



ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2015; 1(12): 1119-1122
www.allresearchjournal.com
Received: 20-09-2015
Accepted: 25-10-2015

Dr. Prasad Sudhir
Associate Professor,
Department of Respiratory
Medicine, Prathima Institute
of Medical Sciences,
Karimnagar, Telangana, India

Dr. Madire Ramulu
Assistant Professor,
Department of Respiratory
Medicine, Prathima Institute
of Medical Sciences,
Karimnagar, Telangana, India

Dr. Chenimilla Nagender Prasad
Professor, Department of
Respiratory Medicine,
Prathima Institute of Medical
Sciences, Karimnagar,
Telangana, India

Corresponding Author:
Dr. Madire Ramulu
Assistant Professor,
Department of Respiratory
Medicine, Prathima Institute
of Medical Sciences,
Karimnagar, Telangana, India

A study on diagnostic value of GeneXpert MTB/RIF in sputum smear negative cases: A tertiary care hospital experience

Dr. Prasad Sudhir, Dr. Madire Ramulu and Dr. Chenimilla Nagender Prasad

Abstract

Background: Pulmonary tuberculosis (PTB) continues to be an important cause of preventable mortality in both developing and developed nations. The cornerstone of TB control remains early diagnosis and treatment. The tuberculosis continues to be one of the greatest killers in the world claiming over 1.5 million deaths in 2014.

Objectives: To study the diagnostic efficacy, the sensitivity, specificity, positive predictive value and negative predictive value of GeneXpert MTB/RIF and these results were compared with the results obtained by culture as reference.

Material & Methods

Study Design: Prospective cross-sectional study.

Study area: Dept. of. Respiratory Medicine, Prathima Institute of Medical Sciences, Karimnagar, Telangana.

Study Period: Jan 2015 - Sep. 2015.

Study population: patients of both sexes who are suspected to have pulmonary tuberculosis clinically and radiologically.

Sample size: study consisted a total of 100 patients.

Sampling method: Simple Random sampling method.

Statistical Analysis: Statistical analysis was performed with SPSS software version 22 services. The analysis of categorical variables was examined by the chi-square test and Fischer's test and value of P of <0.05 was considered significant for all statistical analysis.

Results: The sensitivity of GENEXPERT in Smear negative and Culture positive cases is 74.23%. The specificity of GENEXPERT in Smear negative and Culture positive cases is 90.26%. The PPV of GENEXPERT in Smear negative and Culture positive cases is 81.25%. The NPV of GENEXPERT in Smear negative and Culture positive cases is 86.76%.

Conclusion: GeneXpert is a rapid, reliable, attractive tool with least bio safety concern and requiring minimal training. It is best alternative to conventional methods of tubercular diagnosis which also detects rifampicin resistance simultaneously. And its efficacy is comparable to culture.

Keywords: GeneXpert, Smear negative, rifampicin resistance

Introduction

Pulmonary tuberculosis (PTB) continues to be an important cause of preventable mortality in both developing and developed nations. The cornerstone of TB control remains early diagnosis and treatment. The tuberculosis continues to be one of the greatest killers in the world claiming over 1.5 million deaths in 2014. Yet the disease is not controlled particularly in the developing countries. The major contributing factor for such situation is the delayed diagnosis of this disease.

India has the highest number of TB cases in the world, with over 2 million active TB cases every year [1]. One fourth of the global TB cases occur in India annually [2]. Early and accurate diagnosis is the first critical step in controlling TB. The control of TB is becoming difficult due to slow diagnostic methods with low sensitivity, particularly for the detection of drug resistant forms. Early detection is essential to interrupt transmission and decrease the mortality rate, but the complexity and infrastructural needs of sensitive methods limit their accessibility and effect for diagnosing TB.

The most widely used test smear microscopy is 125 years old and routinely misses half of all cases. The sensitivity of smear microscopy and its inability to detect drug resistance limits its impact on TB control. Culture methods and drug susceptibility testing is complex, time consuming, taking around 6-8 weeks. While patients await diagnosis, they are likely to receive inappropriate or ineffective treatment and consequently disease may progress. This leads to increase in morbidity from tuberculosis and patients continue to transmit drug-resistant TB to others, especially family members and patients resistance may increase to TB drug.

To respond to the urgent need for simple and rapid diagnostic tools in high-burden countries [3]. A new diagnostic test GENEXPERT has been developed which is a rapid, fully automated test based on PCR which detects DNA directly from clinical specimens and also detects rifampicin resistance.

This test is designed to purify, concentrate, amplify and identify targeted rpo B nucleic acid sequences, and delivers result from unprocessed samples within 2hrs of time [4, 5, 6, 7]. The MTB/RIF assay is simple to perform with minimal training, is not prone to cross-contamination, requires minimal biosafety facilities, and has a high sensitivity in smear-negative tuberculosis.

GENEXPERT: It is an automated polymerase chain reaction test. GENEXPERT is a single test that can detect both Mycobacterium tuberculosis complex and rifampicin resistance within 2 hours after starting the assay with minimal hands-on technical time [8].

Unlike conventional nucleic acid amplification tests, in GENEXPERT sample processing, PCR amplification and detection are integrated into a single self- enclosed test unit, which is its cartridge. However, GENEXPERT requires an uninterrupted and stable electrical power supply, temperature control and yearly calibration of the instrument's modules.

Objectives: To study the diagnostic efficacy, the sensitivity, specificity, positive predictive value and negative predictive value of GeneXpert MTB/RIF and these results were compared with the results obtained by culture as reference.

Material & Methods:

Study Design: Prospective cross-sectional study.

Study area: Dept. of. Respiratory Medicine, Prathima Institute of Medical Sciences, Kareemnagar, Telangana.

Study Period: Jan 2015 - Sep. 2015.

Study population: patients of both sexes who are suspected to have pulmonary tuberculosis clinically and radiologically.

Sample size: study consisted a total of 100 patients.

Sampling method: Simple Random sampling method.

Inclusion Criteria

1. Patients coming Prathima Institute of medical sciences with clinical suspicion of pulmonary tuberculosis including symptoms of cough with or without expectoration for >2 weeks, weight loss, fatigue, haemoptysis and loss of appetite.

2. Sputum negative for AFB with radiological evidence for tuberculosis.

Exclusion Criteria

1. Uncooperative patients who will not give sputum for evaluation.
2. Patients associated with co-morbid conditions.
3. Samples received without clinical history.
4. Sputum positive cases.

Ethical consideration: Institutional Ethical committee permission was taken prior to the commencement of the study.

Study tools and Data collection procedure

All the patients are evaluated for the following investigations

1. Routine investigations (CBP, RBS, BU, SC)
2. Chest x-ray.
3. Smear microscopy for AFB in representative samples.
4. Cartridge based nucleic acid amplification test GENEXPERT / GeneXpertin representative samples.
5. Culture of representative samples for mycobacterium tuberculosis.
6. The samples are carried in a specified containers from Department of Pulmonology to District Tuberculosis center by the laboratory technician.
7. Two samples of sputum one spot and the overnight sample will be examined by florescent microscopy.
8. These samples of sputum smear-ve which are suspicious of PTB are submitted to Gene Expert Technique.
9. These samples of sputum smear-ve are also send for culture in solid culture medium.
10. All samples that were culture positive and GeneXpert MTB/RIF negative and samples that were culture negative and GeneXpert MTB/RIF positive were taken. The last result was used for the analysis.

Statistical Analysis

Statistical analysis was performed with SPSS software version 22 services. The analysis of categorical variables was examined by the chi-square test and Fischer's test and value of P of <0.05 was considered significant for all statistical analysis.

Observations & Results

Table 1: Age distribution of all subjects.

Age Group	No. of Cases
20-30 years	4
30-40 years	15
40-50 years	40
50-60 years	30
60-70 years	10

Table 2: Gender distribution of all subjects.

Gender Distribution		
Gender	No of Cases	Percentage
Male	73	73
Female	27	27
Total	100	100

Table 3: GENEXPERT results in all Sputum smear negative samples.

Test	Positive	Negative
AFB	0	100
GENEXPERT	32	68

GENEXPERT detected 32 positive cases out of 100 sputum smear negative samples.

Table 4: Culture results in all Sputum smear negative samples

Test	Positive	Negative
AFB	0	100
Culture	35	65

CULTURE detected 35 positive cases out of 100 sputum Smear negative samples.

Table 5: GENEXPERT results according to smear and Culture in all Sputum smear negative samples.

All Samples	AFB-VE	
	Culture positive	Culture negative
Genexpert Positive	26	6
Genexpert Negative	9	59

	Genexpert
Sensitivity	74.23%
Specificity	90.76%
PPV	81.25%
NPV	86.76%

- The sensitivity of GENEXPERT in Smear negative and Culture positive cases is 74.23%.
- The specificity of GENEXPERT in Smear negative and Culture positive cases is 90.26%.
- The PPV of GENEXPERT in Smear negative and Culture positive cases is 81.25%.
- The NPV of GENEXPERT in Smear negative and Culture positive cases is 86.76%.

Discussion

As Tuberculosis remains one of the deadliest communicable diseases. There are number of tests available for the diagnosis of tuberculosis early detection of tuberculosis, determining drug resistance and prompt treatment is important to decrease the spread, morbidity and mortality of disease. In the RNTCP programme smear is most commonly used for diagnosis.

But the main drawback of it is its low sensitivity, culture although gold standard, takes longer time for positivity. GeneXpert due to its rapidity and sensitivity not only help in early diagnosis and management of tuberculosis especially in patients with high clinical suspicion and history of contact with active tuberculosis patient etc., but also curtail the transmission of the disease. GeneXpert is a simple bench top point of care diagnostic assay that can be performed with minimal training. The results are available within 2 hours, much earlier than the culture which usually takes weeks to come positive.

In this prospective study, we have evaluated the diagnostic yield of GeneXpert to detect MTB in sputum smear negative samples and compared it with culture which was taken as gold standard. Mycobacterial cultures for detection of Mycobacterium tuberculosis was done by using solid culture (Lowenstein Jensen media) in our study. Numbers of studies have demonstrated the utility of GeneXpert in diagnosis of pulmonary tuberculosis in sputum smear negative samples.

In our study among 100 AFB sputum smear microscopy negative samples, 59 samples were negative for all three methods. (Z-N stain, GeneXpert, Culture). In remaining 41 AFB sputum smear negative samples. 26 samples were culture positive and GeneXpert positive. 6 samples were GeneXpert positive and culture negative. 9 samples were GeneXpert negative and culture positive. As cases were evaluated prospectively history of treatment with ATT should be ruled out with low bacterial load. PCR test amplifies any DNA, of live or dead bacilli. Therefore while diagnosing a person with active tuberculosis clinicians need to be very cautious using it as a sole method. Clear history of treatment with ATT is required to avoid false positive results. In our study for AFB negative the Sensitivity and specificity of GeneXpert is 74.23% and 90.76% and PPV and NPV are 81.25% and 86.76%.

In a study done by Monika *et al.* [9], the sensitivity and specificity of GeneXpert in 156 sputum smear negative samples is 79.1% and 93.1% respectively. In a study done by Surendra K Sharma *et al.* [10] the sensitivity and specificity of GeneXpert in 72 sputum smear negative samples is 77.7% and 99.3%. In the study done by Poojan *et al.* [11] the sensitivity and specificity of GeneXpert is 67.7% and 98.2%. In the study done by Sujatha *et al.* [12] the sensitivity and specificity of GeneXpert is 72.5% and 99.2%, In the present study 100 sputum smear negative samples were taken for which sensitivity and specificity is 74.2% and 90.7% respectively.

Sensitivity of GeneXpert in sputum smear negative samples in our study i.e 74.23%. are similar to the results of other studies conducted by Monika *et al.* 79.1% and Surendra *et al.* which is 77.7% respectively.

In a study done by Arzu N Zeka *et al.*, [13] the sensitivity and specificity of GeneXpert in sputum smear negative samples is 74.2% and 99.8% respectively. In a study done by Lombardi *et al.*, [14] the sensitivity and specificity of GeneXpert in sputum smear negative samples is 73.1% and 99.1% respectively. In a study done by Catharina [15] *et al.*, the sensitivity and specificity of GeneXpert in sputum smear negative samples is 72.5% and 99.2% respectively. In a study done by Loannidis *et al.*, [16] the sensitivity and specificity of GeneXpert in sputum smear negative samples is 86.0% and 93.4% respectively. And in present study the sensitivity and specificity of GeneXpert in sputum smear negative samples is 74.2% and 90.7% respectively.

In routine practice, the GeneXpert MTB/RIF test was quite faster 3 - 24 hours than culture, which required 19 days. The GeneXpert MTB/RIF test was positive for 32 of 35 culture-positive samples and 6 of 65 culture-negative samples in our study, the sensitivity of the MTB/RIF test was found to be lower than that of culture.

Our study further strengthens the use of GeneXpert in pulmonary samples as endorsed by WHO. In patients with negative results of smear microscopy but having high clinical or radiological evidence of pulmonary tuberculosis like, sputum smear negative TB. Physicians may exercise their clinical decision to start anti tubercular treatment after sending sputum sample for GeneXpert and Culture. To detect the resistance to ATT drugs. To prescribe appropriate regimen for the patients. And to prevent the spread of PTB, and incidence of MDR TB in our country.

However, GeneXpert does not eliminate the need of conventional microscopy, culture and anti-tubercular drug sensitivity that are required to monitor the progression of

treatment and to detect resistance to drugs other than Rifampicin.

Conclusion

GeneXpert is a rapid, reliable, attractive tool with least bio safety concern and requiring minimal training. And give very fast and accurate results within 2 hours, when compared with smear microscopy and culture which took days to weeks to give positive results. It is best alternative to conventional methods of tubercular diagnosis which also detects rifampicin resistance simultaneously. And its efficacy is comparable to culture.

References

1. Sachdeva KS, Kumar A, Dewan P, Kumar A, Satyanarayan S. New Vision for Revised National Tuberculosis Control Programme (RNTCP): Universal access Reaching the unreached. *Ind J Med Res.* 2012;135 (5):690-4.
2. India, Ministry of Health and Family Welfare, Central TB Division, Directorate General of Health Services, TB INDIA 2014 Revised National TB Control Programme, Annual status report, 2014.
3. Urdea M, Penny LA, Olmsted SS, Giovanni MY, Kaspar P, Shepherd A, *et al.* Requirements for high impact diagnostics in the developing world. *Nature* 2006;444:1:73-9.
4. WHO endorses new rapid tuberculosis test, WHO Media center Press release, Geneva, December, 2010.
5. World health organization-policy statement: automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system. Geneva: world health organization, 2011.
6. Robert B, Elizabeth S, Danica H, JoAnn K, Padmapriya B, Michelle RO, *et al.* Evaluation of the analytical performance of the Xpert MTB/RIF assay. *J ClinMicrobiol.* 2010 Jul;48(7):2495-2501.
7. Helb D, Martin J, Elizabeth S, Catharina B, Ellen W, Ken Ho, *et al.* Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of ondemand, near-patient technology. *J Clin Microbiol* 2010 Jan;48:229-237.
8. Xpert® MTB/RIF, Two-hour detection of MTB and resistance to rifampicin. CGXMTB/RIF-10, [Internet], Rev. A, 2009, April.
9. Monika Agrawal *et al.*, Comparative Study of GeneXpert with ZN Stain and Culture in Samples of Suspected Pulmonary Tuberculosis. *Journal of Clinical and Diagnostic Research.* 2016 May;10(5):DC09-DC12.
10. Sharma SK, Kohli M, Yadav RN, Chaubey J, Bhasin D, Sreenivas V, *et al.* Evaluating the Diagnostic Accuracy of Xpert MTB/RIF Assay in Pulmonary Tuberculosis, 2015. *PLoS ONE* 10(10):e0141011.
11. Poojan Shrestha, Amit Arjyal, Maxine Caws, Krishna Govinda Prajapati, Abhilasha Karkey, Sabina Dongol, *et al.* Buddha Basnyat Tuberculosis Research and Treatment, 2015.
12. Sujatha, sunilnarute, prathibasinghal, Vipul kalley, Gene Xpert for the diagnosis of Tuberculosis, 2/18, 2014.
13. Arzu N. Zeka, Sezai Tasbakan, Cengiz Cavusoglu. Evaluation of the GeneXpert MTB/RIF Assay for Rapid Diagnosis of Tuberculosis and Detection of Rifampin Resistance in Pulmonary and Extra pulmonary Specimens *Journal of Clinical Microbiology.* 2011; Dec;49(12):4138-4141 0095-1137/11/12.00
14. Lombardi G, Di Gregori V, Girometti N, Tadolini M, Bisognin F, Dal Monte P. Diagnosis of smear-negative tuberculosis is greatly improved by Xpert MTB/RIF. *PLoS ONE.* 2017;12(4):e0176186.
15. Catharina Boehme C, Mark Nicol P, Pamela Nabeta *et al.* Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *Lancet* 2011;377:1495-1505.
16. Panayotis Ioannidis, Dimitrios Papaventsis, Simona Karabela, Stavroula Nikolaou, Marina Panagi, Ekaterini Raftopoulou, *et al.* Cepheid GeneXpert MTB/RIF Assay for Mycobacterium tuberculosis Detection and Rifampin Resistance Identification in Patients with Substantial Clinical Indications of Tuberculosis and Smear-Negative Microscopy Results. *Journal of Clinical Microbiology,* 2011, Aug, 3068-307.