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ORIGINAL ARTICLE

# Lower respiratory tract infections among HIV positive and control group in Nepal

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**Abstract** Lower respiratory tract infections (LRTIs) are the most frequent respiratory diseases among HIV infected patients and are frequently the first clinical manifestations of the HIV infections. LRTIs are common not only among the HIV seropositive cases but also the commonest domiciliary and nosocomial infections among the general population. The present study was carried out to determine the comparative prevalence of common bacterial and fungal organism among the HIV positive and control population. This cross sectional study was conducted among 220 people attending National Public Health Laboratory, Kathmandu, Nepal. Out of them 120 were HIV sero-positive and rest were HIV sero-negative. Sputum samples were collected and processed soon after its collection. Macroscopic examination was done to determine the sample integrity. Gram stain, AFB stain and KOH preparation was performed for preliminary identification of the pathogens. Culture was carried out for bacterial and fungal pathogens. Antibiotic susceptibility test (Kirby–Bauer disc diffusion method) was performed from the isolated organisms. The 85 out of 120 HIV sero-positive patients were found to be infected with one or more microbial pathogens. The overall infection rate was found to be significantly lower in HIV seronegative people (27 %). Among HIV seropositive cases prevalence of LRTIs was strongly associated with lower CD4 counts (<200/mm$^3$). The prevalence of mycobacterium tuberculosis was found to be 10 % among HIV/AIDS patients which was significantly higher than among the non-HIV cases (3 %). The bacterial pathogens was observed among 46.6 % of HIV positive and 22.0 % of HIV negative people. Among the positive cases, *K. pneumoniae* was the predominant bacterial pathogens, followed by *E. coli* and *S. pneumoniae*. *C. albicans* was found to be predominant fungal pathogen followed by *Aspergillus* spp. germ tube negative *Candida* spp. and *Penicillium* spp. Similar types of organisms were found to be associated with LRTIs among HIV positive and negative people. The prevalence of both fungal and bacterial infections was significantly higher among HIV seropositive people than HIV seronegative people. All in all, lower respiratory tract illness is significantly higher in HIV/AIDS cases than in HIV seronegative cases.

**Keywords** Lower respiratory tract infections · HIV positive · Bacterial pathogens · Fungal pathogens and tuberculosis

## Introduction

The major causes of morbidity and mortality in HIV infected persons are opportunistic infections which vary from region to region [1]. The lower respiratory tract infections are the most frequent respiratory diseases among HIV infected patients and are frequently the first clinical manifestations of the HIV infections [6]. In Nepal, concerns on LRTIs are increasing among the HIV infected cases because they are being revealed as the important opportunistic infections among these cohorts. LRTIs are common not only among the HIV sero-positive cases but also the commonest domiciliary and nosocomial infections among the general population [9]. The risk of development

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of invasive pneumococcal diseases is higher among the HIV positive cases than the HIV sero-negative population [6]. Diverse types of organisms including Gram positive and Gram negative bacteria, *Mycobacterium* and fungal pathogens are responsible for LRTIs. The etiology of bacterial LRTIs among HIV Patients was reported to be *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebseilla pneumoniae* and *Acinetobacter species*. Bacteria such as *Legionella pneumophila Mycoplasma pneumoniae and Chlamydia pneumoniae* have also been incriminated in HIV associated pneumonia [7, 9, 10]. Fungi such as *Candida albicans*, *Cryptococcus neorformans*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Aspergillus* spp., *Blastomyses dermatitidis*, *Penicillium marneffei*, *Sporothrix schenckii* can cause pulmonary infections in persons infected with HIV [2, 5].

Antiretroviral treatment has greatly reduced the mortality from AIDS related opportunistic infections including LRTIs, by reducing HIV viral load in the blood and by strengthening the immune response, still some degree of immune deficiency remains. So, the risk of developing bacterial and fungal infections of lower respiratory tract remains higher in people with HIV/AIDS than in HIV negative cases. CD4 count less than 200/cumm is frequently associated with higher risk of LRTIs [12]. The present study was carried out to determine the comparative prevalence of bacterial and fungal pathogens among the HIV positive and HIV negative control group.

## Material and methods

### Study population and definitions

The study was carried in HIV reference laboratory at National Public Health Laboratory (NPHL), Teku, Kathmandu, Nepal. This study was conducted among 220 people visiting NPHL for their routine screening for HIV. Out of them, 120 were HIV sero-positive and rests were HIV negative. The seropositive people are not on ART. Cases with confirmed HIV status (positive or negative) and having relevant symptoms of respiratory tract infections were selected for the study. Written consent and questionnaire were taken from each participant.

### Elisa

ELISA test for the detection of antibodies to HIV-1 and HIV-2 in human plasma were used. The bioelisa HIV-1+2 (rec) is a third generation solid phase enzyme immunoassay in which highly purified recombinant antigens are used for the combined detection of antibodies to HIV-1, HIV-2 and HIV-1 subtype O as described in protocol . Finally OD was taken at 450 nm in ELISA plate reader (Bio-Rad) and cut off value were calculated to find out HIV positive and HIV negative.

### CD4+ T-lymphocytes assay

The CD4+ T-lymphocytes count was done by incubating anti-coagulated whole blood with monoclonal antibodies to the CD3+, CD4+ and CD56+ to identify specific cell populations, and then lysing the blood to remove red blood cells. The TruCount method (TriTEST 3-color) uses TruCount tubes which contain a lyophilized pellet containing a known quantity of fluorescent beads. A precise quantity of whole blood was added to the tubes, and the lymphocytes are stained with TriTEST monoclonal antibodies (mAb). The absolute count of a full lymphocyte subset profile (CD3+, CD4+ and CD56+) were determined in four tubes with TriTEST and two tubes with MultiTEST by calculating the ratio of region events for each subset to bead events using the BD Biosciences-developed software, MutltiSET as described in http://www.cdc.gov/.

### Bacteria and fungus isolation and identification

Sputum samples were collected and processed soon after its collection. The quality of the sputum and ET secretion specimens were evaluated according to the criteria given by American Society for Microbiology. According to this, a reliable specimen would have more than 25 leucocytes and fewer than 10 epithelial cells per low power field of microscope [3]. Macroscopic examination was done to determine the sample integrity and Gram staining, AFB staining, KOH preparation were performed for preliminary identification of the pathogens. The digested sputum samples were cultured on Chocolate agar, Sheep Blood agar and MacConkey agar (Oxoid, UK) plates. On the Chocolate agar, bacitracin disk (10 unit) and optochin disk (5 µg) (Oxoid, UK) were placed at primary and secondary inoculation to screen *H. influenzae* and *S. pneumoniae* respectively. The inoculated Chocolate agar plates were incubated in $CO_2$ incubator (10 % $CO_2$) at 37 °C for 24 h while BA and MA plates were incubated at 37 °C for 24 h in aerobic atmosphere. Antibiotic susceptibility test (Kirby–Bauer disc diffusion method) was performed from the isolated organisms. The quality control using American type culture collection (ATCC) done for each microorganism to assure the quality of organism isolation.

### Statistical analysis

Collected data were analyzed and interpreted statistically using graphPad prism version 6.0 and SPSS 17.0. All the

values are expressed as mean±SD and are analyzed using Student's t test which is parametric as well Mann–Whitney test wherever applicable. A value ($P < 0.05$), was considered significant unless stated otherwise (Table 1).

## Results

### Microorganism isolated and CD4 profile of HIV seropositive people

In the 120 HIV positive cases, 85 were found co-infected with one or more microorganism, the co-infection was found to be at a higher rate in males in the 30-40 years age group (Table 2) with male–female ratio of 1.3:1 (Table 3). The 46.6 % of HIV patient were co-infected with bacteria and 36 % with fungus. Among bacteria, *K. pneumoniae* was the predominant bacterial pathogens, followed by *M. Tuberculosis*, *E. coli* and *S. pneumonia*, *S. aureus*, P. *aeruginosa*, *M. catarrhalis* and *H. influenzae* (Table 1). Among fungal infection, *C. albicans* was found to be predominant followed by *Aspergillus* spp., germ tube negative *Candida* spp. and *Penicillium* spp. (Table 1). Among 120 HIV seropositive individuals, the 14 individual has CD4 count less than 200, 17 had CD4 count between 201 and 500 and 89 had CD4 count >500 cells/$mm^3$.

### Microorganism isolated from HIV seronegative people

Among 100 HIV negative cases, only 22 were found to be co infected with bacteria and eight were infected with fungus. The co infection was found at higher rate in >40 years age group. The *K. pneumoniae* and other bacteria including *M. Tuberculosis* among HIV sero positive patients was found to be significantly higher ($P = 0.01$) than in HIV sero negative patients. The overall infection rate was found to be significantly lower in HIV seronegative people (27 %).

Among HIV sero-positive cases prevalence of LRTIs was strongly associated with lower CD4 counts (<200/$mm^3$). Among HIV/AIDS patients (nine out of 12 TB positive patients had CD4 count <200/$mm^3$) which was significantly higher than among the non-HIV cases (3 %). The prevalence of both fungal and bacterial infections was significantly higher among HIV seropositive people than HIV negative people.

Polymicrobial infection was found to be more common among the HIV sero positive cases and *Candida albicans* was the commonest fungal pathogen coexisted with one or more bacterial pathogens. Although insignificant, multidrug resistance pattern was more prominent in HIV infected people than in non-infected cases. Microbial infection was more common among the adult than the children. In all age groups the prevalence of pathogens was higher among HIV cases than the negative ones. In both cases, older age was found to be more susceptible for the infections.

In this study, 65 individuals were studied who living in different rehabilitation centers and 55 individuals were not living in the rehabilitation centers. Among rehabilitation residents 35 (53.8 %) were found to be infected with different bacterial and fungal pathogens and among those who were living in the community, 43 (78.1 %) were found to be infected with different bacterial and fungal pathogens. There was association of LRTI with the residential status of the study population.

**Table 1** Frequency of bacteria and fungus isolates from sputum

| | HIV seropositive (n = 120) | HIV seronegative (n = 100) | OR | 95 % CI | Z statistics | P |
|---|---|---|---|---|---|---|
| Bacteria isolated | | | | | | |
| *K. pneumoniae* | 16 (13.3) | 5 (5.0) | – | – | – | – |
| *M. Tuberculosis* | 12 (10.0) | 4 (3.3) | 0.5548 | 0.2184–1.4095 | 1.238 | 0.02155* |
| *E. coli* | 10 (8.3) | 3 (3.0) | – | – | – | – |
| *S. pneumoniae* | 10 (8.3) | 3 (3.0) | – | – | – | – |
| *S. aureus* | 9 (7.5) | 6 (6.0) | – | – | – | – |
| *P. aeruginosa* | 5 (4.1) | 2 (2.0) | – | – | – | – |
| *M. catarrhalis* | 4 (3.3) | 1 (1.0) | – | – | – | – |
| *H. influenzae* | 2 (1.6) | 2 (2.0) | – | – | – | – |
| Fungus isolated | | | | | | |
| *Candida anbicans* | 16 (13.3) | 3 (3.0) | – | – | – | – |
| *Aspergillus* spp. | 11 (9.1) | 2 (2.0) | – | – | – | – |
| *Candida* spp. | 6 (5.0) | 2 (2.0) | – | – | – | – |
| *Penicillium* spp. | 3 (2.5) | 1 (1.0) | – | – | – | – |

* Significant

**Table 2** Prevalence of pathogens among different age groups

| Age wise distribution | | | |
|---|---|---|---|
| Age group | Co-infection in HIV sero-positive | Co-infection in HIV sero-negative | P |
| <20 years | 8 (33.3) | 4 (20.0) | – |
| 20–30 years | 24 (75.0) | 6 (25.0) | 0.03* |
| 30–40 years | 34 (79.0) | 6 (17.6) | 0.001* |
| >40 years | 18 (85.7) | 11 (50.0) | – |

* Significant

**Table 3** Relative number of pathogens detected

| Sex | Total | | | | Bacteria (%) | | Fungus (%) | | AFB (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | HIV infected | HIV non-infected | OR | CI 95 % | HIV infected (%) | HIV non-infected (%) | HIV infected cases (%) | HIV non-infected (%) | HIV infected (%) | HIV non-infected (%) |
| Male | 69 | 55 | 1.1070 | 0.6483–1.8900 | 32 (46.3) | 12 (21.8) | 16 (23.1) | 5 (9.0) | 6 (10.9) | 2 (3.6) |
| Female | 51 | 45 | | | 24 (47.0) | 10 (22.2) | 20 (39.2) | 3 (6.6) | 4 (8.9) | 1 (2.2) |
| Total | 120 | 100 | | | 56 (46.6) | 22 (22.0) | 36 (30.0) | 8 (8.0) | 12 (10.0) | 3 (3.0) |

## Discussion

Infections with opportunistic pathogens have been one of the hallmarks of AIDS since the beginning of the epidemic. An abundance of research and literature has been dedicated to these opportunistic fungi, viruses, and parasites. Less attention has been given to the bacterial infections complicating the course of persons infected with HIV [4, 8]. Among the opportunistic infections associated with HIV, diseases like pneumonia of bacterial origin occur at a rate many times higher in HIV infected patients than in the general population [13]. In the present study, the bacterial isolates from the HIV infected patients were higher and also polymicrobial etiology in some of these patients is a significant finding, indicating the severity of the infection in this group. More than two third of the patient infected with HIV were found to be suffering from one or more fulminant or latent microbial Lower respiratory tract infections. This is the alarming situation and calls for more attention towards the LRTIs. The pattern of organism isolated and the antibacterial sensitivity was found to be concordant with other studied conducted in various setting.

As per the figures from National AIDS control organization (NACO), bacterial infection constituted 7 % of opportunistic infections and the common organisms encountered in pulmonary infections were *S. pneumoniae*, *H. influenzae* and *S. aureus*. Shailaja et al. has reported 44.28 % of bacterial isolates, among which *K. pneumoniae* was isolated in 32.26 %, *S. pneumoniae* in 25.81 %, *S. aureus* in 12.9 % *P. aeruginosa* in 9.68 %, *M. catarrhalis* in 9.68 % and *M. tuberculosis* in 42.89 % in their study of LRTIs in patients with HIV infections [8]. Tamang et al. from Manipal Teaching Hospital, Pokhara has reported *H. influenzae* in 26.86 %, *S. pneumoniae* in 21.16 % and *M. catarrhalis* in 6.90. Similar organisms were also observed in present study [14]. In HIV negative hospital attending patients, the prevalence of bacterial LRTIs had been reported to be 44.4 %–50.4 % [9, 13]. The lower yield of pathogens in this study as compared to other studies might be attributable to various factors. Natural history of infectious disease in the patient will have already been modified by the use of different type of antibiotics by health professionals at different levels before the patient lands into this tertiary care centre. The use of antibiotics might have played a significant role in culture negativity. Likewise, there is no prudent use of antibiotics in the country and the self-prescribing practice by patients could be another contributing factor.

Polymicrobial etiology was also reported in present study. Polymicrobial infection is slightly higher among the HIV seropositive patients than the normal population. The slightly lower number of mixed infections in our setting could be due to the reason that microbes like *C. pnuemoniae*, *Mycoplasma pneumoniae*, Pneumocystis carinii, were not looked for in this study. Surveillance data from the SAARC region countries, India and Nepal revealed that 83 % and 56 % of patients with AIDS had TB of any organ. Percentage of adult (15–49 years) TB/HIV co-infection is 2.9 in Nepal (Estimates, 2003) .About 55–89 % of AIDS cases in India, were found to be suffering from extensive pulmonary TB. In the present study, *M. tuberculosis* was isolated in 12 HIV patients, out of 120 HIV

seropositive individuals [15]. Although it is less common than bacterial infections serious fungal infections occur in immune-compromised patient, both as new infection and as reactivation of latent disease. Fungal pulmonary infections often precede the appearance of other opportunistics, but frequently co-exist with other pathogens [11]. Though pulmonary candidiasis is documented to be a very rare disease, occurring in late stages of AIDS, oral and oesophageal candidiasis is reported as the second most common (58%) opportunistic infection among HIV patients, from India. In the present study, 36 fungal isolates were recovered from the sputum. *C. albicans* has been identified in 16/36 fungal isolates. To rule out the possibility of oropharyngeal colonization, a common feature among the HIV reactives, those cases with plenty of pseudohyphae on smear examination were considered as significant pathogens.

In conclusion the lower respiratory illness is significantly higher in HIV/AIDS cases than the HIV seronegative cases. The lower the CD4 count the greater is the risk of acquiring LRTIs. *M. tuberculosis* is still the major pathogen causing opportunistic infection among HIV/AIDS patients and *C. albicans* is the major fungal pathogen isolated from patient infected with HIV/AIDS. Emergences of multidrug resistant bacteria are threat for the patient with HIV.

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