Profile of Fungal Lower Respiratory Tract Infections and CD4 Counts in HIV Positive Patients

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Abstract

One hundred and sixty symptomatic confirmed Human Immunodeficiency Virus (HIV)-positive patients, of both sexes with lower respiratory tract 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 antigen detection.

Keywords: AIDS; HIV; CD4; Respiratory tract infections; Opportunistic infections

Introduction

Acquire Immunodeficiency Syndrome (AIDS) caused by the Human Immunodeficiency Virus (HIV) is the most important public health problem of modern times. Though HIV is the causative agent of AIDS, most morbidity and mortality results from opportunistic infections; approximately 80% of these patients are seen to die as a result of such an infection rather than from HIV. Invasive fungal infections are common opportunistic infections associated with significant morbidity and mortality for patients with HIV infection and the risk of invasive fungal infection varies with host immunity as well as environmental exposure [1-3].

The importance of fungal diseases among patients with HIV infection was recognized in the early days of the acquired immunodeficiency syndrome epidemic. Fungal infections were reported in many of the first patients described with a “new acquired cellular immunodeficiency” in 1981 [4]. The spectrum of illness ranges from asymptomatic mucosal candidiasis to overwhelming disseminated infection and life threatening meningitis and fungal pneumonia.

While it is difficult to define the impact of the Human Immunodeficiency Virus (HIV) pandemic on the field of infections, an increase in the number and severity of serious fungal infections has been reported. Fungal disease at any anatomic site accounted for over 20% of the AIDS-defining diseases reported to the Centers for Disease Control (CDC). Because most pulmonary fungal diseases have been considered as AIDS defining, this 20% could be an underestimation of their incidence. Necropsy studies in AIDS patients have showed an incidence of fungal infection of 20% to 49% [5,6]. Fungal pneumonia is an infectious process in the lungs caused by 1 or more endemic or opportunistic fungi. Fungal infection occurs following the inhalation of spores, after the inhalation of conidia, or by the reactivation of a latent infection. Hematogenous dissemination frequently occurs, especially in an immunocompromised host.

The data from India on the etiology and spectrum of fungal infections in HIV/AIDS patients, and the clinical and immunological profile of these patients are scarce. This study was conducted to elucidate the frequency and etiology of various fungal respiratory tract infections in HIV infected patients and its correlation with CD4 cell counts, from north India. In this scenario, knowledge regarding the opportunistic fungal infection will be useful as timely recognition and treatment of Opportunistic Infections (OIs) are the only viable options.

Material and Methods

Study population and design

One hundred and sixty symptomatic confirmed HIV-positive patients, of both sexes having a lower respiratory tract infection were taken as subjects. Patients with confirmed diagnosis of pulmonary tuberculosis and bacterial pneumonia were excluded. Cases were recruited from the outpatient department, wards and the Anti-Retroviral Centre of J.N. Medical College and Hospital from January 2010 to November 2011. All patients were evaluated by a pre-designed protocol covering the patient’s particulars, history, including high-risk behavior, mode of transmission, marital status, partner status, presenting complaints and physical examination.

Collection of specimens, microscopy, culture and identification

Early morning expectorated or induced sputum, Broncho-alveolar-Lavage fluid (BAL) and percutaneous fine-needle aspirates were collected with complete universal precautions according to the patient’s presentation. Relevant methods were used for diagnosis and isolation, which included a battery of tests as per standard procedures [7,8]. The samples were subjected to direct microscopy using Gram and Giemsa staining, KOH mounts, India ink preparations.

Fungal culture was done on Sabouraud dextrose agar, with and without chloramphenicol (16 mg/ml). 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 [9]. Fungal growth was identified by colony morphology, Gram staining, lactophenol cotton blue preparation and Riddle’s slide culture as per standard recommended procedures [10]. Identification & speciation of yeast isolates was done on the basis of germ tube production, morphology on corn meal agar with Tween 80 (Hi-Media), Hi-Crome candida agar (Hi Media), carbohydrate fermentation tests and assimilation tests using yeast nitrogen base agar (Hi Media) as per standard recommended procedures [7-10].

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Direct Immunofluorescence staining for *pneumocystis jiroveci* was done in patients suspected of having PCP. MERIFLUOR -Pneumocystis kit (Meridian Bioscience, Europe) was used for the detection of *Pneumocystis jirovecii* cysts and trophozoites in direct smears of induced sputum and BAL fluid.

**Serology and assessment of immune status**

Serology was performed on the serum 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. platelia™ aspergillus EIA (BioRad, Germany) was used for the detection of aspergillus galactomannan antigen in serum samples. CD4 count was determined for each patient enrolled in our study by flow-cytometry using PartecCyFlow® Counter (Germany) as per the manufacturer’s instructions.

The HIV status of all the patients was confirmed at ICTC, Department of microbiology, JNMC. The HIV antibody status was assessed by three ERS (ELISA, Rapid, and Simple) tests as recommended by the National AIDS Control Organization (NACO), Ministry of Health and Family Welfare, Government of India [11].

**Results**

During the study period, 160 patients with known HIV positive status and having lower respiratory tract infection were enrolled to determine the fungal etiology and their correlation with CD4 counts.

Patients had a mean age of 31.4 ± 13 years with 72% 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 1.4:1 (Table 1).

The sexual mode of transmission was the commonest, seen in 119 (74.4%), followed by blood transfusion and intravenous drug abuse in 13 (8.1%) patients and 2 (1.2%) patients, respectively, while mode of transmission could not be ascertained in 16 (10%) patients.

A definitive microbiological diagnosis of fungal respiratory tract infection was made in 14 (8.7%) out of total 160 patients with lower respiratory tract involvement, which included pulmonary *Aspergillosis* in 4, *Candida pneumonia* in 4, pulmonary *Cryptococcus* in 3 and *pneumocystis jirovecii* pneumonia (PCP) in 3 patients.

Three patients (1.8%) had *pneumocystis* pneumonia (PCP) in our study. Cysts and trophozoites of *P. jirovecii* were detected by immunofluorescence microscopy of induced sputum in all the three cases while two were positive on Giemsa staining. Two patients had CD4 counts <200 cells/ml and one patient had a count as low as 103 cells/ml (Table 2). Two patients had LDH >450 U/L.

Two patients had pulmonary cryptococcosis, while pulmonary cryptococcosis and cryptococcal meningitis was seen in one patient. The CD4 counts of two of these patients were 104 cells/ml and 143 cells/ml, and the patient with both pulmonary cryptococcosis and cryptococcal meningitis had a CD4 count of 67 cells/ml (Table 2).

Galactomannan antigen of aspergillus was detected in sera of 4 patients (positive antigenaemia); all of them were diagnosed as confirmed cases of pulmonary aspergillosis (positive direct microscopy showing dichotomously branching septate hyphae consistent with *Aspergillus* sp, repeated culture and antigen detection). *Aspergillus fumigatus* was recovered from BAL of 3 patients of pulmonary aspergillosis while *aspergillus niger* was isolated from 1 patient (Table 2).

*Candida pneumonia* was seen in 4 (2.5%) patients, on direct microscopy of sputum and percutaneous fine needle aspirates (in 2 out of 4) from these patients abundant budding yeast cells and pseudohyphae were demonstrated on gram’s staining. *Candida albicans* and *candida glabrata* was isolated from 3 and 1 patient respectively. The mean CD4 cell count was 134.2 cells/ml (± 66.7) (Table 2).

Out of 160 HIV positive patients studied 86 (53.7%) patients were placed in the stage 3 and this group comprised the majority of the cases. 65 (40.6%) patients were categorized in stage 4 disease while 7 (4.3%) patients in stage 2 disease. Only 2 (1.2%) patients were classified of having stage 1 disease (Figure 1).

**Discussion**

With the mean age of 31.5 years, 72 % of patients of this study were in the age group of 21 to 40 years, this section of the population is more affected because they are sexually more active and the social structure is patriarchal. Unfortunately, these patients also happen to be in the economically most productive years of their lives. Morbidity and mortality in this age group causes a huge loss to their household and collectively to the Nation. These findings are consistent with the demographic data given by the National AIDS Control Organization (NACO) which reported mean age of 34 years, and also with studies elsewhere in India [12-14].

The sexual mode of transmission was the commonest, seen in 119 (74.4%) in our study, There is some difference between these finding

![Table 2: Distribution various opportunistic fungal infections and Mean CD4 cell counts.](image)

![Figure 1: Distribution of patient according to WHO Stages.](image)
and national data [2] which reports heterosexual route of transmission in 87% of cases, however Vaipayee et al. [15] reported heterosexual route of transmission in 58.9% cases. Sharma et al. [16], found sexual mode of transmission in 41.5% patients and Wadhwa et al. [14] reported it to be 53.3%.

As compared tour study which detected PCP in 3 (1.87%) patients Mishra et al. [17] detected *p. carinii* in 25.34% patients presumed to be suffering from *pneumocystis carinii* pneumonia, this is quite large as compared to our study (%), this may be because of the fact that his study group involved the patients specifically with features of PCP. Earlier reports from India have reported its prevalence to be 17 to 55% [18-20]. In patients with PCP, risk of infection is strongly correlated with CD4 count. In patients with a CD4 count between 201 and 350, a study reported the incidence of PCP to be 0.5% [21]. Incidence of PCP in AIDS patients in developing countries, India, has been low [22,23]. This may be due to lack of diagnosis or prevalence of more virulent conditions, like tuberculosis, leading to pulmonary disease before PCP could manifest [24].

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 [25].

In the present study cryptococcal pneumonia was present in 3 patients and had mean CD4 count of 104.6 cells/ml, one study reported pulmonary cryptococcal infection in 3.3% cases and occurred at mean CD4 count of 144.5 cells/ml [14]. Cryptococcal pneumonia is more severe in patients with HIV. Patients with pulmonary disease frequently progress to disseminated disease [26].

In the present study 4 cases of pulmonary aspergillosis were detected and had mean CD4 count of 95.5 ± 33.3 cells. According to one study when the CD4 count is below 150/mm³, fungal pathogen such as Aspergillus should also be considered Invasive aspergillosis occurs among patients with advanced HIV infection. Patients who have had HIV-associated aspergillosis typically have CD4+ counts<100 cells/μl, a history of other AIDS-defining OIs, and are not receiving ART [27]. Findings of the present study are comparable to this in terms of CD4 count. Patients who have HIV-associated aspergillosis typically have CD4+ counts<100 cells/μl, a history of other AIDS-defining OIs, and are not receiving ART.

**Conclusion**

Fungal infections in HIV positive patients form a large spectrum, from subtle oral candidiasis to life threatening invasive disease. Development of pneumonia is a serious condition and more so in an HIV positive person. Apart from tuberculosis and other bacterial etiologies, fungi account for the substantial proportion in the causative plethora of lower respiratory tract infection. Early diagnosis is essential for effective management of the patients. There are no clinical pathognomonic signs and symptoms specific for fungal infections, especially the deep seated ones, and confident diagnosis relies heavily on a combination of microbiological, histopathological and serological evidence.

**References**

2. NACO, Annual Report 2010-11