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Incidence and significance of opportunistic fungi in leukemia patients in India

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Abstract

Twenty-four patients with acute leukemia were investigated for the incidence of opportunistic fungi. Culture isolations of the sputum and urine samples revealed significant levels of *Candida* in 14 patients; *Candida albicans*, *C. tropicalis* and *C. pseudotropicalis* were the predominant ones isolated. *Aspergillus flavus* was isolated from blood in two cases and *C. albicans* and a black yeast from the blood of another two. Serological studies showed fungal antibodies in seven patients; precipitins against *Candida* were detected in five and *Aspergillus* in two. Both of the *Aspergillus* positive cases and two patients who had rising antibodies against *Candida* died during the course of investigation. In this study 13 of 24 patients developed oral candidiasis.

Introduction

Infection is a frequent complication in patients with leukemia and lymphoma [1, 2]. Although it is difficult to determine which events serve as predisposing factors for fungal infections in these patients, therapy of the underlying disease resulting in the dysfunction of the patient's immune system is considered a major factor. Almost all cytotoxic drugs have adverse effects on the known defense mechanisms of humans – epithelial barriers, lung, neutrophils and other components of the inflammatory response and humoral and cellular immunity (3). It is well established that corticosteroid therapy predisposes to infection and is related to suppression of the immune system (4).

Although leukemia is one of the major diseases of public health importance in India, studies with regard to opportunistic infections, particularly fungal infections, are few. The objective of the present study is to examine the incidence of opportunistic fungi in acute leukemia patients undergoing anti-leukemia therapy at a particular center and to evaluate the significance of the findings.

Material and methods

Twenty-four acute leukemia patients were selected for the study. These patients were receiving the accepted anti-leukemia treatments (i.e., prednisolone, Endoxan, Cytoxinearabinoxide, 6-

Mercaptopurine, Vincristine and Methotrexate) and were found to be neutropenic (200–1000/mm^3) with one or two exceptions. Clinical materials collected for mycological investigations included sputum, urine and blood.

Isolation of fungi from sputum. Freshly expectorated early morning sputum samples, collected after cleaning the mouth and rinsing thoroughly with sterile water before breakfast, were used. Sputum samples of patients who developed oral candidiasis were collected one week after a clinical cure was obtained using oral nystatin therapy. Each sample of the sputum was homogenized with sterile glass beads and 1 ml of the sample was inoculated on Sabouraud dextrose agar (SDA) plates containing chloramphenicol (0.05 mg/ml^{-1}). Eight plates were inoculated in each case and one set of four plates was incubated at 27 °C and another set of four at 37 °C. After 5–7 days, the culture plates were examined for fungi. The intensity of fungal growth was assessed by holding the plates under a well-illuminated light source. Visual scoring was done as follows: No growth 0; minimum growth +; moderate growth ++; heavy growth +++; very heavy growth ++++. Yeast colonies different in color, morphology and growth characteristics were individually subcultured from each plate for identification.

Isolation of fungi from urine. Early morning first urine samples were used for the isolation of fungi. Samples were centrifuged for five minutes and from 1 ml of the sedimented urine, approximately 0.1 ml was pipetted out and inoculated directly onto an SDA plate. Eight plates were inoculated; divided and incubated. Examination of the culture plates, assessment of growth intensity and subculturing were done as described previously.

Isolation of fungi from blood. Whenever a patient had a fever that did not respond to either anti-cancer or anti-bacterial treatments, blood samples were taken for culture isolation of fungi. Either the Biphasic medium or the Sabouraud dextrose broth (both containing chloramphenicol 0.05 mg/ml^{-1}) was used for the isolation of fungi from blood samples. Into each blood culture bottle 2 ml of freshly drawn blood was inoculated aseptically and incubated at 37 °C. From 2–3 blood cultures were prepared from each patient. After 20 days of incubation, the blood culture bottles were examined visually as well as microscopically for fungal growth. The fungi found growing on the medium then were subcultured in pure form for identification.

Identification of fungi. The yeast isolates were identified on the basis of sugar assimilation and fermentation tests [5, 6] and on the basis of morphology on Corn-Meal Agar with Tween-80 or on Eosin Methylene Blue Agar [7]. *Aspergillus* isolates were identified using the methods of Raper and Fennell [8].

Serology. Blood samples were collected from all patients for serological studies. Serological tests were performed with the objective of detecting antibodies to fungi (except for the diagnosis of cryptococcosis) in the sera of the patients using the agar gel double diffusion method [9]. The antigens used were the cytoplasmic antigens of *Candida* (GIBCO Laboratories, USA) and the culture filtrate antigens of *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Histoplasma capsulatum* and *Blastomyces dermatitidis*. For detection of *Cryptococcus neoformans* capsular polysaccharide antigen in the serum of the patients, the Latex Crypto-Antigen System (GIBCO Laboratories, USA) was used.

Oral candidiasis. The incidence of oral candidiasis was assessed on the basis of the clinical picture of the patients, the evidence obtained from direct microscopy in KOH mounts and by culture isolations of the clinical samples obtained from the oral lesions.

Table 1. Fungi isolated from the sputum and urine samples of 24 leukemia patients

Sputum	Urine
Candida albicans (16)	*Candida albicans* (5)
C. tropicalis (4)	*C. krusei* (1)
C. pseudotropicalis (3)	*C. parapsilosis* (1)
C. krusei (1)	*Candida* spp. (1)
C. guilliermondii (1)	*T. cutaneum* (1)
Candida spp. (1)	
Trichosporon cutaneum (1)	
Torulopsis glabrata (1)	
Saccharomyces cerevisiae (1)	

Figures in parentheses indicate the number of patients positive.

Results

a) *The incidence of fungi in sputum, urine and blood samples.* The various fungi isolated from sputum and urine samples are listed in Table 1. Culture isolations yielded fungi from all 24 patients. Often more than one fungus/species was isolated from each sample. *Candida albicans* was isolated from 16 patients, *Candida tropicalis* from 4, *Candida pseudotropicalis* from 3 cases; other fungi were isolated less frequently. While the intensity of growth of *Candida albicans* in the sputa of 9 patients and that of *Candida tropicalis* in two ranged from heavy to very heavy, in the rest of the 13 patients the growth ranged from no growth to minimum to moderate (Table 2). Isolations of urine samples yielded fungi in 14 out of 24 patients; only *Candida* was isolated from all of the positive cases, except in one where *Trichosporon cutaneum* was isolated. In the five *Candida*-positive cases and the *Trichosporon*-positive case, the intensity of growth ranged from heavy to very heavy; in the rest of the cases, growth ranged from medium or no growth. The type of fungi isolated from the sputum and urine samples often were different. With regard to the isolations from blood samples, *Candida* was isolated from one case, *Aspergillus* from two and a black yeast from one. In both the *Aspergillus*-positive cases, the species involved was *Aspergillus flavus*; the fungus was isolated repeatedly in blood cultures.

Table 2. Incidence of *Candida* and oral candidiasis in 24 leukemia patients

Patient number	Intensity of *Candida*		Oral candidiasis
	Sputum	Urine	
1	+++	−	Negative
2	++++	−	Negative
3	+	−	Negative
4	++	++	Positive
5	++++	+++	Positive
6	++	+	Positive
7	++	+	Positive
8	++++	++++	Negative
9	+	−	Positive
10	−	+++	Positive
11	−	+++	Negative
12	+++	−	Negative
13	+	+	Positive
14	++++	+	Positive
15	++++	−	Positive
16	++++	−	Positive
17	++	−	Negative
18	++++	−	Negative
19	++	+++	Negative
20	++++	++	Positive
21	+	−	Negative
22	+	+	Positive
23	++++	−	Positive
24	−	++	Negative

The black yeast isolated could not be identified, since the fungus died on primary subcultures.

b) *Antibodies against fungi.* Fungal antibodies could be detected in 7 out of 24 patients (Table 3); five were positive against *Candida* and two were positive against *Aspergillus*. The patients who were serologically positive for *Aspergillus flavus* also were found to be positive for *Aspergillus flavus* in blood cultures. None of the patients had, however, received any systemic antifungal therapy. In both of the *Aspergillus* positive cases, the patients died within a week after the diagnosis. Although antibodies against *Candida* were observed in five cases, rising titers of antibodies could be detected only in two, and both died subsequently. In the rest of the patients, rising titers of antibodies could not be detected. The antibody level was static in two cases while antibody disappeared in the other case, but the clini-

Table 3. Seropositivity and blood culture results in 24 leukemia patients

Organism	No. of patients positive in serology*	No. of patients positive in blood cultures
Candida	5	1 (*C. albicans*)
Aspergillus fumigatus	–	–
Aspergillus flavus	2	2
Aspergillus niger	–	–
Histoplasma capsulatum	–	–
Blastomyces dermatitidis	–	–
Cryptococcus neoformans	–	–
Others	–	1 (Black yeast)

* Tests were performed for the detection of antibodies in all cases except for cryptococcosis where detection of cryptococcal polysaccharide antigen (Latex Agglutination Test) was done.

cal condition of the patients deteriorated considerably. Further information of these patients was not available as they left the hospital against medical advice and were lost to follow-up.

c) *Incidence of oral candidiasis.* The number of patients who developed oral candidiasis during their stay in the hospital are listed in Table 2. In this study 13 of 24 patients developed oral candidiasis. Except in 3 cases, the type of candidiasis noticed was oral thrush which was controlled within 2–4 days with oral nystatin therapy. In most of these cases, however, the disease relapsed.

Discussion

Recovery of *Candida* from the clinical materials of patients whose resistance to opportunistic infections is reduced by the debilitating illness or therapeutic methods could be regarded as a significant finding [10]. This is particularly true with patients who are neutropenic [11]. According to Body [1], infections considered in toto in acute leukemia were significantly associated with granulocytopenia – 90 per cent of the disseminated fungal infections were seen in patients whose granulocyte count was less than 500/mm^3.

A significant finding in the current study was that almost all the patients investigated in the present study were neutropenic and isolations from all of them yielded opportunistic fungi. Significantly, the intensity of growth of *Candida* in 14 patients ranged from heavy to very heavy in sputum and urine isolations. The reason for a low-level blood culture positivity of *Candida* in this study is difficult to explain. It has been stated, however, that negative blood cultures never exclude the presence of invasive candidiasis [3]. For example, in three studies of patients with acute leukemia at autopsy who had deep candidiasis, negative antimortum blood cultures were seen in 100 per cent [12], 75 per cent [1] and 60 per cent [13]. It also has been stated that in about 50 per cent of the cases of disseminated candidiasis blood cultures were negative [14]. It is likely that absence of detectable candidiasis has been one of the major reasons for the fact that disseminated candidiasis has been primarily a postmortum diagnosis [1, 10, 15–17].

Repeated isolations of *Aspergillus flavus* from the blood samples of 2 patients and also the detection of antibodies against *Aspergillus flavus* in their blood sera clearly indicate systemic *Aspergillus* infections. Interestingly, in both the cases, the species involved was *Aspergillus flavus*. Both of these patients died within a week of diagnosis indicating that the terminal cause of death could be *Aspergillus* infection.

Out of the 24 patients investigated five had detectable antibodies against *Candida* in ID tests. Since serological studies (detection of antibodies) of these patients were done at regular intervals and the baseline titer levels were determined, the clinical significance of a departure from the baseline titer in at least two cases could be assessed (gradual rise in antibody titer levels, i.e.; 1:2, 1:4, 1:8, followed by worsening of the patients condition were suggestive of systemic infection). Blood culture positivity, high antibody titers and rapid and significant changes in antibody titer levels and deterioration of the clinical condition of the patient often indicate deep seated infection [18].

Presentation of oral candidiasis was a common feature in the patients. It is significant that more than 50 per cent of the patients developed oral candidiasis during their hospital stay. In the severely neutropenic patients with frequent disruption of the lining epithelium, the oral mucosa may serve as a portal of entry for systemic invasion [19].

Evidence obtained from the present study indicates that opportunistic fungi, *Candida* and *Aspergillus* in particular, play an important role in the morbidity and mortality of patients of acute leukemia in India – *Candida* seems to have the greatest frequency followed by *Aspergillus*. Similar observations have been reported in other parts of the world [1–3, 10].

We believe that direct demonstration of fungal elements in biopsied tissues is the only means of establishing a definitive diagnosis of invasive fungal infections. However, this is not a practical method in most cases because an invasive procedure would be required. In view of this, the most useful approach for establishing a possible diagnosis of deep fungal infections in leukemia patients would be by positive culture findings in concordence with positive serological tests and clinical findings.

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