Original article:

Prevalence of Human immunodeficiency virus associated bacterial and fungal respiratory tract infections.

***Dr. Meghna S Palewar [1], Dr. A.G Dhanvijay[2] , Dr. S.R More [3]**

[1]Department of Microbiology, BJ Medical college, Pune , Maharashtra, India
[2]Prof . & HOD , Dr. Shankarrao Chavan Government Medical college , Vazirabad,nanded ,Maharashtra , India
[3]Asso. Professor, Dr. Shankarrao Chavan Government Medical college , Vazirabad,nanded ,Maharashtra , India
***Corresponding author**: Email: meghna_palewar@rediffmail.com

Abstract:

Introduction: Among the various opportunistic infections respiratory infections account for upto 70% of AIDS defining illness. Although increasing number of AIDS cases are being reported , spectrum of pathogens in HIV infected is not well known in this part of India **.**Hence present study was undertaken to determine the prevalence of lower respiratory tract infections in 112 HIV seropositive patients for first time from this region .

Methodology : Sputum samples were examined by microscopy and cultured for bacterial, mycobacterial, and fungal pathogens.

Result and conclusion : Out of 112 HIV seropositive cases in this study, pathogenic microorganisms were isolated from 80 cases (71.43%). Monomicrobial infections were noted in 44 cases (55%) and polymicrobial infections in 36 cases (45%). In all 119 pathogens were isolated from 112 HIV reactive cases. 53.78% were bacterial isolates, 16.81% were *Mycobacterium tuberculosis* isolates and 29.41 % were fungal isolates. In this study none of the patients with stage I had any symptoms of respiratory tract involvement, hence were not included in this study. Out of total 22 cases of stage II, pathogens were isolated in 10 cases (45.45%). Similarly out of 54 cases of stage III, pathogens were isolated in 38 cases (70.37%) and out of 36 cases of stage IV pathogens were isolated in 32 cases (88.89%).

Keywords: Human immunodeficiency virus, Acquired immunodeficie ncy disease syndrome ,

Introduction:

The prolonged course of Human immunodeficiency virus infection is marked by a decrease in the number of circulating CD4+ T helper cells and persistent viral replication, resulting in immunological decline and death from opportunistic infections and neoplasm [(1)] Respiratory infections are major cause of morbidity and mortality in persons with HIV infection. Among the various opportunistic infections respiratory infections account for upto 70% of Acquired immunodeficiency disease syndrome defining illness [(2)] . Although increasing number of AIDS cases are being reported , spectrum of pathogens in HIV infected is not well known in this part of India .Thus considering the magnitude of the problem, present study was undertaken to determine the prevalence of various bacterial, mycobacterial and fungal pathogens of lower respiratory tract in HIV seropositive patients for first time from this region . This will also prompt early diagnosis and treatment of respiratory tract infections, reducing the associated morbidity and mortality and improving the quality of life of the patients already suffering miserably .

Subjects and Methods:

The present study was conducted in the Department

of Microbiology, Dr.Shankarrao Chavan College Government medical college and tertiary hospital Nanded during July 2007 to Febuary 2009 after approval by ethical committee . A total of 112 confirmed H.I.V. reactive patients tested with three rapid immunoassay tests (COMBAIDS - RS HIV 1 + 2 Dot immunoassay, HIV 1& 2 Tridot, Retroscreen HIV) suffering from lower respiratory tract infections with symptoms of cough and expectoration were included in the study. The cases were classified into stages one to four on the basis of clinical status of the patients as per revised 2005 clinical staging of HIV/AIDS for Adults and Adolscents[3] .

Three sputum samples, First day - first spot (Deeply coughed direct sample), second day - Early morning and Second day - second spot. All samples were collected in sterile containers. Quality of expectorated sputum sample was assessed by Bartlett's scoring method[4] and unsuitable specimens were excluded. Each sputum sample was divided into two parts, Part 1- Neat sample and Part 2- Concentrated sample by Petroff s method.

a) Part I - Neat sample was subjected for microscopic examination by Gram stain , Saline mount , KOH mount and cultured on Blood agar , MacConkey , Chocolate agar for bacteria and on Sabourard's dextrose agar for fungus [5,6,7]

b) Part II - concentrated sputum sample was subjected to microscopic examination by Ziehl-neelsen stain and cultured on Lowenstein Jensen media [5,6,7] .

The isolated microorganisms on culture were identified by colony characters , staining morphology and biochemical characters [5,6,7] .

Results:

Out of 112 HIV seropositive patients suspected to be suffering from respiratory tract infections, causative organisms for respiratory tract infections were isolated from 80 patients (71.43%) . Of these 80 cases, Monomicrobial infections were noted in 44 cases (55 %) and Polymicrobial infections were detected in 36 cases (45%).

A total of 119 microorganisms were isolated from 80 patients of the HIV reactive group . There were in all , 20 Mycobacterial isolates contributing to 16.81% , 64 bacterial isolates of varied etiology contributing to 53.78% , and 35 fungal isolates contributing to 29.41% of total infection . All 20 mycobacterial isolates were identified as Mycobacterium tuberculosis . Fungal agents were considered pathogenic as they were observed in direct microscopy and isolated in culture repeatedly from sputum samples . Mycobacterium tuberculosis dominated amongst all isolates, followed by Candida albicans as well as Klebsiella pneumoniae (Table No.1). All three patients with Aspergillus isolated had simultaneous coinfection with Mycobacterium tuberculosis in the present study .

In the present study none of the patients with stage I had any symptoms of respiratory tract involvement, hence were not included in this study. Out of total 22 cases of stage II, pathogens were isolated in 10 cases (45.45%). Similarly out of 54 cases of stage III, pathogens were isolated in 38 cases (70.37%) and out of 36 cases of stage IV, pathogens were isolated in 32 cases (88.89%). Thus table 2 shows increased rate of respiratory tract infections from stage II to IV in H.I.V infected patients .There is stastically significant association between increased respiratory tract infections in progressed stages of H.I.V infection (by chi-square test , $X^2 = 10.61$, df=2 , P=0.0049 i.e ($P<0.05$) .

Discussion:

The true incidence and prevalence of the opportunistic respiratory infections in HIV patients is difficult to assess and varies with the population surveyed [(2).] In the present study out of 112 HIV seropositive cases with respiratory tract infections, pathogens were isolated in 80 cases (71.43%) which is comparable to a study conducted by Shailaja etal [(2)] with pathogens isolated in 63 cases (63%) of the 100 HIV reactive cases in the study. In present study organisms were not isolated in thirty two patients . Viral etiology could be a cause in some, since viral aetiology was not studied. Polymicrobial etiology in 36 HIV reactive cases (55%) is a significant finding, indicating the severity of infection in this group. Immunosuppression due to HIV may explain for these polymicrobial respiratory tract infections.

Bacterial pneumonia is a frequent complication of HIV infection, and it often precedes other opportunistic infections [(8)]. In the present study, out of all the pathogens isolated, bacteria other than Mycobacteria constituted 53.78% of total infection as against study by Shailaja [(2)] with bacteria other than Mycobacteria constituting 44.28% of total respiratory tract infection in HIV patients. Thus in the present study, the bacterial isolates from the HIV group were much higher and of varied etiology. This variation could be due to geographic differences and also due to possible hospital acquired superadded infections. Amongst the bacterial isolates other than Mycobacteria, Klebsiella pneumoniae dominated constituting 15.12% of total infection, followed by Staphylococcus aureus in 13.44% and Pseudomonas aeruginosa in 10.08%. Levine etal[(9)] have reported that Staphylococcus aureus is a frequent cause of bacterial pneumonia (7.84%) in HIV infected patients, and can cause pneumonia with high mortality rates . There is rise in incidence of Pseudomonas aeruginosa in HIV infected cases more recently and most of these infections are community acquired[(10)]. In a prospective study of HIV - infected patients from France, 6 of the 33 identified pathogens causing bacterial pneumonia (18%) were Pseudomonas aeruginosa[(11)].

According to Morbidity and Mortality weekly report recurrent pneumococcal pneumonia, either with the same or unrelated serotype, is more common among HIV-1 infected patients, with a rate of 8% -25 % within 6 months[(12)] which correlates with present study where Streptococcus pneumoniae constituted 8.40% of total infection.

Mycobacterium tuberculosis was the commonest isolate among respiratory pathogens (16.80%). This finding correlates with the findings of other authors [(2,13,14)]. Among HIV infected patients with advanced immunodeficiency and pulmonary tuberculosis, cavitary lesions are relatively rare, sputum smears seldom positive for acid fast bacilli, and radiographic infiltrates cannot be used to reliably distinguish between tuberculosis and pulmonary pathogens [(15)]. Thus the prompt diagnosis of pulmonary tuberculosis in persons infected with HIV requires high index of suspicion and access to specialized laboratory techniques such as microscopy, sputum smears and mycobacterial cultures.

In the present study, fungal respiratory tract infections contributed to 29.41% of the total infections. In the presence of oral Candidiasis, etiological role of Candida in pulmonary disease is difficult to establish, when the fungus is recovered from sputum sample. According to Murray and Mills, Candida from sputum is insufficient in diagnosis, unless tissue invasion is also documented histologically[(16).]Though criteria for diagnosis of

pulmonary Candidiasis are controversial , in our study demonstration of budding yeast cells along with pseudohyphae in stained smears, repeated isolation of Candida from sputum sample were used to diagnose Candidial infection. In the present study , Candida albicans contributed to 15.12% and Non Candida albicans contributed to 11.76% of the total isolates .

In a present study there were 3 isolates of Aspergillus species (2.52%) which is comparable to a study by Lanjewar etal[17] with prevalence of pulmonary Aspergillosis as 3% in HIV patients and also to a study by Libbanore etal[18] with a prevalence of 2.1% which usually occurred in patients with CD4 counts < 50 cells/mm^3.

In the present study, respiratory tract infection of HIV seropositive patients in the stage II was 45.45 % (10 of the 22 cases), in stage III was 70.37% (38 of the 54 cases) and that in stage IV was 88.89 %(32 of the 36 cases) Thus with the advancing immunosuppression from stage II to IV, the percentage of the respiratory tract infection was also increasing .

Conclusion:

It is concluded from present study that there is high proportion of respiratory tract infections in patients with HIV infection. Tuberculosis and Candidiasis are the commonest opportunistic pathogens in HIV positive population in this region. Also polymicrobial etiology among HIV reactive patients in this region is a significant finding, indicating severity of infection. Respiratory tract infections increased with advanced immunosuppression. Early diagnosis of opportunistic infections and prompt treatment with specific antimicrobials definitely contributes to increased life expectancy among infected patients delaying the progression to AIDS.

Table No. 1 Showing distribution of 119 microorganisms isolated from HIV infected cases with respiratory tract infections.

Sr. No.	Microorganisms	No. of isolates	Percentage
1	*Mycobacterium tuberculosis*	20	16.80%
2	*Candida albicans*	18	15.12%
3	*Klebsiella pneumoniae*	18	15.12%
4	*Staphylococcus aureus*	16	13.44%
5	*Candida Non albicans*	14	11.76%
6	*Pseudomonas aeruginosa*	12	10.08%
7	*Streptococcus pneumoniae*	10	8.40%
8	*Escherichia coli*	08	6.72%
9	*Aspergillus flavus*	02	1.68%
10	*Aspergillus niger*	01	0.84%
11	Total	119	100

Table No. 2 Showing increased rate of respiratory tract infections from stages II to IV in H.I.V infected patients.

Stage	No.of Cases in study	No.of cases with pathogen isolated	Percentage
1	Nil	Nil	Nil
II	22 (20 %)	10	**45.45 %**
III	54 (48 %)	38	**70.37 %**
IV	36 (32 %)	32	**88.89 %**
Total	112	80	

References:

1) Kumarasamy N , Vallabhaneni Snigdha , Timothy P. Flanigan , Kenneth H. Mayer , Solomon Suniti . Clinical Profile of HIV in India. Indian J Med Res 2005 ; 121: 377- 394.
2) Shailaja VV, Pai LA , Mathur DR, Lakshmi V. Prevalence of bacterial and fungal agents causing lower respiratory tract infections in patien with human immunodeficiency virus infection . Indian J Med Microbiol 2004;22(1) : 28-33.
3) World Health Organization. Interim WHO clinical staging of HIV/AIDS and HIV/AIDS case definitions for surveillance: African region. Switzerland: World Health Organization; 2005.
4) Winn W, Allen S , Janda W,Koneman E, Procop G , Schreckenberger etal . Chapter 1 Introduction to Microbiology : Part I : The Role of the Microbiology Laboratory in the Diagnosis of Infectious Diseases : Guidelines to Practice and Management In : Koneman's Colour Atlas and Textbook of Diagnostic Microbiology, 6th Edition, Philadelphia : Lipipincott Williams and Willkins:.2006 P.17.
5) Colle J.G.,Miles R.S , Watt B , Test for the identification of Bacteria In : Mackie and Mc Cartney Practical Medical Microbiology, Collee J, Fraser A, Marmion B, Simmon A editors , 14 th edition : Churchill Livingstone ; 1996 . P 131-150 .
6) Revised National Tuberculosis Control Programme -- Training Manual for Mycobacterium tuberculosis Culture & Drug susceptibility testing by Central Tuberculosis division Directorate General of Health services Ministry of health & family welfare, Nerman Bhavan , New Delhi. Printed april 2009 : 1-76
7) Milne L.R , Fungi In :Mackie and Mc Cartney Practical Medical Microbiology, Collee J, Fraser A, Marmion B, Simmon A editors , 14 th edition : Churchill Livingstone ; 1996 , P 695-720.
8) Matthew Fei, Laurence Huang . HIV Associated Pneumonia . The Body Complete HIVAIDS Resource , Winter 2008 : 21-31 .
9) Levine SJ, White DA, Fels AO. The incidence and significance of Staphylococcus aureus in respiratory cultures from patients infected with the human immunodeficiency virus. Am Rev Respir Dis 1990 ;141:89-93..
10) Afessa B, Green B. Bacterial pneumonia in hospitilised patients with HIV infection . Chest 2000 ;117 : 1017-

1022

11) Baril L, Astagneau P, Nguyen J ,Similowkski T, Mengual X , Beigelman C,etal . Pyogenic bacterial pneumonia in human immunodeficiency virus - infected patients : A clinical, radiological, microbiological and epidemiology study . Clin. Infect Dis 1998 ; 26 : 964-971.

12) Constance A. Benson, Jonathan E. Kaplan ,Henry Masur, Alice Pau, King K. Holmes . Treating Opportunistic Infections Among HIV- Infected Adults and Adolscents CDC, Infectious Diseases Society of America and National Institutes of Health . Morbidity and Morality Weekly Report ; December 17, 2004 / 53(RR15);1-112

13) Aggarwal Aruna, Arora U, Bajaj R ,Kumari K . Clinico- microbiological Study in HIV Seropositive Patients .JIACM 2005 ;6(2) : 142-145.

14) Ayyagari A, Sharma AK, etal . Spectrum of Opportunistic infections in HIV infected cases in a tertiary care hospital .Indian J Med Microbiol 1999; 17 : 78-80

15) Michael O, Rigsby, Gerald Friedland ,Tubeculosis and Human Immunodeficiency virus Infection In : AIDS Biology Diagnosis ,Treatement and Prevention, , Edited by Vincent T Devita, Samuel Hellman, Steven A Rosenberg, Lippincott Raven publishers , 1997 .

16) Murray JF, Mills J . Pulmonary infectious complications of human immunodeficiency virus infection Part II . Am Rev Respir Dis. 1990 Jun;141(6):1582–1598.

17) Lanjewar DN, Duggal R . Pulmonary pathology in patients with AIDS : an autopsy study from Mumbai. HIV Medicine 2001 ; 2(4) : 266-271.

18) Libanore M, Prini E, Mazzetti M, Barchi E, Raise E, Gritti FM, etal . Invasive aspergillosis in Italian AIDS patients. Infection 2002 ; 30(6) : 341-345

Date of submission: 12 July 2013	Date of Provisional acceptance: 8 Aug 2013
Date of Final acceptance: 22 Aug 2013	Date of Publication: 04 September 2013
Source of support: Nil	Conflict of Interest: Nil