DOI: 10.21276/sjams.2017.5.3.60

Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2017; 5(3D):1031-1037 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Original Research Article

Pattern of pulmonary infection and immunological profile of seropositive HIV with pulmonary tuberculosis patients: A tertiary care hospital based study in Odisha

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Abstract: There is a growing concern regarding opportunistic infections associated with seropositive HIV with pulmonary tuberculosis. Hence the present study was carried out with an objective to find out the pattern of pulmonary infection and their association with immunological profile among the HIV- TB co-infected patients registered in a tertiary care referral unit of Odisha. In this retrospective study the diagnosed cases of pulmonary TB with HIV co-infection, having other pulmonary manifestations registered within June 2013 to May 2016 (three years) were included. The extra pulmonary TB cases were excluded. The detailed basal characteristics, sputum culture characteristics and CD4 count of all included patients were noted. In this study, maximum cases were within age range of 31-45 years (35.29%) and males outnumbered females (M: 73.53%; F: 26.47%). Majority of them were from rural community (92.65%). Only 30.88% of all were on antiretroviral therapy. 58. 82% of all had CD4 count below 200/µl. In sputum culture, only bacterial growth, only fungal growth and mixed bacterial as well as fungal growth was seen in 17.65%, 27.94% and 20.59% of samples respectively. Among all, isolation of mixed organisms, like Streptococcus, Staphylococcus with Klebsiella and Pseudomonas was observed in maximum samples. Next to it, staphylococcus growth was seen in 15.48% samples. Among fungi, Candida albicans was predominant (42.42%) organism isolated in sputum. In 21.21% samples, there was mixed infection of Candida albicans with Candida glabrata, Aspergillus flavus, Candida tropicalis, Candida parapsilosis etc. On bivariate analysis, a significant ($p<0.05$) correlation was observed between CD4 count and bacterial and or fungal infection. From this study it is concluded that CD4 count (immunity status) is the primary determinant of opportunistic pulmonary infection in our setting. So early diagnosis and appropriate treatment of co-infections is needed to reduce morbidity and mortality in TB-HIV patients.
Keywords: Pulmonary Tuberculosis, HIV/AIDS, Co-infection, Opportunistic infection, CD4 count, Bacterial and fungal infection

INTRODUCTION:

Tuberculosis and HIV has been closely associated since the emergence of AIDS. HIV infection is a major contributor of increase incidence of tuberculosis worldwide. Currently according to Global TB report, in 2015, there were an estimated 10.4 million new TB cases worldwide and about 1.2 million of them are living with HIV (11%) [1]. Also globally death due to HIV TB co-infection was estimated to be 400,000 in 2015 [2, 3]. Though India is the second most populous country in the world, one fourth of Global incidence of TB cases occur in India annually. [4] India is one of the thirty TB-HIV co-infection high burden countries [5]. In India, out of total population 1, 310, 000,000. HIV TB incidence is 113,000 with mortality rate 37, 000 in 2015 [5].

Opportunistic infections associated with HIV are one of the important causes of ill health and death in resource poor settings [6-8]. Among various opportunistic infections respiratory tract infection accounts for 70% of AIDS defining illness [9]. Tuberculosis is the most common opportunistic infection and is the cause of death for HIV infected

patients. Similarly, HIV is the important contributor of progression of latent TB infection to active TB disease [10, 11]. The life time risk of tuberculosis in immunocompetent individuals ranges from 5-10% but in HIV TB co-infected individuals there is a 5-15% annual risk of developing active TB disease [12]. Though TB in HIV infection is uncommon in developed countries, it is more common in developing countries like India with poor resource setting [13, 14]. Unlike other opportunistic infections, tuberculosis can occur at any stage of HIV disease and its manifestations depend largely on degree of immunosupression. When CD4 T cell count is more than 200/µl, the disease is more likely to affect upper lobe with infiltrating and cavitary lesions. Atypical pulmonary and extrapulmonary infection is more with progress of immunosupression [15]. There is global evidence for incidence of opportunistic infection increases with degree of immune suppression resulting from HIV infection [7, 8]. Apart from mycobacterium tuberculosis, other atypical mycobacterial infections are also common in HIV infected patients. Many Indian study reports are also available in this regard [16 - 23]. Not only tubercular but also other bacterial, viral, fungal and parasitic infections are common in HIV patients due to low immunity. The low CD4 count is the primary cause of destruction of immune system. Hence chronic HIV infection is associated with variety of opportunistic infection [23, 24]. The respiratory tract infection in HIV patients is caused by Mycobacterium tuberculosis, atypical mycobacteria, Streptococcus pneumoniae, H influenza, Staph aureus, Pseudomonas aerogenosa etc. Earlier studies have also reported Nocardia, Morexeila are other pathogens causing respiratory tract infection in HIV patients. Respiratory infection caused by fungi like candida, Cryptococcus and Aspergillus are not uncommon in HIV [25, 26].

Thus different opportunistic infections in HIV-TB patients differ geographically. Identification of organism causing respiratory tract infection can guide the early intervention and reduce the morbidity in HIV-TB patients. With this background, the present study was carried out to note the organisms causing respiratory illness in HIV - TB Patients with their correlation with immunological profile in a DR - TB centre in south Odisha.

MATERIALS AND METHOD:

The study protocol was approved by Institutional Ethics Committee. This was a retrospective record based study, conducted in the DR - TB centre in collaboration with ART centre of this tertiary care hospital (A referral centre). Our DR-TB centre covers eight districts of South Odisha. All diagnosed HIV seropositive patients co-infected with pulmonary TB (RNTCP and NACO guidelines) registered in both in and outpatient department within the period of June 2013 to May 2016 were included in this study. The data of patients older than 16years (both genders) were extracted from medical records and TB registry. The extra-pulmonary tuberculosis cases and patients having incomplete information were excluded from the study. This cross-sectional study focussed on the baseline characteristics, sputum examination findings and CD4 count of TB HIV co-infected patients at the time when anti-tubercular treatment was initiated.

Specific causative agents of pulmonary infection were diagnosed on the basis of standard clinical definitions and by routine microbiological tests in the department of microbiology of this hospital.[22] The findings of micro-organisms isolated in expectorated sputum or induced sputum samples of all included patients were noted. The induction of sputum was being done using a nebulizer and 3% hypertonic saline for 15 minutes. Detection of bacteria or fungal pathogens was being done using selective and differential media as per standard protocol. CD4 count in blood samples was being done in ICTC of this hospital. All the data were collected in a pre-designed case record form and subjected for statistical analysis [21].

STATISTICAL ANALYSIS:

The data were analysed using statistical package, IBM SPSS statistics for windows, version 20.0. The descriptive statistics were summarised by means, medians, frequencies and percentages according to the type of data. Statistical analysis was done by using unpaired‘t’ test or Mann Whitney U test for continuous and categorical data respectively. Correlation between age, gender, CD4 count and type of organism grown in sputum were determined by bivariate analysis. $P < 0.05$ was considered as minimum level of significance.

RESULT AND DISCUSSION:

A total of 100 TB - HIV co-infected patients were registered during this period of three years, among which 79 patients had other pulmonary infections. Out of them 11 patients were excluded from study because of inadequate sputum content for microbiological testing. Only 68 patients met inclusion criteria and enrolled for analysis. The demographic characteristics are summarised in Table-1. Out of total 68 included cases, 50 were males (73.53%) and 18 were females (26.47%). The median age of males (36.50years, CI :

33.72-39.44) and females (31.50years, CI: 27.98-39.69) were not significantly different. (P>0.05) Again, 50% of cases (n=34) were within the age range of 31-45 years and 35.29% patients were within 16-30 years. There was a significant difference (p= 0.03) in mean body weight of males (44.02 ± 0.94kg, CI: 40.13-45.91) and females (40.11± 1.42kg, CI: 37.10-43.12) 86.76% of them had poor socioeconomic status, 92. 65% were from rural areas and all belong to Hindu families. Only 21 (30.88%) patients were on antiretroviral therapy. 55 cases (80.88%) had chief complain of fever with cough and expectoration. But in 13 cases (19.12%) induced sputum was collected for microbiological tests.

Table-1: Baseline characteristics of study population (n=68)

Profile	Number of cases(n=13)	Percentage	95%Lower CI	95%Upper CI
Age(years)				
16-30	24	35.29	22.84	26.07
31-45	34	50.0	37.38	40.5
46-60	10	14.71	49.82	55.58
Gender (median age)				
Male	50 (36.50)	73.53	33.72	39.44
Female	18 (31.50)	26.47	27.98	39.69
Man Whitney U test (p)	0.40			
Region				
Rural	63	92.65	-	-
Urban	5	7.35		
Religion				
Hindu	68	100	-	-
Other than Hindu	0			
Body weight(kg)(Mean ± SEM)				
Males	44.02±0.94	-	42.13	45.91
Females	40.11±1.42		37.10	43.12
't' test (p)	0.03			
Socio-economic status				
Poor	56	82.35	-	-
Low average	13	19.12		
ART status:				
On ART	21	30.88	-	-
No ART	47	69.12		
Sputum sample				
Expectorated	55	80.88	-	-
Induced	13	19.12		

Table-2: CD4 status of study population with respect to organisms isolated in sputum (n=68)

CD4 count /µl	Number of cases	%	Number of cases showing Bacterial growth	Number of cases showing Fungal growth
1-100	15	22.06	3	8
101-200	25	36.76	11	14
201-300	15	22.06	5	6
301-400	7	10.29	3	4
401-500	4	5.88	2	0
>500	2	2.94	2	0

The median CD4 counts for males and females were 184.0/µl (CI: 166.1-231.1) and 213.0 µl (CI: 155.3-289.7) respectively which were not significantly different. (p=0.53) Among all maximum cases (25,

36.76%) had CD4 counts within 101-200/µl. Equal proportion of total cases (22.06%) showed CD4 count in ranges of 1-100/µl and 201-300/µl **(Table-2).**

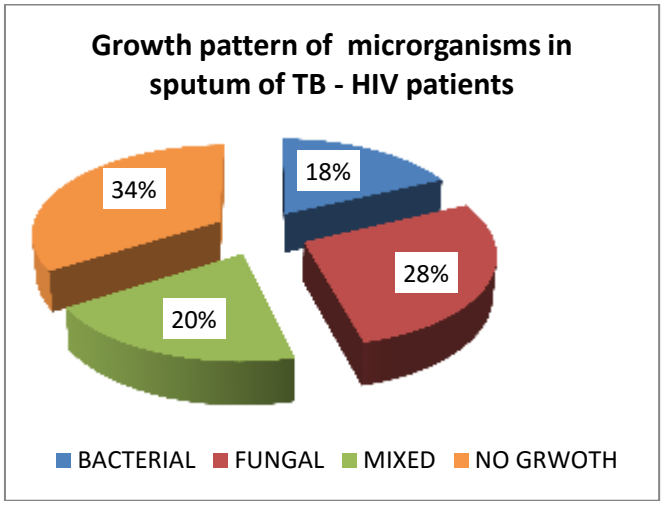

Table-3: Bacterial growth pattern in sputum samples of TB-HIV patients

Bacteria	Number	Percentage
Staph aureus	5	19.23
Streptococcus pneumoniae	4	15.38
Klebsiella	2	7.69
Pseudomonas	2	7.69
Staph aureus +klebsiella	4	15.38
Sp+kl/ pseudomonas/Acinobacter	9	34.61

Table-4: Fungal growth pattern in sputum samples of TB-HIV patients

Fungi	Number	Percentage
Candida albicans alone	**14**	**42.42**
CG alone	**5**	**15.15**
Ca+others (Cg, Cp, Ct, A. fla)	**7**	**21.21**
Ck+others (Cp, A fla, A niger, Cg)	**7**	**21.21**

CG: Candida glabrata, A. fla: Aspergillus flavus,
Ct: Candida tropicalis, Cp: Candida parapsilosis

In this study sample, 33.82% (n=23) of TB HIV co-infected cases showed no growth of microorganisms in sputum culture and was considered as mono infection (TB) in HIV. The sputum culture revealed positive growth of bacterial alone, fungal alone and both bacterial, fungal mixed infection in 12 (17.65%), 19(27.94%) and 14 (20.59%) cases respectively. Thus 45 cases (66.18%) had polyinfection along with mycobacterial tuberculosis **(Fig 1).**

Among 26 samples showing bacterial growth positive, maximum (n=5; 19.23%) showed positive for only Staphylococcus aureus and 15.38% (n=4) specimens had growth of both Staph.aureus as well as Klebsiella pneumoniae. Only Streptococcus pneumoniae growth was observed in 15.38% (n=4) samples. In 7.69% samples K.pneumoniae and in another 7.69%.samples only pseudomonas growth was observed. Rest in 34.61% sputum samples, mixed growth of Streptococcus pneumoniae with other

organisms like pseudomonas, Acinobacter, klebsiella, Staph aureus etc were observed. (Table-3)

Similarly among fungi, alone Candida albicans growth was positive in 42.42% specimens and growth of Candida albicans with other fungi like Aspergillus flavus, Candida glabrata, Candida parapsilosis, in 21.21% of cases. Rest 21.21% sputum specimens showed growth of Candida krusei with fungi like Carapsilosis, Aspergillus fumigatus, Candida glabrata. In 15.15% samples, alone candida glabra growth was observed. (Table-4)

On correlation coefficient bivariate analysis it was observed that there was no significant correlation between age, gender, body weight, status of treatment for HIV (ART) with growth of bacterial and or fungal growth in sputum of HIV TB co-infected cases. But the immunological marker, CD4 count and pattern of microorganism growth in sputum were significantly associated. ($p<0.05$) More number of fungi were isolated in sputum of patients having very low CD4 count (<200/µl).

Tuberculosis and HIV are two major health challenges globally. HIV patients are more vulnerable to different opportunistic infections depending on the immunity status and stages of HIV infection [23]. In the present study, out of total 68 TB - HIV co -infected patients, males outnumbered females and majority were within age range of 31-45 years which might be due to their active sexual life. Such observations were also reported in other Indian studies [22, 27, 28]. Again the women were in younger age range than men in our study population. Probably because of lack of awareness among men and they seek medical advice lately. The high prevalence of HIV TB along with other opportunistic infections among reproductive age group individuals (20-40years), is also reported in the study of Ajit Goswami, 2015 and Ranganathan *et al.;* in 2004 which are 82.7% and 81% respectively [23, 29]. In this study we have included only pulmonary infections still the prevalence is higher than their observations. We have also included the patients who were on with or without ART. In our setting, bacterial pneumonia was less prevalent than pulmonary infection of fungal origin. J. Rubaihayo *et al.;* have also reported the prevalence of bacterial pneumonia in 11.7 and 10. 3% of female and male cases respectively [30]. Again we observed a strong association between low CD4 count and increase rate of pulmonary infection irrespective of ART status. Such observations were also made by V R Charan et al 2015 and Yitayih Wondimeneh *et al.;* in 2012. [22, 31]. Less the immunity more the chance of fungal growth.

In our study, among bacteria, staphylococcus was the most offending agent and streptococcus was next to it. Gr +ve and Gr –ve bacterial growth (mixed infection) was observed in maximum cases. Among fungi, Candida albicans was the most offending agent followed by C krusei. Both of them also caused mixed fungal growth. All the patients were kept on anti TB and anti-HIV treatment. In this study, the sputum culture characteristics of pre and post treatment with ART and anti-TB regimen in individual patients could not be traced and compared. Future long term prospective studies are required in this regard.

CONCLUSION:

Results from the present study show that the frequency of fungal etiology is more than bacterial cause in respiratory tract infections of HIV - TB co-infected patients. Mostly patients with low immunity suffer from bacterial and fungal mixed infection. In our setting, rural people suffer more than that of urban area and lack of education is one of the contributing factors. Improved hygienic practices and early detection as well as timely institution of appropriate interventions for opportunistic infections to can prevent morbidity and mortality. Therefore close attention should be given for proper HIV care programme implementation and health education to reduce disease burden in such areas.

ACKNOWLEDGEMENT:

Authors are grateful to staffs of department of Microbiology of this Institution for giving expert opinion and Mr. Nayan kumar Padhi, employee of our ART centre, for providing data.

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