Original article

Reduction of CD4 count induces opportunistic infections in people living with HIV (PLHIV)

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Abstract:

Objective: Human immunodeficiency virus (HIV), a lentivirus (member of the retrovirus family) causing acquired immunodeficiency syndrome (AIDS), weakens the immune system of the body and hence associates different opportunistic infections. Present study undertook a survey on opportunistic infections.

Materials and Methods: Data were collected from both HIV carriers (CD4 count more than 250/mL of blood) and AIDS patients (CD4 count less than 250/mL of blood).

Results: Analyses of the data revealed that diarrhoea, pulmonary tuberculosis, gland tuberculosis, skin lesions and fever were the common opportunistic infections.

Conclusion: It can be summarized that HIV infected patients having a reduced CD4 count (<250/mL) encounter different opportunistic infections and some of these infections could be continual for long as well.

Key words: HIV; AIDS; opportunistic infections; CD4 count

Introduction:

HIV causes progressive impairment of the body’s cellular immune system leading to the increased susceptibility to tumors, and the fatal conditions known as AIDS¹. AIDS is a disease in which the body's immune system breaks down and is unable to fight off infections, known as ‘opportunistic infections,’ and other illnesses that take advantage of a weakened immune system. The unique feature in the pathogenesis of HIV/AIDS is that the primary target cells for HIV are the immune cells bearing CD4 marker at their surface². HIV infection leads to low levels of CD4 T cells through three main mechanisms: first, direct viral killing of infected cells; second, increased rates of apoptosis in infected cells; and third, killing of infected CD4 T cells by CD8 cytotoxic lymphocytes that recognize infected cells. When CD4 T cell numbers decline below a critical level of 200 cells per µL, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections, for example, pneumocystis pneumonia, toxoplasmosis, cryptococcosis, etc³. In this way, AIDS related mortality and morbidity, which is significantly higher in number as compared to other diseases, is actually due to opportunistic infections rather than HIV itself ⁴. Thus, the success of any HIV/AIDS care and management support relies on effective diagnosis and treatment of opportunistic infections.

In the era of effective antiretroviral therapy (ART), diagnosis and treatment of opportunistic infections is an integral part of this treatment. Diagnosis of CD4 cell count is best validated predictors of likelihood of developing opportunistic infections. Besides, it has great utility in clinical consideration of HIV disease classification and AIDS definition, assessment of prognosis and designing of clinical trials, for e.g. making decision about initiation of

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There are wide ranges of opportunistic infections affecting different systems, for example, respiratory tract infections, gastrointestinal tract infections, urinary tract infections, sexually transmitted infections and disseminated infections. The CD4 cells are likely to co-ordinate a number of immunological functions and as these cells are decreased in immunocompromised patients, the risk and severity of opportunistic infections increases, resulting ultimately in death. Therefore, CD4 count is an important parameter to initiate the prophylaxis of opportunistic infections. When CD4 T cell numbers decline below a critical level of 200 cells per µL, cell-mediated immunity is lost, and infections with a variety of opportunistic microbes appear. The first symptoms often include moderate and unexplained weight loss, recurring respiratory tract infections such as sinusitis, bronchitis, otitis media, pharyngitis, prostatitis, skin rashes, and oral ulcerations.

Based on these suggested data on the assumptions of the influence of CD4 count on the AIDS prevalence, present study was designed with a general objective to explore the relationship between CD4 level and different types of opportunistic infections which might be helpful in the development of the prognosis of different secondary infections in HIV infected patients. Several opportunistic infections were found including diarrhoea, pulmonary tuberculosis, gland tuberculosis, skin lesions, etc. that had been associated with low CD4 count (<250/mL). It was also observed based on the questionnaire that some opportunistic infections had the potential of turning into persistent diseases.

Materials and methods

Sample selection:
This research was a cross-sectional study in which retrospective data and blood samples were collected from 100 people living with HIV (PLHIV) patients. The work was carried out in association with Ashar Alo Society from January 2011 to June 2011. 25% of 400 HIV positive persons registered in the above organization were randomly selected who had CD4 count <250/mL of blood or >250/mL of blood. Sample selection was done by random sampling method using the patients’ lists available in the respective sites. The study was approved by local ethical committee.

Data collection by questionnaires:

Blood sample collection:
Peripheral blood samples were collected from the studied individuals. All blood samples were collected observing universal precautions for venipuncture using the vacuum tube (red top) (Greiner Bio-One, Germany) containing an appropriate anticoagulant K3- Ethylenediaminetetraacetic acid (K3-EDTA). 5 ml blood from each person was collected. Just after collection, the blood was mixed homogenously with the anticoagulant to avoid undesirable clotting. The peripheral blood samples in the EDTA containing tubes were immediately transported to the laboratory in a sampling box with cool packs maintaining the temperature at 2-8 °C. However, in case of delay processing, the samples were stored at 2-8 °C. All specimens were labeled properly with a unique patient identifier, date, and time of collection. Finally, the blood was processed in the laboratory for the determination of CD4 count.

Determination of CD4 count
The determination of CD4 count was done by automated machine (BD FACS Count CD4 reagent tube, USA). To detect the CD4 count, 2.5 ml unlysed whole blood was taken in the CD4 reagent tube followed by the application of the fixative formaldehyde. Then the tube was kept for 1-2 h and was finally transferred to the flow cytometry machine (CD4 counter). The installed software in the machine identifies the lymphocyte populations of interest and calculates absolute CD4 counts (cells/µL of blood).

Sputum specimen collection and processing:
The specimens were collected from all the 100 individuals considered in this study. Patient’s status, age, sex and occupations were considered while collecting the specimens since all these factors are likely to induce infection by HIV. One spot of morning sputum sample was collected in a sterile plastic container. On the initial hospital visit, the patient was pro-
vided a clean, dry, wide-neck, leak-proof container and was requested to cough deeply to produce a sputum specimen. For Ziehl-Neelsen staining to detect TB infections, smear was prepared from yellow purulent portion of the sputum using a bamboo stick. A good smear was spread evenly, 3 cm by 2 cm in size in the middle part of the slide which was neither too thick nor too thin. The sputum was left for 15-30 minutes for air dry. The smear was fixed by placing the slide over the hot plate at 85 °C for about 3-5 minutes.

Ziehl-Neelsen staining:
The smear was covered with carbon fuchsin stain and heated until first vapor was observed. After keeping for 10 minutes, the smear was washed up with clean water. Then it was covered with 25% sulphuric acid for about 3 minutes and washed off with tap water. Finally, after covering with 0.1% methylene blue for maximum 1 minute, the washed and dried smear was examined under bright field microscope under 100× magnification.

Results
Correlation of age, sex, economic and environmental status with the occurrence of HIV:
A total of 100 individuals were considered in the present study irrespective of age and sex. Among the 100 samples, the CD4 count of 50 individuals was <250/mL and the other 50 individuals had CD4 count >250/mL of blood. Persons with CD4 count >250/mL were considered as HIV carriers, whereas those with CD4 count <250/mL of blood were the AIDS patients. The demographic analysis of the study population is given in figures 1 and 2 consecutively to find out the correlation between different demographic variables and HIV infections. The economic and environmental status of the studied population was also analyzed. As shown in figure I, both for the HIV carriers and the HIV patients, the maximum number of subjects were in the age range of 15 to 30 years and 31 to 45 years. According to figure II, male subjects were always found to be higher in number compared to their counterparts female subjects both for the HIV carriers and the HIV patients. This again clearly indicates the association of uncontrolled sexual life style and disrespect to religious notions with the occurrence of HIV as has already been known for long. Our data pinpoints the level of CD4 which could be a new insight in Bangladesh to this existing knowledge on AIDS. However, the subjects should further be analyzed to find out any professional sex workers among them. Figure III shows that the likelihood of HIV occurrence increases with the downward economic and environmental status.

Documentation of opportunistic infections in people living with HIV (PLHIV):
Next, we turned to investigate the level of opportunistic infections in accordance to the number of CD4 in HIV infected patients. Direct interviews of the individuals were taken on the different opportunistic infections they encountered after being diagnosed as either HIV carriers or AIDS patients. They were asked to admit any illness they suffered previously. They were also requested to inform the interviewer if they had been actually suffering from any opportunistic infection during the time of data collection. However, blood and sputum samples were also collected from them to cross check the facts. These specimens were analyzed in the laboratory to find out any opportunistic infection among the subjects. The different infections they have been suffering from are given in figures IV and V, respectively.

Potential of different opportunistic infections to become persistent:
Among the different opportunistic infections, some seemed to be persistent in the AIDS patients. The individuals were asked about the duration of different infections they had been suffering from. All their past and present illnesses were documented (Supplement I). The time interval maintained between the past and present health status of the subjects ranged from 6 to 8 months. Based on the survey, the likelihood of different diseases being persistent is summarized in figure VI. According to this figure, HIV patients have a greater likelihood of continuously suffering from fever followed by pulmonary tuberculosis, skin lesions and cold. So, in order to deal with the secondary infections, more careful therapeutic treatments should be given to these people considering them vulnerable to opportunistic infections. An erroneous treatment may lead to further clinical complicacy such as hypersensitivity.

Discussion
The HIV prevalence rate is low in Bangladesh with less than 0.1% of the population estimated to be HIV positive. The Joint United Nations Programme on HIV/AIDS (UNAIDS) estimates the number of HIV infected people in Bangladesh to be 11,000.
Although the HIV prevalence rate is still low in Bangladesh, behavioral patterns including multiple sex partners and intravenous drug addiction suggest that the number of people infected with HIV could reach epidemic proportions unless major efforts are undertaken to prevent it.

Opportunistic infections continue to cause morbidity and mortality in patients with human immunodeficiency virus (HIV)-1 infection throughout the world. Although hospitalizations and deaths have decreased since the implementation of Anti-retroviral therapy (ART), opportunistic infections still remain a leading cause of morbidity and mortality in HIV-infected persons9-17. Therefore, a retrospective analysis of the different opportunistic infections associated with the AIDS patients could be of utmost importance in the prevention and management of opportunistic infections.

Majority of our people are not aware about the further progression of health hazards (e.g. opportunistic infections) primarily induced by HIV. It is necessary to increase the awareness on opportunistic infections in HIV infected people. Therefore, this type of study would be helpful to reduce the chance of the further onset of opportunistic infections in HIV infected people bearing a low CD4 count (<250/mL of blood).

Present study was conducted to find out different opportunistic infections associated with HIV infection. The samples studied were divided into two groups: HIV carriers (CD4 count >250/mL) and AIDS patients (CD4 count <250/mL). In both HIV carriers and AIDS patients, maximum number of patients was actually in the age range of 16 to 30 years or 31 to 45 years. Again, in both cases, male percentage of being infected with HIV was higher than their female counterparts (figure II). These results correlated with the fact that behavioral patterns influence the rate of HIV infectivity. Again in both the cases it was observed that HIV infection rate decreases with the increasing economic and environmental status.

Analyzing the data collected, it was found that in both the carrier state and the AIDS state, the individuals encountered different opportunistic infections. Among the infections, diarrhea, skin lesions, oral fungal infections and fever were found to be very common. The percentage of their occurrence in the AIDS patients was a bit higher than the HIV carriers (figures IV and V).

Finally, among the opportunistic infections, those with a greater possibility of being persistent were also identified. Persistent infections are chronic infections which last long specially in immunocompromised people such as AIDS patients. As shown in figure 6, fever was found to be the most common and persistent clinical complicity in the AIDS patients followed by pulmonary tuberculosis, skin lesions, cold, oral fungal infections (candidiasis), etc. HIV infects about 0.1% of adult TB patients in Bangladesh6. Moreover, HIV-TB co-infections complicate treatment and care for both diseases. It is so because persons with HIV infection are often taking numerous medications, some of which may interact with anti-TB medications6. The prevalence of tuberculosis documented by this study is almost similar to other studies conducted in different parts of Nepal2. A hospital based study in Western Nepal documented 10.8% prevalence of TB among HIV patients while another similar study in Central Nepal documented 23% prevalence of TB 18-19. However, 57% prevalence of tuberculosis was observed in HIV seropositive patients from Eastern India20. A hospital based study of Far Western Nepal documented 25.9% oral candidiasis, 33.3% esophageal candidiasis in the patients with CD4 count between 101-200/cumm of blood21. Oral candidiasis was found to be the predominant opportunistic infections accounting as high as 32% and was significantly associated with low CD4 count (<200/cumm of blood) 20. All these data also validate our results on the occurrence of candidiasis (34%) as a prominent opportunistic infections associated with AIDS. Yet, 88% candidiasis was identified as the predominant opportunistic infection in HIV patients with a CD4 count of 120/cumm of blood in Eastern India20.

Documentation of opportunistic infections and their potential of occurrences in AIDS patients are also very important. So far our knowledge goes, our study is the first attempt to deal with such a rising issue in Bangladesh. The outcomes could be referred to control and prevent different secondary infections. This would also aid in the treatment policy of these infections as well. However, greater concern should be given to those infections with a higher possibility of being persistent as documented in our study. However, the likelihood of different infections in accordance with age should also be investigated.
In addition, the subjects should also be investigated to determine whether they are suffering from any other sexually transmitted diseases (STDs). Individuals who are infected with STDs are at least 2 to 5 times more likely than uninfected individuals to acquire HIV infection if they are exposed to the virus through sexual contact\textsuperscript{22}. In addition, if an HIV-infected individual is also infected with another STD, that person is more likely to transmit HIV through sexual contact than other HIV-infected persons\textsuperscript{22}. Routine serologic screening for syphilis is recommended at least annually for all sexually active HIV-infected persons. There is an estimated 2 to 5 fold increased risk of acquiring HIV if exposed to that infection when syphilis is present. It should be more frequently screened (every 3–6 months) for those with multiple sex partners, unprotected intercourse, sex in conjunction with illicit drug use, methamphetamine use, or partners who participate in such activities\textsuperscript{23-24}.

Figure I. Bar diagram showing the numbers of studied individuals (A: CD4 count >250/mL and B: CD4 count <250/mL) in different age groups. The samples were categorized in four different age ranges starting from 1 year up to 60 years. The class interval maintained within a single group was 15 years.

Figure II. Number of individuals (A: CD4 count >250/mL and B: CD4 count <250/mL) in different sex; male and female. In both the cases, male candidates were more in number than female candidates.

Figure III. Categorization of the studied individuals in different economic and environmental status. All the samples were categorized in three different groups: poor, average and good. The categorization...
was done for both the HIV carriers (CD4 count >250/mL) and AIDS patients (CD4 count <250/mL). Numbers of individuals having opportunistic infections were greater with the decreasing magnitude of economic and environmental status.

**Figure IV.** Different opportunistic infections in HIV carriers (D: diarrhoea, OFI: oral fungal infection, SL: skin lesion, PTb: pulmonary tuberculosis, GTb: gland tuberculosis, Fe: fever and Cl: cold). According to the figure, fever and pulmonary TB are the highest and lowest prevalent infections among the HIV carriers, respectively. Diarrhoea, skin lesions and oral fungal infections are some other significant infections among the HIV carriers.

**Figure V.** The different opportunistic infections in AIDS patients (D: diarrhoea, OFI: oral fungal infection, SL: skin lesion, PTb: pulmonary tuberculosis, GTb: gland tuberculosis, Fe: fever and Cl: cold). The figure depicts that fever, skin lesions, diarrhoea and oral fungal infections (descending order) are the most prevalent infections.

**Figure VI.** Different persistent infections in AIDS patients. The above mentioned infections were present among the individuals during the time of survey and long before that (~6 months) as well (D: diarrhoea, OFI: oral fungal infection, SL: skin lesion, PTb: pulmonary tuberculosis, GTb: gland tuberculosis, Fe: fever and Cl: cold).

**Conclusion**

In conclusion, this study underscores the importance of the documentation of several opportunistic infections that AIDS patients encounter in Bangladesh. The findings of this study are comparable with other studies conducted throughout the world, especially in Asian countries. This kind of retrospective study could be very beneficial to the present therapeutic practices of the AIDS patients prevailing in Bangladesh.
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