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Pathology

Rapid Diagnosis of Tuberculosis Using Xpert MTB/RIF Assay - Report from a Tertiary Care Hospital in a Hilly City of Northern India

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Abstract | **Original Research Article**

Background: An alarming rise in the global incidence of infections caused by Mycobacterium tuberculosis (MTB) has prompted the need for rapid diagnostic techniques. ***Objective:*** To evaluate the positivity rate of the Xpert MTB/ RIF assay for the detection of M. tuberculosis in pulmonary as well as extra pulmonary specimens. ***Methods:*** During a period of 12 months from January 2017 through December 2017, 3987 clinically TB suspects were enrolled for Xpert MTB\RIF assay in Indira Gandhi Medical College, shimla. The cohort comprised of 1590 suspects of pulmonary TB and 2397 of extra-pulmonary TB (EPTB). The 1590 pulmonary samples include 1150 sputum sample and 440 bronchial-alveolar lavage. The 2397 EPTB samples included pus aspirated from different sites of the body , pleural fluid , ascetic fluid ,peritoneal fluid, synovial fluid aspirate, Peri-nephrotic fluid, gastric lavage, lymph node (LN) aspirate, pericardial fluid, CSF, semen ,endometrial biopsy etc. Xpert MTB/RIF assay was performed on all samples from these patients. ***Results:*** M. tuberculosis (MTB) were detected by Xpert MTB/RIF test in 537(13.47%) out of 3987 samples. 358 out of 1590 pulmonary samples (22.52%) while 179 out of 2397 extra pulmonary samples (7.47%) were positive for MTB by Gene Xpert. Among PTB cases the highest yield of positivity was shown in BAL samples while among EPTB it was for LN Aspirates. Rifampicin resistance was detected in 24 (4.47%) patients. Among these 18 (5.03%) were pulmonary TB Patients and 6(3.35%) were EPTB patients. ***Conclusion:*** Xpert MTB/RIF is a sensitive method for rapid diagnosis of Tuberculosis, especially for PTB case. For countries endemic for TB GeneXpert can serve as a time saving diagnostic modality for detection of M. tuberculosis.

Keywords: Pulmonary and extra-pulmonary Tuberculosis, Gene-Xpert.

INTRODUCTION

TB is the ninth leading cause of death worldwide and the leading cause from a single infectious agent. In 2016, there were an estimated 1.3 million TB deaths among HIV-negative people (down from 1.7 million in 2000) and an additional 374 000 deaths among HIV-positive people. An estimated 10.4 million people fell ill with TB in 2016: 90% were adults, 65% were male, 10% were people living with HIV and 56% were in five countries: India, Indonesia, China, the Philippines and Pakistan. In 2016, there were 600 000 new cases with resistance to rifampicin (RRTB), the most effective first-line drug, of which 490 000 had multidrug-resistant TB (MDR-TB). Almost half (47%) of these cases were in India, China and the Russian Federation [1].

India is the country with the highest burden of TB. As per the Global TB report 2017 the estimated incidence of TB in India was approximately 28,00,000 accounting for about a quarter of the world's TB cases[2]. It is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent TB rather than TB disease [3]. According to WHO, 15% of newly reported TB cases were extrapulmonary[4].

An alarming rise in the global incidence of infections caused by Mycobacterium tuberculosis (MTB) has prompted the need for rapid diagnostic techniques [5]. Inability to rapidly diagnose and treat the affected patients leads to increased morbidity and mortality, development of secondary resistance and ongoing transmission of the disease[6].

Conventionally the diagnosis of pulmonary tuberculosis has been based on clinical scenario, chest X-ray findings, smear microscopy for acid fast bacillus,

or bacterial isolation by culture. In developing countries, out of all the lab investigations, diagnosis still relies heavily on the use of smear microscopy, which has a low sensitivity and specificity as compared to the culture. The microbiological identification of M. tuberculosis by culture remains the gold standard for diagnosis of tuberculosis. However, it does not provide a rapid diagnosis, is a cumbersome procedure and requires sophisticated laboratory facilities of biological safety lab level II/III that cannot be afforded in most of resource limited settings. As an alternate, recent molecular diagnostic techniques are increasingly being promoted owing to their rapid turnaround time and high sensitivity and specificity [7].

The World Health Organization has endorsed the implementation of GeneXpert MTB/ RIF assay for national tuberculosis programs in developing countries. The Xpert MTB/RIF is an automated, user friendly and rapid test based on nested real-time PCR assay and molecular beacon technology for MTB detection and RIF resistance. The results are obtained within a short period of 2 hours. Further on, the technique is not prone to cross-contamination, requires minimal Biosafety facilities and has a high sensitivity in smear-negative pulmonary TB. The diagnosis of EPTB is often difficult to establish, considering that number of bacteria in specimens is often very low, a collection often requires invasive procedures, and it is not easy to obtain multiple samples. In this scenario GeneXpert is a potentially useful tool for extrapulmonary specimens [8, 9].

The RNTCP program is currently scaling up its policy of Universal DST whereby all cases diagnosed with TB will receive a minimum of Rifampicin and Isoniazid resistance testing. For decentralized diagnosis of TB and Rifampicin resistance CBNAAT machines have been provided at district levels. In the year 2017, more than one million CBNAAT tests have been conducted. In addition to the existing 628 Machines, 507 machines have been procured and deployed to cover all districts of the entire country [2, 3].

The purpose of this study was to evaluate the positivity rate of the Xpert MTB/ RIF assay for the detection of M. tuberculosis in pulmonary as well as extrapulmonary specimens and also for rifampicin resistance.

METHODS

A total of 3987 patients were included in this study during a period of 12 months from January 2017 through December 2017. The cohort was comprised of pulmonary (n=1590) and extra-pulmonary (n=2397) TB suspects. Inclusion of pulmonary TB suspects was based on clinical symptoms (productive cough for more than two weeks, persistent low-grade fever, night sweat and weight loss) and radiological findings consistent with tuberculosis. Sputum samples or bronchio alveolar lavage were collected from all these cases depending upon the age. The EPTB suspects were selected on the basis of clinical presentation, radiological findings and histo-pathological evidence. The extra-pulmonary samples were comprised of pus aspirated from different sites of the body, pleural fluid , ascetic fluid ,peritoneal fluid, synovial fluid aspirate, peri-nephrotic fluid aspirate, gastric lavage, lymph node aspirate, ascetic fluid aspirate, pericardial fluid, CSF, semen, endometrial biopsy etc.

Processing of samples

Sputum: All the sputum samples were subjected to:

1. ZN staining for smear microscopy following the WHO recommended protocol.
2. Xpert MTB/RIF assay: Sputum samples were processed directly from Xpert MTB/RIF test, according to manufacturer's protocol. Sample reagent was added in a 2:1 ratio to unprocessed sputum in 15 ml falcon tube and the tube was manually agitated twice during a 15 minute incubation period at room temperature. Then 2 ml of the inactivated material was transferred to the test cartridge by a sterile disposable pipette (provided with kits). Cartridges were loaded into the GeneXpert. The interpretation of data from MTB/RIF tests was software based and not user dependent.

Extrapulmonary samples: EPTB samples were concentrated by cyto-centrifugation at 3000g for 20 minutes and the deposit was processed as for sputum sample using, ZN staining and Xpert MTB/RIF assay.

RESULTS

A total of 3927 clinically TB suspects were enrolled for Xpert MTB\RIF assay at Indira Gandhi medical college, Shimla (Himachal Pradesh) during a period of 12 months from January 2017 through December 2017. The cohort comprised of 43.29 % males, followed by 35.94% females, 11.46 male children (<18 years) and 9.26% female children. Among the total study participants, 491 patients were <18 years of age, while 38.22% were between 18-40 years, 27.19% were 41-60 years and 22.27% were >61 years of age.

Table 1: Age and Gender distribution of the TB Patients

S.No.	Variables	Frequency	Percentage (%)
1.	Age Group		
	<18 Years	491	12.32
	18-40 Years	1524	38.22
	41-60 Years	1084	27.19
	>61 Years	888	22.27
2.	Gender		
	Male	1726	43.29
	Female	1433	35.94
	Male Child	457	11.46
	Female Child	369	9.26

Table-2: Positivity rate and Rif Resistance rate of various PTB & EPTB Samples

S. No.	Type of Sample	Total Number of Sample	Positive For MTB	Positivity Rate (%)	Resistant For Rif (n)	Rif Resistance (%)
1.	Pulmonary Samples					
	Sputum Samples	1150	256	22.26	11	4.30
	BAL (Bronchio-Alveolar-Lavage)	440	102	23.18	7	6.86
	Total	1590	358	22.52	18	5.03
2.	Extra Pulmonary Samples					
	Pleural Fluid	460	33	7.17	---	
	Pus	341	55	16.13	1	1.81
	Lymph Node Aspirate	98	26	26.53	4	15.38
	GIT Samples (Ascetic Fluid, Gastric Lavage , Liver Abscess, Peritoneal Fluid Etc)	813	38	4.67	1	2.63
	Bone & Cartilage Samples (Bone Barbitage, Joint Aspirate, Knee Aspirate, Synovial Fluid Aspirate Etc)	100	7	7.00	---	
	Genitor Urinary Samples (Endometrial Biopsy, Kidney Aspirate, Left & Right Uterine Fluid Aspirate, PCN, Perinephrotic Fluid Aspirate, Semen Etc)	262	3	1.15	---	
	CNS Sample (CSF)	310	14	4.52	---	
	CVS Samples (Pericardial Fluid)	13		23.08	---	
	Total	2397	179	7.47	6	3.35
3.	Grand Total	3987	537	13.47	24	4.47

All the samples (PTB & EPTB) were processed by by Xpert MTB/RIF assay for Mycobacterium tuberculosis (MTB) detection. Among pulmonary samples positivity rates were 22.26% & 23.18% for sputum and BAL respectively. While positivity rate for pleural fluid, pus, LN Aspirate, GIT Samples, Bone & cartilage samples, genitor-urinary samples, CNS Samples and CVS Samples were 7.17%, 16.13%, 26.53%,4.67%,7.00%,1.15%,4.52% and 23.08% respectively(Table 2).

Table-3: Overall results of Gene Xpert

Results	Pulmonary	Extra-pulmonary	Total
No. of samples	1590	2397	3987
MTB Detected	358 (22.52%)	179 (7.47%)	537 (13.47%)
Rifampicin resistance Detected	18 (5.03%)	6 (3.35%)	24 (4.47%)

Out of the total 3987 samples (1590 pulmonary TB, 2397 EPTB) Mycobacterium tuberculosis (MTB) was detected by Xpert MTB/RIF assay in 537 (13.47%) samples, 358 (22.51%) being pulmonary TB suspects and 179 (7.47%) EPTB suspects (Table-3). There is a significance difference

found between detection rate among PTB & EPTB samples (P<0.05). Rifampicin resistance was detected in 24 (4.47%) patients .Among these 18 (5.03%) were pulmonary TB Patients and 6(3.35%) were EPTB patients.

All cases with positive radiological, histological and bacteriological evidence for TB were referred for anti-tuberculosis therapy to the treating physician.

DISCUSSION

Conventional laboratory techniques as ZN smear microscopy for diagnosis of tuberculosis from clinical specimens is less sensitive because large bacillary load (10^5/ml) will be required for a smear to become positive. Moreover the conventional cultures are time consuming and require Biosafety setup and trained laboratory personnels[10].

The GeneXpert MTB/RIF assay is a rapid molecular biology/ gene based assay that can be used close to the point of care by operators with minimal technical expertise. The technique enables diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 hour. The extra advantage is the convenience of sample processing where unprocessed sputum samples as well as clinical specimens from extrapulmonary sites can be directly assayed [10].

In the present study, we have evaluated the diagnostic accuracy of Xpert MTB/RIF assay both for pulmonary and EPTB cases.

In the present study, among pulmonary samples positivity rates were 22.26% & 23.18% for sputum and BAL respectively. While positivity rate for extra pulmonary samples like pleural fluid, pus, LN Aspirate, GIT Samples, Bone & cartilage samples, genitor-urinary samples, CNS Samples and CVS Samples were 7.17%, 16.13%, 26.53%, 4.67%, 7.00%, 1.15%, 4.52% and 23.08% respectively which was significantly less than pulmonary samples. Similar findings were observed in different studies around the world [11-14]. Rifampicin resistance was detected in 18 (5.03%) pulmonary TB Patients and 6 (3.35%). EPTB patients and the difference were statically significant. The study findings were analogous to various studies around the globe [15-17].

So, the present study finding suggested that the Xpert MTB/RIF assay is a useful addition to the diagnostic armamentarium for rapid diagnosis of both pulmonary TB and EPTB as it has greatly shortened the time of detection up to two hours as compared to other techniques. This advantage is translated into clinical management for patients with smear negative TB as the Xpert assay reduces the time to start treatment for several weeks to just a few days.

CONCLUSION

The Xpert MTB test is sensitive and specific for rapid diagnosis of pulmonary and EPTB. This tool has an important diagnostic value for detecting MTB in smear negative cases as it has outperformed ZN microscopy. It can increase the detection of MTB in EPTB by 2-3 times as compared to conventional techniques. We suggest that in addition to its recommended use in MDR cases, its routine use may be extended to screening of smear negative patients with high suspicion of TB and for diagnosis of EPTB. We further recommend, more such studies should be conducted to evaluate the feasibility of using this instrument in our local health care settings.

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