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DIAGNOSTIC ACCURACY OF XPERTMTB/RIF ASSAY IN DETECTION OF PULMONARY TUBERCULOSIS AND RIFAMPICIN RESISTANCE IN COMPARISON WITH CONVENTIONAL METHODS.

Microbiology

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ABSTRACT

Introduction: Tuberculosis is one of the oldest diseases with high morbidity and mortality. India accounts for one- fourth of the global TB burden (2.7million cases).

Material and methods: A total 1056 patients were included in this study. All patients were requested to give two sputum samples, spot sputum sample (at the time of visit) and early morning sputum for mycobacterial testing. All spot samples tested with smear microscopy and Xpert MTB/Rif assay. Rifampicin resistant samples compared with conventional method.

Results: Out of 1056 sputum samples GeneXpert MTB Rif assay detected positive 204 (19.3%), not detected 827 (78.31%). There is significant difference founded for positivity in spot samples and early morning sputum samples (P =0.0026). Rifampicins resistant were 8 (3.9%) andshown 100% sensitivity, specificity with Conventional method.

Conclusion: For diagnosing tuberculosis and detecting Rifampicin resistance GeneXpert MTB/RIF has been especially recommended.

KEYWORDS

XpertMTB/Rif, pulmonary tuberculosis, Rifampicin resistance

INTRODUCTION:

Tuberculosis (TB) is one of the oldest diseases caused by Mycobacterium tuberculosis (MTB) in the world associated with high morbidity and mortality. India accounts for one- fourth of the global TB burden with an estimated 2.79 million incident cases (1). Although it most commonly presents as pulmonary TB (80% of patients), up to 1 in 5 patients present with extra-pulmonary manifestations, including milliary TB, bone and joint TB and TB meningitis. Tuberculosis normally progresses slowly from the latent stage (infection without active disease) to active TB disease, except in HIV co-infected patients where progression can be rapid and fatal.

Rapid Tuberculosis diagnosis can be difficult in clinical practice and early pulmonary TB detection continues to be challenging for clinicians. Prompt diagnosis of active pulmonary TB is a priority for Tuberculosis control, both for treating the individual and for public health intervention to reduce further spread in the community (2).

Mycobacterial Culture is the gold standard for diagnosis of tuberculosis infection and is the first step in detecting drug resistant. However this approach is relatively complex and time consuming (2 months). Sputum acid fast staining is a rapid inexpensive technique to pulmonary tuberculosis but sensitivity specificity low compared to culture. And also pauci-bacillary forms of TB are more commonly identified in patients who are HIV-seropositive (3-5) and in those cases MTB cannot be detected by Microscopic examination. In 2010, WHO endorsed a novel, rapid, automated, cartridge-based nucleic acid amplification test (CBNAAT), the Xpert®MTB/RIF assay (Cepheid, Sunnyvale, USA) (hereafter referred to as Xpert), that can simultaneously detect TB and rifampicin resistance (WHO Policy Xpert 2011), WHO recommends that Xpert be used as the initial diagnostic test in individuals suspected of MDR-TB or HIV-associated TB. This assay doesn't required sample processing but can be used chemically inactivated specimen. Therefore, simple procedure, less time consuming and does not require special technical expertise and biosafety cabinet (6).

This study was to find out diagnostic accuracy of Xpert®MTB/RIF assay in detection of Mycobacterium tuberculosis and Rifampicin resistance as compared to conventional methods (Microscopy and Culture).

MATERIALAND METHODS:

The present study has been conducted at Department of Microbiology, SVIMS, Tirupati, over a period of 11 months (from February 2018 to December 2018). A total 1056 patients were included in this study. All patients were requested to give two sputum samples, spot sputum sample (at the time of visit) and early morning sputum for mycobacterial testing. Along with sputum samples seropositivity was noted.

Specimen Processing: All spot sputum samples separated in to three portions, first portion of sputum samples processed immediately for Xpert®MTB/RIF assay. Second portion of sputum samples processed for ZN staining and third portion of sputum stored for culture. All early morning sputum samples were processed for ZN staining to compare it with spot samples.

Ziehl Neelsen stain: Direct Smear microscopy was performed to investigate presence of acid fast bacilli using conventional ZN staining method. The AFB smear was graded as per RNTCP guidelines: Scanty (1-9/100 fields), 1+ (10-99/100 fields), 2+ (1-10/ fields) and 3+ (>10/field).

Xpert®MTB/RIF assay: The assay was performed using version 4 cartridges according to the manufacturer's recommendations (7). Briefly the sample reagent (containing NaOH and isopropyl alcohol) was added at a 2:1 ratio to clinical specimen to kill the mycobacteria and liquefy the samples. The sample-SR mixture was shaken vigorously and incubated for 10 minutes before being shaken again and kept at room temperature for another 10 minutes. Two ml of the digested material was transferred to the cartridge. The cartridge was subsequently loaded in the instrument where all subsequent steps occurred automatically. Only electronic results were used for comparison.

MTB culture: Only 30sputum samples shown positive (8 Rifampicin resistant and 22 Rifampicin Sensitive) by Xpert®MTB/RIF assay were cultured on Lowenstein Jensen medium according to standard RNTCP guidelines and incubated for up to 8 weeks in 37˚C.

DRUG SUSCEPTIBILITY TESTING: The drug sensitivity testing was done after growth obtained in LJ medium. The sensitivity of the isolates for rifampicin (RIF, 40.0 µg/mL) done by proportion method according to the protocol of National Institute for Research in Tuberculosis, Chennai, India (8).

Statistical analysis: All data arranged in excel spread sheets. Age wise and sex wise distribution compared. Mcnemars test and Chisquare test used to calculate the p value.

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RESULTS:

A total of 1056 patient's samples with suspected TB infection were used to detect TB using GeneXpert and other common methods. Out of 1056 patients 66.4% were males, 33.6% were females.

All Spot sputum samples and early morning sputum samples were processed for acid fast staining. There is significant difference founded for positivity in spot samples and early morning sputum samples (P =0.0026).(Table 1).

Table 1: Comparison of positivity among spot samples and early morning sputum samples.

ZN STAIN	EARLY MORNING SAMPLE		Statistics
SPOT SAMPLES	NEGATIVE	POSITIVE	P=0.0026 (Mcnemars test)
NEGATIVE	885	11	
POSITIVE	0	160	

Out of 1056 sputum samples Gene Xpert MTB Rif assay Detected positive 204 (19.3%), Not detected 827 (78.31%), Error- 18 (1.7%) and Invalid 7 (0.66%) (Figure:1).

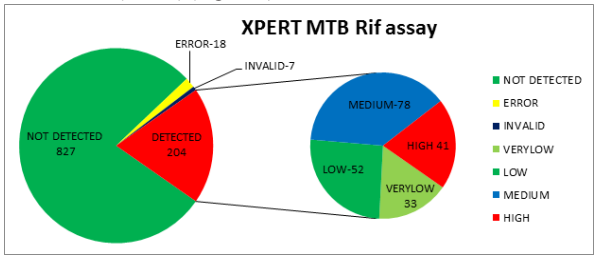

Figure 1: Gene Xpert MTB Rif assay results.

Highest positivity shown in age group range from 21 to 60 years by Gene Xpert Rif assay (Figure 2). Out of 204 GeneXpert positive cases 153 (75%) were males and 51 (25%) were females.

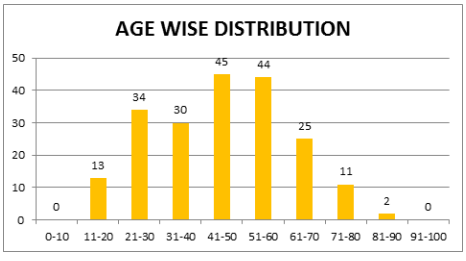

Figure 2: Age wise distribution of all GeneXpert positive cases.

Among 204 GeneXpert positive cases 8 (3.9%) were Rifampicin Resistant. A total of 30 GeneXpert positive sputum samples (8 were Rif resistant and 22 were Rif sensitive) cultured on LJ medium (Gold standard) and Tested for Drug Susceptibility testing (proportion method) for Rifampicin. Culture and DST results shown 100% sensitivity and 100% specificity for GeneXpert results.

Among 1056 spot sputum samples 159 (15%) of samples shown positive by both microscopy and GeneXpert Rif assay and 827 (78.31%) samples shown negative in both microscopy and GeneXpert Rif assay. 45(4.26%) shown positive only in GeneXpert and Negative in Microscopy (p=<0.00000001) (Table: 2).

Table 2: Comparison of sputum positivity by ZN stain and Xpert MTB Rif assay.

ZN stain	GENEXPERT		STATISTICS
SPOT SPUTUM	POSITIVE	NEGATIVE	P=<0.0000001
POSITIVE	159	0	
NEGATIVE	45	827	

Total 192 (18.8%) HIV positive cases noted among 1056 patients. Among all HIV Positive cases 15 patients showed positive by GeneXpert and Microscopy, 157 patients shown negative by both methods. 13 Sputum samples from HIV positive patients shown positivity by GeneXpert and negative by sputum microscopy, significant p value noted for this comparison (P=0.0002649).

DISCUSSION:

Rapid and accurate diagnosis of tuberculosis is a challenge in developing countries (9). Commonly used conventional methods detecting Mycobacterium tuberculosis complex (MTBC) are low sensitivity in clinical specimens [10,11]. Smear microscopy is the current frontline diagnostic test, but lacks sensitivity. Due to the slow growth of *Mycobacterium tuberculosis* and need for sophisticated lab facility, culture is available in reference laboratories. A large number of pulmonary tuberculosis cases remain undiagnosed by traditional sputum microscopy, i.e. diagnostic delays in detection of smear negative suspected pulmonary tuberculosis samples is of major concern. Quality of Sputum has long been assumed to be as a main important predictor of the performance characteristics of diagnostic microbiologic tests, particularly those used to diagnose lower respiratory tract infections. While a recent systematic review found no studies on the influence of sputum quality on the performance of Xpert®MTB/RIF for diagnosis of pulmonary tuberculosis [12]. So Many studies have been done by using molecular techniques for the detection of MTBC, indicating that PCR is a useful and convenient method for rapid diagnosis of tuberculosis from clinical specimens. It is also remarkable method but this method also has some limitations, including the extraction method and PCR inhibitors are associated in some clinical samples.

This study was aimed at carrying out comparative analysis of Ziehl-Neelsen and GeneXpert techniques along with culture for the diagnosis of tuberculosis and detection of rifampicin detection in patients with pulmonary tuberculosis.

For testing of pulmonary tuberculosis, collection of two sputum samples for tuberculosis (TB) diagnosis, with at least one being an early morning (EM) using smear microscopy is recommended by World Health Organization (WHO). In this study we evaluated smear microscopy by spot sputum and early morning sputum samples. There is a significant difference found in both samples (P=<0.0000001),i.e. Early sputum sample is mostly preferable to Microscopic examination for detection Pulmonary tuberculosis.(Table:1)

A highly sensitive and specific tuberculosis diagnostic test would contribute immensely to achieve the 90% reduction in tuberculosis incidence by 2035 as established by the End-TB strategy [13].

In our study, out of 1056 samples 204 (19.31%) samples detected as positive by GeneXpert. Previous studies showed that Xpert®MTB/RIF assay of respiratory samples in the diagnosis of pulmonary TB had a sensitivity of 89% (95% CI: 85%–92%) and specificity of 99% (95% CI: 98%–99%) (10,14). In this study sensitivity and specificity of GeneXpert shown 100%. Tuberculosis can affect any group, in our study 21 – 60 years age groups noted highest positivity by GeneXpert.(Figure:2)

GeneXpert may also be valuable as an add-on test following microscopy for patients who have previously been found to be smear negative [15]. Studies have shown that the test accurately detects 98.2% of smear positive and 72.5% of smear negative cases (16, 17). Smear-negative TB patients could benefit from GeneXpert particularly in those areas where no culture is available. In this study 100% positive for smear positive cases and 22.5% smear negative cases.(Table:2)

Early determination of rifampicin sensitivity is important for the timely detection of multidrug resistant tuberculosis and timely initiation of appropriate therapy in order to reduce the risk of spread and poor outcome. Rifampicin resistant Among 204 GeneXpert positive were 8 (3.9%), Similar studies shown 3% to 10% (10,18,19). All rifampicin resistant GeneXpert samples showed positive 100% by conventional proportion method for drug susceptibility testing.

In our study a total of 192 (18.8%) HIV positive cases noted, 38 patients shown positive by GeneXpert and only 15 shown positive by smear microscopy. Due to paucibacilliary nature of *M. tuberculosis* in Immune suppressed (HIV) patients, smear microscopy examination failed to detect MTB in sputum samples (20).

The main limitation of our study was the relatively low number of samples processed for culture and drug susceptibility testing.

CONCLUSION:

In conclusion, early morning sputum culture has a high incremental diagnostic yield for suspected pulmonary tuberculosis patients. For

diagnosing tuberculosis and detecting rifampicin resistant strains the GeneXpert MTB/RIF is an assay with a high overall specificity and sensitivity. It is a rapid method that could complement the reference methods. Due to paucibacilliary nature of mycobacterium tuberculosis commonly identified in patients with HIV-seropositive and sputum acid fast bacilli (AFB) smear negative cases GeneXpert has been especially recommended.

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