PATTERN OF SYSTEMIC FUNGAL INFECTIONS AND ANTIMICROBIAL SUSCEPTIBILITY OF ISOLATES IN CANCER PATIENTS

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ABSTRACT

Fungal pathogens are implicated as an important cause of morbidity and mortality in cancer patients. In Pakistan the pattern of systemic fungal infections in cancer patients is not known. The present study was done to determine the pattern of systemic fungal infections and antimicrobial susceptibility of fungal isolates in cancer patients in Rawalpindi. It is a non-interventional descriptive study carried out from May to October 2003 at the Armed Forces Institute of Pathology, Rawalpindi. Blood, urine, stool and sputum specimens were collected for culture isolation of fungi from 73 febrile cancer patients. Antifungal susceptibility of the isolates to amphotericin B, fluconazole and itraconazole was determined by Etest and broth macrodilution technique. Forty-five fungi were isolated from 28 patients. Twenty isolates were Candida albicans, 21 were non-Candida albicans yeasts: C. tropicalis (n=13), C. glabrata (n=5), C. kefyr (n=3), Rhodotorula rubra (n=1) and three were Aspergillus fumigatus. All the isolates of C. albicans were susceptible to itraconazole; one was resistant to fluconazole while 3 isolates showed intermediate resistance to amphotericin B. The non-Candida albicans were generally more resistant: all the isolates of C. kefyr were resistant to amphotericin B, two isolates of C. tropicalis were resistant to fluconazole and three isolates of C. glabrata were resistant to itraconazole. Isolates of A. fumigatus were susceptible to amphotericin B but resistant to fluconazole. Systemic fungal infections in cancer patients by non-Candida albicans are on the rise and they are generally more resistant than C. albicans. Antifungal susceptibility testing must be performed in these cases in order to improve survival and decrease morbidity. Itraconazole can be used for prophylaxis of fungal infections in these patients.

Keywords: Cancer, haematologic malignancy, fungal infections, anti-fungal susceptibility testing.

INTRODUCTION

In the last few decades, fungi have emerged as important human pathogens especially among the immunocompromised patients. Invasive fungal infections are important cause of morbidity and mortality in cancer patients. These patients suffer prolonged periods of neutropenia due to anticancer therapy and fungal pathogens encountered in these situations have often disseminated by the time they are recognized clinically [1]. Fungaemic patients have a two-fold increased risk of dying compared to bacteraemic patients. In the United States, 25% of patients with malignancy have invasive fungal infections. Majority of the fungi responsible for these infections are Candida spp. and Aspergillus spp. [2].

With increasing number of centres offering cancer treatment in Pakistan, systemic fungal infections are going to assume increasing importance as a major cause of morbidity and mortality among these patients. However, very few studies have attempted to explore the subject and the incidence of systemic fungal infections in our cancer patient population is not known [3, 4]. Prompt and effective treatment is required to counter these potentially life-threatening infections. In order to formulate appropriate

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empiric antimicrobial therapy for these patients, it is essential to be aware of the local pattern of infections, causative fungi and their antimicrobial susceptibility, which vary in different centres. We planned this study to determine the frequency of systemic fungal infections in cancer patients and the antimicrobial susceptibility pattern of the isolates at two tertiary care units in Rawalpindi.

SUBJECTS AND METHODS

The study was conducted from May to October 2003 at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi in collaboration with the Armed Forces Bone Marrow Transplant Centre, Rawalpindi and the Oncology units of Combined Military Hospital, Rawalpindi. Cancer patients of all ages and both sexes admitted to the hospital for chemotherapy, radiotherapy or bone marrow transplantation were included. Patients who developed fever were empirically administered parenteral amikacin and amoxicillin-clavulanic acid after collecting the appropriate samples for culture. Vancomycin was substituted for amoxicillin-clavulanic acid if there was no response after 72 hours and imipenem was substituted for amikacin and the antiviral agent acyclovir was added to the regimen if there was still no response after a further period of 24-72 hours. If the patient still failed to respond to treatment after 24-72 hours, specimens for fungal culture were collected and an antifungal agent (amphotericin B, itraconazole or fluconazole) was added.

Patients who were already febrile at the time of admission, or were taking antifungal treatment or had evidence of non-fungal infections (and had responded to the initial treatment regimen) were excluded from the study. Sampling technique was non-probability convenience.

Specimen Collection and Processing

Sputum, urine, stool and blood specimens were collected for culture. Sputum specimens were collected irrespective of the time. Sputum was induced with expectorants where its production was scanty. Tracheal aspirate and broncho-alveolar lavage fluid were collected depending upon the clinical condition of the patient. Mid-stream and clean-catch urine specimens were collected in sterile culture bottles. If the patient was catheterized then it was collected aseptically by standard urine collection technique. Stool was collected in wide-mouthed sterile containers. Blood for fungal cultures was collected aseptically from peripheral veins from two different sites. Specimens like cerebrospinal fluid and other body fluids were collected if there was a particular indication of the system involvement. The specimens were delivered to the laboratory within half an hour for processing.

Sputum specimens were digested and homogenized by the N-acetyl-L-cysteine-Na-citrate method and decontaminated with 4% NaOH [5]. Sputum and stool samples were cultured quantitatively. A growth of 106 CFU/mL was taken as significant [6]. Urine, CSF and other body fluids were centrifuged before further dealing.

Microscopy

Wet films for identification of fungal hyphae were prepared from deposits of urine, sputum and various body fluids. Smears were also examined after Gram and lactophenol cotton blue staining. Giemsa and silver methanamine staining were done on sputum, broncho-alveolar lavage fluid and tracheal aspirates for identification of Pneumocystis carinii trophozoites.

Culture

Sputum, urine and stool specimens were inoculated on plain Sabouraud's dextrose agar, Sabouraud's agar with chloramphenicol and Sabouraud's agar with cycloheximide and were incubated at 22-280 C. Blood was inoculated into two bottles of tryptic soya diphasic medium and incubated at 22-280 C. Growth was observed daily for up to 14 days. Subculture on Sabouraud's agar was carried out if there was any visible turbidity in tryptic soy diphasic medium. Negative cultures were repeated if the clinical condition indicated persistent infection.

Identification of Fungal Isolates

The isolated fungi were identified as either yeast or mold by Gram and lactophenol cotton blue staining. Yeasts were presumptively identified as Candida albicans by the germ tube test while other Candida spp. were identified by production of pseudohyphae and arrangement of blastoconidia after inoculation onto cornmeal agar and incubation at 22-280 C for 48 hours. Further

identification of Candida spp. was done by using API 20 C AUX (bioMerieux SA, Lyon, France). Urease test and latex agglutination test were performed for the identification of Cryptococcus spp. Molds were identified on the basis of growth rate, appearance, colony pigmentation, growth in media containing cycloheximide and arrangement of conidia and hyphae.

Anti-fungal Susceptibility Testing

Minimum inhibitory concentrations (MICs) of amphotericin B, fluconazole and itraconazole were determined against all the fungal isolates. Etest (AB Biodisk, Solna, Sweden) was performed for yeast only. MICs against mold were determined by broth macrodilution technique of NCCLS [7] and MIC breakpoints for susceptibility against various anti-fungal drugs were interpreted according to the recommendation of Rex et al [8]. Two-fold dilutions of the antifungal agents from 128 to 0.03 μg/mL were prepared with the working suspension of the inoculum. The tubes were incubated at 360 C for 48 hours. MIC endpoints were defined as the lowest concentration causing growth of <20% of the control level for fluconazole, and as the lowest concentration causing growth of <5% of the control level for amphotericin B. Quality control strains of yeast (Candida albicans ATCC 90028) and mold (Paecilomyces variotti ATCC 22319) were tested in the same manner with each batch [9].

RESULTS

A total of 73 individuals were included in the study. Forty-five fungi were isolated from 28 patients. Out of these, 21 were males while 7 were females. Nine patients had solid tumours and 19 had haematological malignancies (table-1). The frequency of systemic fungal infection in our study population was 38%.

The total fungus culture isolates were 45. Out of these, forty-two isolates were yeasts and 3 were molds. Candida albicans was the predominant fungus isolated (n=20). Aspergillus fumigatus was isolated from the sputa of Chronic Myeloid Leukaemia (CML) cases only. However, yeasts were also isolated from all the cases of CML (table-1). In 11 patients, fungus was isolated from more than one anatomical site and in 4 cases it was isolated from three sites. Sputum specimens yielded the maximum number of fungal isolates (n=20), followed by urine (n=12), faeces (n=12) and blood (n=1). The type of fungus isolates from various specimens is depicted in (table-2).

Twenty-three patients were receiving chemotherapy and the same number of patients was receiving antibiotics. Steroids were being administered to 15 individuals while one patient was receiving radiation therapy when the fungus was isolated (table-3). Mean total leukocyte count (TLC) in the patients yielding fungal isolates was 16.65×109/L (range 0.1-80.8×109/L, 95% CI: 8.2-25.24×109/L) while the mean absolute neutrophil count was 5×109/L (range 0.02-26.75×109/L, 95% CI: 2.92-7.17×109/L). Only four cases (two of Acute Lymphocytic Leukaemia (ALL) and two of solid tumours) had neutropenia of <500/μL. Candida spp. was isolated from sputa of all the four cases.

Among the 20 Candida albicans isolates, three isolates showed intermediate resistance to amphotericin B (MIC 2.0μg/mL) while the rest were susceptible to this drug. One isolate was resistant to fluconazole (MIC >256μg/mL). All Candida albicans isolates were susceptible to itraconazole (table-4).

Among the 22 non-Candida albicans isolates: C. tropicalis (n=13), C. glabrata (n=5), C. kefyr (n=3), and Rhodotorula rubra (n=1), all the isolates of C. kefyr were resistant to amphotericin B (MIC >32 μg/mL), while two isolates of C. tropicalis were intermediately susceptible (MIC 2 μg/mL) to the same drug. Two isolates of C. tropicalis were resistant to fluconazole (MIC >256μg/mL) while three isolates of C. glabrata were susceptible dose dependant (MIC 16-32μg/mL). Three isolates of C. glabrata were resistant to itraconazole (MIC >1μg/mL), while three isolates (two isolates of C. glabrata and one isolate of C. tropicalis) showed susceptible dose dependent antifungal susceptibility pattern (MIC 0.25-0.5μg/mL) to this drug. Amphotericin B and itraconazole revealed very low MICs against all the isolates as compared to fluconazole, except Candida kefyr and Candida glabrata. Candida kefyr was resistant to amphotericin B while Candida glabrata was resistant to itraconazole (table-4).

One isolate of Rhodotorula rubra was isolated from urine. Its MICs against amphotericin B, fluconazole and itraconazole were 2 μg/mL (intermediate), >256 μg/mL (resistant) and 1.5

μg/mL (resistant) respectively. All three isolates of Aspergillus fumigatus were sensitive to amphotericin B (MIC 0.03 μg/mL) but were resistant to fluconazole (MICs>64μg/mL) (table-4)

DISCUSSION

The incidence of opportunistic fungal infections has increased in the recent years. Majority of these infections occur in immunocompromised hosts, reflecting the impact of organ transplantation, intensive cancer chemotherapy and radiotherapy. The common fungal isolates in this setting are Candida spp. and Aspergillus spp. Butt and Karamat have reported a 23% incidence of fungal pneumonia in cancer patients with Candida albicans and Aspergillus spp. as the main fungal pathogens [3].

For many years, Candida albicans was the most common species causing infection but recently other species have emerged as frequent causes of systemic infection. Wingard in a study on fungal infections in cancer patients has reported that Candida tropicalis was responsible for 25% of infections while Candida albicans accounted for 54% of the cases [10]. However, Zepeloin et al, [11] and Strickland-Marmol et al, have noticed increasing trend of isolation of non-Candida albicans particularly after the introduction of fluconazole [12]. In our study almost half the isolates were non-Candida albicans yeasts (n=21) and there was no significant difference between the isolation rates of the Candida albicans and non-Candida albicans (p=0.98). Rhodotorula rubra is another yeast that is now being increasingly isolated from blood and catheter sites [13]. We isolated Rhodotorula rubra from the urine sample of a female who was not catheterized. The possibility of rare fungal pathogens should always be kept in mind, as the management protocols are quite different in these infective agents.

In recent years, an increase in infections by Aspergillus spp. has been reported in cancer patients while candida infections have declined [14]. However, this trend was not observed in our study and Aspergillus fumigatus was isolated from sputa of only three patients. One of the important risk factors for the Aspergillus spp. infection is severe granulocytopenia lasting for more than 10 days [15]. This degree of granulocytopenia is frequently encountered in situations like bone marrow transplant recipients. In our setup, fluconazole is routinely administered prophylactically to every patient undergoing bone marrow transplantation. Since we had excluded all patients on antifungals, the number of patients with severe granulocytopenia in our study population was low. This might be responsible for the small number of Aspergillus isolates in our study.

Amphotericin B was active against most of our fungal isolates. However six isolates showed intermediate resistance to this drug while all three isolates of C. kefyr were resistant (MIC >32 μg/mL). Until now resistance to amphotericin B has been reported only in C. lusitaniae, C. parapsilosis and C. kefyr [13]. In our study one isolate of C. albicans was resistant to fluconazole, whereas two non-Candida albicans isolates were resistant and three had higher MICs (sensitive dose dependent). This is consistent with published data in which one of the causes of emergence of systemic fungal infections due to non-Candida albicans has been reported to be the use of fluconazole prophylaxis in cancer and bone marrow transplant patients [14].

Itraconazole generally showed good MICs against most of the fungal isolates (0.006-3.0μg/mL). The susceptibility patterns of the two azoles (fluconazole and itraconazole) were not similar. In our study the isolates showing resistance to fluconazole were generally susceptible to itraconazole and vice versa. This is in consistent with Hazen’s observation that one triazole cannot be used to predict the efficacy of the other triazole [13].

Comparing the two triazoles, it is clear that itraconazole is superior to fluconazole as clinical fungal isolates in our study were more susceptible to itraconazole than fluconazole (table-3). Overall, amphotericin B showed least resistant and intermediate results but the emergence of newer fungal agents like Candida kefyr and Candida tropicalis necessitates the antifungal susceptibility testing of isolates in critically ill cancer patients.

CONCLUSION

The trend of systemic fungal infections in our cancer patients is changing. Newer fungal agents are now implicated as causing severe life threatening infections. Infections by non-Candida albicans are on the rise and these fungi are generally more resistant than Candida albicans.

Table-1: Types of fungal isolates in various malignancies

	Type of malignancy					
	ALL	**AML**	**CLL**	**CML**	**Lymphomas**	**Others**
Candida albicans (n=20)	6	5	3	3	1	2
C. tropicalis (n=13)	2	7	-	-	1	3
C. kefyr (n=3)	2	-	-	-	-	1
C. glabrata (n=5)	1	2	-	-	1	1
Rhodotorula rubra (n=1)	-	-	-	-	-	1
Aspergillus fumigatus (n=3)	-	-	-	3	-	-

ALL = Acute lymphocytic leukemia *AML = Acute myeloid leukemia*
CLL = Chronic lymphocytic leukemia *CML = Chronic myeloid leukemia*

Table-2: Type of fungal isolates from various specimens

Fungus	Specimens			
	Sputum	**Urine**	**Faeces**	**Blood**
Candida albicans (n=20)	7	4	8	1
C .tropicalis (n=13)	6	4	3	-
C. glabrata (n=5)	2	2	1	-
C. kefyr (n=3)	2	1	-	-
Rhodotorula rubra (n=1)	-	1	-	-
Aspergillus fumigatus (n=3)	3	-	-	-
Total	20	12	12	1

Table-3: Clinical status of patients yielding fungal isolates in various malignancies

Malignancy	**Chemotherapy**	**Neutropenia <500/µL**	**Steroids**	**Antibiotics**	**Radiotherapy**
ALL (n=6)	6	1	4	3	-
AML (n=9)	7	-	5	9	-
CLL (n=1)	1	1	1	1	-
CML (n=3)	2	-	1	3	-
Lymphoma (n=3)	2	-	1	2	-
Others (n=6)	5	2	3	5	1
Total (n=28)	23	4	15	23	1

ALL = Acute lymphocytic leukemia *AML = Acute myeloid leukemia*
CLL = Chronic lymphocytic leukemia *CML = Chronic myeloid leukemia*

Table-4: Susceptibility pattern of fungal isolates against amphotericin B, fluconazole and itraconazole (n=45)

	Amphotericin B susceptibility No. of isolates (MIC range in µg/mL)			Fluconazole susceptibility No. of isolates (MIC range in µg/mL)			Itraconazole susceptibility No. of isolates (MIC range in µg/mL)		
	S	**I**	**R**	**S**	**SDD**	**R**	**S**	**SDD**	**R**
C. albicans (n=20)	17 (0.03-1.0)	3 (2.0)	-	19 (1.5-8.0)	-	1 (>256)	20 (0.006-0.032)	-	-
C. tropicalis (n=13)	11 (0.5-1.0)	2 (1.5-2.0)	-	11 (0.038-4.0)	-	2 (>256)	12 (0.016-0.125)	1 (0.75)	-
C. kefyr (n=3)	-	-	3 (>32)	3 (3.0-4.0)	-	-	3 (0.016-0.19)	-	-
C. glabrata (n=5)	5 (0.75-1.0)	-	-	2 (12.0)	3 (24-32)	-	-	2 (0.4-0.75)	3 (1.5-8.0)
R. rubra (n=1)	-	1 (2.0)	-	-	-	1 (>256)	-	-	1 (3.0)
A. fumigatus (n=3)	3 (0.03)	-	-	-	-	3 (>64)	-	-	-

S = Susceptible *I = Intermediate resistance* *SDD = Susceptible dose dependant*
R = Resistant *MICs = Minimal inhibitory concentrations*

Antifungal susceptibility testing must be performed in all cancer patients in order to improve survival and decrease morbidity. Based upon our results we recommend that itraconazole should be used for prophylaxis of fungal infections in these patients. Clinicians and microbiologists must work closely for timely diagnosis and prompt treatment of systemic fungal infections among cancer patients.

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