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AIDS-related opportunistic mycoses seen in a tertiary care hospital in North India

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Sixty symptomatic confirmed human immunodeficiency virus (HIV)-positive adult patients, of both sexes, suspected of having a fungal infection were taken as a study population, and the clinicomycological profile was correlated with the immunological status of the patients with particular reference to CD4 counts. Relevant samples were collected and subjected to direct microscopy, fungal culture and serology. CD4 counts were determined by flow cytometry. Patients belonged to the age group of 17–65 years, with a male : female ratio of 4.8 : 1. Heterosexuality was the commonest mode of transmission. Candidiasis was the most common diagnosis (41.7 %), followed by cryptococcosis (10.0 %), and pneumocystinosis and aspergillosis (8.3 % each). Two cases of histoplasmosis were also diagnosed. A low mean CD4 count of $<$200 cells μl^{-1} was seen with most fungal infections. A total of 73 % of patients belonged to World Health Organization (WHO) stage 4, while 23.33 % belonged to stage 3. Thirty one patients (51.67 %) belonged to Centers for Disease Control and Prevention (CDC) stage C3. Various fungal infections correlated well with the mean CD4 counts. It was difficult to correlate statistically WHO and CDC staging because of the small sample size. However, it was possible to assess to a limited extent the possibility of using clinical diagnosis to predict the status of progression of HIV infection in a resource-poor outpatient setting.

INTRODUCTION

AIDS caused by the human immunodeficiency virus (HIV) is the most important public health problem of modern times (Rosen, 1994). As per the global estimate of the World Health Organization (WHO) and Joint United Nations Programme on HIV/AIDS (UNAIDS) in December 2005, the total number of patients with HIV in 2005 was 40.3 million, with 4.9 million newly infected people and 3.1 million AIDS-related deaths (UNAIDS/WHO, 2005). HIV made a delayed entry into India, but its spread has been very rapid and at present is in an advanced stage of the epidemic in some states of the country (NACO, 1999).

Though HIV is the causative agent of AIDS, most morbidity and mortality in AIDS patients results from opportunistic infections; approximately 80 % of these patients are seen to die as a result of such an infection rather than from HIV. Mostly the infections seen in AIDS patients are endemic to the geographical region, and involve many organs and organ systems simultaneously with a tendency to disseminate (George *et al.*, 1996; Sivaraman *et al.*, 1992).

There are major differences in the spectrum of opportunistic infections in India and in the West (White & Zaman, 1992; Aquinas *et al.*, 1996). Limited studies from India have shown that tuberculosis is the most common opportunistic infection, followed by a host of other bacterial, parasitic, viral and fungal infections. Various mycoses form the bulk of opportunistic infections in AIDS patients and are increasing in the form of an epidemic parallel to the AIDS epidemic (Mirdha *et al.*, 1993). The data from India on the spectrum of fungal infections in HIV/AIDS patients, and the clinical and immunological profile of these patients are scarce. Hence our aim was to study the regional profile of fungal opportunistic infections and their correlation with the immunological profile of the patients.

Abbreviations: ART, anti-retroviral treatment; CDC, Centers for Disease Control and Prevention; HIV, human immunodeficiency virus; PCP, pneumocystis pneumonia; UNAIDS, Joint United Nations Programme on HIV/AIDS; WHO, World Health Organization.

METHODS

Study population and design. Sixty symptomatic confirmed adult HIV-positive patients, of both sexes, suspected of having a fungal

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infection were taken as subjects. Cases were recruited from the outpatient department, wards and the Anti-Retroviral Treatment Clinic of Lok Nayak Hospital and GB Pant Hospital, New Delhi. All patients were evaluated by a pre-designed protocol covering the biodata, history, including high-risk behaviour, mode of transmission, marital status, partner status, presenting complaints and physical examination.

Microscopy, culture and identification. Depending on the clinical symptoms, relevant clinical samples were collected with complete universal precautions and relevant methods were used for diagnosis and isolation, which included a battery of tests as per standard procedures (Forbes *et al.*, 2002; Milne, 1996). The samples were subjected to direct microscopy using Gram and Giemsa staining, KOH mounts, India ink preparations, Gomori methenamine silver staining and toluidine blue O staining depending on the type of specimen and the suspected infection in the patient.

Fungal culture was done on Sabouraud dextrose agar, with and without chloramphenicol (16 μg ml^{-1}), brain heart infusion agar and 5 % sheep blood agar. Specimens were streaked in duplicate; one set of inoculated slants was incubated at 25 °C and the other at 37 °C, and they were examined every other day for growth up to 4–6 weeks before discarding as negative. Samples inoculated on blood agar were incubated for 24–48 h and samples on brain heart infusion agar were incubated for 1–2 weeks (Forbes *et al.*, 2002; Koneman *et al.*, 1997). Fungal growth was identified by colony morphology, Gram staining, lactophenol cotton blue preparation and Riddle's slide culture as per standard recommended procedures (Moore & Jaciow, 1979).

Identification & speciation of yeast isolates was done on the basis of germ tube production, morphology on corn meal agar with Tween 80 (Hi Media), HiCrome candida agar (Hi Media), carbohydrate fermentation tests and assimilation tests using yeast nitrogen base agar (Hi Media) as per standard recommended procedures (Forbes *et al.*, 2002; Koneman *et al.*, 1997; Moore & Jaciow, 1979).

Serology and assessment of immune status. Serology was performed on the serum/cerebrospinal fluid samples collected from the patients using antigen detection by latex agglutination for *Cryptococcus*, using the cryptococcal antigen latex agglutination system (CALAS) (Meridian Bioscience), and the *Aspergillus* sp. using Pastrorex aspergillus (Sanofi Diagnostics Pasteur), direct immunofluorescence for detection of *Pneumocystis jiroveci* in sputum/bronchioalveolar lavage samples using MERIFLUOR-pneumocystis kit (Meridian Bioscience) and antibody detection by immunodiffusion for *Histoplasma* sp. and *Blastomyces* sp. using the ID-fungal antibody kit (4AG – *Aspergillus*, *Blastomyces*, *Coccidioides*, *Histoplasma*) from IMMY Immuno Mycologics as per the manufacturers' instructions. CD4 count was determined for each patient enrolled in our study by flow cytometry using the fluorescent activated cell sorter BD FACS Count system (Becton Dickinson) as per the manufacturer's instructions.

RESULTS AND DISCUSSION

During the 15 month study from February 2005 to April 2006, 60 patients with known HIV positive status and suspected fungal infections were enrolled to determine the spectrum of fungal infections and their correlation with CD4 counts.

Patients had a mean age of 34 ± 9 years with 82 % patients in the age group of 21–40 years, the most productive age group of the country. The male : female ratio in our study was 4.8 : 1. Two of our patients were intersexes (Table 1).

Table 1. Demographic profile of study population

Demographic profile	No. of males (%)	No. of females (%)
Age group		
15–20	0 (0)	1 (10)
21–25	4 (8.3)	1 (10)
26–30	16 (33.3)	4 (40)
31–35	11 (22.9)	2 (20)
36–40	8 (16.6)	1 (10)
41–45	4 (8.3)	1 (10)
46–50	1 (2.1)	0 (0)
51–55	2 (4.2)	0 (0)
56–60	1 (2.1)	0 (0)
61–65	1 (2.1)	0 (0)
Marital status		
Married	39 (81.2)	9 (90)
Unmarried	9 (18.8)	1 (10)
Partner status		
Positive	13 (33.3)	9 (90)
Negative	8 (20.5)	0 (0)
Not known	18 (46.2)	0 (0)

Our findings are consistent with the data given by the National AIDS Control Organization (NACO) and in studies elsewhere in India (Kothari & Goyal, 2001; Kumarasamy *et al.*, 1995). While the males belonged to a wider age spectrum, the females were a considerably younger population, and most of them acquired infection from their spouses, reflecting the male dominance in Indian society and emphasizing an increased need for awareness and counselling of both the spouses. The sexual mode of transmission was the commonest, seen in 32 (53.3 %) patients (heterosexuality accounting for 94 % of these), as seen in other studies in India (Kothari & Goyal, 2001; Kumarasamy et al., 1995; Kaur *et al.*, 1992), followed by intravenous drug abuse and blood transfusion in 5 and 3 patients (8.3 and 5 %), respectively, while mode of transmission was not known in 18 (30 %) patients.

The common presenting complaints were weight loss (88.3 %), loss of appetite (75 %) and fever (71.7 %), similar to other studies from India (Kothari & Goyal, 2001; Kaur *et al.*, 1992) (Fig. 1). A history of tuberculosis (60 %) was the most common past history elicited, as reported in other studies from India (Kothari & Goyal, 2001; Kumarasamy *et al.*, 1995; Vajpayee *et al.*, 2003).

Lymphopenia and anaemia were noted in 23.3 and 65 % of patients, respectively, followed by leucopenia in 11.6 % of patients, as also seen in a study in Vellore (Kaur *et al.*, 1992), while two patients had pancytopenia with haemoglobin levels of 3.8 g dl^{-1} and 6.5 g dl^{-1}. Neutropenia was seen in 5 (8.3 %) patients.

From 60 patients, 140 samples were collected and processed (Table 2). The gastrointestinal system was

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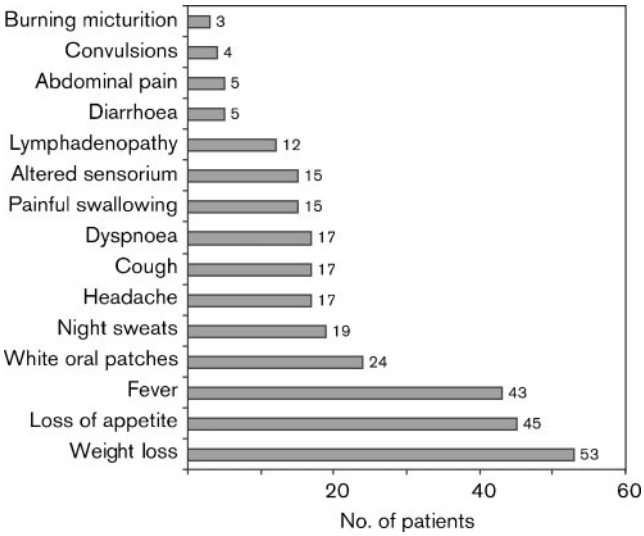

Fig. 1. Signs and symptoms at presentation.

involved in 33 (55 %) patients, the respiratory system in 21 (35 %), followed by the central nervous system in 17 (28.3 %) and genitourinary involvement in 2 (3.3 %) patients. Four patients (6.7 %) showed skin involvement (two with scabies, one with pyoderma and one with anti-retroviral treatment (ART)-associated hyperpigmentation). Thirteen patients (16.7 %) showed multi-system involvement.

Oral candidiasis was the most common form of candidiasis, 22 cases (mean CD4 count 222.5 ± 133.7 cells μl^{-1}), 3 among the 22 patients also had oesophageal candidiasis (mean CD4 count 85 ± 78 cells μl^{-1}), 1 out of 22 also had *Candida* diarrhoea and 1 out of 22 also had candiduria. Three cases were diagnosed as *Candida* pneumonia (mean CD4 count 161 ± 73.08 cells μl^{-1}) (Table 3). A CD4 count of <200 cells μl^{-1} and a viral load of $>10\,000$ copies ml^{-1}, along with factors like tobacco consumption, poor oral hygiene and xerostomia, have been shown to facilitate the occurrence of oral lesions in these individuals (Bravo *et al.*, 2006). This may also play a role in patients in our study due to the abundance of such associated factors in them. Also the severity of lesions has been shown to increase with a fall in CD4 counts in a study from India (Lattif *et al.*, 2004). At a CD4 count of <50 cells μl^{-1}, oesophageal thrush becomes common. Two of our patients with oesophagitis had CD4 counts <50 cells μl^{-1}, which is in agreement with most studies (Maenza *et al.*, 1996). *Candida albicans* (59.3 %) was the commonest *Candida* spp. isolated, followed by *Candida glabrata* (14.8 %) and *Candida parapsilosis* (11.8 %), while *Candida guilliermondii, Candida krusei, Candida lipolytica* and *Candida tropicalis* were isolated from one patient each. Non-*albicans Candida* species were isolated only from oral thrush. Studies from the USA have reported *C. tropicalis* and *Candida dubliniensis* as the common non-*albicans Candida* spp. in thrush, but none of our isolates was *C. dubliniensis* (Bravo *et al.*, 2006; Martinez *et al.*, 2002). All three patients with oesophageal candidiasis had infection with *C. albicans*, the most common *Candida* spp. reported in invasive candidiasis (de Repentigny *et al.*, 2004; Phillips *et al.*, 1996).

Six patients had cryptococcosis. One patient presented with both pulmonary and central nervous system cryptococcosis, while cryptococcal meningitis and pulmonary cryptococcosis alone were seen in four patients and one patient, respectively. The CD4 counts of five of these patients were $\leqslant 200$ cells μl^{-1}, and three among these five had a CD4 count <100 cells μl^{-1} (mean CD4 count 138.8 ± 92.11 cells μl^{-1} in cryptococcal meningitis) (Table 3). The incidence of cryptococcal meningitis was seen to be 8.3 %, similar to the data from the USA, where it was estimated to be 6.1–8.5 % (Currie & Casadevall, 1994).

Five patients (8.3 %) had pneumocystis pneumonia (PCP) in our study. Three patients had CD4 counts <200 cells μl^{-1} and one patient had a count as low as 94 cells μl^{-1} (Table 3). Incidence of PCP in AIDS patients in developing countries, including India, has been low (Udwadia *et al.*, 2005; Nissapatorn *et al.*, 2003). This may be due to lack of diagnosis or prevalence of more virulent conditions, like tuberculosis, leading to pulmonary disease before PCP

Table 2. Correlation between fungi isolated/detected and clinical specimens

Fungus	Total	Oral swab	Sputum	CSF	Urine	Stool	FNAC /Bx	Blood
Candida spp.	27	22	3	0	1	1	0	0
C. albicans	16	11	3	0	1	1	0	0
Non-*albicans Candida*	11	11	0	0	0	0	0	0
P. jiroveci	5	0	5	0	0	0	0	0
Cryptococcus neoformans	7	0	2	5	0	0	0	0
S. cerevisiae	2	1	0	0	0	1	0	0
Aspergillus niger	5	0	1	0	0	0	0	4
R. rubra	1	1	0	0	0	0	0	0
Histoplasma capsulatum	2	0	0	0	0	0	1	2*

CSF, Cerebrospinal fluid; FNAC/Bx, fine needle aspiration cytology/ biopsy.
*Histoplasmosis was diagnosed in one patient by histopathology of lymph node FNAC, as well as antibody detection from blood.

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Table 3. Opportunistic fungal infections and CD4 correlation

Fungal infection (no. of patients, %)	Mean CD4 count (cells μl^{-1})	Range (cells μl^{-1})
Oral candidiasis (22, 36.7)	222.5 ± 133.7	37–564
PCP (5, 8.3)	188.4 ± 94.5	94–326
Cryptococcal meningitis (5, 8.3)	138.8 ± 92.11	66–294
Aspergillosis (5, 8.3)	151.4 ± 93.91	29–268
Candida pneumonia (3, 5)	161 ± 73.08	77–210
Oesophageal candidiasis (3, 5)	85 ± 78	37–175
S. cerevisiae gastrointestinal infection* (1, 1.6)	100	–
Histoplasmosis (2, 3.3)	315.0 ± 35.35	290–340
Cryptococcal pneumonia (2, 3.3)	144.5 ± 31.31	87–202
R. rubra oral ulcers (1, 1.6)	52	–
Candiduria (1, 1.6)	210	–
Candida diarrhoea (1, 1.6)	256	–

*This patient had oral thrush as well as severe diarrhoea due to *S. cerevisiae*.

could manifest (Abouya *et al.*, 1992). Interestingly, the frequency of PCP has decreased in both developed and developing countries due to a combination of chemoprophylaxis with ART. The early AIDS-related mortality due to other causes may also reduce the rate of this disease (Chariyalertsak *et al.*, 2001).

Four patients were diagnosed as having probable cases of invasive aspergillosis (positive antigenaemia), while one patient, with underlying neutropenia (CD4 count 29 cells μl^{-1}), was diagnosed as a proven case of invasive pulmonary aspergillosis (positive direct microscopy, culture and antigen detection). Histoplasmosis was suspected in two cases. One patient (CD4 count 340 cells μl^{-1}) had oral thrush with axillary lymphadenopathy, which showed a non-tubercular chronic granulomatous reaction on histopathology, while the other patient (CD4 count 290 cells μl^{-1}) presented with hepatosplenomegaly with pancreatitis and HIV nephropathy. One case of *Rhodotorula rubra* oral ulcers, and one case with thrush and diarrhoea due to *Saccharomyces cerevisiae* were also diagnosed. No case of blastomycosis was seen (Tables 2 and 3).

Tuberculosis was the most common non-fungal aetiology seen in 18 patients (30 %), others included non-fungal diarrhoeas (5 %), cytomegalovirus oesophagitis (5 %), bacterial pneumonias (3.3 %), HIV encephalopathy (3.3 %), cerebral toxoplasmosis (1.6 %), aphthous ulcers (1.6 %) and HIV nephropathy (1.6 %).

In our study, only 14 (23.3 %) patients were on antiretroviral therapy (Fig. 2). The CD4 counts ranged from 29 to 564 cells μl^{-1}. The median CD4 count was 157 cells μl^{-1} and the mean was 182.8 ± 117.25 cells μl^{-1}. Thirty-six (60 %) patients had CD4 counts <200 cells μl^{-1}, with CD4 counts <100 cells μl^{-1} in 33.3 % and <50 cells μl^{-1} in 8.3 % patients depicting a major population with severe immunosuppression. This finding is important as most fungal infections and tuberculosis seen in our patients are AIDS-defining illnesses manifesting at significantly lower CD4 counts (CDC, 1985).

Various fungal infections were compared with the mean CD4 counts in these patients and they correlated well (Table 3). A total of 73 % of our patients belonged to WHO stage 4, while 23.33 % were in stage 3. Only two patients (3.33 %) belonged to stage 2 while none of our patients belonged to stage 1 (Fig. 3). Out of 14 patients in stage 3, with no AIDS-defining illnesses, it was seen that 6 patients had a CD4 count of <200 cells μl^{-1}, and were in a danger of disease progression in the absence of ART prophylaxis had their CD4 counts not been measured. Thirty-one patients (51.67 %) were in Centers for Disease Control and Prevention (CDC) stage C3, followed by sixteen (26.67 %) in CDC stage C2. All 47 cases with 49 AIDS-defining illnesses or episodes belonged to stage C2 and C3 with CD4 count of <500 cells μl^{-1}, and none belonged to stage C1. However, five patients with

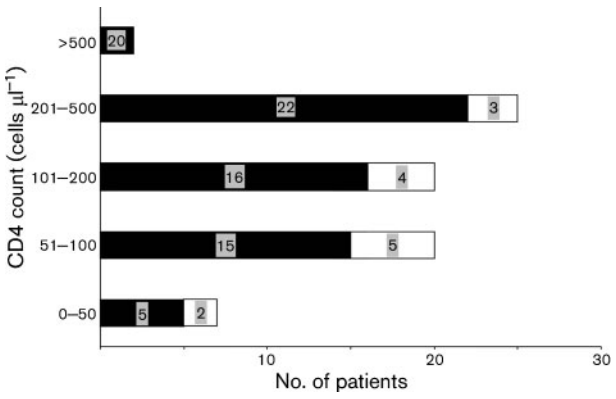

Fig. 2. CD4 profile and correlation of ART with CD4 profile. Black bars, total number of patients; white bars, number of patients on ART.

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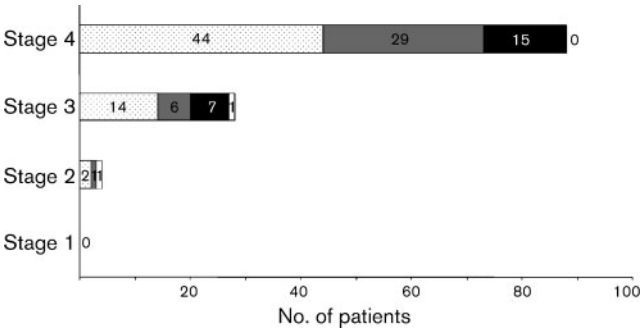

Fig. 3. WHO stages and correlation with CD4 counts. Spotted bars, total number of patients; grey bars, number of patients with CD4 count <200 cells μl^{-1}; black bars, number of patients with CD4 count 201–500 cells μl^{-1}; white bars, number of patients with CD4 count >500 cells μl^{-1}.

no AIDS-defining illnesses were grouped in B3 with CD4 count <200 cells μl^{-1}, and had the advantage of getting ART and prophylaxis started in the absence of clinical conditions. The majority of AIDS-defining illness were tuberculosis (36.7 %), invasive candidiasis (18.4 %), cryptococcosis (12.2 %), PCP (10.2 %), cytomegalovirus oesophagitis (6.1 %), and histoplasmosis, HIV encephalopathy and recurrent bacterial pneumonias in 4 % each, and cerebral toxoplasmosis in 2 %. None of the patients belonged to CDC stages A1, A2 or A3. This again proves the lack of awareness regarding the clinical spectrum and the various presentations of AIDS, in addition to the lack of diagnostic facilities at the peripheral health centres, due to which patients present very late to the tertiary health centres.

It was difficult to correlate statistically WHO and CDC staging because of our small sample size. However, we could assess to a limited extent the use of clinical diagnosis to predict the progression of HIV infection in a resource-poor setting, in a developing country like ours, as this study highlights the relationship between various clinical conditions and CD4 counts. However, it was realized that a long-term planned study might help in better interpretation and use of CD4 counts, and help in the provision of guidelines on possible points of intervention with prophylactic algorithms against various opportunistic infections in our setup.

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