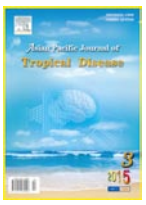
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading doi: 10.1016/S2222-1808(14)60660-5

Surveillance of tuberculosis co-infection among HIV infected patients and their CD4$^+$ cell count profile

Sonali Jain[1], Amit Kumar Singh[2*], Ravinder Pal Singh[1], Jyoti Bajaj[3], Ajit Shriram Damle[3]

[1]*Department of Microbiology, Gold Field Institute of Medical Sciences and Research, Faridabad, Haryana, India*

[2]*Department of Microbiology, Mayo Institute of Medical Sciences, Barabanki, Uttar Pradesh, India*

[3]*Department of Microbiology, Government Medical College, Aurangabad, Maharashtra, India*

PEER REVIEW

Peer reviewer

Prof. Gopa Banerjee, Department of Microbiology, KGMU, Lucknow-226003, India.
Tel: +91 9415109154
E-mail: gopa.banerjee31@rediffmail.com

Comments

The paper is well written. Authors determined the occurrence of tuberculosis among HIV infected patients and their CD4$^+$ cell count profile. The results are interesting.
Details on Page 237

ABSTRACT

Objective: To determine the occurrence of tuberculosis (TB) among HIV infected patients and their CD4$^+$ cell count profile.

Methods: A total of 263 HIV-seropositive patients either hospitalized or reporting to the anti-retroviral therapy centre for follow up were included in the study. HIV-seropositive patients were then investigated clinically, radiologically and microbiologically for diagnosis of pulmonary TB as per the revised guidelines issued by WHO. Two early morning sputum samples were collected from patients, and isolation and identification of *Mycobacterium tuberculosis* was done as per standard protocol.

Results: Out of them 41 (15.6%) were TB positive and 222 (84.4%) were TB negative. Among TB positive patients, smear positive and culture positive patients were 19 (7.2%), while smear negative and culture positive were 9 (3.4%). Mean CD4$^+$ T cell counts for HIV-seropositive and HIV-related TB patients were 156.14 cells/μL and 197.24 cells/μL respectively.

Conclusions: The clinical manifestations in HIV-related TB patients are quite variable and lead to difficulties in diagnosis. Thus radiological evaluation along with proper laboratory investigation in all clinically suspected cases of tuberculosis is the key for prompt diagnosis and treatment.

KEYWORDS

HIV-related tuberculosis, HIV-seropositive, Smear positive pulmonary tuberculosis, Smear negative pulmonary tuberculosis

1. Introduction

Tuberculosis (TB) has been a major public health problem in India and other developing countries. India, being among the 22 high TB burden countries in the world, contributes 20% of global incidence annually. It has been estimated that approximately 40% of Indian population is infected with the TB bacilli and about 10% of them will develop the disease during their lifetime. The annual incidence of TB cases in 2011 is estimated to be 2.3 million cases in India out of 9 million TB cases globally. The estimated annual incidence and prevalence are 2.3 million and 3.1 million cases respectively. If left untreated, one sputum positive patient can infect 10-15 persons per year. On the other hand, improper treatment can develop drug resistance and potentially untreatable forms of TB[1].

TB is one of the most common opportunistic infections and an important cause of mortality among people living with HIV infection. This may be attributed to difficulty in diagnosis and treatment due to factors such as co-toxicity,

*Corresponding author: Amit Kumar Singh, Assistant Professor, Department of Microbiology, Mayo Institute of Medical Sciences, Barabanki, Uttar Pradesh, India.
Tel: 09235 555773
Fax: 05248 227676
E-mail: amit.singh.dr@gmail.com
Foundation Project: Supported by National AIDS Control Organisation, India for HIV testing.

Article history:
Received 23 Jun 2014
Received in revised form 25 Jun, 2nd revised form 27 Jun, 3rd revised form 29 Jun 2014
Accepted 10 Jul 2014
Available online 25 Jul 2014

drug interactions, pill burden and co-morbidity. On the other hand, HIV infection increases the risk of progression of latent TB infection to active TB disease, thereby increasing mortality rate in lack of proper and timely treatment of HIV and TB, and risk of reactivation of TB even after successful treatment[1,2].

India ranks 2nd in the world with 130000 HIV-related TB patients and it accounts for about 10% of the global burden. This is coupled with unequal distribution of prevalence within country which is a challenge for joint delivery of integrated services. Studies suggest that an integrated approach to TB and HIV services is necessary in effective management of infection. Studies also indicate that emphasis needs to be on early diagnosis linked to TB and HIV treatment[1,3].

$CD4^+$ cells play an important role in the control of *Mycobacterium tuberculosis* (*M. tuberculosis*) infection, as decrease in the number of these cells in HIV-infected individuals increases the risk of both primary and reactivation of latent disease. Following initial infection the risk of development of active TB disease in immuno-competent individuals is approximately 10% in lifetime, whereas in individuals with HIV co-infection the annual risk may increase up to 10%, and decline in $CD4^+$ T cell count rises the risk of reactivation of TB[4]. HIV infected persons are at risk of TB throughout their course of disease, even after they respond to anti-retroviral therapy (ART). Thus the present study was aimed to determine the occurrence of tuberculosis among HIV infected patients and their $CD4^+$ cell count profile.

2. Materials and methods

The study was conducted in Department of Microbiology, Government Medical College, Aurangabad, Maharashtra, India from January 2007 to December 2008. HIV-seropositive patients (n=263) either hospitalized or reporting to the ART centre for follow up were included in the study. HIV-seropositive status of the patients was confirmed under a predetermined protocol according to WHO/UNAIDS/NACO Strategy III[5]. Initially patients were screened by spot/rapid test kits. Patients with positive result in rapid tests were confirmed by ELISA test before reporting positive. Pre-test and post-test counselling were done and data were collected in a preformed questionnaire.

HIV-seropositive patients were then investigated clinically, radiologically and microbiologically for diagnosis of pulmonary TB as per the revised guidelines issued by WHO[6]. If one sputum smear was positive for acid fast bacilli and laboratory confirmation of HIV infection or strong clinical evidence of HIV infection, it was considered as a case of smear-positive pulmonary TB. While, if two sputum smears were negative for acid fast bacilli but radiographic abnormalities were consistent with active TB and laboratory confirmation of HIV infection or strong clinical evidence of HIV infection along with positive anti-tubercular treatment response or culture positive for *M. tuberculosis*, it was considered as a case of smear-negative pulmonary TB. Symptoms suggestive of pulmonary TB includes persistent cough for 3 weeks or more, usually with expectoration which may be accompanied by one or more of the following symptoms such as weight loss, chest pain, tiredness, shortness of breath, fever, particularly with rise of temperature in the evening. In our study culture was done in both of smear-positive and smear-negative pulmonary TB cases to determine the smear positivity rate.

Two early morning sputum samples were collected from each patient under guidance in a vacant room with open windows. Patients were asked to rinse the mouth before the sample collection. After collection the samples were examined and processed within 4-5 h. All samples were subjected to concentration and decontamination by modified Petroff's method[7]. A smear was made, fixed and stained using Ziehl-Neelsen staining. Microscopic examination of smear was done under oil-immersion field and the positive samples were graded as per Revised National Tuberculosis Control Programme (RNTCP) guidelines[8].

Culture was done by inoculating the concentrated samples on both Lowenstein Jensen medium with and without *p*-nitrobenzoate (PNB), 500 μg/mL and incubated at 37 °C temperature and examined weekly for 8 weeks before reporting negative. Identification of *M. tuberculosis* was done on its basis of cultural characteristics, Ziehl-Neelsen staining, catalase test and nitrate reductase test as mentioned in Table 1 and Figure 1. Positive and negative controls of tubercle and non-tubercle bacilli used were *M. tuberculosis* H37Rv, ATCC 25618 and *Mycobacterium terrae*, ATCC 15755 respectively. For estimation of $CD4^+$ T cell counts by flow cytometry, 3 mL of blood was also collected in an ethylene diamine tetraacetic acid vacutainer from these patients.

Table 1
Characteristic features of *M. tuberculosis*.

Test	Results
Colony morphology	Visible colonies seen after 1-4 weeks of incubation at 37 °C. Dry, greyish white, cauliflower-like growth with no pigment production up to 4 weeks.
Growth on LJ medium with PNB	Growth is inhibited on LJ medium with PNB.
Catalase test	Catalase test is not heat-stable.
Nitrate reductase test	Nitrate reductase test is positive.

Ethical approval was taken from Ethical Committee, Government Medical College, Aurangabad, Maharashtra, India. Prior to counselling a verbal consent was also taken from HIV infected individuals. Anti-tubercular treatment was also started on patient diagnosed as a case of pulmonary TB. Statistical analysis was done by using *Chi*-square test. A P value of <0.01 was considered significant.

Table 2

Distribution of pulmonary TB among HIV-seropositive patients. *n* (%).

TB positive or negative	Distribution
1.TB positive	41 (15.6)
a) Smear-positive pulmonary TB	30 (11.4)
i. Sputum smear positive, culture positive	19 (7.2)
ii. Sputum smear positive, culture negative	11 (4.2)
b) Smear-negative pulmonary TB	11 (4.2)
i. Sputum smear negative, culture positive	9 (3.4)
ii. Sputum smear negative, culture negative (diagnosed by clinico-radiological features and positive ATT response)	2 (0.8)
2.TB negative	222 (84.4)

Percentage in parenthesis represent out of total HIV-seropositive patients. ATT: Antitubercular therapy.

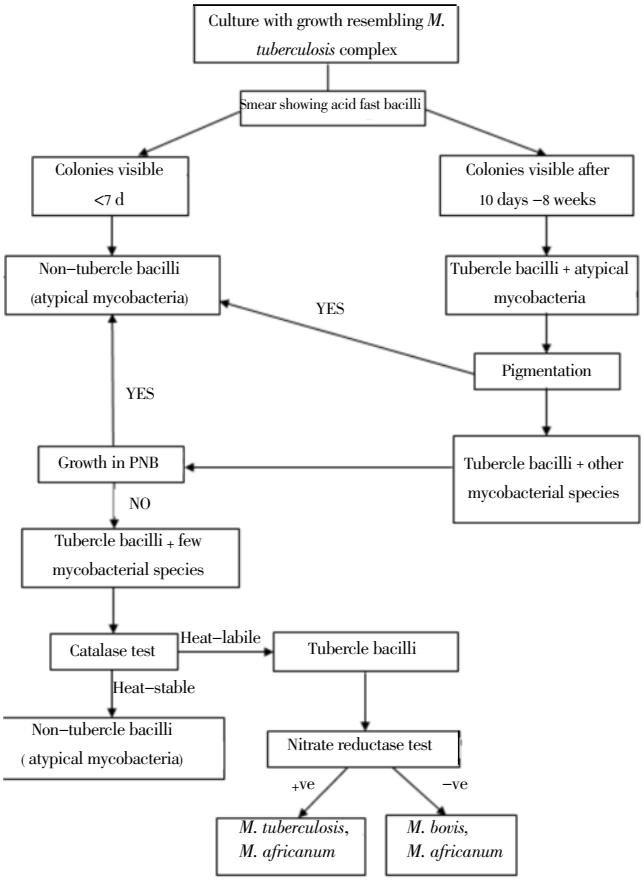


Figure 1. Flowchart for identification of *M. tuberculosis*.

3. Results

A total of 263 HIV-seropositive patients were investigated for pulmonary TB. Out of them 41 (15.6%) of patients were TB positive and 222 (84.4%) were TB negative. Among TB positive patients, smear positive and culture positive patients were 19 (7.2%), while smear negative and culture positive were 9 (3.4%) as documented in Table 2. Two cases were diagnosed on the basis of clinco-radiological features and positive antitubercular therapy response.

Among 263 HIV-seropositive patients, 189 (71.9%) were males and 74 (28.1%) were females. TB co-infection was more common in males (78.1%) compared to females (21.9%). Fever, weight loss and cough were found to be significantly associated with HIV and TB co-infection (Table 3). $CD4^+$ T cell counts for all HIV-seropositive and HIV-related TB patients are documented in Table 4. Mean $CD4^+$ T cell counts for HIV-seropositive and HIV-related TB patients were 156.14 cells/μL and 197.24 cells/μL respectively.

Table 3

Clinical features in HIV-seropositive patients with TB co-infection.

Variables		TB positive (*n*=41)	TB negative (*n*=222)	Total (*n*=263)	Statistical analysis	
					OR (*CI*)	*P* value
Clinical symptoms	Fever	31 (75.6%)	88 (39.6%)	119 (45.2%)	4.72 (2.20-10.11)	0.0004
	Weight loss	27 (65.8%)	55 (24.8%)	82 (31.2%)	5.85 (2.86-11.95)	<0.0001
	Cough	25 (60.9%)	47 (21.2%)	72 (27.4%)	5.81 (2.87-11.77)	<0.0001
	Headache	11 (26.8%)	57 (25.7%)	68 (25.9%)	1.06 (0.49-2.25)	0.88
	Chronic iarrhea	10 (24.3%)	87 (39.2%)	97 (36.9%)	0.50 (0.23-1.07)	0.07
Sex	Male	32 (78.1%)	157 (70.7%)	189 (71.9%)	1.47 (0.66-3.25)	0.34
	Female	9 (21.9%)	65 (29.3%)	74 (28.1%)		

P value <0.01 was considered significant.

Table 4

$CD4^+$ T cell count of HIV-seropositive cases and HIV-related TB cases. *n* (%).

$CD4^+$ T cell count	HIV-seropositive cases	HIV-related TB cases
>200 cells/μL	47 (17.8)	6 (14.6)
<200 cells/μL	216 (82.2)	35 (85.4)
<100 cells/μL	131 (49.8)	18 (43.9)

4. Discussion

TB is a major challenge to the healthcare professionals in developing countries. TB is one of the most common co-infections associated with HIV infection. The incidence of TB has significantly increased worldwide due to emergence of HIV infection. Limited resources to combat the infection and its endemicity are major reasons for increasing incidence of HIV-related TB in developing countries.

In the present study prevalence of HIV-related TB cases was found to be 41 (15.6%). The study showed a higher prevalence of co-infection among male patients 32 (78.1%) as compared to females 9 (21.9%). Bhaumik *et al.* and Giri *et al.* observed an incidence of TB in 17% HIV-seropositive patients while studying the pattern of opportunistic infections in Tripura and Loni respectively[9,10]. Shrivastava and Shrivastava demonstrated a prevalence of 20% among 305 HIV-seropositive patients referred from Integrated Counseling and Treatment Center to TB clinic of Southern India[11]. A high prevalence were observed by Takalkar *et al.*

(52.3%) and Madkar *et al.* (59%)[12,13]. There is a wide variation in the prevalence of TB co-infection among HIV patients in different geographical location of India. This variation may be attributed to a difference in diagnostic capabilities of different center especially for extra-pulmonary TB and lack of standard to compare.

Several studies showed different rates of HIV-related TB prevalence ranging from 7.7%-28% in different countries worldwide[14-17]. WHO estimated a 29% TB-related deaths worldwide related to HIV infection, whereas contribution of TB was 26% of the estimated deaths due to HIV infection[18]. Approximately 1.4 million new cases of HIV-related TB and 0.5 million deaths was also estimated. Sub-Saharan African countries accounted for an approximately 80% of the estimated global burden of HIV-related TB, followed by 10% contribution of Southeast Asian countries. Nearly one-third of the global HIV-related TB burden was accounted by South Africa[19].

Thirty out of 41 HIV-related TB were smear positive showing a sensitivity of 73.1% which was higher as compared to previous data which ranges from 43% to 51%[20]. Out of 11 smear negative TB, nine were culture positive indicating use of culture as an additional measure to increase the sensitivity of smear microscopy for detection of HIV-related TB.

In the present study the commonest clinical feature associated with HIV-related TB cases were fever (75.6%) followed by weight loss (65.8%) and cough (60.9%). Headache (26.8%) and chronic diarrhea (24.3%) were the symptoms not significantly associated with these cases. These findings were comparable with the data regarding the clinical features associated with HIV-related TB cases[21]. Similar to the finding in present study, recent studies have also noted that few proportion of HIV-related TB cases are either asymptomatic or have fewer symptoms, especially in developing countries where the burden of both TB and HIV infection are very high[22,23]. Furthermore, studies have also shown that TB in patients with advanced HIV infection and low $CD4^+$ T cell counts have atypical radiographic features, lesser cavitary pulmonary lesions or normal chest radiograph in comparison to HIV-unrelated TB patients[24-28].

$CD4^+$ T cell count <200 cells/μL was seen in 82.2% of HIV-seropositive patients with 49.8% of the patients had $CD4^+$ T cell count <100 cells/μL. Thirty five (85.4%) HIV-related TB patients had $CD4^+$ T cell count <200 cells/μL. This finding is consistent with study conducted in different regions of country[29,30].

The present study showed a lower prevalence of TB among HIV infected patients as compared to studies conducted in different regions of the country indicating a variation in geographical distribution of the co-infection. This may also be attributed to lack of better inter sectoral coordination between organizations dealing with these two diseases. Hence, it would be advisable that for early diagnosis, proper management and cure of HIV-related TB cases a simultaneous workup is needed for both of these entities. Furthermore the clinical manifestations in HIV-related TB patients are quite variable and lead to difficulties in diagnosis. Thus radiological evaluation along with proper laboratory investigation in all clinically suspected cases of tuberculosis is the key for prompt diagnosis and treatment.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Tuberculosis has been a major public health problem in India and other developing countries. India, being among the 22 high TB burden countries in the world, contributes 20% of global increase annually. TB is one of the most common opportunistic infections as an important cause of mortality among people living with HIV infection. CD4 cell plays an important role in the control of *M. tuberculosis* infection. A decrease in $CD4^+$ T cell count increases risk of reactivation of TB. Thus the present study was aimed to determine number of occurrence of tuberculosis among HIV infected patients and their CD4 cell count profile.

Research frontiers

CD4 cell count plays an important role in the control of *M. tuberculosis* infection as a decrease in counts leads to reactivation of disease. HIV infected persons are at risk of TB throughout the course of disease even after they respond to ART.

Related reports

In the present study presence of HIV related TB cases was found to be 41(15.6%). Srivastava *et al.* demonstrated a presence of 20% among 305 HIV patients infected from Integrated Counseling and Treatment Center to TB clinic in Southern India. Thirty out of 41 HIV related TB with smear positive showing a sensitivity of 73% which was higher as compared to previous data which ranges from 43%-51%.

Innovations & breakthroughs

The study correlation of HIV with tuberculosis is rare in Northern India. Manifestation in HIV related TB patients are quite variable and lead to difficulties in diagnosis. This paper was presented in this issue very nicely.

Applications

Radiological evaluation with proper laboratory diagnosis help in proper diagnosis and treatment.

Peer review

The paper is well written. Authors determined the occurrence of tuberculosis among HIV infected patients and their $CD4^+$ cell count profile. The results are interesting.

References

[1] Central TB Division. TB India 2013. Revised national TB control programme-annual status report. New Delhi: Central TB division, Ministry of Health and Family Welfare, Government of India; 2012. [Online] Available from: http://www.tbcindia.nic.in/pdfs/tb%20india%202013.pdf [Accessed on 2nd April, 2014]

[2] Lüthi B, Diacon AH. [Tuberculosis and HIV-features of the co-infection]. *Ther Umsch* 2011; **68**(7): 389-394. German.

[3] World Health Organization. Global tuberculosis report 2013. Geneva: WHO; 2013, p. 8-10. [Online] Available from: http://www.who.int/tb/publications/global_report/en/ [Accessed on 11th April, 2014]

[4] Piggott DA, Karakousis PC. Timing of antiretroviral therapy for HIV in the setting of TB treatment. *Clin Dev Immunol* 2011; doi: 10.1155/2011/103917.

[5] National AIDS Control Organisation. National strategies and algorithms for HIV testing. In: *Guidelines for HIV testing*. New Delhi: National AIDS Control Organisation; 2007, p. 78-83.

[6] World Health Organization. Improving the diagnosis and treatment of smear-negative pulmonary and extra-pulmonary tuberculosis among adults and adolescents. Recommendations for HIV-prevalent and resource-constrained settings. Geneva: WHO; 2007, p. 5. [Online] Available from: http://www.who.int/hiv/pub/tb/pulmonary/en/ [Accessed on 11th November, 2013]

[7] Buijtels PCAM, Petit PLC. Comparison of NaOH-*N*-acetyl cysteine and sulfuric acid decontamination methods for recovery of mycobacteria from clinical specimens. *J Microbiol Methods* 2005; **62**: 83-88.

[8] Central TB Division. Revised national tuberculosis control programme: laboratory network. Guidelines for quality assurance of smear microscopy for diagnosing tuberculosis. New Delhi: Central TB Division, Ministry of Health and Family Welfare, New Delhi; 2005, p. 40. [Online] Available from: http://tbcindia.nic.in/pdfs/RNTCP%20Lab%20Network%20Guidelines.pdf [Accessed on 23rd March, 2014]

[9] Bhaumik P, Debnath K, Sinha B. Spectrum of opportunistic infections among HIV/AIDS patients of Tripura. *J Indian Acad Clin Med* 2013; **14**(3-4): 218-221.

[10] Giri PA, Deshpande JD, Phalke DB. Prevalence of pulmonary tuberculosis among HIV positive patients attending antiretroviral therapy clinic. *N Am J Med Sci* 2013; **5**(6): 367-370.

[11] Shrivastava SR, Shrivastava PS. HIV-tuberculosis interface: a comparison of collateral prevalence of HIV and tuberculosis in an urban health centre. *Ann Trop Med Public Health* 2013; **6**: 290-296.

[12] Takalkar AA, Saiprasad GS, Prasad VG, Madhekar NS. Study of opportunistic infections in HIV seropositive patients admitted to Community Care Centre (CCC), KIMS Narketpally. *Biomed Res* 2012; **23**(1): 139-142.

[13] Madkar SS, Vankudre AJ, Nilekar SL. Spectrum of opportunistic infections in HIV-AIDS patients. *Indian J Community Health* 2012; **24**(3): 184-187.

[14] Iroezindu MO, Ofondu EO, Hausler H, Wyk BV. Prevalence and risk factors for opportunistic infections in HIV patients receiving antiretroviral therapy in a resource-limited setting in Nigeria. *J AIDS Clini Res* 2013; doi: 10.4172/2155-6113.S3-002

[15] Olaniran O, Hassan-Olajokun RE, Oyovwevotu MA, Agunlejika RA. Prevalence of tuberculosis among HIV/AIDS patients in Obafemi Awolowo University Teaching Hospital Complex Oauthc, ILE -IFE. *Int J Biol Med Res* 2011; **2**(4): 874-877.

[16] Ige OM, Sogaolu OM, Ogunlade OA. Pattern of presentation of tuberculosis and the hospital prevalence of tuberculosis and HIV co-infection in University College Hospital, Ibadan: a review of five years (1998-2002). *Afr J Med Med Sci* 2005; **34**: 329-333.

[17] Getahun H, Gunneberg C, Granich R, Nunn P. HIV Infection-associated tuberculosis: the epidemiology and the response. *Clin Infect Dis* 2010; **50**(Suppl 3): S201-S207.

[18] World Health Organization. Global tuberculosis control: surveillance, planning, financing. WHO report 2008. Geneva: WHO; 2008. [Online] Available from: http://www.who.int/tb/publications/global_report/2008/download_centre/en/ [Accessed on 20th March, 2014]

[19] World Health Organization. Global tuberculosis control: surveillance, planning, financing. WHO report 2007. Geneva: WHO; 2007. [Online] Available from: http://www.who.int/tb/publications/global_report/2007/download_centre/en/ [Accessed on 20th March, 2014]

[20] Cattamanchi A, Dowdy DW, Davis JL, Worodria W, Yoo S, Joloba M, et al. Sensitivity of direct versus concentrated sputum smear microscopy in HIV infected patients suspected of having pulmonary tuberculosis. *BMC Infect Dis* 2009; **9**: 53.

[21] Sterling TR, Pham PA, Chaisson RE. HIV infection-related tuberculosis: clinical manifestations and treatment. *Clin Infect Dis* 2010; **50**(Suppl 3): S223-S230.

[22] Mtei L, Matee M, Herfort O, Bakari M, Horsburgh CR, Waddell R, et al. High rates of clinical and subclinical tuberculosis among HIV-infected ambulatory subjects in Tanzania. *Clin Infect Dis* 2005; **40**: 1500-1507.

[23] Kall MM, Coyne KM, Garrett NJ, Boyd AE, Ashcroft AT, Reeves I, et al. Latent and subclinical tuberculosis in HIV infected patients: a cross-sectional study. *BMC Infect Dis* 2012; **12**: 107.

[24] Geng E, Kreiswirth B, Bruzynski J, Schluger NW. Clinical and radiographic correlates of primary and reactivation tuberculosis: a molecular epidemiology study. *JAMA* 2005; **293**(22): 2740-2745.

[25] Besen A, Staub GJ, da Silva RM. Clinical, radiological, and laboratory characteristics in pulmonary tuberculosis patients: comparative study of HIV-positive and HIV-negative inpatients at a referral hospital. *J Bras Pneumol* 2011; **37**(6): 768-775.

[26] Pepper T, Joseph P, Mwenya C, McKee GS, Haushalter A, Carter A, et al. Normal chest radiography in pulmonary tuberculosis: implications for obtaining respiratory specimen cultures. *Int J Tuberc Lung Dis* 2008; **12**(4): 397-403.

[27] Badie BM, Mostaan M, Izadi M, Alijani MAN, Rasoolinejad M. Comparing radiological features of pulmonary tuberculosis with and without HIV infection. *J AIDS Clini Res* 2012; **3**: 188.

[28] Padyana M, Bhat RV, Dinesha M, Nawaz A. HIV-tuberculosis: a study of chest x-ray patterns in relation to CD4 count. *N Am J Med Sci* 2012; **4**: 221-225.

[29] Agarwal SK, Makhija A, Singh NP, Prabhakar A, Baveja UK. Tuberculosis in HIV/ADIS patients in a tertiary care hospital in Delhi. *Indian J Tuberc* 2003; **50**: 163-165.

[30] Gagiya A, Doctor N, Gamit S, Patel A, Patel K, Patel P. Manifestations of tuberculosis in HIV/AIDS patients and its relationship with CD4 count. *Int J Med Sci Public Health* 2014; **3**: 215-218.