www.jmscr.igmpublication.org Impact Factor 3.79
ISSN (e)-2347-176x

IGM Publication Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Fungal Infection in Paediatric Cancer Patients of a Tertiary Care Hospital in North India

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ABSTRACT

Introduction- *Paediatric cancer patients undergoing chemotherapy are at high risk of developing fungal infection which remains a major cause of morbidity and mortality. But the burden of fungal infection in most of our population is still not well known. Our study was aimed to estimate burden and outcome of fungal infection in paediatric cancer patients.*

Objectives -*To identify different fungal isolates and their antifungal susceptibility pattern in these patients.*

Methodology- *This study was a hospital based observational study conducted in Department of Microbiology and Paediatric cancer unit, King George's Medical University, Lucknow, UP for a period of one year. In this study we analysed 209 symptomatic paediatric cancer patients.*

Result- *Out of 209 patients 78 fungal isolates were isolated from different clinical samples. Candida species were the leading pathogen (62/78) followed by Aspergillus species (16/78). A total of 19 patients of invasive fungal infection were diagnosed from positive blood culture. Only one patient was having both candidemia and candiduria from enrolled patients.*

Conclusion- *Though incidence of fungal infection is lower than bacterial infection but mortality rate remains high due to fungal infection. So it is necessary to introduce efficient methods of prevention, diagnosis and treatment of fungal infections in paediatric cancer patients.*

INTRODUCTION

Fungal infection remains an important cause of morbidity and mortality in immunocompromised paediatric cancer patients.[1] The widely known risk factors for fungal infections in these patients are prolonged fever, severe neutropenia, administration of wide range antibiotics, corticosteroid therapy, aggressive chemotherapy and stem cell transplantation.[2,3,4] The buccal mucosa is very sensitive to cytotoxic chemotherapeutic drugs which may predispose to oral candidiasis.[5] and sometimes fungal septicaemia.[6] Fungal septicaemia is frequently associated with oral candidiasis in immunocompromised patients. Most of the invasive infections in neutropenic cancer patients are caused by *Candida albicans and Aspergillus spp.* However, some studies observed that *non-albicans Candida spp*. and previously uncommon opportunistic fungal pathogens such as *Fusarium spp. zygomycetes and dematiaceous moulds* infection are increasing in these patients.[7,8,9] while a previous study also showed *candida spp*. remains the most common fungal pathogens account for 75% of fungal infections and most of which have been attributed to *C. albicans*.[10] Recently, other *candida spp*. such as *C. tropicalis, C.parapsilosis and C. lusitaniae* are also implicated in fungal infections in immunocompromised individuals.[11] However bacterial infections are more common in this population, even though fungal infections are prevalent in paediatric cancer patients and lead to poor prognosis. Therefore evaluation of epidemiology, prevention and appropriate therapy for fungal infection are paramount steps in successful management to decrease morbidity and mortality of these patients. Many previous studies have considered also to start empirical antifungal therapy in risk patients for successful management.[2,3,12] In many studies most of the clinical and epidemiological data are generally derived from studies on adults, and only few retrospective studies have been designed to analyse the risk factors and survival in fungal infections of paediatric cancer patients.[13,14,15] The present study was aimed to collect data on epidemiology, susceptibility pattern of antifungal drugs and outcome of fungal infections in these patients followed in one year period at Department of Microbiology with collaboration of Paediatric Oncology Unit KGMU, Lucknow, India.

MATERIAL AND METHODS

This study was a hospital based prospective observational study of one year duration from June 2012 to April 2013. Our study was designed to evaluate the incidence of fungal infections to identify the different types of fungi and associated risk factors that influence the outcome of such infections. The samples were collected according to the physicians request from 209 symptomatic paediatric cancer patients. These patients were treated with chemotherapy and showed clinical manifestations of fungal infection such as fever with or without neutropenia, steroids usage, high dose cytarabine, invasive medical devices, patient with localised site of infection, difficulty or burning sensation when urinating, ulcerative

lesions with white creamy deposits in the oral cavity. Neutropenia was defined as an absolute neutrophil count (ANC) of 500/ml and fever was defined as a single axillary-equivalent temperature of 38.5^{0}C or two separate axillary-equivalent temperatures of 38^{0}c measured at least 1hour apart during a 6-hour period. We collected blood samples from all neutropenic patients who had persistent fever for more than 5 days and were not responding to antibiotics. Few patients received prophylactic antifungal therapy with fluconazole 200 mg/day for five to ten days. Peripheral venous blood samples were collected aseptically in biphasic blood culture media containing brain heart infusion broth and agar. Oral samples were obtained by swabbing the palate and buccal mucosa. Other samples were broncho-alveolar lavage (BAL) fluid, sputum, cerebrospinal fluid (CSF) and urine, gastric aspirate, and sterile body fluids (ascetic fluid, pleural fluid). All samples were processed immediately after collection by standard methods for mycological diagnosis (direct examination and isolation in culture media) at the Medical Mycology Laboratory, department of microbiology, KGMU, Lucknow. The direct examination was performed on the fresh samples (without clarification and staining) or clarified with 10% to 20% potassium hydroxide solution when necessary. For isolation, culture was done on two separate tubes of sabouraud dextrose agar (SDA) media with chloramphenicol (50mg/L), and then incubated at 37^{0}C and 28^{0}C in an aerobic atmosphere (for yeasts and moulds isolation) and examined daily for 21 days. The biphasic fungal blood culture bottle inoculated with blood was incubated at 37^{0}C aerobically and maintained for up to 4 weeks to see for fungal isolation before they were considered as negative. Yeasts were identified with recommended tests like Germ tube formation in pooled human serum, chlamydospore formation on corn meal agar with 1% tween 80, characteristic colony color on Hi-crom candida agar (Hi Media, Mumbai, India), sugar fermentation and assimilation profiles. The characteristic macroscopic and microscopic morphological features with slide culture were the mainstay to identify filamentous fungi. Antifungal susceptibility testing was done for all clinical isolates by disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) document M44-A3 for yeast and for moulds CLSI has developed a reference method for disk diffusion antifungal susceptibility testing of nondermatophyte filamentous fungi (moulds; CLSI documents M51-A and M51-S1). Agar plates (90 mm in diameter) containing Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg of methylene blue per ml (GMB) at a depth of 4.0 mm were used. The antifungal tested include the Azoles (Fluconazole 25µg, Voriconazole 1µg), Polyenes (Amphotericin B10µg), Echinocandins (Caspofungin 5µg). Quality control (QC) was performed in accordance with CLSI document M44-A3 for yeast by using Candia albicans ATCC 90029 and C. krusei ATCC 6258.

RESULT

A total of 209 children with Onco-hematological malignance were studied. The mean (SD) age of

the patient was 7.52(3.8) yrs. There were 159 males and 50 females with male: female ratio 3.2:1. The most common underlying haematological malignancy was ALL 45.50%, AML 8.98%, Wilm's tumour in 8.38%, Hodgkins lymphoma (HL) in 8.10%cases, Nonhodgkins lymphoma (NHL) in 5.08% cases, Ewing's sarcoma in 5.68%, Rhabdomyosarcomain in 5.08%, Neuroblastoma in 4.49%, Retinoblastoma in 3.59%, CML in 2.09% and other tumours 2.39%. From 334 clinical samples 78 positive strains were isolated from the following specimens: blood (19), urine (20), sputum (9), gastric aspirate (8), oral swab (7), pus (6), pleural fluid (4), CSF (3) and ascetic fluid (2). Out of 78 strains 62 (79.48%) yeasts and 16 (20.52%) moulds were isolated from different clinical samples. C. tropicalis (43.59%) was the predominant species isolated followed by C. albicans (16.66%), C. glabrata (7.69%), C. kefyr (3.85%), C. guilliermondii (3.85%) and Rhodotorula (3.85%). While Aspergillus fumigates (12.82%) was the commonest among moulds followed by Aspergillus flavus (7.69%). [Table 1] During the course of this study, incidence of invasive fungal infection (IFI) due to blood stream infection was 5.68%. The most common cause of invasive fungal infection was Candidemia (3.59%) followed by aspergillous (1.49%) and rhodotorula (0.59%) blood stream infection. C. tropicalis was the predominant species (75%) during candidemia, followed by C. albicans (8.3%). ALL (63%) was the most common underlying disease. Thus IFI was more common in patients with haematological malignancy. Out of 209 patients one patient with candidemia also had candiduria due to C. tropicalis. All these IFI patients were febrile and neutropenic. Our study showed that incidence fungal infection of the central nervous system was diagnosed in 3.3% of children treated for acute leukaemia and solid tumours. Fever, headache and convulsions were the presenting features. All the isolates were yeast no mould infection was diagnosed in these CNS infected patients. Out of three, one was C.albicans and 2 were C.tropicalis. The most frequent etiological agent of CNS infection was nonalbicans spp.

The present study analysed drug susceptibility data of clinical isolates and found that candida was 15.7% resistance to fluconazole where as 8.3% C. tropicalis isolated were resistant to fluconazole. Antifungal susceptibility test for isolates of Rhodotorula could not be performed as the isolate failed to grow on MHA plate. Resistence in aspergillous species for fluconazole was 32.16%. No resistence was observed against the other antifungal tested. Three strains of C. tropicalis were found resistant to fluconazole while other isolates were susceptible to all azole and polyene groups of antifungals tested among the blood stream infection causing isolates. Our study also revealed a better outcome of fungal infections in paediatric patients with solid tumours and lymphomas in comparisons to acute leukaemia. Further prospective study with a larger sample size might be needed to determine the associations more clearly.

Table

Sample	Fungal isolates from different samples								
	Total isolates	*C. tropicalis*	*C. albicans*	*C. glabrata*	*C. kefyr*	*C. guilliermondii*	*Rhodotorula*	*Aspergillus fumigatus*	*Aspergillus flavus*
Blood	19	9	1	0	1	1	2	3	2
CSF	3	2	1	0	0	0	0	0	0
Urine	20	11	4	3	1	0	1	0	0
Oral Swab	7	5	1	0	0	1	0	0	0
Pleural Fluid	4	0	0	1	0	0	0	2	1
Pus	6	1	1	1	0	0	0	1	2
Sputum	9	2	3	0	1	1	0	2	0
Gastric Aspirate	8	1	4	0	0	0	0	2	1
Ascitic Fluid	2	1	1	0	0	0	0	0	0
Total	78	32	13	5	3	3	3	10	6

DISCUSSION

With recent advances in therapeutics, the duration of survival of cancer patients has improved with appropriate therapy and general care. Post-chemotherapy neutropenia is the major risk factor responsible for the development of fungal infection in paediatric cancer patients. Persistent fever not responding to the antibiotics is first symptom of developing progressing fungal infection in Onco-haematological disorder patients with immunosuppressive therapy.[16] Only few studies has been estimated the burden of fungal infections in paediatric cancer patients.[17-21] but with our best knowledge there are no study from north India estimating the burden and outcome of fungal infection in paediatric haemato-oncology patients. In our study incidence of invasive fungal infection in these patients was 5.68% which is similar to the data reported by the literature which indicates incidences varying from 0-20%.[4,22,23]

The most common agents responsible for IFI were *Candida* and *Aspergillus spp*. These invasive fungal infections were associated with a high morbidity and mortality rate which also affect life health care by burdening costs. Our study is also accordance with other previous studies which revealed *invasive Candida infection (ICI)* is more frequent than *invasive Aspergillus infection (IAI).* Candida spp. is the third most common agent implicated in healthcare-associated bloodstream infections (ICI) in paediatric population.[24-27] Many studies have strong recommendations concerning prophylactic treatment for IFI.[28] In our study out of 19 positive blood samples for fungal infection 5(26.32%) patients were having solid tumours and 14(73.68%) were with malignant haematology. According to our study Candidemia was more common in patients with haematological malignancy and most common underlying

malignancy was ALL (12/19, 63%). Importantly, many previous studies showed presence of candiduria prior to development of Candidemia indicating a probable ascending route of infection.[29-31] In present study the same Candida species was detected in urine and blood of one patient indicating the occurrence of candiduria as possible route of systemic infection. Among yeasts, Candida non albicans (CnA) species was the most frequently isolated like few other studies.[32] The most frequently isolated candida non- albicans (CnA) spesies was C .tropicalis, accounting for 43.59% followed by C. albicans accounted for only 16.66%. Like other studies our study showed that moulds were responsible for high morbidity and mortality associated invasive fungal infection and *A. fumigates* was most frequently isolated mould which lead to most frequent complication i.e. aspergillosis.[33] Previously *Rhodotorula spp.* has been considered non-pathogenic which now emerged as opportunistic pathogens with ability to colonise and infect patients suffering from haematological malignancies. In our study we isolated rhodotorula spp. in blood and urine samples with incidence of 2/140 (1.4%) and 1/89 (1.1%) respectively. This incidence of fungemia (1.4%) was accordance to many recent studies in the USA which demonstrated the incidence of fungemia caused by rhodotorula was between 0.5 to 2.3%.[34] According to many studies most cases of rhodotorula fungemia were associated with central catheters in patients with hematologic malignancies.[35] In our study both the patients of rhodotorula fungemia were on central catheter and had haematological malignancy i.e. ALL. If fever is not subsided, even on administration of broad-spectrum antibiotics in paediatric cancer patients on chemotherapy, this should be an indication of prophylaxis with antifungal.[36] The most commonly prescribed antifungal medication used for both primary and secondary prevention are triazoles, including fluconazole in patients undergoing immunosuppressive therapy.[37-39] While some studies showed prophylaxis with antifungals in paediatric cancer patients on chemotherapy is often ineffective.[40,41] One of the most important aspects for successful management of paediatric cancer patients with fungal infection is early diagnosis and prompt introduction of antifungal therapy to reduce incidence of fungal infection and hence the mortality rate.

CONCLUSION

Our study demonstrated that the fungal infections were associated with increased morbidity and mortality in paediatric cancer patients. Hence active surveillance should be there to control and diagnose fungal infection early. This study also highlights the emergence of antifungal drug resistance which is probable due to the complexity of predisposing factors. So efforts should be made to develop a framework for early detection and appropriate management of life-threatening fungal infection in paediatric cancer patients.

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