

Original Research Article

Flash Diagnostic Tool for MDR TB in HIV Era

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Abstract: 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). The sputum samples are received from patients attending ICTC clinic at Osmania General Hospital, Hyderabad. Sputum specimen was processed for TB detection by Cartridge Based Nucleic Acid Amplification testing (CB-NAAT) using Xpert MTB/RiF assay technology. A total of 125 HIV patients were in the study. Out of these patients 20 MTB cases were detected. Of these 20 cases 18(90%) cases were sensitive to Rifampicin (RIF) and 2(10%) cases were showing resistance to Rifampicin (RIF) drug. Co-existence of HIV and tuberculosis is high and there is high Prevalence of MDR tuberculosis in HIV patients. The Gene Xpert MTB/RIF assay is a novel integrated diagnostic device that performs sample processing and real-time PCR analysis in a single hands-free step for the diagnosis of tuberculosis and rapid detection of RIF resistance in clinical specimens.

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

INTRODUCTION

Tuberculosis is the commonest opportunistic infection in HIV-infected patients in developing countries including India. The prevalence of tuberculosis among HIV patients in various parts of India has been increasing steadily. The dual epidemic of tuberculosis and HIV is a significant problem in the developed and developing countries. The HIV pandemic has altered both the epidemiology of tuberculosis and the measures for approaching its control. WHO estimates that more than 7 million people, 98% of whom are in the developing world, are co-infected with HIV and tuberculosis. The incidence of tuberculosis in HIV infected patients is about a hundred-fold than that in the general population. It is estimated that 60-70% of HIV-positive persons will develop tuberculosis in their lifetime. Approximately, 50% of adult Indian population is infected with Mycobacterium tuberculosis and the spread of HIV infection has led to a potentially explosive increase in the number of cases of tuberculosis. TB is the leading cause of death among people living with HIV, accounting for one in five HIV related deaths. In 2012

some 3,20,000 people died of HIV-associated TB[1]. Globally the numbers of HIV-associated TB deaths are increasing. People living with HIV are facing emerging threats of drug-resistant TB such as multi-drug resistant (MDR-TB) and extensively drug resistant TB (XDR-TB). About 1.8 million new cases of tuberculosis are occurring annually in India, whereas the pool of HIV-infected individuals is quite large. Tuberculosis is the only major opportunistic infection in HIV infected individuals which can spread through the air from a HIV positive person to a HIV negative person. The emergence of drug resistance and development of multidrug resistant tuberculosis (MDR TB) has become a new but significant obstacle for TB control. As Rifampicin resistance is an important indicator for drug resistant TB, rapid diagnosis of tuberculosis and detection of Rifampicin (RIF) resistance are essential for knowing the magnitude of problem & early management of drug resistance[2]. The rapid detection of M. tuberculosis in infected patients is essential for disease management, because the high risk of transmission from person to person and emergence of MDR-TB and extensively drug resistant tuberculosis

and detection of rifampicin resistance is also important because it is an important indicator for detection of drug resistant tuberculosis including MDR-TB patients [3]. Culture is the “gold standard” for final determination, but it is time consuming and may take up to 2 to 8 weeks. Although smear microscopy for acid-fast bacilli (AFB) is rapid and inexpensive, it has poor sensitivity and a poor positive predictive value (PPV). Thus, rapid identification, which is essential for earlier treatment initiation, improved patient outcomes, and more effective public health interventions, relies on nucleic acid amplification techniques. Molecular assays have been established to allow the prediction of drug resistance in clinical specimens within 1 working day and are potentially the most rapid methods for the detection of drug resistance. The GeneXpert MTB/RIF assay is a novel integrated diagnostic device that performs sample processing and real-time PCR analysis in a single hands-free step for the diagnosis of tuberculosis and rapid detection of RIF resistance in clinical specimens [3,4].

Objective

- The objectives of this study were use of cartridge based nucleic acid amplification testing.
- To find out the co- prevalence and the trend of tuberculosis infection among HIV patients.
- To determine the prevalence of MDR Tuberculosis in HIV positive patients.

MATERIALS AND METHODS

This study is done at Osmania general hospital, Hyderabad, India. Sputum samples are received from HIV patients attending ICTC clinic. HIV infection was diagnosed using Rapid kit tests (SD Bioline HIV ½ 3.0 Rapid kit for screening and confirmed using combaid-s Advantage-ST HIV 1+2 Immuno dot Test Kit and HIV 1/2/0 Tri-line Human Immunodeficiency Virus Rapid Test Device). CD4+T cell counts were determined by flowcytometry technique using BD FACSCount TMreagent kit. A total of 125 sputum samples of the patients irrespective of their ART treatment and signs and symptoms of Tuberculosis were included in the study. 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 proforma containing details of patients like Name, Address, Age; Sex 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 [4,5].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 .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; The manual steps involved in the assay are adding reagent to liquefy sputum and sample loading .The test procedure is made biosafe by tuberculocidal property of the assay’s sample reagent.

RESULTS

Of total 125 HIV confirmed cases, included in study, MTB was detected in 20(16%) patients as shown in [Fig-1].

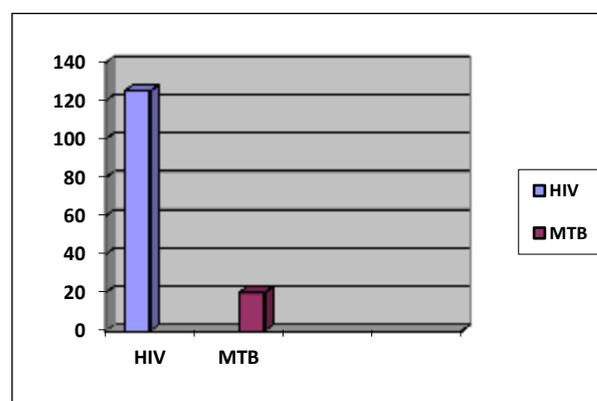


Fig-1: HIV (125), MTB (20)

Out of these 20 patients which were reported MTB positive -infected cases 18 (90%) cases were sensitive to rifampicin (RiF) and 2(10%) cases were showing resistance to Rifampicin (RiF) Drug as shown in [Fig-2].

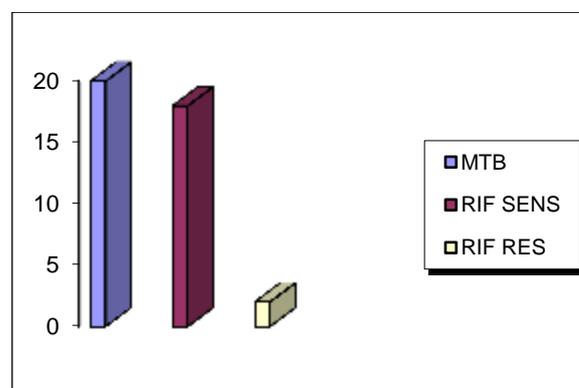


Fig-2: MTB (20), RIF –S (18), RIF-R (2)

Sex wise distribution showed that 71(56.8%) were male and 54 (43.2%) were Females.[Fig 3]

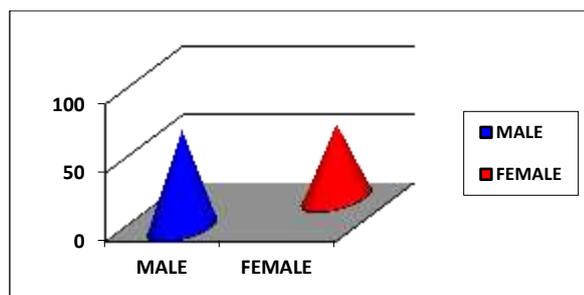


Fig-3: Male (71): Female (54)

DISCUSSION

This study was conducted to evaluate the role of CBNAAT in diagnosing pulmonary tuberculosis in PLHIV and detection of rifampicin resistance among these patients. People living with HIV need early diagnosis and treatment of active TB disease. Xpert MTB/RIF rapid test is recommended as the initial diagnostic test for people living with HIV who have suspected TB. At least one-third of the 35.3 million people living with HIV worldwide are infected with latent TB. Persons co-infected with TB and HIV are 29.6 times (27.1 – 32.1) more likely to develop active TB disease than persons without HIV [6]. TB is the most common presenting illness among people living with HIV, including those who are taking antiretroviral treatment. There were an estimated 1.1 million HIV positive new TB cases globally in 2012.

In given study 16% patients were found to be HIV–TB co-infected, of which 71(56.8%) were males and 54(43.2 %) were females. Male predominance has been observed in other studies from Punjab [6, 7]. In a Study conducted in AIIMS-New Delhi for period of six years, HIV-TB co-infection was diagnosed in 33% patients of which 81.3 % were male [8,9]. This male predominance may be due to their migration for employment within and outside the state thereby subjecting them to risk behaviour. Majority of female are illiterate and are house-wives who don't even have easy access to healthcare facilities, and HIV and TB both being social stigma often go unreported [10]. The present study shows 16% of MTB cases in PLWH, which is in accordance with the study conducted by Anuradha *et al.* [11].

High prevalence of MDR-TB was detected among HIV positive cases (15.78%) which in accordance with the study done by Sethi *et al.* 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%) [12].

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. The system can be operated under diverse environmental conditions, with minimally trained staff and least biosafety concerns. But every packaged deal has its own share of negativities and in this, device maintenance, annual calibration of instrument modules. WHO recommends CBNAAT for diagnosis of pulmonary tuberculosis and detection of rifampicin resistance, especially in PLHIV and re-treatment cases who are at risk of MDR-TB [11, 12].

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