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A STUDY OF GENE XPERT IN SCREENING OF SPUTUM IN HIV POSITIVE PATIENTS PRESENTING TO TERTIARY CARE CENTRE

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ABSTRACT

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Introduction: Present study was conducted to study Gene Xpert in screening of sputum in HIV positive patients, to estimate the burden of drug resistance TB in HIV patients and to estimate the prevalence of MDR TB in HIV positive patients.

Methods: RNTCP is currently using Gene Xpert to screen all HIV positive patients to diagnose pulmonary TB and rifampicin resistance. In this study, pulmonary samples referred to state level intermediate reference laboratories (IRLs) Indore between January 2015 to December 2015 were investigated and subjected to Gene Xpert.

Results: A total of 3033 pulmonary specimens were included in the study undergoing Gene Xpert; out of which 604 specimens were from HIV positive patients. In 85 (14.07%) HIV positive patients (out of 604) MTB was detected and RIF was sensitive (14.07%). In 06 (0.99%) HIV positive patients MTB, was detected and RIF resistance was found.

Conclusion: Screening of pulmonary samples with Gene Xpert in all HIV positive patients has enormous scope in early diagnosis and treatment of TB in terms of active case finding of patients with drug resistant tuberculosis. The results are available in less than 2 hours. This leads to less transmission of disease with reduced morbidity.

Key words: Tuberculosis, MDR Tuberculosis, HIV, Gene Xpert

INTRODUCTION

Tuberculosis (TB) continues to be one of the greatest killers in the world due to infectious disease, claiming over 1.4 million deaths in 2011[1]. In the global tuberculosis report (2014), WHO reported that in 2013, 9 million people developed TB, including 1.1 million cases among people who were HIV positive[2]. 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[1].

Twenty five percent of global annual TB incidents occur in India making it the highest Tuberculosis burden country[2]. In December 2010, WHO recommended use of a new Cartridge Based Nucleic Acid Amplification test (CB-NAAT), named GeneXpert system[1]. The Xpert Mycobacterium Tuberculosis / Rifampicin 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*[3-4]. It can detect TB along with rifampicin resistance in less than two hours, directly from untreated sputum samples[3,5].

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[1,3,5] . Many people with HIV TB coinfection die from TB because these patients are paucibacillary and diagnosis is delayed. Gene Xpert overcomes these limitations as it offers accurate and rapid diagnosis of active TB[3].

The most common method to diagnose pulmonary TB is still sputum AFB smear. Sputum AFB smear by fluorescent technique can detect TB in 20-80% cases[6]. Sufficient bacillary load is a must for Sputum AFB smear. Furthemore, it cannot detect drug resistance. As the numbers of bacilli in sputum of severely immunosuppressed HIV patients is low, TB often goes undetected with sputum AFB smear[5].

A more sensitive approach to diagnosis is to culture sputum samples, which can include testing for drug resistance. However, such techniques require expensive and sophisticated laboratory infrastructure and staff, and it can take weeks or months to obtain results.

Present study was conducted to study Gene Xpert in screening of sputum in HIV positive patients and to estimate the burden of drug resistance TB in people living with HIV (PL HIV).

MATERIAL & METHODS

In this study, pulmonary samples obtained during the clinical routine and sent to state level Intermediate reference laboratories (IRLs) INDORE M.P., between 01 January 2015 to 31 December 2015 were investigated.

A total of 3033 pulmonary specimens (sputum, bronchoalveolar lavage, bronchoscopic aspirate, postbronchoscopic sputum) were included in the study that were found negative on sputum smear microscopy. Detailed consent was taken from every patient before performing bronchoscopy. Out of which 604 samples were from HIV positive patients.

(As per meeting of RNTCP national expert committee (January 2013), decision was taken to use Gene Xpert for rapid identification of Multi drug resistant Tuberculosis and improve case detection among all PL HIV patients)[7].

All the samples were subjected to the four cartridge based Gene Xpert test that works on a molecular level to identify mycobacterium tuberculosis. Samples were collected in a small tube. From the tube, the sample was fed into the machine. The machine looks for the DNA specific to the TB bacterium. If there are TB bacteria in the sample, the machine will detect their DNA and automatically multiply it. This technique is called PCR (polymerase chain reaction), and allows the machine to also look at the structure of the genes[8-10]. This is important to detect if a TB bacterium has developed resistance to drugs. The DNA of the TB bacterium is, in a way, like a long string of different colours. If one or more of the colours change (if there is a mutation in the DNA), then the bacterium can become resistant to certain TB drugs. The Gene Xpert was used to test for resistance to one of the most common TB drugs, Rifampicin.

The data collected was analyzed and statistical analysis was done with chi square test with 2 x2 contingency table. The result was expressed in proportions.

RESULTS

A total of 3033 pulmonary specimens were included in the study undergoing Gene Xpert; out of which 604 (19.91%) specimens were from HIV positive patients. In 664 (27.33%) patients out of total 2429 specimens from HIV negative patients, MTB was detected and Rifampicin was sensitive. In 85 (14.07%) HIV positive patients (out of 604), MTB was detected and RIF was sensitive. In 46 (1.89%) patients out of total 2429 HIV negative patients, MTB was detected and Rifampicin was resistant and in 06 (0.99%) HIV positive patients, MTB was detected and RIF resistance was found. Mycobacterium tuberculosis was detected in 710 (29.23%) out of total 2429 HIV negative patients screened with Gene Xpert. Mycobacterium Tuberculosis was detected in 91 (15.06%) out of total 604 HIV positive patients screened with Gene Xpert.

Table 1: Gene Xpert in HIV positive and HIV negative patients

Variables	HIV +ve (%)	HIV –ve (%)	Total (%)
Total n	604 (19.91)	2429 (80.08)	3033
TB –ve	513 (84.9)	1719 (70.76)	2232 (73.59)
TB +ve	91 (15.06)	710 (29.23)	801 (26.4)
Rifampicin S	85 (14.07)	664 (27.33)	749 (24.69)
Rifampicin R	06 (0.99)	46 (1.89)	52 (1.71)

The prevalence of Drug Resistant Tuberculosis in HIV positive patients was found to be statistically insignificant (p value 0.966698) with chi square test when compared to HIV negative patients.

The case detection of Mycobacterium Tuberculosis with Gene Xpert in HIV positive patients was found to be statistically significant (p value -0.13; less than 0.0001) with chi square test when compared to screening of HIV negative patients with Gene Xpert.

DISCUSSION

In our study, Gene Xpert gave an extra edge over diagnosis of pulmonary TB in sputum smear negative HIV patients. Diagnosis of TB before gene X pert was a troublesome task and often needed long time. Gene Xpert gives the results in 2 hours only. This somehow leads to less transmission of disease and fewer deaths. On the other hand resistant bacilli to Rifampicin in a given sample can be detected.

In 2010, Boehme CC et al found in his study that a single, direct MTB/RIF test identified 551 of 561 patients with smear-positive tuberculosis (98.2%) and 124 of 171 with smear-negative tuberculosis (72.5%)[11]. In 2011, Marlowe et al found in his study that out of 90 sputum smear negative patients subjected to Gene Xpert, 31 (34.4%) were found to be positive for Mycobacterium Tuberculosis[12]. This is in accordance with our study. In 2010, Helb D, et al found Gene Xpert to be positive for Mycobacterium Tuberculosis in 109 patients that were found to be sputum smear negative[13].

A similar study for sputum negative TB patients was conducted at our centre in which 72 sputum smear negative patients were included and broncho-alveolar lavage (BAL) taken by doing a bronchoscopy which was then subjected to Gene Xpert test. From these 72 patients 34 came out to be positive on Gene Xpert test further proving the efficacy of test on pulmonary samples[14]. Furthermore study conducted by Avashia S et al concluded that Gene Xpert was very effective in detecting rifampicin resistance in extrapulmonary samples[15].

In 2014, Nguyen T et al found Rifampicin resistance in 3.7% of HIV positive patients with Tuberculosis[16]. In 2009, Rajsekaran S et al found that HIV was found to coexist with 14.2 % of Multi drug resistant TB patients[17]. We found Rifampicin resistant in 0.99 % of HIV positive patients. Furthermore Rajsekaran S et al found that HIV coinfection increasing trend was observed among MDR-TB patients to the tune of 12.3%, 14.7%, 17% and 12.6% during 2004, 2005, 2006 and 2007 respectively (p = 0.81). On the contrary, Deivanayagam CN et al in 2002 found that HIV seropositivity among MDR TB patients was 4.42% and MDR TB was detected in 33.9% HIV positive patients[18].

Gene Xpert gives results within 2 hours. A person can know on the same day whether or not she has TB. It is also very important and good that the Gene Xpert can detect if the TB of the person is resistant to Rifampicin. If the health care worker knows from the start that the TB of a person is resistant to Rifampicin, she can choose other drugs to treat the TB effectively.

CONCLUSION

Screening of pulmonary specimens of all HIV positive patients with Gene Xpert has enormous scope in terms of active case finding of new tuberculosis patients and furthermore those with drug resistant tuberculosis. Though the prevalence of drug resistant tuberculosis in HIV positive patients was no more than with HIV negative patients; still timely detection of drug resistance in HIV leads to reduced morbidity in view of ongoing HIV TB coinfection epidemic. Gene xpert has emerged as a boon in these patients with confusing clinical scenario. The results are available in less than 2 hours.

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