Evaluation of role of interferon gamma release assays in the diagnosis of latent tuberculosis in human immunodeficiency virus-infected patients

Rajender Singh, Nazish Fatima[1], Indu Shukla[1], Mohammed Shameem[2]

Department of Microbiology, HIMS, SRHU, Jollygrant, Dehradun, Uttarakhand, Departments of [1]Microbiology and [2]TB and Chest Disease, JNMC, AMU, Aligarh, Uttar Pradesh, India

Address for correspondence:
Dr. Rajender Singh, Department of Microbiology, HIMS, SRHU, Jollygrant, Dehradun, Uttarakhand, India.
E-mail: panwar.rajendra@gmail.com

Abstract

Introduction: Tuberculosis (TB) is the most common opportunistic infection in human immunodeficiency virus (HIV)-infected individuals. The risk of eventually developing active TB from latent TB infection (LTBI) is about 10% per year in HIV-positive patients in contrast to 10% lifetime risk in HIV-negative patients. Until recently, the tuberculin skin test (TST) was the only tool available for diagnosing LTBI. Interferon-gamma release assays (IGRAs) were recently developed and address many of the limitations of TST test, especially in immunocompromised state. **Aims and Objectives:** (1) To determine the prevalence of latent, active pulmonary, and multidrug-resistant (MDR)-TB among HIV-positive patients in and around Aligarh region; (2) sensitivity and specificity of TST and IGRAs for diagnosis of LTBI in HIV positive patients; and (3) to assess drug resistance and mutational patterns of the clinical isolates of MDR-TB in HIV-TB co-infection. **Materials and Methods:** A cross-sectional study was done on all the patients attended the ICTC centre, JNMC, AMU Aligarh, seropositive for HIV, i.e. 469 (sample size) for the study period of 2 years from October 2015 to October 2017. All 469 HIV-positive patients were screened for latent and active pulmonary TB. Diagnosis of TB (active and latent) was made using clinical, radiological, and microbiological tests. TST and IGRA testing along with CD4 cell counts were also determined. Line probe assay was also done to assess drug resistance and mutational patterns of MDR-TB in HIV patients. **Results:** In our study, prevalence of HIV infection was 5.04%. Sixty-seven (14.28%) patients were as active TB (HIV-TB co-infection), out of which only one patient (1.49%) was confirmed as MDR-TB, 117 (24.94%) were diagnosed as LTBI. It was also evaluated that IGRA has more sensitivity (75%) and specificity (76%) than TST with sensitivity of 71.7% and specificity 66%. **Conclusion:** As there is no gold standard test for latent TB, longitudinal follow-up is needed to interpret discordant test results. There is a need to interpret negative QFT results with caution and to test for latent TB at higher CD4 counts, if possible. Interferon gamma assays can become better tool for diagnosis of especially for latent TB. However, more research study required for establish their relevance, especially in immunocompromised states.

Key words: Active tuberculosis, human immunodeficiency virus-tuberculosis coinfection, interferon-gamma release assays, latent tuberculosis

INTRODUCTION

Tuberculosis (TB) is the most common opportunistic infection in human immunodeficiency virus (HIV)-infected individuals and is responsible for one-third of HIV-associated deaths.[1] In India alone, 2.5 million people are currently infected with HIV, of whom 40% are also coinfected with TB.[2] HIV increases the risk of re-activation of latent TB infection (LTBI) from approximately 0.04 cases per 100 person years[2] to as high as more than 10/100 person years.[3-5] Until recently, the tuberculin skin testing (TST) was the only tool available for diagnosing LTBI. The risk of developing active TB in people with a positive TST is well defined,[6,7] and there is strong evidence in people with and without HIV coinfection that isoniazid prevention therapy (IPT) reduces this risk.[8] However, false-positive TST results can occur among persons who have been given the Bacille Calmette–Guérin (BCG) vaccine or who have been exposed to nontuberculous mycobacteria and false-negative results can occur in persons with impaired cellular immunity.[9] Interferon-gamma release assays (IGRAs) were recently developed and address many of these limitations. Previous systematic reviews have shown that, compared with the TST, IGRAs have a higher specificity in low TB incidence settings, correlate better with surrogate measures of *Mycobacterium tuberculosis* (MTB) exposure, and have no cross reactivity with the BCG vaccine.[10] However, these reviews did not specifically assess the performance of IGRAs in HIV-infected individuals. Hence, the aim of our study is also focused on the sensitivity and specificity of IGRAs for diagnosing latent TB in high prevalent areas like in our country as most of study done on low TB endemic countries and prevents conversion of latent to active TB using IPT. With the following background, we undertook the present study to determine the prevalence of latent, active pulmonary, and multidrug-resistant (MDR) TB among HIV-positive patients in and around Aligarh region, to determine the sensitivity and specificity of TST and IGRAs for the diagnosis of LTBI in HIV-positive patients.

MATERIALS AND METHODS

The present study was cross-sectional prospective study conducted in the ICTC center, Department of Microbiology, J. N. Medical College and Hospital, AMU, Aligarh, for period of 2 years from October 2015 to October 2017. Written and informed consent was taken from all 469 patients (convenience sampling methods) who were HIV seropositive confirmed in ICTC, Department of Microbiology, JNMC, AMU, Aligarh. Along with TST (Mantoux), blood samples were taken from all 469 study participants for IGRAs testing. Sputum of symptomatic TB-suspected HIV-positive patient was sent for sputum microscopy, culture, drug sensitivity testing done in Culture and DST Laboratory (RNTCP certified), Department of Microbiology, J. N. Medical College AMU, Aligarh. Line probe assay (LPA) was also done to assess drug resistance and mutational patterns of MDR-TB in HIV patients. A detailed clinical history was recorded from each patient after taking proper written consent from the patients. Proper ethical clearance was obtained through the Institutional ethical committee of JNMC, AMU Aligarh.

SPSS software version was used for all data analysis and Chi-square test had been performed for sensitivity and specificity for IGRAs and TST (Mantoux) test (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY).

Criteria for selection of patients: We divided our study groups on the basis of the following mentioned definitions. Active TB diagnosis: The patient was diagnosed with active TB when the presence of MTB in a clinical sample was laboratory confirmed by acid-fast bacilli on microscopy, by growth in culture or by a nucleic acid amplification test. LTBI diagnosis: It was given to patients with a positive IGRA (QFT) and no indication of current or previous active TB. Participants with a prior active TB, which could explain a positive IGRA and with no signs or symptoms of active TB at baseline, were classified as prior active TB. Finally, those that had received preventive TB therapy before entering the study were defined as prior LTBI. No TB: Patients with a negative baseline IGRA and no previous or current TB infection were classified as no TB.[11]

Cases included in the study were 469 HIV-confirmed patients with clinically suspected active pulmonary TB cases, asymptomatic patients with history of risk exposure, and suspected MDR-TB cases. Control group included fifty healthy individuals, BCG vaccinated without HIV, without TB, and with no personal/family history of TB or no risk factor of exposure/contact. All the cases of extrapulmonary TB, end-stage renal disease, leukemia/lymphoma, pregnant, and lactating patients and who had TST received in the past 16 months of their sample collection (for TST and IGRA) were excluded from the study.

All the patients reporting at ICTC, Department of Microbiology, JNMC, for the HIV status testing were

assessed by three ERS (ELISA, Rapid, and Simple) tests as recommended by the National AIDS Control Organization, Ministry of Health and Family Welfare, and Government of India (Guidelines of HIV testing, 2017). The CD4 cell counts of all the patients were estimated by Flow-Cytometry using Partec CyFlow Counter (Germany) done in ART centre. Quantiferon TB Gold (QFT-G, manufactured by Cellestis Limited, Carnegie, Victoria, Australia) test was performed on the study subjects according to the provided kit insert. TST was performed in all the study subjects according to standard protocol.[12]

Sputum of all HIV-positive patients was stained by Ziehl–Neelsen staining and Fluorescent staining. Culture was done with Modified Lownstein-Jensen's medium along with biochemical identification such aslike Niacin test, catalase test, and Growth on Para Nitro Benzoic acid (PNB est) for detection of MTB. 1% Proportional method was followed for determining drug susceptibility of mycobacteria for anti-TB drugs, namely, rifampicin, isoniazid, ethambutol, and streptomycin. LPA (GenoMTBDR*plus*VER 2.0 by HainLifescience, Germany) and GeneXpert MTB/RIF (or CBNAAT) test were performed (according to manufacturer's instruction) to determine the resistance pattern of anti-TB drugs in study group.

RESULTS

Out of the 9293 blood samples collected from patients suspected of HIV infection at the ICTC, Aligarh, 469 (5.04%) confirmed HIV positive during the study period. Two hundred and eighty-nine (61.62%) were males and 180 (38.38) were females. Majority of 168 (35.82%) patients were in the age group of 25–34 years and 161 (34.32%) in age group of 35–49 years. Sexual transmission (61.19%) followed by blood transfusion (12.15%); use of infected syringes and needles (6.82%); by vertical transmission, i.e., (3.41%) were observed. On the basis of history of exposure/contact, clinical, and radiological grounds, 184 HIV-positive patients were suspected to be suffering from TB. One hundred and seventy-six (95.65%) were having a history of TB contact and 52 (28.26%) did not have the history of BCG vaccination. Abnormal chest X-ray findings were seen in 59 (32.06%) out of 184 suspect TB cases [Table 1]. Table 2 depicts comparative results of various tests performed to reach at the diagnosis of active TB among the study group from the total suspected pulmonary TB cases (*n* - 184) according to which 32 (17.39%) were positive by microscopy, 59 (32.06%) had suggestive chest

X-ray finding, 33 (17.93%) were culture positive from sputum samples, along with adjunctives tests such as TST and IGRA with 9.23% and 10.32% positivity, respectively. We concluded 67 out of 184 patients as having active TB (case definition, CDC, 2013) among HIV patients (HIV-TB coinfection group). One MDR-TB patient (1.49%) was confirmed from 67 suspected MDR-TB (Cat. 7 according to PMDT criteria) patients in HIV-TB coinfection cases after performing LPA and CBNAAT test. LPA showed mutation at MUT 3 band in *rpo B* gene and MUT 1 band in *kat* gene, respectively, was found. DST (drug susceptibility test) performed by 1% proportion method for the above patient showed resistance to all first-line anti-TB drugs. Out of 469 HIV-positive patients, 117 were diagnosed as LTBI on the basis of IGRA and/or TST positivity with no current and previous diagnosed TB as defined in classification of the study group [Table 3]. The table also shows more IGRA positivity (24.94%) than TST (22.38%). Eighteen (3.83%) patients out of total 469 study participants (HIV positive) showed indeterminate results for IGRA test. On studying the demographic profile of LTBI patients, being underweight was the most common risk factor, followed by history of close contact [Table 4]. IGRA has more sensitivity (75%) and specificity (76%) than TST with a sensitivity of 71.7% and specificity 66% in our study. We estimated good concordance (as К =0.892) between the two tests for diagnosis of LTBI in HIV-positive patients or comparison of baseline CD4 levels with

Table 1: Demographic profile of human immunodeficiency virus positive cases suspected of pulmonary tuberculosis (*n*=184)

Clinical presentation	Frequency (%)
Fever	70 (38.04)
Cough	66 (35.86)
Weight loss	55 (29.89)
Breathing difficulty	48 (26.08)
Abnormal chest X-ray	59 (32.06)
Absence of h/o BCG vaccination	52 (28.26)
History of TB contact	176 (95.65)

TB=Tuberculosis; BCG=Bacille Calmette-Guérin

Table 2: Comparative results of various tests among human immunodeficiency virus-positive cases suspected of tuberculosis (*n*=184)

Tests	Positive (%)
Microscopy (ZN and fluoroscent staining)	32 (17.39)
Chest X-ray findings	59 (32.06)
Culture	33 (17.93)
IGRA	19 (10.32)
TST	17 (9.23)

ZN=Ziehl-Neelsen; IGRA=Interferon gamma release assays; TST=Tuberculin skin test

Table 3: Results of tuberculin skin test and interferon gamma release assays in the screening latent tuberculosis infection cases among human immunodeficiency virus positive patients

Test Performed	Total	Positive in active TB (%)	Positive in suspected LTBI (%)
TST (*n*=469)			
Positive	122 (26.01)	17 (3.6)	105 (22.38)
Negative	347 (73.98)	132 (28.14)	215 (45.84)
IGRA (*n*=469)			
Positive	136 (28.99)	19 (4.05)	117 (24.94)
Negative	315 (67.16)	114 (24.30)	201 (42.85)
Indeterminate	18 (3.83)	0 (0)	18 (3.83)
TST+ and IGRA+			102 (21.74)
TST− and IGRA−			329 (70.14)

LTBI=Latent tuberculosis infection; IGRA=Interferon gamma release assays; TST=Tuberculin skin test; TB=Tuberculosis

Table 4: Demographic profile of latent tuberculosis infection patients in our study group (*n*=117)

Risk factors	Present	Absent/unknown	Total
History of close contacts/family history	43 (36.75)	74 (63.24)	117
Silicosis	7 (5.98)	110 (94.01)	117
Fibronodular disease on chest X-ray	10 (8.54)	107 (91.4)	117
Diabetes mellitus	19 (16.23)	98 (83.76)	117
Smoking	33 (28.20)	84 (71.79)	117
Use of corticosteroid	9 (7.69)	108 (92.30)	117
Underweight	71 (60.68)	46 (39.31)	117
H/o BCG vaccination	105 (89.74)	12 (10.25)	117

LTBI=Latent tuberculosis infection; BCG=Bacille Calmette-Guérin

TST and IGRA results in LTBI cases, TST and IGRA results were less positive in patients with baseline CD4 count level <200/μl (36.19% and 28.20%, respectively) as compared to those cases who had CD4 count >200/μl (63.8% and 71.79%, respectively). It also shows more indeterminate result (66.66%) in IGRA test among LTBI patients with CD4 count level <200/μl, while less indeterminate result (33.33%) in IGRA test among LTBI patients with CD4 count level <200/μl.

DISCUSSION

LTBI is a subclinical infection with MTB without clinical, bacteriological, or radiological evidence of the disease. Effective treatment of latent TB in HIV-infected individuals offers an opportunity to prevent significant morbidity and mortality. However, accurate diagnosis of LTBI in HIV-infected individuals is challenging. Individuals with advanced immunosuppression are those most at risk of re-activation of LTBI and are a key priority for diagnosis and treatment. Until recently, the TST was the only tool available for diagnosing LTBI. Of the 9293 patients attending the ICTC centre, Department of Microbiology, JNMC and Hospital referred from various outpatient departments during the period of October 2015 to September 2017 screened for the HIV status, a total of 469 patients turned HIV positive indicating a prevalence of HIV infection in and around Aligarh region to be 5.04%. Out of 184 suspected patients, sputum microscopy was found positive in 32 patients (17.39%), while Rao *et al*. found 27.45% positivity.[13] In our study, sputum culture positivity was seen around 17.93% among the 184 suspected pulmonary TB cases which is slightly higher to another study done by Isaakidis *et al*. showed 12% sputum culture positivity.[14] Only one patient, i.e. 1.49% was confirmed MDR-TB positive by LPA and CBNAAT (nucleic acid amplification test). Isaakidis *et al*. estimated the much higher MDR-TB, i.e. 25% in Mumbai ART centre.[14]

The dual burden of HIV and TB/drug resistant-TB in India is significantly high with a combined rate of 5.2%, ranging from 0.4% to 28.8% in various studies, with increasing trends noted in states having a higher burden of HIV infection.[15-18] Swaminathan *et al*. estimated 4.2% MDR-TB in HIV patients in their study.[18]

Out of 469 HIV-positive patients, 117 (24.94%) were diagnosed as LTBI on the basis of IGRA and/or TST positivity with no current or previously diagnosed TB as defined in classification of the study group, with more IGRA positivity (24.94%) than TST (22.38%). In our study, 18 (66.66%) cases showed indeterminate results with IGRA test. When used to diagnose LTBI, rates of indeterminate results with the QFT-IT have ranged from <1 to 11% as reviewed by Cattamanchi *et al*. Cheallaigh *et al*. found a statistically significantly lower rate of indeterminate results with IGRA test. 2% of QFT-IT results were indeterminate, compared to 7% of T-SPOT. TB results.[19] Talati *et al*. in their study observed indeterminate IGRA results as much as 14% of patients with T-SPOT and 18% of patients with QFT-3G.[20] Other studies in HIV-seropositive individuals have also found that around 10% of patients have an indeterminate result.[21]

Currently, the WHO strongly recommends at least 6 months of isoniazid preventive therapy (6INH, 9INH, and 12INH) for HIV-infected patients and suggests a continuous 36INH regimen as the surrogate treatment, especially in regions with high HIV and TB prevalence. People who have been recently infected with TB have a high risk of reactivation, and those who are close contacts

of people with active TB have a high possibility of having been infected within the past 2 years.[22] Studies have reported that the reactivation rate of TB is 15 times greater for those who have been recently infected (<2 years).[23,24] On comparison of sensitivity and specificity of IGRA and TST for LTBI in HIV infection in our study, it seems that IGRA has more sensitivity (75%) and specificity (76%) than TST with sensitivity of 71.7% and specificity 66% among LTBI in HIV patients [Table 5]. While in the control groups (HIV negative with no TB with BCG vaccinated), sensitivity and specificity for IGRA were 6% and 94%; and 56% and 44% for TST. Most studies tells about sensitivity of IGRAs test on active TB which is an immunocompromised state itself thus underestimated sensitivity in LTBI patients for both TST and IGRAs test were obtained in these studies. Meta-analysis done by Pai *et al*. performed in low- and high TB-burden countries and found a pooled sensitivity of 78% (95% confidence interval, 73%–82%) for QuantiFERON-TB Gold.[25] In 216 Japanese nursing students without any TB exposure history and with BCG vaccination, specificity for TST, and Quantiferon were 64.6% and 98.1%, respectively.[26] In a Korean study, QuantiFERON®-TB was 96% specific and TST was 49% specific.[27] Another study found that QuantiFERON®-TB was negative in all fifty healthy medical students (74% BCG vaccinated).[28] TST was positive in 36% of them. All studies indicates better IGRAs specificity than TST for diagnosis of LTBI and despite the limited data, there may have a significant role of IGRAs in identifying TB infection either active or latent in immunocompromised state.[29-32]

In our study, good concordance was observed between IGRA and TST with good strength of agreement of K = 0.892 for the diagnosis of LTBI

in HIV patients [Table 6]. In studies from Africa, concordance between TST and IGRAs has been fair.[33] Other studies from countries with a low prevalence of TB have found poor concordance between TST and QFT-3G.[34]

On comparing baseline CD4 levels with TST and IGRA levels in our study, we found that TST and IGRA were more positive in those patients with baseline CD-4 count level >200/μL i.e., 67 (63.80%) and 84 (71.79%) patients, respectively, while 38 (36.19%) and 33 (28.20%) patients were positive for IGRA and TST with baseline line CD4 count levels <200/μL respectively. We found more indeterminate IGRA results 12 (66.66%) in patients with CD-4 count <200/μL. Studies have shown that TST is more reliable at CD 4 counts >100/μL.[35] Talati *et al*. wanted to define a similar CD4 count cutoff for IGRAs. However, limiting analysis to patients with higher CD4 counts did not improve concordance in their study population. They found that an indeterminate results was associated with a CD4 count of <200/μL.[20]

Limitation of the study

The sensitivity and specificity of the IGRA and TST for the diagnosis of LTBI in immunocompromised state are not so reliable due to lack of gold standard test and also require more follow-up studies in future which was not done in our study. Further observational and follow-up studies required in larger sample size to determine the sensitivity and specificity of IGRAs and their comparison with TST.

CONCLUSION

The lack of a gold standard makes it difficult to determine which diagnostic test is more sensitive or specific for LTBI. Given the result of our study and the limited data currently available in the literature, it is unclear if IGRAs can be used for the diagnosis of LTBI in HIV-infected individuals. Some authors have suggested a combined approach of TST and IGRA.[36] We feel this would lead to confusion for physicians when there are discordant results. Question that remains are whether in high incidence countries there is a better concordance between IGRAs and TST; can we define a lower interferon-gamma cuff value for patients who are immunocompromised or have HIV; is there a CD4 cutoff value below which these test are no longer useful; and is IGRA positivity an accurate predictor of progression to active TB. Accurate diagnosis of LTBI in HIV-infected individuals is challenging.

Some studies emphasized that CD4 count level had an impact over the result of IGRAs test.[33, 37-39]

Table 5: Comparative evaluation of tuberculin skin test and interferon gamma release assays in respect to sensitivity, specificity among latent tuberculosis infection in study group (*n*=117)

Test Performed	TST (%)	IGRA (%)
Sensitivity	71.7	75
Specificity	66	76

IGRA=Interferon gamma release assays; TST=Tuberculin skin test

Table 6: Degree of concordance between tuberculin skin test and interferon gamma release assays

Test Performed	IGRA+ (*n*=117)	IGRA−
TST+ (*n*=105)	102	3
TST−	15	329

K=0.892. IGRA=Interferon gamma release assays; TST=Tuberculin skin test

Patients with indeterminate results will require retesting, testing with a larger volume of blood (in order to obtain more), or use of a different diagnostic test. IGRA test can be used in the community as a routine to prevent the conversion of latent to active TB by chemoprophylaxis but more beneficial in active TB cases however more studies require in latent TB cases especially in immunocompromised state in community settings.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Raviglione MC, Harries AD, Msiska R, Wilkinson D, Nunn P. Tuberculosis and HIV: Current status in Africa. AIDS 1997;11 Suppl B: S115-23.
2. Horsburgh CR Jr., O'Donnell M, Chamblee S, Moreland JL, Johnson J, Marsh BJ, *et al*. Revisiting rates of reactivation tuberculosis: A population-based approach. Am J Respir Crit Care Med 2010;182:420-5.
3. 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;320:545-50.
4. Holmes CB, Wood R, Badri M, Zilber S, Wang B, Maartens G, *et al*. CD4 decline and incidence of opportunistic infections in Cape Town, South Africa: Implications for prophylaxis and treatment. J Acquir Immune Defic Syndr 2006;42:464-9.
5. Komati S, Shaw PA, Stubbs N, Mathibedi MJ, Malan L, Sangweni P, *et al*. Tuberculosis risk factors and mortality for HIV-infected persons receiving antiretroviral therapy in South Africa. AIDS 2010;24:1849-55.
6. Comstock GW. How much isoniazid is needed for prevention of tuberculosis among immunocompetent adults?. Int J Tuberc Lung Dis 1999;3:847-50.
7. Golub JE, Pronyk P, Mohapi L, Thsabangu N, Moshabela M, Struthers H, *et al*. Isoniazid preventive therapy, HAART and tuberculosis risk in HIV-infected adults in South Africa: A prospective cohort. AIDS 2009;23:631-6.
8. Smieja MJ, Marchetti CA, Cook DJ, Smaill FM. Isoniazid for preventing tuberculosis in non-HIV infected persons. Cochrane Database Syst Rev 2000;1999 (2):CD001363.
9. Jasmer RM, Nahid P, Hopewell PC. Clinical practice. Latent tuberculosis infection. N Engl J Med 2002;347:1860-6.
10. Pai M, Riley LW, Colford JM Jr. Interferon-γ assays in the immunodiagnosis of tuberculosis: A systematic review. Lancet Infect Dis 2004;4:761-76.
11. Pullar ND, Steinum H, Bruun JN, Dyrhol-Riise AM. HIV patients with latent tuberculosis living in a low-endemic country do not develop active disease during a 2 year follow-up; A Norwegian prospective multicenter study. BMC Infect Dis 2014;14:667.
12. Centers for Disease Control and Prevention. Targeted tuberculin testing and treatment of latent tuberculosis infection. MMWR 2000;49:1-54.
13. Rao GC, Rajeswari GK, Kalyani N, Kalyani JS, Reddy MS, Vasudha K. Study of clinical, microbiological and radiological correlation of TB: HIV co-infection. J Evol Med Dent Sci 2015;4:905-14.
14. Isaakidis P, Das M, Kumar AM, Peskett C, Khetarpal M, Bamne A, *et al*. Alarming levels of drug-resistant tuberculosis in HIV-infected patients in metropolitan Mumbai, India. PLoS One 2014;9:e110461.
15. Williams BG, Granich R, Chauhan LS, Dharmshaktu NS, Dye C. The impact of HIV/AIDS on the control of tuberculosis in India. Proc Natl Acad Sci U S A 2005;102:9619-24.
16. Paramasivan CN, Venkataraman P. Drug resistance in tuberculosis in India. Indian J Med Res 2004;120:377-86.
17. Deivanayagam CN, Rajasekaran S, Venkatesan R, Mahilmaran A, Ahmed PR, Annadurai S, *et al*. Prevalence of acquired MDR-TB and HIV co-infection. Indian J Chest Dis Allied Sci 2002;44:237-42.
18. Swaminathan S, Paramasivan CN, Ponnuraja C, Iliayas S, Rajasekaran S, Narayanan PR. Anti-tuberculosis drug resistance in patients with HIV and tuberculosis in South India. Int J Tuberc Lung Dis 2005;9:896-900.
19. Cheallaigh CN, Fitzgerald I, Grace J, Singh GJ, El-Eraki N, Gibbons N, *et al*. Interferon gamma release assays for the diagnosis of latent TB infection in HIV-infected individuals in a low TB burden country. PLoS One 2013;8:e53330.
20. Talati NJ, Seybold U, Humphrey B, Aina A, Tapia J, Weinfurter P, *et al*. Poor concordance between interferon-gamma release assays and tuberculin skin tests in diagnosis of latent tuberculosis infection among HIV-infected individuals. BMC Infect Dis 2009;9:15.
21. Karam F, Mbow F, Fletcher H, Senghor CS, Coulibaly KD, LeFevre AM, *et al*. Sensitivity of IFN-gamma release assay to detect latent tuberculosis infection is retained in HIV-infected patients but dependent on HIV/AIDS progression. PLoS One 2008;3:e1441.
22. Parekh MJ, Schluger NW. Treatment of latent tuberculosis infection. Ther Adv Respir Dis 2013;7:351-6.
23. Landry J, Menzies D. Preventive chemotherapy. Where has it got us? Where to go next? Int J Tuberc Lung Dis 2008;12:1352-64.
24. Sutherland I. Recent studies in the epidemiology of tuberculosis, based on the risk of being infected with tubercle bacilli. Adv Tuberc Res 1976;19:1-63.
25. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: An update. Ann Intern Med 2008;149:177-84.
26. Mori T, Sakatani M, Yamagishi F, Takashima T, Kawabe Y, Nagao K, *et al*. Specific detection of tuberculosis infection: An interferon-γ–based assay using new antigens. Am J Respir Crit Care Med 2004;170:59-64.
27. Kang YA, Lee HW, Yoon HI, Cho B, Han SK, Shim YS, *et al*. Discrepancy between the tuberculin skin test and the whole-blood interferon gamma assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. JAMA 2005;293:2756-61.
28. Kobashi Y, Obase Y, Fukuda M, Yoshida K, Miyashita N, Oka M. Clinical reevaluation of the QuantiFERON TB-2G test as a diagnostic method for differentiating active tuberculosis from nontuberculous mycobacteriosis. Clin Infect Dis 2006;43:1540-6.
29. Chapman AL, Munkanta M, Wilkinson KA, Pathan AA, Ewer K, Ayles H, *et al*. Rapid detection of active and latent tuberculosis infection in HIV-positive individuals by enumeration of *Mycobacterium tuberculosis*-specific T cells. AIDS 2002;16:2285-93.
30. Pathan AA, Wilkinson KA, Klenerman P, McShane H, Davidson RN, Pasvol G, *et al*. Direct *ex vivo* analysis of antigen-specific IFN-γ-secreting CD4 T cells in *Mycobacterium tuberculosis*-infected individuals: Associations with clinical disease state and effect of treatment. J Immunol 2001;167:5217-25.
31. Brock I, Weldingh K, Leyten EM, Arend SM, Ravn P, Andersen P. Specific T-cell epitopes for immunoassay-based diagnosis of *Mycobacterium tuberculosis* infection. J Clin Microbiol 2004;42:2379-87.

32. Dheda K, van Zyl Smit R, Badri M, Pai M. T-cell interferon-γ release assays for the rapid immunodiagnosis of tuberculosis: Clinical utility in high-burden vs. low-burden settings. Curr Opin Pulmon Med 2009;15:188-200.

33. Rangaka MX, Wilkinson KA, Seldon R, Van Cutsem G, Meintjes GA, Morroni C, *et al*. Effect of HIV-1 infection on T-cell–based and skin test detection of tuberculosis infection. Am J Respir Crit Care Med 2007;175:514-20.

34. Jones S, de Gijsel D, Wallach FR, Gurtman AC, Shi Q, Sacks H. Utility of quantiFERON-TB gold in-tube testing for latent TB infection in HIV-infected individuals. Int J Tuberc Lung Dis 2007;11:1190-5.

35. Fisk TL, Hon HM, Lennox JL, Reyn CF, Horsburgh CR Jr. Detection of latent tuberculosis among HIV-infected patients after initiation of highly active antiretroviral therapy. AIDS 2003;17:1102-4.

36. Luetkemeyer AF, Charlebois ED, Flores LL, Bangsberg DR, Deeks SG, Martin JN, *et al*. Comparison of an interferon-gamma release assay with tuberculin skin testing in HIV-infected individuals. Am J Respir Crit Care Med 2007;175:737-42.

37. Kabeer BS, Sikhamani R, Swaminathan S, Perumal V, Paramasivam P, Raja A. Role of interferon gamma release assay in active TB diagnosis among HIV infected individuals. PloS one 2009;4:e5718.

38. Balcells ME, Pérez CM, Chanqueo L, Lasso M, Villanueva M, Espinoza M, *et al*. A comparative study of two different methods for the detection of latent tuberculosis in HIV-positive individuals in Chile. Int J Infect Dis 2008;12:645-52.

39. Raby E, Moyo M, Devendra A, Banda J, De Haas P, Ayles H, *et al*. The effects of HIV on the sensitivity of a whole blood IFN-gamma release assay in Zambian adults with active tuberculosis. PLoS One 2008;3:e2489.