Prevalence of HIV in Clinically Suspected Cases of Pulmonary Tuberculosis

RAJESH BAREJA, VASHISHTH MISHRA, RAHUL KUMAR GOYAL, RABINDRA NATH BEHARA

ABSTRACT

Context: Tuberculosis (TB) and Human Immunodeficiency Virus (HIV) infection have been inextricably bound together from the early years of the HIV epidemic.

Aims and objective: A prospective study was conducted to evaluate the prevalence of HIV infection in patients with tuberculosis, describing and comparing the characteristics of pulmonary TB cases with or without the presence of HIV infection.

Materials and Methods: A total of 75 clinically suspected patients of TB were asked for sputum samples for acid-fast bacilli (AFB) staining and culture that were processed according to Revised National Tuberculosis Control Program guidelines. One 5 mL blood sample each was taken for HIV testing and processed according to the guidelines of National Aids Control Organisation.

Results: Out of 75, nine (12%) patients were found to be HIV positive. The male female ratio among HIV positive cases was 2:1. Four out of nine HIV-positive patients and one among 66 HIV-negative had candidiasis (p <0.001). Three of the HIV-positive patients had chronic diarrhea compared to only one patient in the HIV-negative group (p <0.05). The lone patient having herpetic skin lesion was found to be HIV-positive. Among the HIV co-infected patients, there was only one patient who was both smear and culture positive, rest were negative. On the other hand, among the 66 HIV-negative TB patients, 25 (37.88%) were positive on both smear and culture. Two (3.03%) patients were positive on culture but negative on smear examination. Radiological involvement of multiple lung zones (p < 0.05) and presence of associated mediastinal lymphadenopathy were found to significant higher in HIV co-infected TB patients.

Conclusion: Universal HIV testing of TB cases should be encouraged for more effective treatment, care and prevention programs.

INTRODUCTION

Tuberculosis (TB) and Human Immunodeficiency Virus (HIV) infection have been inextricably bound together from the early years of the HIV/AIDS (Acquired Immunodeficiency syndrome) epidemic. Of the 15 countries with the highest rates of TB/HIV co-infection among adults, 12 are in Africa and the others in Asia, including India, Myanmar and Thailand. Africa has recorded HIV infection rates of 50 % among TB patients [1]. The increasing global burden of tuberculosis has been related to HIV/AIDS. This is due to the fact that the risk of developing active TB disease among co-infected persons is 10% per year, making HIV the most potent risk factor for progression to active TB disease [2-4].

Dual infection with Mycobacterium tuberculosis (MTB) and HIV is associated with a number of important infections between the infective agents. Firstly, HIV-induced immunosuppression increases the likelihood of reactivation of latent TB. This adds to the reservoir of infectious cases of TB in community [2]. On the other hand, infection with MTB up-regulates the immune system, which leads to the activation of T cells and activated T cells, in turn, produce more HIV virions than quiescent T cells. Higher HIV viral loads increase the rate of disease progression and also increase HIV infectiousness [5].

The co-infection of HIV and MTB is associated with major diagnostic problems since HIV infection often leads to extra pulmonary and smear negative pulmonary tuberculosis. X-rays abnormalities, which are not specific for TB in HIV-negative patients, are even more non-specific, with only minor abnormalities or abnormalities that do not look like classical tuberculosis, in the HIV-infected. In addition, patients infected with HIV have frequent illnesses with pulmonary involvement caused by agents other than MTB [6].

The behaviour pattern of TB/HIV association differs from region to region. In view of this and the absence of any data on this aspect in this region, the present study was conducted with a view to evaluating the prevalence of HIV infection in patients with tuberculosis, describing and comparing the
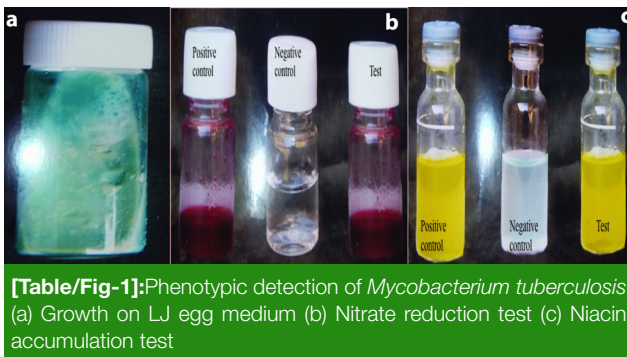
[Table/Fig-1]:Phenotypic detection of *Mycobacterium tuberculosis* (a) Growth on LJ egg medium (b) Nitrate reduction test (c) Niacin accumulation test

[Table/Fig-2]: Detection of HIV antibodies using three different methods (a) HIV COMB test (b) HIV TRIDOT test (c) Microlisa-HIV ELISA test

characteristics of pulmonary TB cases with or without the presence of HIV infection.

MATERIALS AND METHODS

This prospective study was carried out in the Department of Microbiology, over a period of twelve months, from November 2011 to October 2012, at a tertiary care hospital that also operates as a centre for the Revised National Tuberculosis Control Program (RNTCP). All clinically suspected fresh cases of pulmonary TB, smear negative or positive cases, were included in the study. Patients receiving or who received anti-TB treatment in the previous month, patients with extra pulmonary tuberculosis and non-consenting patients were excluded from this study. Ethical clearance for the study was obtained from the institutional ethical committee. A total of 75 patients were included and were asked for sputum samples for acid-fast bacilli (AFB) staining and culture according to RNTCP guidelines [7]. One 5 mL blood sample each was taken for HIV testing according to NACO (National Aids Control Organisation) guidelines [8].

All clinical sputum specimens were digested and decontaminated by the N-acetyl-L-cysteine-NaOH method with a final NaOH concentration of 1% [9]. After centrifugation, AFB smears were made for all clinical specimens studied using the sediment and stained with the Ziehl-Neelsen acid-fast method. The remaining sediment was suspended in 1-2 mL of sterile 0.67 M phosphate buffer (pH 6.8) and vortexed for 15 s. This suspension was used for inoculation of egg-based Lowenstein-Jensen (LJ) media. The processed specimen (0.1 mL) was inoculated on LJ egg medium and incubated at 37ºC for a maximum of eight weeks and read once weekly. The identification process of MTB comprised the phenotypic identification of cultures of acid-fast bacilli grown on solid medium based on the combination of observation of colony morphology [Table/Fig-1(a)], inability to grow on a culture medium containing PNB (p-nitrobenzoate) and results of biochemical tests specific for thermo labile catalase, nitrate reduction [Table/Fig-1(b)] and niacin accumulation test [Table/Fig-1(c)] [8]. Reference strains such as Mycobacterium tuberculosis (H37Rv) and Mycobacterium intracellulare (ATCC 13950) were used for quality control. All the culture and biochemical media were purchased from Hi-Media, Mumbai
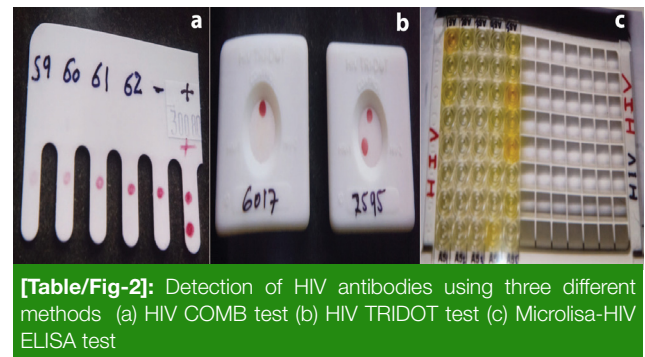
and processed according to the manufacturer instructions.

The serum samples were analysed for the presence of HIV antibodies as per NACO guidelines. HIV testing was carried out according to the following algorithm using three test kits, viz. HIV COMB (J.Mitra & Co.) [Table/Fig-2(a)], HIV TRIDOT (Biomed industry) [Table/Fig-2(b)] and Microlisa-HIV ELISA (J.Mitra & Co.) [Table/Fig-2(c)]. Chi-square test using SPSS version 11.0 and Mc Nemar's test were applied for statistical analysis.

RESULTS

In the current study, a total of 75 patients were recruited. Out of 75, nine (12%) patients were found to be HIV positive. The male female ratio among HIV positive cases was 2:1. The clinical features were almost similar between HIV-infected and non-infected patients. In both the groups, evening rise of temperature was the commonest symptom, followed by weight loss, cough exceeding two weeks, sputum and anorexia. However, weight loss and dyspnoea were much more common in the HIV-infected TB patients compared to their HIV-negative counterparts, though the difference did not reach the level of statistical significance ($p > 0.05$) [Table/Fig-3].

Three types of opportunistic infections were observed. Among them, candidiasis and chronic diarrhea were observed in both groups, though their incidence was much higher in HIV-positive TB patients. Four out of nine HIV-positive patients and one among 66 HIV-negative had candidiasis ($p < 0.001$). Three of the HIV-positive patients had chronic diarrhea compared to only one patient in the HIV-negative group ($p < 0.05$). The lone patient having herpetic skin lesion was found to be HIV-positive.

Five types of risk behaviours were observed among these patients. Sexual promiscuity was recorded 6.06% and 55.55% in HIV-negative and HIV-positive TB patients respectively [Table/Fig-4].

It was observed that among the HIV co-infected patients, there was only one patient who was both smear and culture positive rest were negative. On the other hand, among the 66 HIV-negative TB patients, 25 (37.88%) were positive on both smear and culture. Two (3.03%) patients were positive

Symptoms Total (n=75)	HIV negative (n=66)	HIV positive (n=9)	p value
Cough > 2 weeks	25 (37.88%)	3 (33.33%)	p > 0.05
Sputum	14 (21.21%)	2 (22.22%)	p > 0.05
Dyspnoea	5 (7.58%)	1 (11.11%)	p > 0.05
Chest pain	6 (9.09%)	0 (0%)	p > 0.05
Haemoptysis	2 (3.03%)	0 (0%)	p > 0.05
Weight loss	38 (57.58%)	7 (77.78%)	p > 0.05
Anorexia	23 (34.84%)	3 (33.33%)	p > 0.05
Evening pyrexia	54 (81.81%)	8 (88.89%)	p > 0.05

[Table/Fig-3]: Profile of clinical symptoms among the recruited patients

Total (n=75)	HIV negative (n=66)	HIV positive (n=9)	p value
Sexual promiscuity	4 (6.06%)	5 (55.55%)	p < 0.001
Multiple blood transfusion	3 (4.54%)	0	p > 0.05
Intravenous drug abuse	0	1 (11.11%)	p > 0.05
Homosexual	1 (1.51%)	1 (11.11%)	p > 0.05
HIV-positive spouse	0	2 (22.22%)	p < 0.05

[Table/Fig-4]: Profile of risk behaviour among the recruited patients

on culture but negative on smear examination. A total of 39 (59.09%) patients were negative on both smear and culture examination.

Radiological involvement of multiple lung zones (p < 0.05) and presence of associated mediastinal lymphadenopathy were found to significant higher in HIV co-infected TB patients. Cavitary lesions, on the other hand, were significantly more common among HIV-negative TB patients (p < 0.05).

DISCUSSION

India is home to a third of the global cases of tuberculosis and the problem is now being further complicated by the alarming spread of HIV [6]. TB may also influence HIV evolution. Proinflammatory cytokine production by tuberculous granulomas (in particular TNF-α) has been associated with increased HIV viraemia, which might accelerate the course towards severe immunosuppression [10].

In the present study, there was a distinct shift in the age and sex profile of subjects between HIV-positive and HIV-negative TB patients. While in the HIV-negative group, the median age was 45 years (range of 24-75 years) and the male female ratio was 4:1, the corresponding values in the HIV co-infected group were 34 years (range of 22-55 years) and 2:1 respectively. Liberato et al. (2004) reported that the greatest impact of the rise in TB incidence has been on people between 25 and 45 years of age, since this is the age group predominantly affected

by HIV [11]. A higher frequency of individuals was observed under 50 years of age in the HIV seropositive group, whilst the proportion of patients over 50 who were infected with TB but not HIV was 2.28 times greater than the numbers who were HIV positive. However, contrary to our findings, this group found that there was higher frequency of male individuals among pulmonary TB patients, in general, and the frequency was even greater among TB cases where HIV infection was also present [11]. Higher prevalence of HIV in a population is associated with a younger population of TB patients, having a relatively higher representation of females. It has, however, been suggested by Bellamy et al. (2000) that there exists a relationship between TB susceptibility and chromosome X [12].

The present study showed HIV-positivity rate of 12% among the recruited patients. A study conducted in New Delhi on 555 patients with TB demonstrated an HIV seropositivity of 9.4%, vs. an overall seropositivity in this same hospital of 0.4% from 1994-1999 [13]. Mohanty et al. (1995) observed HIV prevalence rate of 30% among TB patients in Mumbai whereas the same was found to be still higher at 40% in Northern Thailand [14,15].

Though in the present study weight loss and evening pyrexia were the most common symptoms encountered in both HIV-positive and HIV-negative TB patients, the frequency of these symptoms were higher in the former group than in latter. These findings, hence, confirm to the reports published jointly by the Central TB Division and National AIDS Control Organization, Government of India [16,17]. However, among HIV infected patients, cough is reported less frequently, probably because of weak cough reflex due to debilitated condition of patients in advanced disease and absence of cavitations, dominant interstitial/military lesions which do not communicate with the bronchi and less endobronchial irritation. A number of other studies have also reported that weight loss and fever were most frequently encountered among patients co-infected with TB and HIV, supporting the hypothesis that tuberculosis as a disease is more difficult to diagnose in HIV positive patients due to change in its usual clinical pattern [18,19].

In the present study, the distribution of all the opportunistic infections known to be associated with HIV/AIDS was significantly higher in the HIV-positive TB patients compared to HIV-negative ones. Akin to our findings, the Central TB Division and National AIDS Control Organization has also recorded oral and oesophageal candidiasis as the second most common opportunistic infection reported from India, second only to pulmonary TB, and seen in 58% of HIV-positive cases. Chronic diarrhea has also been documented as a common manifestation in AIDS cases. Some of the other opportunistic infections reported from India are Herpes zoster, Toxoplasmosis, Pneumocystis carinii pneumonia, CMV retinitis and Cryptococcal meningitis [17].

Among the different risk factors, sexual promiscuity, Injection drug use (IDU), male homosexual behaviour and presence

of HIV-positive spouse were found to be the significant risk factors associated with HIV-positivity in this study. Regional differences are observed in India. IDU is most relevant in the northeast, while heterosexual transmission has been found to drive the large epidemic in other parts of the country [20]. Studies from Latin America countries have also confirmed that risk factors for HIV infection were independent of the presence of tuberculosis [21].

In the present study, sputum smear positivity for AFB was significantly lower in HIV-positive cases than in their HIV-negative counterparts. This is an agreement with a number of studies reported from several countries across the globe, all of which reported that HIV-positive individuals are less likely to be smear positive than HIV-negative individuals [22,23]. The frequency of smear positive individuals has been demonstrated to correlate with the immune status. A relatively better immune status is associated with smear-positive disease, whereas increased impairment of immunity carries higher chances of sputum smear negativity [24]. Liberato et al. (2004) have also reported that over 50% of co-infected patients in Brazil were negative for acid-fast bacilli in the sputum [11]. The frequency of culture positivity of sputum samples on LJ media among HIV co-infected patients compared to their HIV-negative counterparts was significantly low. Though mycobacterial culture is recommended for diagnostic purposes in smear negative pulmonary tuberculosis its yield is comparatively lower in HIV-positive TB patients due to lower bacterial concentration in their sputum samples. A study from Brazil reported that patients who were HIV seropositive had a lower frequency of positive sputum culture for Mycobacterium tuberculosis in comparison with those who were HIV-negative [11]. In another study conducted in Brazilian reference hospital, among 171 HIV/TB patients, only 96 (56.14%) had been subjected to acid-fast staining and LJ culture test. Only 18 of these 96 patients were positive in both the acid-fast staining and LJ culture [25].

It has been generally observed that progression of immunosuppression correlates with chest radiographic abnormalities in HIV infected TB patients. Prior to gross derangement of CD4+ lymphocyte count, the chest radiograph resembles typical pulmonary TB. As immunosuppression worsens, chest X-rays more often show atypical findings such as pulmonary infiltrates affecting the lower lobes, intrathoracic lymphadenopathy and some time even a normal chest radiograph [26]. In the light of these observations, in this study, the absence of cavity lesions, associated mediastinal lymphadenopathy and multiple zone involvement in HIV co-infected patients could be explained by the advance nature of immunosuppression in them. The relative lack of smear and culture positivity in co-infected patients could also be explained by the same logic. A study from Ethiopia, however failed to observe a significant relationship between cavity lesions on chest X-ray and HIV serostatus, though cavities were found to be more common in smear positive patients, irrespective of their HIV status [27].

CONCLUSION

HIV co-infection of TB patients is a significant problem that presents unique treatment problems and poses a public health threat. This study found a high prevalence of HIV co-infection in adult TB patients. Risk factors predisposing to HIV infection were prevalent in both the HIV-positive and HIV-negative groups. Universal HIV testing of TB cases should be encouraged to develop a more comprehensive picture of TB/HIV co-infection so that public health officials can deliver more effective treatment, care and prevention programs.

REFERENCES

[1] Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. *JAMA.* 1999; 282: 677-96.

[2] Selwyn PA, Hartel D, Lewis VA, Schoenbaum EE, Vermund SH, Klein RS, et al. A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. *N Engl J Med.* 1989; 320 (9): 545-50.

[3] Pape JW, Jean SS, Ho JL, Hafner A, Johnson WD Jr. Effect of isoniazid prophylaxis on incidence of active tuberculosis and progression of HIV infection. *Lancet.* 1993; 342: 268-72.

[4] Hopewell PC. Impact of human immunodeficiency virus infection on the epidemiology, clinical features, management, and control of tuberculosis. *Clin Infect Dis.* 1992; 15: 540-47.

[5] Villacian JS, Tan GB, Teo LF, Paton NI. The effect of infection with mycobacterium tuberculosis on T cell activation and proliferation in patients with and without HIV co infection. *J Infect.* 2005; 51: 408-12.

[6] Narain JP, Lo YR. Epidemiology of HIV-TB in Asia. *Indian J Med Res.* 2004; 120: 277-89.

[7] Culture of Mycobacterium tuberculosis and drug susceptibility testing on solid medium. Revised National TB Control Programme, Central TB Division, *Ministry of Health & Family Welfare, New Delhi,* India. 2009: 28-54.

[8] National AIDS Control Organization. Guidelines on HIV testing, *Ministry of health and family welfare, NewDelhi, India.* 2007: 38-53.

[9] Kent PT, Kubica GP. Public health mycobacteriology - a guide for the level III laboratory, Department of Health and Human Services/Centers for Disease Control and Prevention, *Atlanta,* 1985; 207-10.

[10] Garrait V, Cadranel J, Esvant H, Herry I, Morinet P, Mayaud C, et al. Tuberculosis generates a microenvironment enhancing the productive infection of local lymphocytes by HIV. *J Immunol.* 1997; 159: 2824–30.

[11] Liberato IR, de Albuquerque MF, Campelo AR, de Melo HR. Characteristics of Pulmonary tuberculosis in HIV seropositive and seronegative patients in a northeastern region of Brazil. *Rev Soc Bras Med Trop.* 2004; 37: 46-50.

[12] Bellamy R, Beyers N, McAdam KP, Ruwende C, Gie R, Samaai P, et al. Genetic susceptibility to tuberculosis in Africans: A genome-wide scan. *Proc Natl Acad Sci USA.* 2000; 97: 8005-09.

[13] Sharma SK, Aggarwal G, Seth P, Saha PK. Increasing HIV seropositivity among adult tuberculosis patients in Delhi. *Indian J Med Res.* 2003; 117: 239-42.

[14] Mohanty KC, Basheer PMM. Changing trend of HIV infection and tuberculosis in a Bombay area since 1988. *Indian J Tuberc.* 1995; 42: 117-20.

[15] Yanai H, Uthaivarovit W, Panich V, Sawanpanyalert P, Chaimanee B, Akarasewi P, et al. Rapid increase in HIV-related tuberculosis, Chiang Rai, Thailand, 1990-1994. *AIDS.* 1996; 10: 527-31.

[16] Vermund SH, White H, Shah SA, Altaf A, Kristensen S, Khanani R, et al. HIV/AIDS in Pakistan: has the explosion begun? *J Pak Med Assoc.* 2006; 56 (1): S 1-2.

[17] Central TB Division and National AIDS Control Organization. *Training module for medical officers on TB/HIV. NewDelhi, India.* 2005: 7-13.

[18] Pozniak AL, Macleod GA, Ndlovu D, Ross E, Weinberg MJ. Clinical and chest radiographic features of tuberculosis associated with Human Immunodeficiency Virus in Zimbabwe. *Am J Resp Critical Care Med*. 1995; 152: 1558-61.

[19] Trajman A, B Neto E, Belo MT, Teixeira EG, Selig L, Ferrari G, Branco MM. pleural tuberculosis and human immunodeficiency virus co-infection. *Int J Tuberculosis Lung Dis*. 1997; 1: 487-88.

[20] Chandrashekaran P, Dallabetta G, Loo V, Rao S, Gayle H, Alexander A. Containing HIV/AIDS in India: the unfinished agenda. *Lancet Infect Dis*. 2006; 6: 508-21.

[21] Kritski A, Dalcomo M, Bianco RD, Melo FF, Pinto W, Schechther M, Castelo A. Assciacao tuberculose e infeccao pelo HIV no Brazil. *Boletin de la Oficina Sanitaria Panamericana.* 1995; 118: 542-54.

[22] Elliot AM, Halwiindi B, Hayes RJ, Luo N, Tembo G, Machiels L, et al. The impact of human immunodeficiency virus on presentation and diagnosis of tuberculosis in a cohort study in Zambia. *J Trop Med Hyg.* 1993; 96: 1-11.

[23] Johnson JL, Vjecha MJ, Okwera A, Hatanga E, Byckwaso F, Wolski k, et al.. Impact of human deficiency virus type-1 infection on the initial bacteriologic and radiographic manifestations of pulmonary tuberculosis in Uganda. *Int J Tuberc Lung Dis.* 1998; 2: 397-04.

[24] Idemyor V. HIV and tuberculosis co-infection: Inextricably linked liaison. *J Natl Med Assoc.* 2007; 99: 1414-19.

[25] Carvalho BM, Monteiro AJ, Pires NRJ, Grangeiro TB, Frota CC. Factors related to HIV/tuberculosis coinfection in a Brazilian reference hospital. *Braz J Infect Dis.* 2008; 12: 281-86.

[26] Central TB Division and National AIDS Control Organization. *Training module for medical officers on TB/HIV. NewDelhi, India*. 2005: 20-31.

[27] Kassu A, Mengistu G, Ayele B, Diro E, Mekonnen F, Ketema D, et al. Co infection and clinical manifestations of tuberculosis in human immunodeficiency virus infected and uninfected adults at a teaching hospital, northwest Ethiopia. *J Microbiol Immunol Infec.* 2007; 40: 116-22.

AUTHOR(S):
1. Dr. Rajesh Bareja
2. Dr. Vashishth Mishra
3. Dr. Rahul Kumar Goyal
4. Dr. Rabindra Nath Behara

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Microbiology, SRMSIMS, Bhojipura, Bareilly, Uttar Pradesh, India.
2. Assistant Professor, Department of Microbiology, SRMSIMS, Bhojipura, Bareilly, Uttar Pradesh, India.
3. Professor, Department of Microbiology, SRMSIMS, Bhojipura, Bareilly, Uttar Pradesh, India.
4. Professor, Department of Microbiology, SRMSIMS, Bhojipura, Bareilly, Uttar Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Rajesh Bareja,
Assistant Professor, Department of Microbiology, SRMSIMS, Bhojipura, Bareilly-243202, U.P., India.
Phone : :+91-9458705236, +91-9034882188
E-mail : rajeshbareja@gmail.com