Original Research Article

Microbiological profile of respiratory tract infections among HIV-infected and HIV-non infected patients: A comparative study

Rajeev Ramanlal Shah*

Associate Professor, Department of Microbiology, Shri Aurobindo Medical College and PG Institute, Indore, Madhya Pradesh, India
*Corresponding author email: **dr.rajeevrshah@gmail.com**

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Abstract

Background: The respiratory tract infections are among the first few secondary infections commonly seen in HIV infected patients. Hence, this study was undertaken to document the occurrence of bacterial and fungal respiratory tract infections in HIV infected patients.

Materials and methods: Expectorated and induced sputum samples were collected from 961 HIV-reactive patients and 300 HIV-nonreactive patients and processed for routine bacterial and fungal pathogens.

Results: Out of 961 samples, 532 (55.36%) showed presence of routine bacterial and fungal pathogens. While 300 HIV non-infected RTI patients showed 73 (24.33%) bacterial or fungal pathogens. Among the findings, *Mycobacterium tuberculosis* constituted the highest number, 209 (21.14 %), followed by other bacterial infections, 117 (18.41%), and fungi, 66 (3.03%). The study showed that males were infected with HIV more than females and most of them belonged to the adult age group in the prime of their working life. Weight loss followed by fever and cough were the most common symptoms.

Conclusion: When pathogens from both HIV infected and non-infected groups are compared and probability value was calculated it was found highly significant with $p < 0.001$. *M. tuberculosis* is the most common opportunistic pathogen with probability value of <0.02 followed by bacterial pathogens ($p<0.5$) causing respiratory tract infections in HIV, when they were compared with findings of HIV non-infected RTI patients. HIV/RTI infected patients mean CD4 count was 339.10 $\pm$ 84.0 cells/µL and mean CD4 count of identified pathogen was 263.85 $\pm$ 94.47 cells/µL, i.e. with lowering of immune status higher chances of opportunistic infections.

Key words

HIV, Bacterial RTI, CD4 count, *Mycobacterium tuberculosis,* Prevalence.

Introduction

Acquired Immunodeficiency Syndrome (AIDS) caused by *Human Immunodeficiency Virus* (HIV). HIV infection is a global pandemic, with cases reported from virtually every country [1]. HIV has posed a major challenge to public health in the present time. Among the various opportunistic infections, respiratory infections account for up to 70% of AIDS defining illness [2]. Their relative importance differs in different parts of the world [3] HIV is now in pandemic form and treatment of HIV with HAART has decreased morbidity and delayed the mortality up to 20-30 times.

HIV made a delayed entry into India, but its spread has been very rapid and at present, it is in an advanced stage of the epidemic [4]. Though HIV is the causative agent of AIDS, most morbidity and mortality in AIDS patients results from opportunistic infections. About 80% of these patients are seen to die as a result of such an infection rather than from HIV [5, 6]. The spectrum of HIV-related respiratory diseases has evolved since initial years of the epidemic [7].

Out of the global total population living with HIV infection, 95% live in developing countries. Changes in epidemiology have influenced the spectrum of respiratory tract infections in HIV-infected population. The increasing proportion of patients with AIDS, who are intravenous drug users and members of racial or ethnic minorities, correlate with increasing cases of bacterial pneumonia and tuberculosis (TB) [8].

Material and methods

The present study got approval from the ethical committee of Government Medical College, Surat and even got permission of GSACS, Ahmedabad.

A predesigned and pretested questionnaire was used to collect data on socio-demographic profile. Blood samples of these subjects were tested for HIV. The HIV-infected patients were all diagnosed as HIV reactive as per the NACO guidelines [9]. In the patients found HIV sero-positive even, CD4 count was calculated on FACS count, by flow cytrometry method (Becton Dickinson) method from their blood samples.

Three consecutive early morning sputum samples were collected and even samples were concentrated before reporting negative for AFB from 961 HIV infected patients and 300 HIV sero-negative subjects who had complaint of cough and fever for more than one week. Sputa samples were collected in a sterile wide mouthed container. The quality of the expectorated sputum was assessed both by macroscopic and microscopic examination. Any sample that was thin, watery, and with no purulent matter was considered unsuitable for further processing. Bartlett's scoring method was used for microscopic evaluation of the expectorated sputum [10]. A sputum was considered unsuitable if it had a final score of 0 or less. All unsuitable specimens were discarded and a repeat specimen was collected.

Case definition for T

Cases were defined as patients with both HIV sero-positive as well as having complaints of cough and fever for more than one week or in other words suffering from respiratory tract infections (RTI) at the time of sputum and data collection. One patient was included only once.

Definition for C1 (Control group)

C1 Control group was defined as patients with respiratory tract infection (RTI) but sero-negative for HIV at the time of sample and data collection.

Sputum smear microscopy

The most frequent method of TB detection involved microscopic examination of sputum for

acid-fast bacilli (AFB) [11]. Microscopy had the advantage of being inexpensive, relatively rapid to perform, and specific in most settings. However, to be considered smear positive a specimen needs to contain approximately 10^5 mycobacteria per milliliter. The sensitivity of sputum microscopy in HIV infection ranges from 43 to 51 per cent [12], and in many resource-limited settings with high rates of co-infection, the sensitivity may be much lower [13]. Methods that improved speed or sensitivity include fluorescence microscopy [14] and alternative specimen processing methods, such as concentration, bleach sedimentation and same-day sputum collection (so called front loading) strategies [14, 15]. Any procedure for digestion or liquefaction followed by centrifugation, prolonged gravity sedimentation, or filtration increased sensitivity by 13 to 33 per cent over direct microscopy, when culture was used as the reference standard [14].

Expectorated sputum was used to detect the bacterial and fungal pathogens and induced sputum was used for detection of trophozoites and cysts of *P. carinii*. Quality of the expectorated sputum was assessed both by macroscopic and microscopic examinations. Specimens which were clear, thin, and watery with no purulent material were rejected. Microscopically, Bartlett's scoring method was used to assess the quality of the sputum. Smears were prepared and subjected to Gram's staining, Ziehl-Neelsen staining (20% H_2SO_4 and 1% H_2SO_4), and Toluidine O stain. KOH mount was done for fungi.

The specimens were cultured on 5% Sheep Blood agar, MacConkey agar, heated Blood agar, Lowenstein Jensen Media(only in limited number of cases), and Sabouraud's dextrose agar. The plates were incubated at 37°C for 18 to 24 hours in humid air plus 5 to 10% CO_2. Sabouraud's dextrose agar slopes were incubated in duplicates at 28°C and another at 37°C for 4 weeks and observed for growth at intervals. Lowenstein Jensen media was incubated at 37°C for 4 weeks and observed for growth. Identification of the organisms was conducted according to standard microbiological procedures [16, 17]. Antibiotic sensitivity testing was done by Kirby Bauer disc diffusion methods according to CLSI guidelines [18]. Antibiotic discs were selected as per CLSI (Clinical and Laboratory Standards Institute).

Candida in the sputum was regarded as pathogen only after obtaining the same strain in repeated samples in pure growth, observing numerous polymorphonuclear leukocytes on Gram's stain of the sputum samples along with pseudohyphae. All *Candida* obtained in Sabouraud's dextrose agar was then processed for identification of species. Germ tube test was done. All *Candida* were inoculated on corn meal agar and incubated at 25°C to demonstrate chlamydospore formation and to look for typical morphology. Sugar assimilation test was done on Yeast Nitrogen Base agar for further speciation, using the following sugars: glucose, maltose, sucrose, lactose, trehalose, raffinose, and galactose.

The CD4 count of the patients included in the study was done. The method used was Flow cytometry method and done by FACS count.

Results

Prevalence of pathogenic bacterial and fungal isolates from both HIV seropositive (T) and HIV seronegative (C1) groups was as per **Table – 1**. Microbial profile of patients of both the groups studied (HIV reactive and HIV non-reactive patients) was as per **Table – 2**. Polymicrobial isolation from patients of both the groups was as per **Table – 3**. In the present study, polymicrobial infections were observed with *Klebsiella pneumoniae* and *Candida albicans* were detected from 23 (2.39%) patients from HIV sero positive T group patients followed by *M.tuberculosis* and *Candida albicans* mixed infection from 14 (1.46%) patients from the same T group patients (**Table - 3**). Comparison of Mean CD4 of patients of various groups was as per **Table – 4**.

Table - 1: Prevalence of pathogenic bacterial and fungal isolates from both HIV seropositive (T) and HIV seronegative (C1) groups.

Identified Pathogens	HIV +VE RTI +VE (T) Patients (n=961)		HIV -VE RTI +VE (C1) patients (n=300)		X^2	P Values
	(n)	%	(n).	%		
M. tuberculosis	209	21.75	27	9.00	4.79	0.02
Other bacteria	177	18.41	37	12.33	1.11	0.5
Fungal agents	66	6.87	04	1.33	2.9	0.1
Polymicrobial	80	8.32	7	2.33	2.71	0.1
Total no. of patients with pathogens	**532**	**55.36**	**73**	**24.33**	**10.9**	**0.001**

Table - 2: Microbial profile of patients of both the groups studied (HIV reactive and HIV non-reactive patients).

Pathogenic Isolates	HIV +VE RTI +VE (T) Patients (n=961)		HIV -VE RTI +VE (C1) patients (n=300)	
	(n)	%	(n)	%
Klebsiella pneumonia	51	5.31	18	6.00
Pseudomonas aeuroginosa	42	4.37	07	2.33
Strepococcus pneumonia	23	2.39	10	3.33
Escherichia coli	19	1.98	0	0
Staphylococcus aureus	17	1.77	0	00
Streptococcus pyogenus	14	1.46	0	0
Acinetobacter	11	1.14	0	0.00
Candia albicans	47	4.89	4	1.33
Candida (non-albicans)	63	6.56	12	4.00
Aspergillus	3	0.31	0	0.00
Total bacterial pathogens	**177**	18.42	**37**	12.33

Table - 3: Polymicrobial isolation from patients of both the groups.

Microorganisms	HIV +VE RTI +VE (T)		HIV -VE RTI +VE (C1)	
	(n)	%	(n)	%
M. tuberculosis + Candida (NCAC)	17	10.25	1	6.3
M. tuberculosis + Candida albicans	6	3.75	1	6.3
M. tuberculosis + S. Pneumonia	12	1.25	0	0.33
Klebsiella pneumoniae + Candida(NCAC)	9	5.63	0	-
Klebsiella pneumoniae + Candida albicans	5	3.13	0	-
Klebsiella pneumoniae + Pseudomonas	10	1.04	2	0.67
K. pneumoniae + Escheria coli	5	0.52	0	0.00
K. pneumoniae + Staphylococcus aureus	3	0.31	1	0.33
Pseudomonas + Candida (NCAC)	4	2.5	0	-
Pseudomonas + Candida Albicans	3	1.88	1	-
Proteus + Candida (NCAC)	4	2.5	0	-
Proteus + Candida albicans	2	1.25	0	-
Total polymicrobial (Mix) infections	**80/961**	**5.20**	**6/300**	**2.00**

Table - 4: Comparison of Mean CD4 of patients of various groups.

Organisms	Mean CD4 cells/µL	Standard Deviation	Cases	%
Pulmonary tuberculosis	198.52	±32.25	244	25.39
Extra-pulmonary TB	104.89	±47.09	64	06.66
All TB cases	151.71	±72.62	308	32.05
Patients excluding only pulmonary TB	408.40	±202.23	653	67.95
Candida albicans	257.12	±82.96	63	6.55
Candida (NCAC)	499.73	±196.24	97	10.09
Candida albicans + Candida (NCAC)	404.20	±193.14	160	16.65
Other patients excluding only Candida + NCACs	437.26	±231.5	801	83.35
All organism including PMTB	263.85	±94.47	501	52.13
All patients with identified organism	288.62	±94.47	612	63.68
Only bacterial RTI	**223.07**	**±83.21**	**466**	**48.49**
Only fungal RTI = Candida albicans + Candida (NCAC) + Aspergillus	**377.29**	**±268.29**	**66**	**6.87**
Atypical Bacterial RTI (No isolates with PMNLs seen)	**389.22**	**±89.93**	**87**	**09.05**
Polymicrobial RTI	**392.26**	**±87.14**	**80**	**08.32**
All RTI patients without identified pathogens (Probable Viral RTI)	**502.97**	**±114.89**	**262**	**27.26**
Total patients	**339.10**	**±84.0**	**961**	**100**

Discussion

In the present study most common mode of acquisition of HIV infection was the heterosexual route, 773 (80.43%). In the present study 101 (10.51%) patients had acquired HIV from their mothers through placental barrier, followed by 46 (4.79%) patients who acquired the infection through blood transfusion,

During a newly begun course of treatment with abacavir, asthma could also be seen due to hypersensitivity. Dyspnea (13%), cough (27%) and pharyngitis (13%) are common symptoms [19]. In present study we found only 5.20% (50/961) patients of RTI with HIV who recently started HAART (calculated from date of starting HAART, which should be within three months) and nearby CD4 count of 200, who might could have allergic RTI after newly started HAART. So we can say that in this part of world, HAART is well tolerated and HAART induced hypersensitivity-RTI were found in very small number of patients Some patients even develop pulmonary infiltrates. T-20 seems to increase the risk of bacterial pneumonia, at least among smokers. Dyspnea and tachypnea are also seen in lactic acidosis secondary to nuke therapy. In addition, pulmonary symptoms after institution of HAART might result from the Immune Reconstitution and Inflammatory Syndrome (IRIS). The list of etiologies includes a number of infective and non-infective causes [20]. Low CD4+ T-cell count and high viral load are risk factors.

In the present study, RTI were observed in 595 (61.08%) HIV patients, while they were observed in 374 (38.92%) full blown AIDS patients, who signified that most RTI occurred early before any other secondary infections were seen or AIDS had been established. These result matches well with the statement that RTI are the first secondary infections observed even before full blown AIDS can establish.

The occurrence of respiratory infections in HIV-reactive patients is well documented. In our study, among the respiratory pathogens, *M. tuberculosis* constituted the maximum number, 244 (25.39%), followed by other bacterial infections, 177 (18.41%) and fungi, 66 (6.87%). Most of the infections seen in AIDS are endemic to that geographical region. *M. tuberculosis* is endemic in India and thus, is commonest opportunistic infection in India [9].

In a study conducted at YRG Care, Chennai, TB was the most common opportunistic infection, with pulmonary TB affecting 35% of the HIV-positive group and extrapulmonary TB in 11% of them [21]. In the present study 244 (25.39%) cases of pulmonary TB were found while 64 (6.66%) cases were of extra-pulmonary TB.

In a study conducted by Shailaja, et al. [22] 44.3% were bacteria, 42.9% were mycobacteria, and 12.8% were fungi. Bacterial pathogens were *K. pneumoniae, S. pneumoniae, S. aureus, P. aeruginosa, Moraxella catarrhalis, Escherichia coli,* and *N. asteroides.*

In the present study, the other bacterial isolates were *K. pneumoniae, P. aeruginosa, S. pneumoniae, S. aureus, Actinomyces. C. albicans and C.non-albicaans (NCAC)* were the fungal isolates. Polymicrobial etiology was noticed in eight (8.33%) of the patients indicating the severity of infection in these patients.

P. aeruginosa pneumonia is often community acquired and is associated with high mortality. Dropulic, et al. in their study on the clinical manifestations of *P. aeruginosa* infection among patients with AIDS found that of the 73 episodes of *P. aeruginosa* infections, 13 were that of pneumonia [23].

In the present study, there were 42 (4.37%) isolates of *P. aeruginosa* causing pneumonia in the HIV infected patients. Whiile they were isolated from seven patients (2.33%) in HIV non-infected patients. Pseudomonas was even isolated with Candida in the seven (0.73%) patients of T group of HIV-infected patients.

K. pneumoniae were isolated from 51 (5.31%) of HIV -infected patients, but they were isolated from 18 (6.00%) patients of C1 group of HIV non-infected RTI patients. *S. pneumoniae* were isolated from 23 (2.39%) patients from T group, while from C1 group they were isolated from only 10 (3.33%) of RTI patients. Acinetobacter were isolated from 11 (1.14%) patients of HIV infected patients group only, suggesting that immunosupression favored their growth.

During the last few decades, there has been an increased incidence of Candidiasis due to the emergence of pandemic of AIDS. Candidiasis is undoubtedly the most common fungal infection in HIV-infected individuals. It had been observed in recent years that *Candida albicans* had been replaced by Non candida albicans candida (NCAC) as primary and the most important fungal pathogen in causing opportunistic fungal infections in HIV sero-positive patients [24, 25]. Although *C. albicans* remains the second most common causative agent, an increasing incidence of non-albicans species of *Candida* has been seen in the last few years [1]. Introduction of fluconazole and itraconazole has increased the incidence of non-albicans *Candida*. These species include *C. tropicalis, C. krusei, C. glabrata, and C. parapsilosis* [1].

PCP was not isolated in this study. This was also the observation made by Shailaja, et al [22]. It is the most common AIDS-defining illness in the developed world. Unlike in the West, the prevalence of PCP is low or negligible in India. In India, very low rates (5 to 7%) [22, 23] of PCP have been reported. Some reasons for this could be the predominance of other pulmonary diseases like TB, or due to the extensive use of cotrimoxazole in the prophylaxis of PCP in HIV-reactive individuals with CD_4 count of less than 200 cells/µl.

Although HIV infection is most closely associated with altered cell-mediated immunity (which is manifested by a decrease in CD4

count), a number of additional immune deficits may occur in association with HIV infection [26, 27] including a poor antibody response due to B cell dysfunction and defects in chemotaxis, phagocytosis, and intracellular killing by monocytes, macrophages, and neutrophils [27]. In addition, HIV-infected individuals may experience impairment of local defenses, manifested by a depression of specific IgA at the mucosal surfaces [26]. These immune abnormalities all contribute to an increased risk of bacterial infection among HIV-infected persons, particularly by encapsulated bacteria, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*.

The immune status of the patients was assessed using CD4 count. The CD4 count of HIV-positive patients who were diagnosed to have TB was low. The mean CD4 count was 198.52 $\pm$ 32.25 cells/µl in this group of patients.

The HIV-positive patients who did not have TB had a mean CD4 count of 408.40 cells/µl. This finding correlates with the statement of association of TB with low CD4 count. HIV-reactive patients who had a CD4 count of more than 408 cells/µl were not positive for TB but had other bacterial infections. Those who had TB had a CD4 count of less than 250 cells/µl, with mean count of 198.52 cells/µl, which demonstrated that TB is common in HIV patients when their immune status is low.

Also, mortality was high in those patients with very low CD4 count, thus proving the association of CD4 count with the immune status of the body. Patients with CD4 counts less that 200 cells/µl are 19 times more likely to die than those with CD4 counts greater than 350 cells/µl [18]. If we consider RTI without unidentified pathogens in HIV infected patients, and co-related their PMNLs count in sputum, 87% were non-significant for PMNLs count but the mean CD4 count was 502 $\pm$293.89, it means majority of them had viral etiology of RTI and in other small group they could be anaerobes or fastidious bacterial pathogens caused RTI at very high CD4 count, but most of these RTI must be minor like common cold and not life threatening. But HIV patients might have risk of serious candidal RTI infections at may 499.76 $\pm$196.24 according to our study. So, the result of present study microbiologically favored that HAART should be started earliest at 500 cells/µl. CD4 cell count, but should not be delayed below 350 cells/µl CD4 count, to avoid life threatening TB and LRTI infections. It may vary from patient to patient depending on their having severity of opportunistic infection and CD4 count (**Table - 4**).

In spite of these much damages to immunity of respiratory tract, when we compared RTI due to viruses in HIV non-infected 300 individuals their prevalence seems to be increased almost double as in their sputa also, around 94% did not show significant PMNLs. So we could say that almost 47.87% (460/961) patients in HIV infected patients might be having viral etiology of RTI, while in non-HIV infected group probable viral etiology could be around 85.67% (257/300). So in other words we could say that prevalence of viral RTI had been decreased in HIV infected patients. Could this be due to excessive production of interferon among HIV-infected patients which might be responsible for low prevalence of viral RTI in HIV-infected patients or due to shifting of RTI from viral to bacterial very fast?

Conclusion

Respiratory tract infections are among the first secondary infections seen in HIV-infected patients appeared at as much high mean CD4 count as 502.97 $\pm$ 114.89. With the starting HAART early (Highly effective antiretroviral therapy), one can maintain CD4 count level high so vastly reduce the morbidity and mortality due to RTI in HIV-reactive patients.

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