Original Research Article

Utility of GeneXpert as a diagnostic tool for extra pulmonary tuberculosis

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Abstract

Background: India has the highest tuberculosis (TB) burden with high rates of MDR TB. Conventional TB culture considered as the gold standard often leads to considerable delay in diagnosis. GeneXpert newer diagnostic modality for TB not only detects *Mycobacterium tuberculosis* but also rapidly detects rifampicin resistance. Additional studies from various patient population is needed to reinforce the need of GeneXpert for diagnosis of extra pulmonary TB (EPTB). **Aims:** To evaluate the utility of GeneXpert for the detection of *Mycobacterium tuberculosis* (MTB) in extrapulmonary specimens. **Settings and Design:** A randomized prospective study at a tertiary care hospital in Mumbai over a period of one year. **Methods and Material:** 100 suspected EPTB specimens [pus, tissue, pleural fluid, ascitic fluid, synovial fluid, cerebrospinal fluid (CSF) and urine] were subjected to AFB smear, culture by LJ and MGIT and GeneXpert as per standard protocols and results were compared. **Statistical Analysis:** Significance of differences among categorical variables was examined by the Chi-square test and Fischer's exact test **Results:** AFB smear positivity was only 35%, while using GeneXpert, overall MTB detection rate and rifampicin resistance rate was 50% and 14% respectively. Sensitivity and specificity of GeneXpert were 89.58%, and 86.54% respectively with PPV- 86.00% and NPV- 90.00%. **Conclusions:** GeneXpert when compared with culture, shows high sensitivity and specificity for the diagnosis of EPTB.
Key-Words: Extrapulmonary tuberculosis, GeneXpert, Rifampicin resistance

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Received Date: 10/06/2018 Revised Date: 02/07/2018 Accepted Date: 14/08/2018
DOI: https://doi.org/10.26611/1008722

Access this article online

Quick Response Code:

Website: www.medpulse.in

Accessed Date: 20 August 2018

INTRODUCTION

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) is still one of the world's deadliest communicable diseases. It typically affects the lungs but can affect other sites like lymph nodes, meninges, kidney, spine etc. As per WHO global report 2015, India has the highest TB burden accounting for 23% of the global cases with an estimated incidence of 2.2 million. Majority of the patients in India presents with pulmonary TB, however 0.27 million cases were notified as new extrapulmonary tuberculosis (EPTB).[1] In 2014, globally, 3.3% of new cases and 20% of previously treated cases had Multidrug Resistant TB (MDR-TB) which resulted in approximately 1,90,000 deaths. In India, the estimated prevalence of MDR-TB is about 2.2% in new cases and 15% in retreatment cases.[1] Indian studies have documented as high as 19% rates of MDR-TB in case of EPTB patients.[2] Conventional TB diagnosis relies on smear microscopy, isolation of the causative organism on culture, radiological/ histological findings and clinical suspicion. The diagnosis of EPTB cases is challenging due to inadequate volume and paucibacillary nature of the biological samples.[3,4]. Conventional culture considered as the gold standard often leads to considerable delays, compromising patient care and outcomes. In 2010, WHO endorsed a novel, rapid, automated, cartridge-based nucleic acid amplification test (CBNAAT), GeneXpert or Xpert® MTB/RIF assay (Cepheid, Sunnyvale, USA) for diagnosis of pulmonary TB and simultaneous detection of rifampicin resistance to be completed within 2 hours.[5,6]

How to cite this article: Basu Shaoli, Baveja Sujata, Swami Anjali M, Taklikar Shripad. Utility of GeneXpert as a diagnostic tool for extra pulmonary tuberculosis. *MedPulse International Journal of Microbiology*. August 2018;7(2): 10-13.
https://www.medpulse.in/Microbiology/

This test has high sensitivity and specificity[6] and can be used by operators with minimal technical expertise. Moreover it is not prone to cross-contamination and requires minimal biosafety facilities.[7,8] In 2013, updated recommendations suggested that GeneXpert should be used in addition to conventional microscopy and culture as the initial diagnostic test in adults and children suspected of having MDR-TB, HIV-associated TB or suspected TB meningitis.[9] Additional studies from various patient population is needed to reinforce the need of Xpert MTB/Rif test for the diagnosis of EPTB and detection of Rifampicin resistance.[10,11] Hence this study was done to test the utility of the GeneXpert system for the detection of MTB in extrapulmonary specimens and its comparison with conventional methods.

METHODS AND MATERIAL

A prospective study was conducted in Department of Microbiology at a tertiary care hospital in Mumbai from January 2014 to December 2014 on 100 randomly selected suspected cases of extrapulmonary TB. Inclusion criteria included age > 18 years, patients with high suspicion of EPTB based on history and clinical findings. Exclusion criteria included age < 18 years, exclusive pulmonary TB and patients already on antituberculosis treatment (ATT). The study was approved by institutional ethics committee. Written informed consent was obtained from the patient prior to participation in the study. A detailed history including the demographic profile, presenting complaints, past history, co-morbidities and treatment details was recorded. On basis of clinical findings, clinically relevant samples were collected under aseptic precautions in a sterile container. Samples included were pus, tissue, pleural fluid, ascitic fluid, cerebrospinal fluid (CSF), urine and synovial fluid from knee aspirate. All samples were immediately transported to the laboratory. If not processed immediately it was stored in refrigerator at 4^{o}C for a maximum of 48 hours. Processing of samples was carried out in Bio Safety Cabinet (BSC) 2 and level 2 biosafety practices were followed. Each sample was divided equally into 2 parts, one used for smear and culture and the other for GeneXpert. First part of the sample was decontaminated by NALC NaOH method and sediment was used for microscopy for acid-fast bacillus (AFB) by Ziehl-Nelsen [ZN] staining and culture on egg based solid medium (Lowenstein–Jensen) and liquid medium (MGIT [Mycobacteria Growth Indicator Tube] 960 culture; BD Microbiology Systems) as per standard protocol with quality control. Second part of all samples was used for GeneXpert. They (except CSF) were processed directly (tissue specimens were chopped into very small pieces) by the addition of a 2:1 volume of SR buffer. For CSF, equal volume of sample reagent was added to the CSF. 2 ml of the sample mixture was added directly to the GeneXpert cartridge. The cartridge was loaded into the GeneXpert instrument following the manufacturer's instructions and results were interpreted.

Statistical Analysis: Statistical analysis was performed with the software package: PASW statistic 18 for Windows. The significance of differences among groups of categorical variables was examined by the Chi-square test and Fischer's exact test for large and small samples respectively. A value of p of ≤ 0.05 was considered significant for all statistical analyses.

RESULTS

All 100 samples suspected of EPTB were tested for *Mycobacterium tuberculosis* (MTB) by GeneXpert and compared with conventional methods [includingconventional microscopy, solid culture (LJ) and liquid culture (MGIT)] for detection of MTB. Out of 100 cases, 63% were in the age group of 18-30 years followed by 31-45 years (24%). Overall the mean and median age was 31 years and 26 years (18-77 years) respectively. 50% were males (male to female ratio = 1:1); however, there was higher female preponderance in the age group 18-30 years. Hospitalised patients (61%) were in significantly higher proportion as compared to outpatients (39%). Out of 100 cases of suspected EPTB, 8% were HIV positive. Overall, AFB smear positivity by Ziehl-Neelsen (ZN) stain was seen in 35% of specimens. Using LJ medium, only 34% were culture positive whereas 66% did not show any growth. Out of 100, only 95 specimens were subjected to MGIT due to insufficient sample; wherein culture positivity by MGIT was observed only in 45 (47.37%). Overall, using GeneXpert, MTB was detected in 50% of TB suspected specimens. GeneXpert test also provided a semi-quantitative report of the number of DNA copies detected in the specimen. Out of 50 detected positive by GeneXpert, 14%, 66%, 16% and 4% of specimens were detected as very low, low, medium and high bacillary load respectively. Moreover, MTB positivity was significantly higher when MGIT culture and GeneXpert were used as compared with smear and LJ culture. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of GeneXpert as compared with AFB smear, culture on LJ and MGIT are mentioned in Table 1. Overall, considering culture as gold standard, sensitivity and specificity of GeneXpert were 89.58%, and 86.54% respectively with PPV- 86.00% and NPV- 90.00% ($p<0.01$*). GeneXpert detects MTB along with Rifampicin resistance which is an added advantage. Among the 50 GeneXpert positive specimens, 14% showed Rifampicin resistance.

Table 1: Comparison of GeneXpert with AFB smear, LJ and MGIT culture

MTB detected by Gene Xpert	Positive	Negative	Total	Sensitivity	Specificity	PPV	NPV	p value by Chi square test
AFB Smear								
Yes	33	17	50					
No	02	48	50	94.28	73.84	66	96	**<0.01***
Total	35	65	100					
L J Culture								
Yes	32	18	50					
No	02	48	50	94.12	72.73	64	96	**<0.01***
Total	34	66	100					
MGIT Culture								
Yes	40	05	45					
No	05	45	50	88.89	90	88.89	60	**<0.01***
Total	45	50	95					
Total Culture (LJ/MGIT)								
Yes	43	07	50					
No	05	45	50	89.58	86.54	86	90	**<0.01***
Total	48	52	100					

*p value is significant

DISCUSSION

TB is one of the leading causes of death due to an infectious etiology. In India, TB accounts for one death every 2 minutes.[1,12] A major hindrance to the diagnosis of EPTB is it's atypical presentation, often simulating neoplasia and/or inflammatory disorders. Difficulty in sampling from the extrapulmonary sites and the paucibacillary nature of the specimens also make EPTB a diagnostic challenge.[3,4,13] Dependency on smear microscopy in these samples may lead to higher false negative rates due to the low sensitivity of this technique. MTB culture is quite a prolonged technique, requiring well-trained laboratory personnel and thereby can cause delay in diagnosis and delay in treatment as well. GeneXpert as recommended by WHO in 2013 helps in early diagnosis of EPTB along with detection of rifampicin resistance.[9] In the present study, out of the 100 specimens tested, MTB was detected in 50% of the EPTB suspected cases using GeneXpert. These results are comparable with the study by Zeka *et al.*[14] in 2011 which showed GeneXpert positivity of 54.17% (26/48) amongst the extrapulmonary samples. Similarly Vadwai V. *et al.*[10] from Mumbai also demonstrated comparable findings with GeneXpert positivity of 42.8% (228/533) amongst the 533 extrapulmonary samples. However in 2014, Scott *et al.*[15] from South Africa reported a lower positivity of 22% from the 1175 extrapulmonary samples using GeneXpert. In the present study we compared GeneXpert with total culture (LJ/MGIT) positive specimens. We found 90% of total culture positive specimens was also detected positive by GeneXpert, but it failed to detect in remaining 10% culture positive specimens. Also, 87% of culture negative specimens were also detected negative using GeneXpert. However, 13% of the culture negative specimens were detected positive by GeneXpert. These detection rates by GeneXpert was found to statistically significant ($p< 0.01$). One of the possible reasons for non-detection of specimens by GeneXpert even though when they were positive on culture could be due to reduced numbers of *M. tuberculosis* in the specimen, dilution by Sample Reagent (SR) buffer, or too harsh a treatment by SR buffer. The tissue samples were minced into pieces and well mixed and homogenised before inoculating into culture, hence yielding better results in these paucibacillary specimens which probably could not be picked by GeneXpert as there it was directly inoculated with SR buffer. On the other hand, detection of some samples by GeneXpert even though being culture-negative can be explained due to paucibacillary nature of extrapulmonary specimens. Also there is a tendency of *M. tuberculosis* to form clumps which leads to an uneven distribution of the bacilli, as a result, it is not picked up on culture. Loss of viable bacilli during NALC-NaOH processing (decontamination) is also contributory. These GeneXpert positive but culture negatives samples, however, showed clinical/ cytological features or radiological evidence highly suggestive of TB reflecting true disease. In the present study, GeneXpert when compared with culture showed sensitivity of 89.58%; specificity of 86.54%; Positive Predictive Value (PPV) of 86.00% and Negative Predictive Value (NPV) of 90.00%. These results were comparable with the study by Causse M. *et al.*[16] which showed GeneXpert sensitivity of 95% and specificity of 100% in EPTB samples. Moreover, Malbruny *et al.*[17] in 2011 showed GeneXpert having PPV of 85.7% and NPV of 97.3%. In the study by Ozkutuk N.

et al.[18] in 2014 showed, overall sensitivity, specificity, positive and negative predictive values of GeneXpert assay for extrapulmonary specimens as compared to culture as 58.2%, 98.4%, 66.7% and 97.7% respectively. These study findings are discordant with the present study. A study by Vadwai V. *et al*.[10] detected rifampicin resistance using GeneXpert in 19.30% (44/228) of the positive cases which was concordant with the present study in which 14% of the GeneXpert positive were detected with Rifampicin resistance.

CONCLUSION

GeneXpert assay when compared with culture, shows high sensitivity and specificity for the diagnosis of EPTB. It has a short turnaround time and also simultaneously detects rifampicin resistance within 2 hours. Thus, the GeneXpert assay could be a useful addition to the diagnostic armamentarium for rapid diagnosis of extrapulmonary tuberculosis. Further studies involving larger subsets of populations should be undertaken. Also since the possibility of false positive and false negative results cannot be ruled out, clinical correlation with the report is important.

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Source of Support: None Declared
Conflict of Interest: None Declared