

## Original Research Article

# Spectrum of opportunistic infections with correlation to CD4 counts in newly diagnosed HIV seropositive cases

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### ABSTRACT

**Background:** A rise in HIV/AIDS is observed in resource poor countries like India despite successful implementation of control programmes. Most of these deaths recorded in cases of AIDS are because of opportunistic infections [OI] and other malignancies. The reason may be attributed to the effective destruction or decrease in CD4 cells which play a pivotal role in immune system. OI cause substantial morbidity and hospitalization, economical loss to the society and shorten the survival time of HIV patient. The objective of this study was to evaluate the different type of infections and identify the frequent pathogens affecting the HIV patients who are attending a tertiary care hospital in India. The clinical profile of these patients was studied and proportion of CD4 counts with respect to their type of infection and pathogen is also evaluated.

**Methods:** A prospective cross sectional study was conducted for one year period. Clinical samples were collected from all the newly diagnosed cases of HIV and performed various staining techniques and cultured on appropriate culture media. All the isolates were identified as per standard guidelines. Serological evaluation for IgM antibodies for toxoplasma was done by ELISA. CD4 counts were estimated by FACS.

**Results:** Tuberculosis was the commonest (67%) OI in HIV cases, followed by candidiasis (61.5%), respiratory tract infections (50%), gastro intestinal tract infections (44%) and meningitis (38%) in our study. The mean CD4 cell counts in the study was 267.11 cells/ $\mu$ l. The commonest fungal pathogen was *C.albicans* and *Cryptosporidium parvum* the parasitic pathogen. The mean CD4 cell counts were lesser in parasitic infections when compared to bacterial and fungal infections. Six cases of *P.jiroveci* pneumonia were identified in our study. Mortality was recorded among the HIV cases with CD4 cell counts <50 cells/ $\mu$ l.

**Conclusions:** Early diagnosis and prompt treatment of OIs contributes to increased life expectancy among infected patients, delaying the progression to AIDS. This study helps the clinicians in proper guidance to come up with right diagnosis and early response to manage the patients in resource poor countries like India.

**Keywords:** AIDS, CD4 counts, HIV, Opportunistic infections, Tuberculosis

### INTRODUCTION

Globally a decline in incidence and prevalence of HIV has been observed through implementation of various measures like successful awareness programmes and health education systems with active participation of governmental and nongovernmental organizations.<sup>1</sup> A

rise in HIV/AIDS is observed in resource poor countries like India despite successful implementation of control programmes. However, the rate of mortality still pose a problem to health care system in developing countries like India. Most of these deaths recorded in cases of AIDS are because of opportunistic infections (OI) and other malignancies.<sup>2</sup> The reason may be attributed to the

effective destruction or decrease in CD4+ cells which play a pivotal role in immune system. The incidence of HIV associated OI have declined in developed countries by effective implementation of anti-retroviral therapy (ART). But the relative frequencies of these opportunistic infections, causative pathogens vary in different countries and also in different places of same country. OI cause substantial morbidity and hospitalization, economical loss to the society and shorten the survival time of HIV patient. They also affect the quality of life of HIV affected individuals by increasing morbidity.<sup>3</sup> Around the world OI has reduced in HIV individuals by implementation of ART, which may be due to reduction in the viral load of HIV thereby boosting the immune system. In addition, measures to treat and prevent OI become essential if ART stops working because of poor adherence to the regimen and development of drug resistance if noted. CD4 count has shown to be an effective predictor in assessing the development of OI in HIV seropositive individuals. It is absolutely necessary to have knowledge about the type of OI and the pathogens distributed in the region. Effective management and treatment of these infections not only improves the quality of life but also helps in prevention of transmissible diseases like tuberculosis etc.<sup>4</sup> The type of infections and the spectrum of pathogens responsible have been documented in many studies conducted in China, Africa, Korea, Thailand and Bangladesh. Studies about the distribution of opportunistic pathogens among people living with HIV in India have been reported and are limited to place and region. The present study was aimed to evaluate the different type of opportunistic infections and identify the frequent pathogens affecting the HIV patients who are attending a tertiary care hospital in India. The clinical profile of these patients was studied and proportion of CD4 counts with respect to their type of infection and pathogen is also evaluated.

## **METHODS**

The present study was conducted at Narayana Medical College General and superspeciality hospital, a tertiary care hospital for a period of one year from March 2015 to February 2016. Details of the study were informed and written consent was obtained from all the participants in the study after explaining the details and were treated as per the guidelines. The participants were followed up and monitored for development of any complications thereafter.

Study population and design: Two hundred patients (n = 200) of both sexes, newly diagnosed HIV seropositive attending the outpatient departments of general medicine, respiratory medicine and admitted in wards of hospital who fulfilled the inclusion criteria were included in the study. A predesigned protocol was followed for evaluation including the Socio demographic and biodata, clinical examination, mode of transmission, presenting complaints were noted.

## ***Inclusion criteria***

- Age above 20 years
- Diagnosed as HIV seropositive by standard NACO guidelines
- No history of other co morbidities like Diabetes, Hypertension etc.
- No History of tuberculosis and on anti-tuberculosis treatment.

HIV testing was done with pre-and post- test counseling and records were maintained. HIV status was confirmed by following NACO guidelines by performing HIV rapid test and ELISA. Strict confidentiality was maintained regarding the data. The study was approved by the institutional research and ethical committee of the hospital.

## ***Diagnosis of Opportunistic infections***

OI were diagnosed as per the criteria laid down by center for disease control and prevention (CDC).<sup>6</sup> Depending upon the system and organ involvement, as decided by the physician, specimens which included swabs, pus, sputum, CSF, blood, lymph node aspirates and stool were sent to laboratory after collection by following universal precautions and standard collection procedures. The samples were subjected to microscopy by staining procedures like grams, Zeihl-Nelson (Zn), Modified acid fast staining for cryptosporidium, silver methenamine staining for Pneumocystis jiroveci and Indian ink staining for cryptococcus, and cultured on appropriate culture media (blood agar, MacConkey agar, chocolate agar, Lowenstein-Jensen medium and Sabourad's dextrose agar, BHI broth) depending on the specimen. The growth on the media was subjected to a set of standard biochemical tests as per CLSI guidelines. The isolate was identified and reported.

## ***Identification of mycobacteria***

Sputum specimens positive by microscopy (direct/concentrated) or suspected with Mycobacteria were inoculated on Lowenstein-Jensen media [Hi-media, Mumbai] and incubated at 37°C for 4-8 weeks. Growth on media was confirmed by Zn staining and Niacin test. Isolate negative for Niacin test was considered as Atypical Mycobacteria. Mycobacteria ATCC 25177 was used as control strain.

## ***Identification of parasites***

Watery stool specimens from diarrhea patients were collected in leak proof containers and subjected to modified acid fast staining to identify cryptosporidium parasite. On microscopy parasite was stained pink-red. The strain was compared with the referral strain cryptosporidium (Microbiology QC slides, Himedia SL-45-10; Himedia). Suspected cases of Cerebral toxoplasmosis identified by the physician with

neurological involvement, and suggestive computed tomography and magnetic resonance imaging studies of the brain. Anti-toxoplasma IgM antibodies were estimated by serological ELISA kit (Biomérieux, USA).

#### Estimation of CD4 counts

The CD4 counts of HIV seropositive subjects were estimated by FACS caliber flow cytometer (Beckton-Dickinson, USA) and noted as per NACO guidelines 7. Dual color immune phenotyping was performed using standard whole blood methodology. The estimation of viral load is an important predictor in monitoring and assessment of response to ART, but it was not done in our study because of heavy cost.

Serological evaluation: Suspected cases of cerebral toxoplasmosis were identified by the physician with neurological involvement, and suggestive computed tomography and magnetic resonance imaging studies of the brain.<sup>8</sup>

#### Statistical analysis

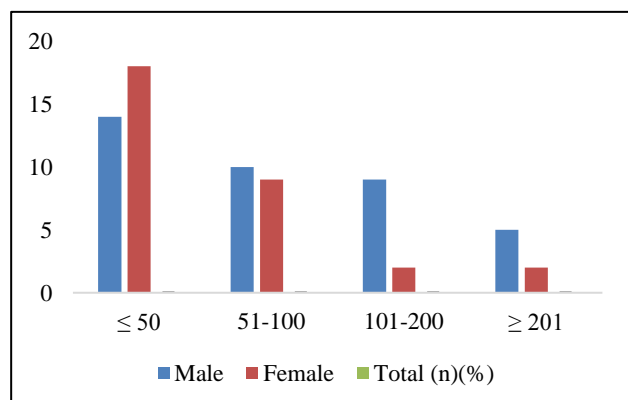
All the data was entered in Microsoft excel data sheet and analyzed. The mean, median and STD deviation was calculated regarding continuous variables.

#### RESULTS

Patient profile: In our study, 200 HIV seropositive (186-HIV-1 and HIV-2- 14) cases with OI were studied, of whom 115 were males (57.5%) and females 85 (42.5%). In both males and females, the predominant age group was 41 -60 years (males: 28% and females: 21.5%) and overall in the study 49.5% (99/200). The male to female ratio was 1.35 :1. Mean age of the study group was 42.19 (SD: 11.51) years. Heterosexual mode of transmission was the commonest with 83% followed by 6.5% by blood transfusion, 2% homosexual route and 8.5% other route (Unknown, needle prick etc). Mean CD4 cell count of the study group was 267.11 cells/ $\mu$ l (Table 1).

**Table 1: Demographic profile of study group.**

	Male (n)	Female (n)	Total (n) (%)
No of HIV seropositive patients	115	85	200
<b>Age in years</b>			
20-40 years	53	35	88 (44%)
41-60 years	56	43	99 (49.5%)
$\geq$ 61 years	6	7	13 (6.5%)
<b>Mode of transmission</b>			
Heterosexual	94	72	166 (83%)
Blood transfusion	8	5	13 (6.5%)
Homosexual	4	0	4 (2%)
Others	9	8	17 (8.5%)
<b>CD4+ lymphocyte counts/<math>\mu</math>l</b>			
$\leq$ 50	28	34	62 (31%)
51-100	35	25	60 (30%)
101-200	20	12	32 (16%)
$\geq$ 201	32	14	46 (23%)



**Figure 1: Distribution of CD4 counts and Deaths of enrolled cases.**

Symptomatic presentation of the cases is represented in Figure 1. Fever was the commonest (88%) followed by weight loss (82%), acute cough (<3 weeks duration) 62%, Chronic cough 46% and oral thrush was observed in 49% and diarrhea in 38% of cases. Lymphadenopathy, headache and altered sensorium, neck rigidity were other associated symptoms. 19% were found to be asymptomatic.

Table 2 represents the distribution of OI with relation to their mean CD4 cell counts. Tuberculosis was the commonest OI in our study with an overall 67% (134/200), pulmonary cases were 68.66% (92/134), extra pulmonary 31.34% (42/134) and 38 of the cases were having both pulmonary and extra-pulmonary tuberculosis. Second followed by candidiasis 61.5%

(123/200), with oral candidiasis in 87.8% (108/123) and esophageal candidiasis was observed in 15 cases (12.2%) in our study. 98 (49%) of cases were diagnosed with respiratory tract infections (other than Mycobacterium tuberculosis) and 37% were of bacterial etiology and 10% were of fungal pathogens. 6 cases were identified with

Pneumocystis jiroveci pneumonia and all were with CD4 counts <50 cells/ $\mu$ l. Meningitis was observed in 76(38%) cases with bacterial constituting 58, fungal 12 cases and parasitic (Toxoplasma) in 6 cases. Gastrointestinal infections were observed in 44% (88/200), with parasitic etiology in 76 cases and bacterial in 12 cases.

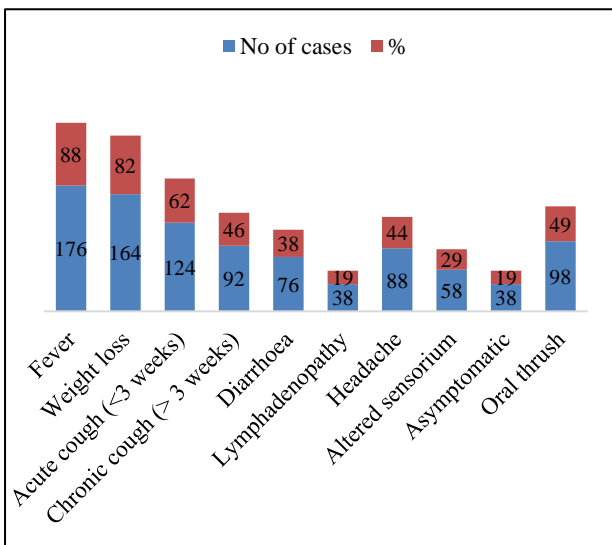
**Table 2: Distribution of opportunistic disease and CD4 cell count.**

Type of OI	No. of cases	Mean CD counts
<b>Tuberculosis (n = 134) 67%</b>		
Pulmonary	92	74.5 (1-312)
Extra pulmonary	42	67.5 (1-276)
Both	38	65.5 (1-254)
<b>Meningitis (n = 76) 38%</b>		
Bacterial	58	72.3 (1-302)
Fungal	12	61.2 (1-238)
Parasitic (Toxoplasma)	6	38.2 (1-168)
<b>Respiratory Infections (n = 100) 50%</b>		
Bacterial	74	70.2 (1-294)
Fungal	20	60.4(1-228)
<b>Pneumocystis carinii pneumonia</b>	6	35.8 (1-154)
<b>Candidiasis (n = 123) 61.5%</b>		
Oral thrush	108	45.3 (1-186)
Esophageal Candidiasis	15	40.2(1-176)
<b>Gastrointestinal Infections (n = 88) 44%</b>		
Bacterial	12	54.2(1-212)
Parasitic	76	41.6(2-180)

**Table 3: Microbial profile of HIV seropositive patients.**

Type of Pathogens	Number	%
<b>Bacterial respiratory pathogens</b>		
Mycobacterium tuberculosis	92	23.1
Streptococcus pneumoniae	12	3.0
Klebsiella pneumoniae	24	6.0
Pseudomonas sp	18	4.5
Staphylococcus aureus	22	5.5
Escherichia coli	12	3.0
<b>Fungal pathogens</b>		
Candida albicans	78	19.6
Candida krusei	11	2.8
Candida tropicalis	9	2.3
Aspergillus fumigatus	12	3.0
Aspergillus niger	8	2.0
Cryptococcus neoformans	18	4.5
Pneumocystis carinii	6	1.5
<b>Intestinal parasites</b>		
Cryptosporidium parvum	28	7.0
Isospora	8	2.0
Entamoeba histolytica	22	5.5
Giardia lamblia	18	4.5
<b>Total</b>	<b>398</b>	

Table 3 clearly represents the microbial isolates in our study. A total of 398 isolates including bacterial, fungal and parasitic were isolated. Mycobacterium tuberculosis forms the predominant bacterial isolate (23.1%) followed by others *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas* and *Escherichia coli* at 3%, 6%, 5.5%, 4.5% and 3% respectively. Among the fungal isolates *Candida albicans* was the major isolate with 19.6% and other candida species isolated were *C. krusei* (2.8%) and *C. tropicalis* (2.3%). *Aspergillus fumigatus* (3%), *Aspergillus niger* (2%), *Cryptococcus neoformans* (4.5%) and *Pneumocystis jirovecii* (1.5%) were other fungal pathogens in our study. *Cryptosporidium parvum* (7%) was the major parasitic pathogen followed by *Entamoeba histolytica* (5.5%), *Giardia lamblia* (4.5%) and *Isospora belli* (2%) in our study. In diarrheal cases, some of the patients presented with mixed bacterial and parasitic infections (*Escherichia coli* and *Entamoeba histolytica*).



**Figure 2: Clinical presentation of study population.**

Figure 2 represents the mortality of the cases with correlation of CD4 counts. A total of 69 deaths were recorded with males 38 and females 31. Majorities (51.61%) were with CD4 counts <50/ $\mu$ l indicating that CD4 counts is better predictor in mortality. Seven deaths were recorded with CD4 counts >200, with 4 cases of tuberculous meningitis and 3 cases with multi organ involvement.

## DISCUSSION

Although HIV is implicated as the causative agent of AIDS, the mortality and morbidity associated is because of OI which occur because of lowered humoral and cellular immune mechanisms of the patient. Most of the deaths reported in AIDS cases are because of OI.

In our study the common age group was 41-60 years (49.5%) which is in conformity with findings of Singh A

et al 2003, SK Sharma et al 2004 and Chakravarty J et al with 92%, 89% and 77% in the same age group.<sup>9-11</sup> Heterosexual route of transmission was common mode of acquisition, as observed universally and all the females who acquired were monogamous. However, the possibility of polygamy cannot be ruled out in Indian scenario due to social linkages and traditions. 6.5% of cases, acquired by blood transfusion which is similar to report of NACO which reported as 3.5%.<sup>12</sup>

As per the WHO report 2015, TB leads the OI among HIV affected individuals and kills almost three fourths of affected cases. 13 Several studies globally and in India has reported tuberculosis as the most common OI and kills nearly a quarter of million every year.<sup>14,15</sup> Similar findings were reported from our study with 67% incidence of tuberculosis. In our study, out of total 134 cases of tuberculosis, pulmonary tuberculosis accounted for 68.66% cases, extra pulmonary in 31.34% cases and 28.36% with both pulmonary and extra-pulmonary cases. Kumaraswamy et al in his study reported a higher prevalence of extra pulmonary tuberculosis (49%) than pulmonary which is contrary to the findings of our study.<sup>16</sup> Mean CD4 count observed in cases of pulmonary tuberculosis in our study was 74.5 (1-312) cells/ $\mu$ l, extra pulmonary tuberculosis was 67.5 (1-276) cells/ $\mu$ l and 65.5 (1-254) cells/ $\mu$ l in cases of both, these findings are on par with findings of Shahapur PR et al.<sup>17</sup> The HIV positive patients with extra pulmonary tuberculosis had lower mean CD4 counts than those with pulmonary tuberculosis. Some studies reported candidiasis as the most common OI in their studies. In our study, it was second most common OI with prevalence of 61.5%, with oral thrush 87.8% and esophageal candidiasis in 12.2%, which is on par with findings of Singh et al (59%), and several studies have reported the incidence up to 70%.<sup>18</sup> The mean CD4 cell counts in cases with candidiasis was 45.3 cells/ $\mu$ l (1-186) in cases with oral thrush and 40.2 cells/ $\mu$ l (1- 176) in esophageal candidiasis. Most of the studies reported candidial infections in HIV cases at CD4 counts <200 cells/ $\mu$ l which is same as findings in our study. Other fungi isolated from HIV patients were *Aspergillus* sp, from respiratory tract infections. The prevalence of respiratory infections was 49% in our study with mostly bacterial pathogens (74) and fungal pathogens (20) and *Pneumocystis jirovecii* pneumonia (PCP) was observed in 6 cases. Most of the studies in India reported PCP among CD4 counts <50 cells/ $\mu$ l, in our study the mean CD4 count among these cases was 35.8 (1-154) cells/ $\mu$ l. The prevalence of PCP among HIV in Indian studies is less documented when compared with industrialized nations.<sup>19</sup> In our study the prevalence of *Pneumocystis jirovecii* was 0.6% among all respiratory pathogens.

Bacterial meningitis was reported in 58 cases in our study and *Pseudomonas aeruginosa*, *Staphylococcus aureus* were the common pathogens. 18 cases of *Cryptococcus neoformans* were identified with 12 from meningitis and 6 from cutaneous manifestations. The mean CD4 count of

patients with bacterial meningitis was 72.3 (1-302) cells/ $\mu$ l and with Cryptococcal meningitis 61.2 (1-238) cells/ $\mu$ l indicating that CD4 counts in HIV patients with fungal meningitis are lower than bacterial meningitis cases. Six cases of toxoplasmic encephalitis were diagnosed based on Ig M ELISA and imaging studies, with mean CD4 cell counts 38.2 (1-168) cells/ $\mu$ l. Studies documenting the presence of toxoplasma in HIV positive patients are very limited. The prevalence of cryptococcal meningitis has been well reported from western population when compared to Indian studies. The findings in our study were on par with findings of Bharathi et al and Ravindre kaur et al who reported the prevalence of Cryptococcus as 3.8% and 6% in their studies.<sup>20,21</sup>

Gastrointestinal infections are very recurrent and troublesome in HIV patients. The prevalence of gastrointestinal OI in our study was 44% with parasitic 76 cases and bacterial 12 cases; however 6 cases had mixed bacterial and parasitic infections. The mean CD4 counts among HIV positive cases with parasitic gastrointestinal pathogens were 41.6 (2-180) cells/ $\mu$ l and bacterial were 54.2 (1- 212) cells/ $\mu$ l. *Cryptosporidium parvum* was the predominant pathogen causing diarrhea in our study (7%) followed by *Entamoeba histolytica* (5.5%), *Giardia* (4.5%) and *Isospora* (2%). Several studies from India and other parts of the world also reported the same.<sup>22,23</sup> To summarize Tuberculosis (67%) was the most common OI in our study followed by candidiasis (61.5%), respiratory tract infections (50%), gastrointestinal tract infections (44%) and meningitis (38%). Findings of our study were on par with many of the studies globally and in India. However, some of the studies mentioned candidiasis as the most common OI, however the type of OI in HIV patients are dependable on variable factors like the hosts immune response, duration of ART and response of the individual to ART. The mean CD4 counts of the cases were less in parasitic infections when compared to bacterial and fungal infections indicating that CD4 counts play an important role in predicting the response of ART. Early diagnosis and prompt treatment of OI contributes to increased life expectancy among infected patients, delaying the progression to AIDS.<sup>24</sup>

## CONCLUSION

Study highlights the importance of understanding the type of OI prevalent in the region and CD4 counts associated with the infections. This study helps the clinicians in proper guidance to come up with right diagnosis and early response to manage the patients in resource poor countries like India.

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