settings. Colonization with *Candida* has been shown to precede fungemia in many instances, and is regarded as an independent risk factor for systemic fungal infection (14, 15). In the present study, neutropenia, aggressive cytotoxic chemotherapy, steroid therapy, and broad-spectrum antibiotic usage were the major risk factors.

With the introduction of azole therapy in the early 1900s, there has been a declining trend in the proportion of *C. albicans* among the total clinical *Candida* isolates (14). Resurgence of *C. albicans* to pre-fluconazole levels has recently been reported (16). In the present study, *C. albicans* was the dominant organism among the superficial and systemic infection isolates, representing 58.6% of all *Candida* spp. isolated.

There is evidence linking prior exposure to triazoles and the emergence of resistance in *Candida* spp. (8,14). Of the five *Candida* strains demonstrating fluconazole resistance, four were from cases of oropharyngeal candidosis, and complete remission was observed upon treatment with ketoconazole. None of these patients were on antifungal prophylaxis, and the resistance observed independent of triazole exposure probably reflected a selection of resistant isolates from the environment. An interesting finding of our study was the susceptibility of all isolates to ketoconazole, suggesting that azole cross-resistance is not inevitable. The low resistance observed in our study could have been due to the reduced use of this drug over the last decade and the consequent selection of fluconazole-resistant and ketoconazole-susceptible strains. The hydrophobicity of ketoconazole and the operation of different azole-resistance mechanisms might contribute to the absence of cross-resistance between the two azoles (17).

Antifungal susceptibility testing has come of age in guiding physicians in the selection of antifungal therapies (12). The disk diffusion method, used for testing the resistance of *Candida* spp. to triazoles, provides qualitative results 24 h sooner than the standard NCCLS M27A method for testing yeasts; in addition, the disk diffusion method offers an alternative, simple, rapid, reproducible, and cost effective approach (12). The disk diffusion procedure using Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg/ml methylene blue has been shown to have a very good correlation with the microdilution NCCLS M27A method (18).

The present study emphasizes the significance of candidosis in immunocompromised hosts such as children with onco-hematological diseases; the complexity of predisposing factors, especially those promoting drug resistance, therefore needs to be considered in this context. It has become necessary to test patients for *Candida* in colonization and disease states and to analyze antifungal susceptibility patterns in order to develop a framework for the appropriate and timely management of life-threatening fungal infections.

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