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Original Research Article

Scenario of mycobacterial and fungal infection in HIV seropositive patients and their co-relation with CD4 count in Western Uttar Pradesh

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ABSTRACT

Background: HIV infection is defined by sero-conversion and the detection of HIV-specific antibodies. Emergence and pandemic spread of acquired immunodeficiency syndrome is due to the exposure to human immunodeficiency virus (HIV). A decrease in CD4 count is at least partially responsible for the profound immunodeficiency that leads to various OIs in HIV- infected persons. When the CD4 count falls below 200cells/µL, there is irreversible breakdown of immune defence mechanism and patient become prey to a variety of human opportunistic pathogens. HIV positive patients must receive infections screening and access medical care before onset of advanced immunosuppression.
Methods: In this study, total 230 HIV positive patients were selected during 18 months of study period. CD4 counts were estimated of all HIV positive cases. Positive HIV patients were investigated further to detect mycobacterial and fungal opportunistic infections. They were subjected to routine microscopy such as KOH mount, India ink, Gram's staining for suspected fungal infection and ZN staining method for suspected mycobacterial infection. For fungal infection, samples were inoculated in two Sabouraud Dextrose Agar followed by different biochemical test and LPCB mount; for mycobacterial infection, samples were cultured on LJ medium followed by biochemical test.
Results: In our study, maximum patients presented with complain of fever (90.43%), weight loss (73.91%) followed by loss of appetite (35.65%), breathlessness (33.91%), coughing (28.69%) and chest pain (22.17%). Overall prevalence of OIs (*Mycobacterium* and fungal) was 93 (40.43%) among 230 HIV positive patients. Among OIs 63(27.39%) patients were detected as having Mycobacterial infection and 41(17.82%) as had opportunistic fungal infections. Maximum OIs were related to patients with CD4 count 0-200 cells/µL followed by 201-400 Cells/µL. Most common OIs, among mycobacterial and opportunistic fungal infection were *M. tuberculosis* (50 isolates) and *Candida spp*. (26 isolates) respectively.
Conclusions: This study provides important information about the risks of OIs at lower CD4 counts among HIV positive patients. These results highlight the need for early screening of HIV infected patients for opportunistic infections. There is also need to increase awareness in healthcare providers in order to improve decisions regarding prophylaxis for prevention of OIs and appropriate therapeutic intervention.

Keywords: CD4 counts, HIV, OIs, LPCB mount, ZN staining

INTRODUCTION

Human immunodeficiency virus (HIV) types, derived from primate lentiviruses, are the etiologic agents of AIDS. HIV infection is defined by seroconversion and the detection of HIV - specific antibodies.[1] In the 21st century, AIDS is one of the most important public health problem worldwide. National adult HIV prevalence was 0.26% (0.22-0.32%) among general population in India.[2] HIV specific T lymphocytes are present in the peripheral

blood of these HIV exposed seronegative individuals, suggesting that cell mediated immunity could have a protective role in the prevention of OIs. In most individual who seroconvert, HIV infection is followed by a long period of clinically asymptomatic latency which ultimately results in the development of AIDS.[1]

If HIV infected patients are left untreated, they develop fatal opportunistic infections as a result of HIV-induced deficiencies in the immune system.[3] People with advanced human immunodeficiency virus (HIV) are vulnerable to infections called "opportunistic infections" (OIs) because organisms take advantage of the opportunity offered by a weakened immune system. OIs cause substantial morbidity and hospitalization, necessitate toxic and expensive therapies, and shorten the survival of people with HIV infection.[4] A decrease in $CD4^+$ count is at least partially responsible for the profound immunodeficiency that leads to various OIs in HIV-infected patients.

When the CD4 count falls below 200cells/µL, there is irreversible breakdown of immune defence mechanism and patient become prey to a variety of human opportunistic infections like bacterial, viral, fungal, parasitic infections such as tuberculosis, candidiasis, herpes zoster, pneumocystis *jirovecii* pneumonia, cytomegalovirus (CMV) etc.[3] The spectrum of opportunistic infections in the HIV infected subjects varies from one region to another.[5]

Currently, the initiation of primary prophylactic therapies for OIs is based chiefly on the absolute CD4 count, which has shown to be an excellent predictor of the short term overall risk of developing acquired immunodeficiency syndrome (AIDS) among HIV-infected patients.[4] Hence, present study focused on HIV positive patients who visited ART centre at UPUMS, for symptoms suggestive of Mycobacterial and/or opportunistic fungal infection. Prevalence of OIs and their association with CD4 count was also determined in our study.

METHODS

This prospective study was done on patients visiting ART centre at UPUMS, Saifai. HIV positive patients were investigated for mycobacterial (typical/atypical) and opportunistic fungal infections in the Department of Microbiology, UPUMS, Saifai, Etawah.

Inclusion criteria

All patients who were registered in ART centre at UPUMS, Saifai.

Exclusion criteria

Patient who were not registered in ART centre and patients who did not give consent.

Study period and sample size

Total 230 individuals were selected during the period of 18 months.

Sample collection

According to patient's clinical presentation, samples were collected and submitted in our department. Among 230 patients, dual sample were collected from 9 (4%). All the samples were divided into two halves, one half was used for Mycobacterial infection and second half was used for detection of fungal infection. Depending upon the patient's clinical features various samples were collected which included sputum (31%) followed by BAL fluid (19%), pleural fluid (11%), pus and swabs (13%), while blood (1%), urine (3%), genital swab (1%), ascetic fluid (4%), E.T. aspirate (5%) were less in number (Figure 1).

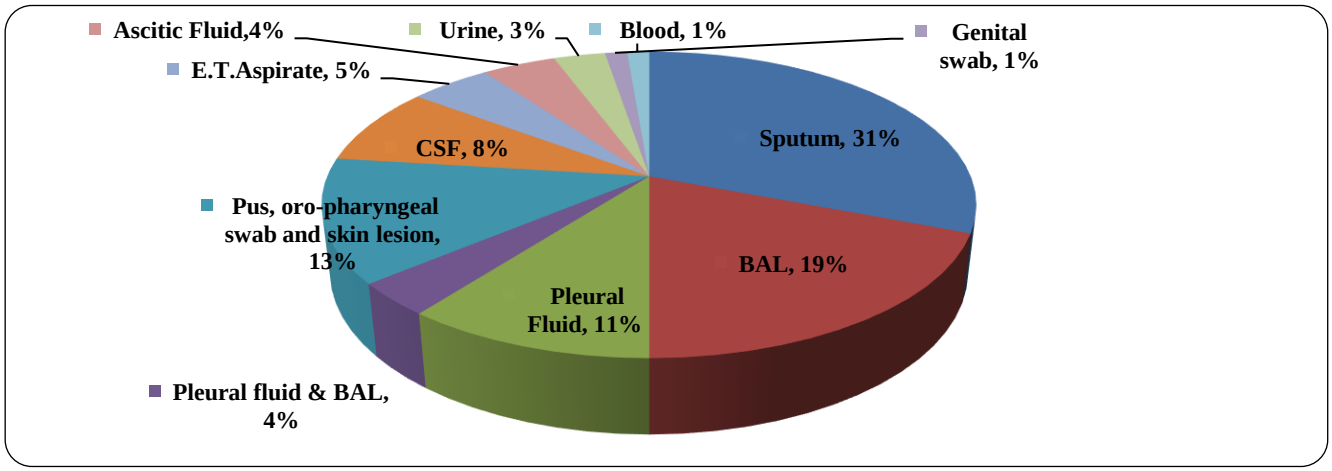

Figure 1: Distribution of samples among patients (N-230 patients).

Sample processing

- CD4 counts of all HIV seropositive patients were estimated using Alere Pima™ CD4-automated, image based immune haematology test machine.
- For tuberculosis infection: Standard ZN staining method was used for suspected mycobacterial infection. Initially homogenization was done for all clinical samples like body fluid, tissue to release the mycobacterium contained in them.
- Then specimen was decontaminated with the Petroff's method.
- Then pellets were used for culture on LJ medium
- Isolated mycobacterial colonies were further identified by AFB staining and appropriate biochemical test (catalase test, nitrate reduction test, niacin test etc).

For fungal infection

Microscopy of KOH mount, India ink, Gram's staining were performed. Each sample was inoculated in two Sabouraud-Dextrose Agar and incubated at 25^0 C and 37^0 C followed by LPCB mount and different biochemical test (germ tube test, sugar assimilation test, sugar fermentation test, chlamydospore formation on corn meal agar, urease test).

RESULTS

According to inclusion and exclusion criteria, total 230 individual were selected during the period of 18 months. All the patients were taking antiretroviral therapy (ART) as per NACO guidelines. The mean age of all patients was 34.23±13.78 years with minimum age 3 years and maximum age 71 years. Out of 230 individuals enrolled in our study, 166 were male and females were 64. The mean age ±SD of male was 33.09±13.7 years with minimum age of 5 years and maximum age of 70 years and females with 34.59±14 years with minimum of 4 years and maximum of 71 years.

In our study population, maximum patients presented with complain of fever (90.43%), weight loss (73.91%) followed by loss of appetite (35.65%), breathlessness (33.91%), coughing (28.69%) and chest pain (22.17%) etc. (Figure 2). Patients with suspected oral candidiasis came with white coated tongue and mouth ulcer.

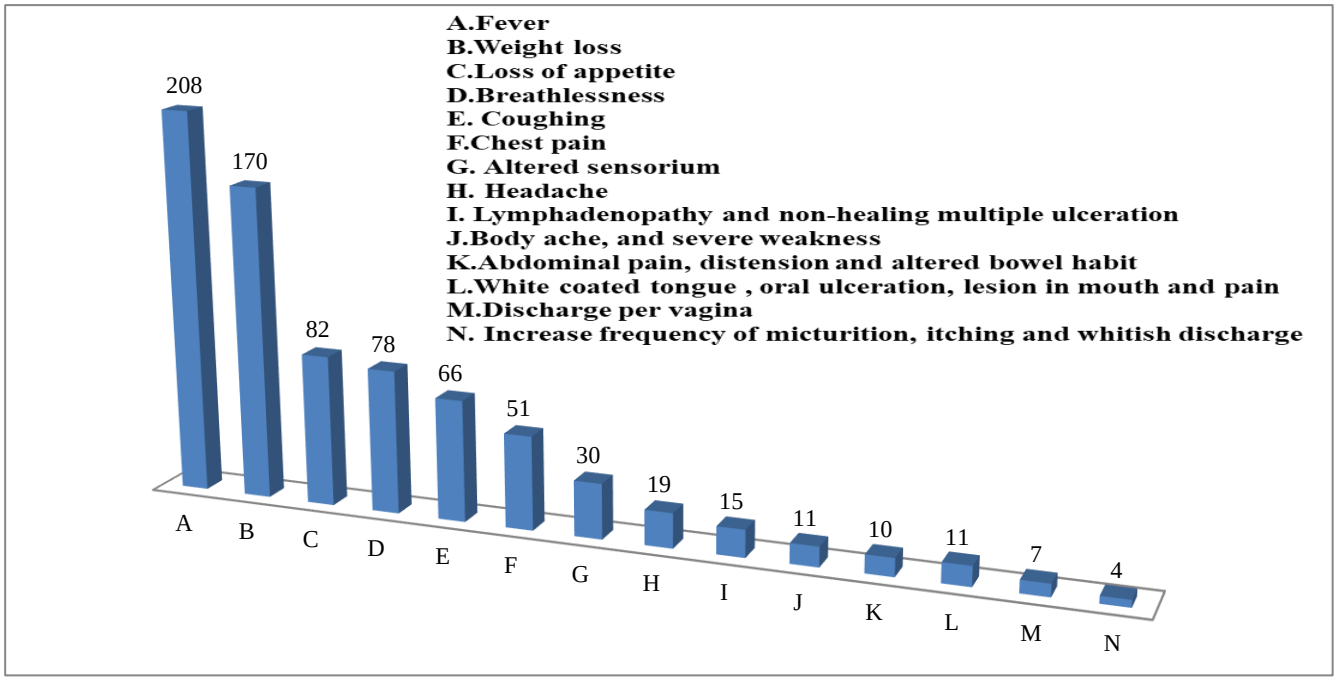

Figure 2: Clinical presentation of patients.

Detection of mycobacterial infection

Total 63 (27.39%) patients were detected as having mycobacterial infection either by microscopy or culture method. Among positive cases, 33 (13.86%) samples were positive by ZN smear, while 61 (25.63%) samples were positive by culture. Two samples were positive by smear microscopy but did not grow on culture and 30 samples were culture positive and smear negative. Maximum isolates were from BAL (8.26%) and sputum (6.52%), while genital swabs, urine and blood were found negative for *Mycobacterium* infections (Figure 3).

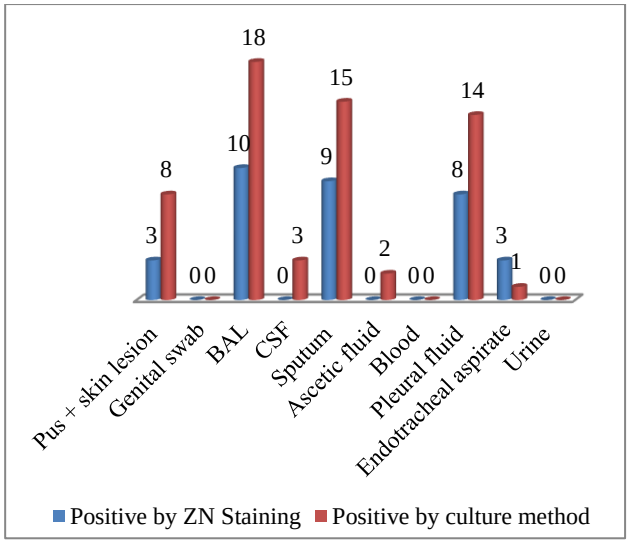

Figure 3: Results of ZN staining and culture.

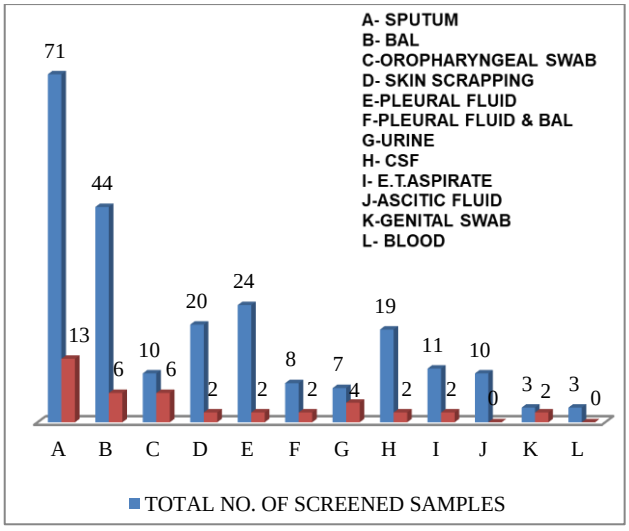

Figure 4: Distribution of fungal OIS in clinical sample.

Differentiation and identification of grown isolates into MTBC and MOTT

On the basis of biochemical reaction 50 (81%) isolates were identified as *Mycobacterium tuberculosis* and 13 (19%) isolates were identified as MOTT.

Detection of opportunistic fungal infection

All 230 patients were screened for Opportunistic fungal infection. Maximum fungal isolates were from sputum (18.30%) followed by BAL fluid (13.63%) and oro-pharyngeal swab (13.63%) (Table 1 and Figure 4).

Table 1: Distribution of fungal isolates among various clinical samples.

Clinical samples	Fungal species	Number
sputum	*Candida spp.*	11
	Aspergillus spp.	2
BAL fluid	*Candida spp.*	3
	Aspergillus spp.	2
	Alternaria spp.	3
Pleural fluid	*Aspergillus spp.*	2
Skin	*Aspergillus spp.*	1
	Penicillium spp.	1
Oro-pharyngeal swab	*Candida spp.*	6
Urine	*Candida spp.*	4
E.T. aspirate	*Candida spp.*	2
Genital swab	*Candida spp.*	2
CSF	*Cryptococcus spp.*	2

In our study, most common fungal isolates were *Candida spp.* 26(68.2%) followed by *Aspergillus spp.* 7(17.07%). We observed *Cryptococcus spp.* (4.87%), *Alternaria spp.* (7.31%) and *Penicillium spp.* (2.43%) as a less commonly isolated species (Figure 5).

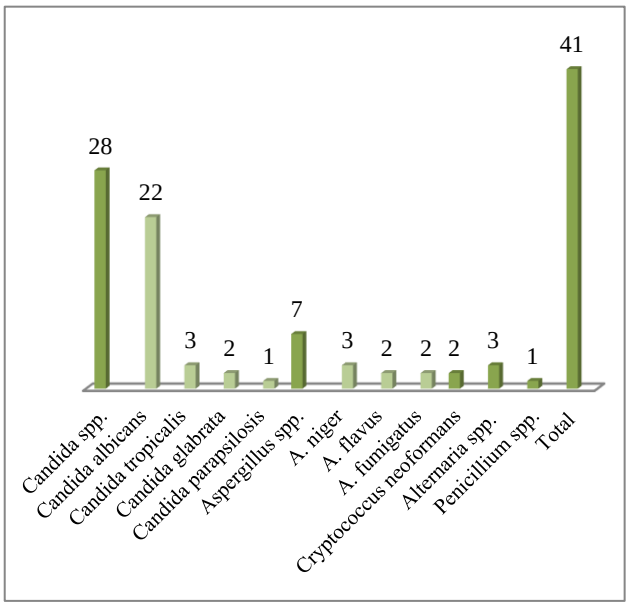

Figure 5: Species distribution of grown fungal isolates.

Among all positive samples, 15 (37%) samples were positive by KOH mount as well as grew on SDA and only 2 (5%) samples showed capsulated budding yeast cell on India ink. 26 (58%) samples of total fungal infections were only grown on SDA. All samples were further identified by LPCB mount.

Prevalence of opportunistic infection in our study population

Among 230 patients, 93 (40.43%) were infected by OIs either by *Mycobacterium spp.* or fungus. OIs frequency was significantly high in age group 31-40 (15.21%) years, followed by age group 21-30 (11.73%) years (Table 2). In our study, 13.04 % of patients were infected by both mycobacterial and opportunistic fungal infection.

Table 2: Distribution of OIs with age group.

Age groups (yrs)	With OIs	Without OIs	Total
<10	3(1.30%)	9(3.9%)	12(5.21%)
11-20	6(2.60%)	14(6.08%)	20(8.69%)
21-30	27(11.73%)	35(15.21%)	62(26.95%)
31-40	35(15.21%)	43(18.69%)	78(33.91%)
41-50	12(5.21%)	19(8.26%)	31(13.47%)
>50	10(4.34%)	17(7.39%)	27(11.73%)
Total	93(40.43%)	137(59.56%)	230(100%)

The average age ±SD of individuals with OIs was 35±12.4years with mean CD4 count 223.88±143.7cells/µL; whereas it was 33.69±14.7 years for those without any OIs with mean CD4 count 304.03±178.28 cells/µL. Infected males presented with average age ±SD (4.8±12.9) with mean CD4 count ±SD 218.3±130.9 and infected females presented with average age ±SD (35.78±9.7) with Mean CD4 count ±SD 246.9±190.86 (Table 3).

On the analysis of Table 4, the variation of infection with CD4 count was found to be statistically significant (p-value=0.0005). When compared to patients with CD4 count of <200cells/ µL, patients with CD4 count 200-400cells/µL was found to be protective twice (odds ratio observation), i.e. no risk of having infections. The rest of opportunistic infection reduced by 4 times in patients with CD4 count >400cells/µL. We found maximum prevalence of OIs as Mycobacterial (15.60%) and fungal infection (9.10%) at CD4 count 0-200cells/µL.

Table 3: Demographic details of HIV positive individuals enrolled in the study.

Gender	Individuals (n=230)					
	With OIs			Without OIs		
	N=93	Age ±SD (yrs)	CD4 counts Mean±SDCells/µL	N=137	Age ±SD (yrs)	CD4 counts Mean±SD Cells/µL
Males	75	34.8±12.9	218.3±130.9	91	33.46±14.336	302±186.60
Females	18	35.78±9.7	246.9±190.86	45	34.13±15.5	306.3±162.4

Table 4: CD4 counts of individuals with or without OIS.

CD4 count range cells/µL	With OIs	Without OIs	OR	CI	*P*-value
0-200	57	52	1		X^2=15.16 *P*=0.0005
201-400	28	51	2	1.1-3.62	
>400	82	347	4	1.98-10.98	

OR- odd ratio, CI- confidential interval

DISCUSSION

Opportunistic infections are the life-threatening manifestations caused by the various pathogens. According to NACO module-2015; in developing countries like India, *Mycobacterium tuberculosis* ranks as the most common infection seen in AIDS cases.[2] In present study we screened 230 patients, in which we detected 93 (40.43%) patients either infected by *Mycobacterium*, fungus or both. In our study, overall prevalence of mycobacterial and fungal infection was 27.39% and 17.82% respectively. It was similar to study of Dabla V et al, which showed prevalence of *Tuberculosis* 28.07% and *Candidiasis* 18.79%.[6] Prevalence of opportunistic infection varied from region to region. As studied by Chavan VR et al, prevalence was 48.06% and study of Agarwal SG et al, showed prevalence of 44.14%.[7,8] In our study group, majority of patients were male, with male to female ratio of total patients was 2.59:1 consistent with the study of Badiee P et al, in 2010 (Figure 2). Preponderance of males may be due to their migration to the metropolitan cities in search of work. Staying away from their spouse for longer period and philandering habit of males might have resulted in acquiring HIV infection.[9]

In our study, we found significantly high rate of OIs in 21-30 years (33.87%) followed by (24.35%) in 31-40 years of age group. Study of Srirangraj et al, also showed high rate of OIs in similar age group patients among South East Indian population. This might be due to highly active sexual activity of this age group and heterosexual contact was seen to be major route of transmission of HIV.[10] Raviglione et al, showed high OI burden could be associated to malnutrition, overcrowding, poor hygiene and lack of a good public health infra-structure.[11] Among *Mycobacterium* infection, maximum isolates were from BAL (8.26%) followed by sputum (6.52%), while genital swabs, urine and blood were negative. Maximum isolates from BAL might be due to no cavitation present in HIV patients; this may lead to less isolation of *Mycobacterium* from sputum. In this study, pulmonary infection occur in 41 (17.22%) cases and extra pulmonary infection occur in 27 (11.34%) cases, while 5(2.13%) involved both

pulmonary and extra pulmonary infection. Similar findings were reported by Agarwal SG in 2015.[7]

In current study, maximum fungal isolates were from sputum (31.70%) followed by BAL fluid and oro-pharyngeal swab (14.63%). Among fungal isolates, *Candida spp*. (68.29%) were the commonest followed by *Aspergillus spp.* (17.07%) similar to study of Ramesh K et al, in 2015.[4] *Candida* spp. were isolated maximally from oropharyngeal swab (60%), Urine (57.14%) and sputum (15.49%). Thus oro-pharyngeal candidiasis is the most common opportunistic fungal infection reported as supported by study of Kaur R et al, in 2016 and Jain S et al in 2014.[9,12] Other isolated spp. in our study were *Alternaria spp.* (7.31%), *Cryptococcus spp.* (4.87%) and one *Penicillium spp.* (2.43%). According to study of Jain S et al, in 2014, CNS Cryptococcosis is one of the most important risk factor associated with HIV infection contributing to a high morbidity and mortality among HIV infected patients.[12] In our study, 2 samples were positive for *Cryptococcus spp*. with CD4 count below 100cells/µL.

When CD4 counts of these individuals with or without OIs were taken into consideration, 40.43% of total population had opportunistic infection. In our study group, 61.29% of total opportunistic infection occurred in patients with CD4 count <200cells/µL. Among Mycobacterial infection, 57.14% occurred in CD4 count of <200cells/µL and in fungal infection, 51.21% occurred in CD4 count of<200cells/µL. When compared to patients with CD4 count of <200cells/ µL, patients with CD4 count 200-400cells/µL was found to be protective twice (odds ratio observation), i.e. no risk of having infections. Prevalence of opportunistic infection reduced by 4 times in patients with CD4 count >400cells/µL. similar to study of Ramesh K et al, OIs followed regular trend with respect to CD4 count in our study.[12] High prevalence rate in our study may also be due to the fact that study was conducted on patients of ART centre and these were screened patient referred directly from various adjoining primary health centre.

CONCLUSION

This study provides important information about the risks of OIs at lower CD4 counts among HIV positive patients. All HIV positive patients have high prevalence of *Mycobacterium* and opportunistic fungal infections compared to normal population. So, all sero-positive patients must be screened for OIs (especially *Mycobacterium* and fungus) to reduce morbidity and mortality. This highlights the need for early screening of HIV infected patients for opportunistic infections. There is also need to increase awareness in healthcare providers in order to improve decisions regarding prophylaxis for prevention of OIs and appropriate therapeutic intervention that will eventually improve the quality of life in HIV infected patients.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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