INTRODUCTION

People with advanced human immunodeficiency virus (HIV) are vulnerable to infections called "opportunistic infections" (OIs) because they take advantage of the opportunity offered by a weakened immune system. Since the beginning of the HIV epidemic, OIs have been recognized as common complications of HIV infection (1-3). OIs cause substantial morbidity and hospitalization, necessitate toxic and expensive therapies, and shorten the survival of people with HIV infection (4,5). A decrease in CD4+ count is at least partially responsible for the profound immunodeficiency that leads to various OIs in HIV-infected persons (6).

The introduction of antiretroviral therapy (ART) has dramatically reduced the incidence of OI among HIV-positive people who have received ART; however, around the world, millions of people living with HIV in resource-poor communities have no access to ART (1). Even where ART drugs are available, they do not entirely remove the need for preventing and treating OI. In addition, measures to prevent and treat OIs become essential if ART stops working because of poor adherence, drug resistance or other factors. Providing prevention and treatment of OIs not only helps HIV-positive persons to live longer, healthier lives, but it can also help to prevent tuberculosis (TB) and other transmissible OIs from spreading to others. At present, the initiation of primary prophylactic therapies for OIs is based chiefly on the absolute CD4+ count, which has been shown to be an excellent predictor of the short-term overall risk of developing AIDS among HIV-infected patients (7).

The relative frequencies of specific opportunistic diseases may vary in different countries and even in different areas within the same country (8). The identification of such pathogens is very important for HIV and AIDS case management. As the number of AIDS patients increases in India, little information is available about the prevalence of OIs in different parts of India, considering the vastness of the country. We conducted this retrospective analysis to document the present scenario of the OIs among the HIV-infected patients in the eastern part of India along with their CD4 count, which had not been done previously.

MATERIALS AND METHODS

Study population: We reviewed the case records of 125 HIV-infected patients admitted between April 2006 and March 2007 at Calcutta Medical College Hospital, Kolkata West Bengal, and of referred patients from Apex Clinic, Calcutta Medical College Hospital, a referral center for patients of HIV infection or AIDS. Our observations included only periods of hospitalization; we did not investigate patients’ records after their discharge from the hospital. The patients included in the study were from different states of eastern India such as Orissa, Bihar and Jharkhand and from West Bengal. Their HIV status was confirmed by three ERS (Enzyme-linked immunosorbent assay [ELISA], Rapid, Simple), an ELISA (viz., HIV ELISA, Rapid test) and Western blot as recommended by the National AIDS Control Organization (NACO), Ministry of Health and Family Welfare, Government of India (9). The admitted patients were referred to us because they presented symptoms presumed to be due to HIV infection or because they were admitted to the hospital to investigate signs or symptoms of unknown origin, such as prolonged fever (10).

The study methods comprised pretest counseling, informed...
Diagnoses of opportunistic diseases: For the diagnosis of OIs, routine microbiology smears, cultures and serology were performed. Different samples were collected depending on patient symptoms and clinical presentation under universal aseptic precautions in suitable sterile containers for the routine diagnosis. Sputum, stool and blood samples were collected and used for the isolation, culture and identification of species of the pathogen. OIs were diagnosed according to the criteria suggested by the Centers for Disease Control and Prevention (CDC) (11). Sabouraud’s dextrose agar (SDA) was used as transporting media for isolation of Candida causing oral candidiasis to the HIV patients. Pseudohyphae and budding yeast were characteristic findings. The appearance of the lesion and presence of yeast forms on microscopic examination of the oropharynx were sufficient evidence to confirm the diagnosis (12). The most prevalent and obtained pure culture of Candid albicans spp. was compared with referral strain C. albicans ATCC 10231, and yeast cultures were compared with referral strain Saccharomyces cerevisiae ATCC 2601.

Cases of TB were classified as definite if the culture for Mycobacterium tuberculosis was positive for acid-fast bacilli. The decontaminated sputum sample from the diagnosed TB patients was further studied for obtaining a pure culture of M. tuberculosis growing on prepared LJ slants (Himedia, Mumbai, India). The positive pure cultures grew yellow colonies on the slants. The isolated pure culture of M. tuberculosis spp. was compared with referral strain M. tuberculosis ATCC 25177.

Stool specimens from all diarrhea patients (following WHO criteria of watery stool for at last 48 h prior to investigation) were processed (13) and examined microscopically for the presence of Cryptosporidium parvum, a zoonotic pathogen that causes chronic watery diarrhea. Modified Ziehl-Neelsen (AFB staining) was the staining technique used for staining of Cryptosporidium. Cryptosporidia and other coccidia stained pink-red. The isolated pure culture of Cryptosporidium spp. was compared with a referral strain Cryptosporidium (Microbiology QC slides, Himedia SL45-10; Himedia).

Enteric bacterial flora from the stool samples of diarrhea patients were isolated using differential selective screening media such as UTI agar (urinary tract infection agar; Himedia, Mumbai, India) after documenting their clinical manifestations, including intestinal flu, inflammation-associated cramping, abdominal pain, nausea and vomiting. Different enteric pathogens that exhibit a particular colony color facilitate the identity of particular microorganisms such as Escherichia coli (pink-magenta), Proteus mirabilis (light green), Enterococcus fecalis (blush green), Staphylococcus aureus (cream), Pseudomonas aeruginosa (colorless) and C. albicans (pin point white). TCBS agar (thiosulfate citrate bile salt sucrose; SRL, Mumbai, India) was used for isolation of Vibrio cholerae and other enteropathogenic Vibrio.

Cryptococcal meningitis was confirmed by clinical symptoms and signs as well as the detection of cryptococcal capsular antigen (Cryptococcus antigen latex agglutination Test; Remel, Lenexa, Kans., USA).

Toxoplasmic encephalitis was diagnosed in the presence of at least two of the following findings: a history of neurological symptoms, neurological signs at admission or suggestive computed tomography scan or magnetic resonance imaging of the brain (20, 21). A response to anti-Toxoplasma (IgM) antibodies, which was detected by a serological commercial kit (Equipar s.r.l., Sarona NO, Italy), gives a satisfactory result.

The viral OIs, including cytomegalovirus (CMV) infection, which was primarily diagnosed by the common manifestations including pneumonia, retinitis (an infection of the eyes), blindness and gastrointestinal disease (22), was confirmed by serological diagnosis (ELISA, IgM; Equipar s.r.l.). Infection with herpes simplex virus (HSV) and hepatitis B virus (HBV) was primarily diagnosed on clinical grounds and also confirmed by the abovementioned serological diagnosis (ELISA, IgM: Equipar s.r.l.; ELISA, HBsAg: Biomerieux, Boxtel, The Netherlands). The VDRL test (Trust antigen; Span Diagnostics, Surat, India) was done to detect the occurrence of syphilis causing Treponema pallidum.

Evaluation of whole blood CD4+ lymphocyte count: The CD4+ count of the HIV-seropositive subjects (n = 125) was done at the discretion of the treating physicians. The CD4+ T-cell percentage and CD4+ counts were estimated by FACS Calibur flow cytometer (Becton Dickinson, San Jose, Calif., USA). Dual-color immunophenotyping was performed using standard whole blood methodology.

Statistical analysis: The data was analyzed using MINITAB statistical software version 13.1. A regression model was fitted by using the data to analyze the effect of co-variables (i.e., age, sex, mode of infection, CD4+) on different response variables (i.e., different OI). The null hypothesis (H0 hypothesis) was also been tested. A P-value > 0.05 was regarded as statistically significant. The mean, median, mode and standard deviation were calculated using the same software.

RESULTS

Patient profile: In this study, 125 HIV (HIV-1 subtype) patients with OI were studied, of whom, 105 (84%) were male and 20 (16%) were female. The majority (52%) of patients were 31-40 years old followed by 27, 19 and 1% for the age groups 21-30, ≥31 and ≤20 years old, respectively. The predominant mode of transmission of HIV was by heterosexual transmission, accounting for 64% of cases, followed by other routes (Table 1). The distribution of the study population according to CD4 cell count/cumm of blood was a maximum 36% of the population with 101-200 CD4 cell count, followed by 23, 22 and 17% of patients with ≥201, 51-100 and ≤50 CD4 cell count, respectively.

Yeast, HBV and venereal disease are remarkably absent in female subjects (Fig. 1), which is a significant finding from an epidemiological point of view. The follow-up of the case studies revealed that 7 cases (5.6%) out of 125 HIV-infected patients expired during the later period of their treatment.

Ten (8%) of the study patients were receiving ART. Dermatological reactions were found as drug-related complications in patients with HIV infection. The most serious disorder, Steven-Johnson syndrome (SJS), was found to occur in 5 (4%) HIV-infected patients after intake of a combination of rifampicin-isoniazid after beginning treatment for recurrent pulmonary TB occurring as an OI (14). The intention of the regression model and null hypothesis is to test the effect of different covariates on different response variables. Using the P-value method, one can conclude whether a given covariate has an effect on the response variable. From our data analysis it is clear that age, sex, mode of consent, blood withdrawal, clinical evaluation and recording of demographic information (such as age, sex, race, marital status, occupation and present address).
infection and CD4+ count (co-variates) have effects on the OIs (response variables) of our study subjects, i.e., they have >0.05 P-value. The median CD4+ count observed in the study population was 120.

Prevalence of different OIs: The distribution of different OIs according to the male-female ratio is shown in Figure 1, and the prevalence of different OIs related to CD4 cell count of the study population is shown in Figure 2.

Among the spectrum of OIs observed, oral candidiasis (OC) emerged as the most frequent infection, developing in 88% of patients whereas oral yeast (Saccharomyces)-infection was found in 5.6% of patients.

TB emerged as the second most prevalent infection, developing in 57% of subjects. The diagnosis of TB was definite for all the suspected patients, and both pulmonary (69.4%) and extra pulmonary (16.6%) types were seen. The HIV-positive patients with extra pulmonary TB had lower median CD4+ counts (46/μl blood) than those of HIV-positive pulmonary TB patients (105/μl blood) which are the most common location of the disease. No evidence of multidrug-resistant M. tuberculosis was identified in this study. All the patients responded well to the standard anti-tubercular therapy.

C. parvum (43.1%) was the pathogen most frequently isolated from the diarrheic patients. Various enteropathogenic species of Vibrio were found in about 47% of the immunocompromised patients, while simultaneously the presence of bacterial pathogens from Enterobacteriaceae was 40% and E. coli was found in 42% of cases.

Table 1. Patients characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients (n = 125)</th>
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<tbody>
<tr>
<td></td>
<td>Male (%)</td>
</tr>
<tr>
<td>No. of HIV-seropositive patients</td>
<td>105 (84)</td>
</tr>
<tr>
<td>Age in years (mean ± standard deviation)</td>
<td>(35.6 ± 6.77)</td>
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<tr>
<td>≤20</td>
<td>1 (0.8)</td>
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<tr>
<td>21-30</td>
<td>27 (21.6)</td>
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<tr>
<td>31-40</td>
<td>55 (44)</td>
</tr>
<tr>
<td>≥41</td>
<td>22 (17.6)</td>
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<tr>
<td>Mode of transmission of HIV</td>
<td></td>
</tr>
<tr>
<td>Heterosexual transmission</td>
<td>80 (64)</td>
</tr>
<tr>
<td>Homosexual transmission</td>
<td>6 (4.8)</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>3 (2.4)</td>
</tr>
<tr>
<td>Intravenous drug abuse</td>
<td>20 (16)</td>
</tr>
<tr>
<td>Frequent needle prick</td>
<td></td>
</tr>
<tr>
<td>Vertical transmission</td>
<td></td>
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<tr>
<td>Unsterilized injection equipments</td>
<td>6 (4.8)</td>
</tr>
<tr>
<td>CD4+ lymphocyte count/μl (mean ± standard deviation)</td>
<td>(439.45 ± 222.11)</td>
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<tr>
<td>≤50</td>
<td>18 (14.4)</td>
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<tr>
<td>51-100</td>
<td>25 (20)</td>
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<tr>
<td>101-200</td>
<td>38 (30.4)</td>
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<tr>
<td>≥201</td>
<td>24 (19.2)</td>
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Fig. 1. Male-female ratio of the study subjects with corresponding opportunistic infections (OIs).

Fig. 2. Prevalence of different opportunistic infections (OIs) related to CD4 cell count of the study population. 1, Candida; 2, Yeast; 3, M. tuberculosis; 4, Cryptosporidium; 5, Cryptococcus; 6, Enterobacteriaceae; 7, E. coli; 8, Vibrio sp.; 9, cytomegalovirus; 10, HBsAg; 11, herpes simplex virus; 12, syphilis.
Among the viral OIs, CMV infection was the most predominant; 45% of subjects were positive for CMV infection among 125 HIV-seropositive study subjects. The other viral infections found in the study population were HSV (7.2%) and HBV (5.6%).

Meningitis was the most common form of disseminated cryptococcosis, and it developed in 4% of the study patients. All cases were diagnosed by clinical and neurological symptoms with confirmation by laboratory investigations.

Very few other OIs were found, viz., herpes zoster virus (HZV) infection, syphilis from T. pallidum, etc., among the patients investigated.

Interestingly, not even a single case of malignancy was found to occur among the investigated patients.

**DISCUSSION**

Although HIV is the initial causative agent in AIDS, most of the morbidity and mortality seen in immunocompromised patients results from OIs that take advantage of the lowered cellular and humoral defenses of the patient.

In the present study, the patients presented with more than one symptom, the most common being asthma, fatigue, loss of weight, fever, chronic cough, loss of appetite and continuing diarrhea. Most of the patients were of lower socio-economic status, living away from family and had gone in search of employment in metropolitan areas. They were working as taxi drivers, carpenters, goldsmith, laborer in building construction, mechanics, etc. They returned home when they were not able to work anymore and while investigating the cause of their presenting symptoms were found to be HIV-seropositive.

In the present study, the 31- to 40-year-old age group (52%) was found to be most commonly involved. These findings were consistent with those of other Indian studies (15). The major mode of HIV transmission was heterosexual contact, found in 64% of cases, with some reports of homosexual transmission (5%), which was in contrast to other Indian studies but similar to some extent with transmission modes reported in the Western population (16). Transmission by intravenous drug users accounted for 20% of cases. Transmission by blood transfusion accounted for 3% of infections, which was similar to the finding of the NACO study (3.8%) (15). The other important category of transmission was unsterilized injection equipment, which accounted for 8% of cases. In remote places of our country, such as in the villages, patients tend to report a history of receiving injectable drugs administered by unqualified medical practitioners where adequate sterilization measures are not always followed.

The most important observation in this study was that none of the study subject was free of at least a single OI, so the overall prevalence rate of OIs could be rated as 100%. This could be secondary to associated malnutrition, overcrowding, poor hygiene and lack of a public health infra-structure in India (17). This 100% prevalence rate may be due to the fact that this study was conducted at a major metropolitan government hospital where the patients seek care mostly when referred from different peripheral health care centers, i.e., when complications set in.

The important finding related to CD4 count revealed that irrespective of the different infections, the patients always exhibited a greater prevalence of OIs when the CD4 count was below 200, i.e., when they were in the AIDS stage, compared to the lower prevalence of the same infection among patients with HIV seropositivity (i.e., CD4 count above 200). Another important finding was that ART-treated patients did not differ much from patients not receiving ART with regard to developing OIs. These observations highlight the importance of performing investigations on people dying with HIV in resource-limited developing countries like India where region-specific disease patterns may be observed. Very often, these OIs represented not new infections but the reactivation of old infection (18). A high prevalence (45%) of CMV-infection was found in our investigation, which is one of the most common viral OIs in patients with AIDS (19,20). An important characteristic of CMV infection was that some patients had no specific clinical symptoms and the CMV infections were often indistinguishable from other types of infection. The common unifying feature of CMV disease in the immunocompromised patient was the presence of fever, and approximately 65% of cases developed hearing defects and perceptual organ damage such as optic atrophy and blindness.

The lower median CD4 count (120/μl) seen in our study may be due to later detection of the infections, reflecting an infrastructural inadequacy of laboratory facilities at the peripheral health centers. At these centers, diagnoses may remain uncertain, and treatment may be held up until significant immunosuppression has already set in, at which point patients are referred to metropolis health care centers or hospitals.

Our study highlights that candidial infection is the most common OI, followed by TB and diarrhea, all of which are presumed to result from our geographic, climatic and socioeconomic conditions. Early diagnosis of OIs and prompt treatment definitely contributes to increased life expectancy among infected patients, delaying the progression to AIDS. Despite the moderate (n = 125) number of patients included in our study, we believe the data obtained here provide some important background information that can form the basis of future, more elaborate and systematic studies. Furthermore, the data shown can be a valuable means of determining the range and relative frequency of infectious diseases, and this can potentially have an immediate impact on patient care by suggesting appropriate interventions based on the results.

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**REFERENCES**


