EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 4
339-342
Year: 2017

DIAGNOSIS OF TUBERCULOSIS IN PLHIV PATIENTS BY USING THE XPERT® MTB/RIF ASSAY: A CLINICAL STUDY

Dr. Atul Kharate[1], Dr. Vijay Agrwal[2], Dr. Vijay Chhajlani[3], Dr. Jyoti Khurana*[4], Neha Dawar[5], Amit Verma[6], Dhwani Patel[7] and Nagmi Aliya Ansari[8]

[1]MBBS, DTCD, MCAI, STO, Madhya Pradesh Directorate of Health Services, MP.
[2]MBBS, DTCT, Superintendent, MRTB Hospital, Chest Centre Indore, M.P.
[3]MBBS, DTO, Indore M.P.
[4]PhD, MSc, Applied Microbiology, I/C CBNAAT IRL MRTB Hospital, Chest Centre Indore, MP.
[5]M.Sc., EQA Microbiologist, IRLMRTB Hospital, Chest Centre Indore M.P.
[6]B.Sc.Microbiology, LT, IRLMRTB Hospital, Chest Centre Indore M.P.
[7]M.Sc Biotechnology, LT, IRLMRTB Hospital, Chest Centre Indore M.P.
[8]M.Sc. Microbiology, DMLT,LT, IRL MRTB Hospital, Chest Centre Indore M.P.

***Corresponding Author: Dr. Jyoti Khurana**
PhD, MSc, Applied Microbiology, I/C CBNAAT IRL MRTB Hospital, Chest Centre Indore, MP.

Article Received on 23/01/2017 **Article Revised on 13/02/2017** **Article Accepted on 05/03/2017**

ABSTRACT

Objective: Tuberculosis (TB) is one of the commonest opportunistic infection and the leading cause of death in HIV patients in developing countries. HIV infection is a well recognised risk factor for both activation of initial infection and reactivation of latent infection. This study was done to find out the co-prevalence and the trend of HIV infection among tuberculosis patients and to determine the prevalence of MDR Tuberculosis in HIV positive patients using Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, United States). **Materials and Methods:** The sputum samples are received from Indore M.P, India. Sputum samples of the patients with symptoms suggestive of pulmonary tuberculosis including both new cases and on treatment are received from the various DMCs and hospitals. Sputum specimen was processed for TB detection by Cartridge Based Nucleic Acid Amplification testing (CB-NAAT) using Xpert MTB/RiF assay technology. **Results:** The study period is from 2015 to August 2016. A total of 4595 patients with symptoms suggestive of pulmonary tuberculosis including both new cases and on treatment. Out of these patients 1087 were reported MTB detected. Out of these 4595 patients 953 were reported HIV positive and out of 953 patients 127(13.3%) were reported positive for (HIV +TUBERCULOSIS). Of these 127 cases 118(92.9%) cases were sensitive to rifampicin (RIF) and 9(7%) cases were showing resistance to rifampicin (RIF) Drug. **Conclusion:** Co-existence of HIV and tuberculosis is high and there is high Prevalence of MDR tuberculosis in HIV patients.

KEYWORDS: Co-infection, Cartridge based nucleic acid amplification testing, Human immunodeficiency virus (HIV), Pulmonary, Rifampicin, Tuberculosis.

INTRODUCTION

Human immunodeficiency virus (HIV) associated tuberculosis (TB) remains a major global public health challenge, with an estimated 1.4 million patients worldwide. Co-infection with HIV leads to challenges in both the diagnosis and treatment of tuberculosis. Further, there has been an increase ingrates of drug resistant tuberculosis, including multi-drug (MDR-TB) and extensively drug resistant TB (XDRTB), which are difficult to treat and contribute to increased mortality. Because of the poor performance of sputum smear microscopy in HIV-infected patients, newer diagnostic tests are urgently required that are not only sensitive and specific but easy to use in remote and resource-constrained settings.[1]

In the global tuberculosis report (2015), WHO reported that in 2014, Worldwide, 9.6 million people are estimated to have fallen ill with TB in 2014: 5.4 million men, 3.2 million women and 1.0 million children. Globally, 12% of the 9.6 million new TB cases in 2014 were HIV-positive. 9 million people developed TB, including 1.1 million cases among people who were HIV positive. At the same time, global burden of multidrug-resistant TB (MDR-TB) was estimated to be 480,000 cases leading to estimated 210,000 deaths.[2]

HIV and *Mycobacterium tuberculosis* have a synergistic interaction; each accentuates progression of the other. Clinical presentation of TB in early HIV infection

resembles that observed in immunocompetent persons. In late HIV infection, however, TB is often atypical in presentation, frequently causing extra pulmonary disease. These factors coupled with low sputum smear-positivity, often result in a delayed diagnosis.[3]

The incidence of tuberculosis (TB) is currently increasing in HIV-infected patients living in Africa and Asia, where TB endemicity is high, reflecting the susceptibility of this group of patients to mycobacteria belonging to the TB group. In this population, extension of multiple resistance to anti-tuberculous drugs is also a matter of anxiety. HIV-induced immunosuppression modifies the clinical presentation of TB, resulting in atypical signs and symptoms and more frequent extrapulmonary dissemination.[4]

Sputum smear microscopy is inefficient due to its variable sensitivity particularly in patients with sputum smear-negative and/or extrapulmonary disease, and drug-resistant TB. Latest generation liquid culture diagnostics and molecular line probe assays are costly and cannot be performed in resource-limited settings, due to need for biosafety measures and specialised staff.[5] Besides technical expertise and biosafety concerns, Lowenstein-Jensen (LJ) method, "the gold standard test", takes several weeks to produce result causing delayed onset of treatment.[6] In December 2010, WHO recommended use of a new Cartridge Based Nucleic Acid Amplification test (CB-NAAT), named GeneXpert system.[2] The Xpert MTB/RIF assay employs five distinct molecular beacons (nucleic acid probes), each labelled with a differentially coloured fluorophore and responding to a specific nucleic acid sequence within the *rpoB* gene of *M. tuberculosis*.[5,7] It can detect TB along with rifampicin resistance in less than two hours, directly from untreated sputum samples.[7,8]

Revised National TB Control Programme (RNTCP) is also currently using Xpert MTB/RIF to diagnose pulmonary TB, paediatric TB, extrapulmonary TB and rifampicin resistance and Multi Drug Resistance Tuberculosis in high risk populations like HIV positive as recommended by WHO under 2013 policy recommendations.[2,7,8]

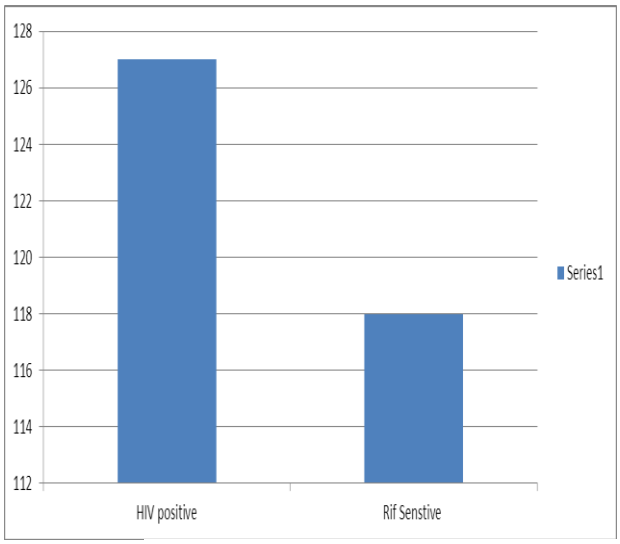
MATERIALS AND METHODS

This study is done at IRL MRTB Hospital M.P., India. The sputum samples are received from Indore M.P. The study period is from 2015 to August 2016. A total of 4595 patients with symptoms suggestive of pulmonary tuberculosis including both new cases and on treatment. All specimens were collected in pre-sterilized falcon tubes with three layer packing system, after through rinsing of the oral cavity with clean water. Samples along with prescribed Performa containing details of patients like Name, Address, Age, Sex, HIV status, and Name of the referring centre was received in the Microbiology Laboratory.

TB detection was done by Xpert MTB/RiF assay, made by Cepheid-Sunnyvale-USA. Sputum specimens were processed according to the GeneXpert Dx system operator manual given by Central TB division, Government of India, Guidance document for use of cartridge based nucleic acid amplification test (CB-NAAT) under RNTCP.[8,9] The assay is designed for extraction, amplification and identification of *rpoB*gene of *M. tuberculosis* as it accounts for more than 95% of mutations associated with rifampicin resistance), ensuring high degree of specificity by use of three specific primers and 5 unique molecular probes.[10] The number of positive beacons, their detection timing (indicated by rise of fluorescent signal above a predetermined baseline cycle threshold) and the results of sample processing controls, allow the test to distinguish among the following results: no TB; TB detected, rifampicin resistance detected; TB detected, no rifampicin resistance detected; TB detected, rifampicin resistance indeterminate; and an invalid result.[5,7] Xpert MTB/RIF cartridge is a disposable, single self-enclosed test unit in which all steps of NAAT i.e. Sample processing, PCR amplification and detection are automated and integrated. The manual steps involved in the assay are adding reagent to liquefy sputum and sample loading.[11] The test procedure is made biosafe by tuberculocidal property of the assay's sample reagent [7].

RESULTS

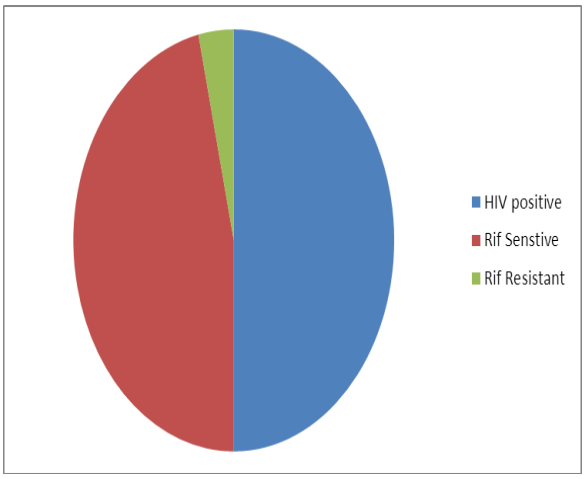
A total of 4595 patients with symptoms suggestive of pulmonary tuberculosis including both new cases and on treatment. Out of these patients 1087 were reported MTB detected. Out of these 4595 patients 953 were reported HIV positive and out of 953 patients 127(13.3%) were reported positive for (HIV +TUBERCULOSIS).fig -1.



Table/Fig-1: Total HIV TB positive cases & Rif Sensitive cases

Out of these 1087 patients which were reported MTB positive 127(11.6%) were reported positive for (HIV +TUBERCULOSIS). Of these 127 Co-infected cases 118 (92.9%) cases were sensitive to rifampicin (RiF) and

9(15.78%) cases were showing resistance to rifampicin (RiF) Drug as shown in Table/Fig-2:



Table/Fig-2: TB-HIV Co-infected, cases sensitive to rifampicin (RiF) and cases showing resistance to rifampicin (RiF)

DISCUSSION

Globally, 51% of notified TB patients had a documented HIV test result in 2014.[12] India accounts for about 10% of the global burden of HIV-associated TB. The mortality in this group is very high and every year: 42000 people die every year among TB/HIV co-infected patients.[12]

In given study 11% patients were found to be HIV–TB co-infected.As per the WHO Global Report on Tuberculosis 2013, India accounts for 64,000 MDRTB cases out of 300,000 cases estimated globally to occur among the notified pulmonary TB cases annually.[12] High prevalence of MDR-TB was detected among HIV positive cases (15.78%) which in accordance with the study done by Sethi et al., at PGIMER, Chandigarh for a span of 41 months who reported significantly higher association of MDR-TB (27.3%) with HIV seropositive patients as compared to HIV seronegative patients (15.4%).[13]

Cartridge Based Nucleic Acid Amplification testing (CB-NAAT) is a new operational system recommended by WHO. Results from 12 single centre evaluation studies with varying design and study population and reviewed by WHO reported the sensitivity in detecting TB from 70%-100% in culture positive patients and around 60% in those with smear negative disease and specificity ranging from 91%-100% and average rifampicin sensitivity and specificity around 98% and 99%.[8]

Studies from different part of world have reported high sensitivity and specificity by using Xpert MTB/RIF test based on this assay. In a study by Raizada et al., that covered a population of 8.8 million across 18 sub-district level tuberculosis units (TU) in India, Overall 28% TB cases were bacteriologically confirmed, of which 27.6% TB cases were detected on Xpert MTB/RIF against smear positivity rate of 12.9%. However, of 9 Xpert MTB/RIF negative and culture positive cases, 8 were detected on smear microscopy too. Positive predictive value (PPV) for rifampicin resistance detection by Xpert MTB/RIF was 97.7%.[14] Similar study on HIV positive adults referred to DOTS centre with pulmonary symptoms suggestive of tuberculosis Singh and co-authors concluded that "CBNAAT is easily done, valid, more accurate and reliable alternative to sputum microscopy for detection of pulmonary tuberculosis in HIV patients".[15]

CONCLUSION

In the rapidly evolving era of TB and HIV co-infection, updated knowledge as well as changing research priorities, particularly with respect to new TB diagnostics is the need of the hour. The Gene Xpert system can be used as the initial diagnostic test in individuals suspected of having MDR-TB or HIV associated TB as it can test 4 modules with capacity to perform 15 to 20 tests in one working day and the result is available in less than 2 h and hence screening capacity of healthcare centre can be increased. Use of Xpert has significantly increased TB finding and it has also significantly increased MDR case finding and cost wise it is much less than performing culture and DST. The system can be operated under diverse environmental conditions, with minimally trained staff and least biosafety concerns.

Authors' contributions statement: AK designed and supervised the study, provided research laboratory. VA scientifically advised the study.VC, reviewed the literature, JK prepared, preliminarily drafted the manuscript & edited the final version of the manuscript. ND,AV,DP & NAA practically carried out the study. All authors reviewed and approved the final version of the manuscript.

REFERENCES

1. Padmapriyadarsini C, Narendran G & Soumya Swaminathan S. Diagnosis & treatment of tuberculosis in HIV co-infected patients. 2011; Indian J Med Res 134(6): 850-865.
2. Tuberculosis. WHO Global Tuberculosis Report 2015 20th Edition. http://www.who.int/tb/publications/global_report/gtbr15_main_text.pdf?ua=1.
3. Sharma, S.K, Mohan, A & Kadhiravan, T. HIV-TB co-infection: Epidemiology, diagnosis & management. Indian J Med Res 2005; 121: 550-567.
4. Aaron L, Saadoun D, Calatroni I, Launay O, Mémain N, Vincent V, Marchal G, Dupont B, Bouchaud O, Valeyre D, Lortholary O. Tuberculosis in HIV-infected patients: a comprehensive review. Clin Microbiol Infect. 2004; 10(5): 388-98.
5. Weyer K, Mirzayev F, Migliori GB, Gemert WV, D'Ambrosio L, Zignol M. Rapid molecular TB

diagnosis: evidence, policy making and global implementation of Xpert MTB/RIF. ERJ. 2013; 42(1): 252–71.[PubMed].
6. Walusimbi S, Bwanga F, De Costa A, Haile M, Joloba M, Hoffner S. Meta-analysis to compare the accuracy of GeneXpert, MODS and the WHO 2007 algorithm for diagnosis of smear-negative pulmonary tuberculosis. BMC Infectious Diseases. 2013; 13: 507. [PMC free article] [PubMed].
7. Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF Assay for the Diagnosis of Pulmonary and Extrapulmonary TB in Adults and Children: Policy Update. Geneva: World Health Organization; Issued date 2013. [PubMed].
8. Guidance document for use of Catridge Based-Nucleic Acid Amplification Test (CB-NAAT) under Revised National TB Control Programme (RNTCP) issued central TB division, directorate general of health services September 2013.
9. Laboratory Services for Progammatic Management of Drug Resistant Tuberculosis, Ch.4. Guidelines on Programmatic Management of Drug Resistant TB (PMDT) in India. Revised National Tuberculosis Control Programme. May 2012.
10. Xpert MTB/RIF implementation manual: technical and operational 'how-to'; practical considerations. Report no. WHO/HTM/TB/2014.1. [PubMed].
11. Talbot EA, Pape JW, Sundaram L, Boehme CC, Perkins MD. Transforming TB Diagnosis: Can Patients and Control Programs Afford to Wait? Am J Trop Med Hyg. 2012; 87(2): 202–04. [PMC free article][PubMed].
12. Reach the Unreached. TB INDIA 2014, Revised National TB Control Programme, ANNUAL STATUS REPORT. May 10. 2014.
13. Sethi S, Mewara A, Dhatwalia SK, Singh H, Yadav R, Singh K, et al. Prevalence of multidrug resistance in *Mycobacterium tuberculosis* isolates from HIV seropositive and seronegative patients with pulmonary tuberculosis in north India. BMC Infectious Diseases. 2013; 13: 137. [PMC free article] [PubMed].
14. Raizada N, Sachdeva KS, Nair AS, Kulsange S, Gupta RS, Thakur R, et al. Catching the missing million: experiences in enhancing TB & DR-TB detection by providing upfront Xpert MTB/RIF testing for people living with HIV in India. PLoS One. 2015; 10(2): e0116721. [PMC free article] [PubMed].
15. Singh AK, Karmakar D, Jha AK. Compararative accuracy Of geneXpert/CBNAAT (Cartridge Based Nucleic Acid Amplification test)-TB and sputum microscopy in diagnosis of Pulmonary Tuberculosis in HIV positive patients and a meta analysis of existing literature. Conference: ASICON 2013: National CME, At Kolkata.