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ACCURACY AND IMPACT OF GENEXPERT MTB/RIF ASSAY FOR THE DIAGNOSIS OF SMEAR-NEGATIVE TUBERCULOSIS USING BRONCHOALVEOLAR LAVAGE FLUID IN A TERTIARY CARE HOSPITAL IN KOLKATA, INDIA

Microbiology

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ABSTRACT

Early detection is mainstay for tuberculosis treatment. Fifty-nine of 148 smear-negative bronchoalveolar lavage samples detected very low concentrations of M. tuberculosis in GeneXpert assay (38.86%); 56 were cultured on extended incubation and grew M.tuberculosis. Seven showed Rifampicin resistance(4.73%) by GeneXpert assay also confirmed by phenotypic method. The sensitivity, specificity, PPV, NPV of GeneXpert were 100%, 96.74%, 94.92% and 100% respectively.

KEYWORDS

Tuberculosis; Bronchoalveolar lavage; GeneXpert; BACTEC MGIT

INTRODUCTION:

Tuberculosis remains a constant threat for the entire world. World Health Organization (WHO) documents 8 million cases and 1.3 million deaths each year[1]. One of the main challenges for adequate control is delay in case detection and early treatment, thereby interrupting transmission chains. The resistance towards two major drugs (Rifampicin and Isoniazide) had rang the alarm among the scientists that consideration of a more sensitive and quicker diagnostic tool is necessary for the proper management[2]. The development of the GeneXpert assay (Cepheid, Sunnyvale, CA, USA), brought promise in terms of increased case detection[2,3]. GeneXpert is the only fully automated and heminested real-time DNA-based PCR test which can detect both Tuberculosis and rifampicin resistance (RR)[2,3]. The PCR target, an 81-base-pair region of the *rpoB* gene, is the rifampicin resistance determining region (RRDR)[2,3]. The GeneXpert assay was highly effective for identification of rifampin-resistant strains in smear-negative samples[4]. The reactions take place in a single-use cartridge, thus without cross contamination and with the turnaround time of the test being less than two hours[5,6].

OBJECTIVE OF STUDY:

The objective of this study was to analyse the efficacy of GeneXpert for detecting tubercle bacilli in smear negative bronchoalveolar lavage fluid (BAL) in a suggestive patient group belonging to Indian subcontinent.

STUDY METHODOLOGY:

In this retrospective study, BAL fluids from 148 patients were collected over the period of one year (September 2014 to October 2015) and was tested for *Mycobecteruim tuberculosis*. This study was conducted at Peerless Hospitex Hospital and Research Center using samples of bronchial aspirates. We collected 148 BAL samples from the adult patients who met the following inclusion criteria:

i) clinical suspicion of tuberculosis and
ii) smear negativity by Ziehl-Neelsen method.

RESULT AND ANALYSIS:

148 specimens were subjected to GeneXpert MTB/Rif analysis and 59 of them were positive in very low concentrations of Mycobacterium tuberculosis (38.86%). Also, 7 of them showed resistance to Rifampicin (4.73%). Five of these patients received treatment for TB before. All those 148 samples were allowed to grow in rapid culture BD BACTEC (MGIT) which is considered as the gold standard test for detection of TB. However, none of the samples showed any signs of growth within the set time frame of 8 weeks in MGIT. But, upon prolonged incubation (12th week onwards), 56 BAL samples which came positive in GeneXpert, showed growth in MGIT culture. Three out of 59 samples did not grow at all. Seven resistant cases were also confirmed by phenotypic drug sensitivity testing in MGIT. The remaining 89 cases which were also negative in GeneXpert detection, also did not grow in prolonged MGIT culture. This confirms that GeneXpert can detect TB cases with 100% sensitivity and 96.74% specificity as shown in Table 1. The statistics for the calculation was done using freely available online calculator using default parameters (https://www.medcalc.org/calc/diagnostic_test.php).

Table 1. Assessment Of Sensitivity And Specificity Of Genexpert Method For Detection Tb From Bronchoalveolar Lavage (bal)

Statistics	True positive (a)	False negative (b)	False positive (c)	True negative (d)
Number (n)	56	0	3	89
Sensitivity (a/a+b)	100% (95% CI = 93.62%-100.00%)			
Specificity (d/c+d)	96.74% (95% CI = 90.77%-99.32%)			
Positive Predictive Value (a/a+c)	94.92% (95% CI = 85.85%-98.94%)			
Negative Predictive Value (d/b+d)	100% (95% CI = 95.94%-100.00%)			

DISCUSSION:

This report demonstrates that GeneXpert plays a pivotal role in identifying TB from the smear negative cases which were otherwise would have been missed even after bronchoscopy and subsequent microscopy. Zeka et al found 68.8 % sensitivity and 100% specificity in smear-negative pulmonary specimens using GeneXpert method . In another study conducted by Ioannidis et al, the sensitivity and specificity for microscopically negative specimens were 86.3%, 93.0% respectively . But in our study, we have only considered smear negative BAL specimens and there were three false positive cases detected (Table 1). Although MGIT culture is considered as the "gold standard" for final determination, but it is slow and may take up to 2 to 8 weeks for detection . None of our very low concentration isolates grew in 8 weeks in this culture. Growth was indicated only after 12 weeks of incubation. Lee et al established that the sensitivity and specificity of the GeneXpert assay using bronchial washing or BAL fluid for the diagnosis of pulmonary tuberculosis (PTB) were 81.6%, and 100% respectively with positive predictive value (PPV) and negative predictive value (NPV) being 100% and 92.1%, respectively . Almost every study performed to date and meta-analysis has indicated consistently high specificity of 99% using this is method . Theron et al also suggested that MTB/RIF mediated detection of TB cases are more accurate and rapid than smear microscopy . Drug resistance surveys in several states of India have revealed that the prevalence of drug resistant TB is 2–3% among new cases and 12-17% among reinfection cases[12].

CONCLUSION:

In the present study we are reporting the efficient detection of TB using GeneXpert MTB/RIF assay in Indian population. To our knowledge, this is the first report of TB diagnosis from smear negative BAL samples from Indian population using GeneXpert MTB/RIF assay. Our study demonstrates that the GeneXpert is as good as MGIT detection system in terms of accuracy with the advantage of extremely short detection time and availability of rifampicin susceptibility result simultaneously. Detection of drug resistant cases from smear negative BAL samples and prolonging the set conventional incubation time for MGIT culture highlights the importance of the finding.

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CONFLICT OF INTEREST:

Authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION:

AKS performed the the bronchoscopy and supplied the samples. PC and DC collected the samples and subsequent data over the period of the study. DG critically analyzed the patients and wrote the manuscript. SG and DG formulated the idea of the study, performed the statistical analysis and organized the manuscript. SM and SC helped in analyzing patient profile.

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