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Pulmonary Medicine

TO STUDY CORRELATION BETWEEN RESULTS OF GENE EXPERT AND AFB CULTURE IN BRONCHIAL ASPIRATE SAMPLES IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS

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ABSTRACT Tuberculosis remains one of the deadliest communicable diseases. There are number of tests available for the diagnosis of tuberculosis but conventional microscopy has low sensitivity and culture although gold standard, but takes longer time for positivity. On the other side, Nucleic acid amplification techniques due to its rapidity and sensitivity not only help in early diagnosis and management of tuberculosis especially in patients with high clinical suspicion like immunocompromised patients, history of contact with active tuberculosis patient etc., but also curtail the transmission of the disease.

KEYWORDS : tuberculosis , microscopy , culture , immunocompromised, sensitivity

Introduction :

According to the global tuberculosis report 2014 of world health organization (WHO) , Tuberculosis remains one of the worlds' deadliest communicable diseases that is caused by mycobacterium tuberculosis . The disease usually affects the lungs (pulmonary tb) and spread by air transmission from people with pulmonary tb . In 2013 , out of the estimated global annual incidence of 9 million TB cases / year . Early diagnosis is imperative for early patient management and successful patient outcomes . False negative results and misdiagnosis of TB suspects are common in developing nations , as most TB control programmes use ZN smear microscopy , which has poor sensitivity and multiple visits Are required that leads to higher default.

Mycobacterial culture , although considered as the gold standard but is slow and usually takes 2-6 weeks time to yield a final result and requires proper infrastructure and technical expertise There are number of nucleic acid amplification methods that have been developed for rapid detection and identification of MTB in clinical specimens of pulmonary and extra pulmonary tuberculosis cases . These techniques not only provide the advantage of rapidity of diagnosis but also detect even low MTB genomic copies in various specimens More recently, the WHO endorsed the GeneXpert (Xpert® MTB/Rif assay) for the diagnosis of TB . The GeneXpert utilizes a DNA-PCR technique for simultaneous detection of Mycobacterium tuberculosis and Rifampicin resistance related mutations. It is the first fully automated bench top cartridge based nucleic acid amplification (CB-NAAT) assay for TB detection that includes all necessary steps of DNA PCR. It gives results within 2 hours. Diagnostic accuracy of GeneXpert for pulmonary TB has been reported high . Patients with high risk of tuberculosis like presumptive HIV-associated TB patients and pediatric presumptive including extra pulmonary cases in whom AFB smear examination is usually negative, are the most likely to be benefited from GeneXpert .

AIM: To evaluate the sensitivity, specificity, positive predictive value and negative predictive value of Nucleic acid amplification assay (GeneXpert) using Bronchial aspirate samples in patients with suspected pulmonary tuberculosis and compare with Acid Fast Bacilli (AFB) culture.

MATERIALS AND METHODS:

INCLUSION CRITERIA : Patients with clinical suspicion of pulmonary tuberculosis including symptoms of cough with or without expectoration for > 2 weeks , weight loss , fatigue , hemoptysis and loss of appetite

EXCLUSION CRITERIA :

1. SAMPLES RECEIVED WITHOUT CLINICAL HISTORY
2. SAMPLES RECEIVED WITHOUT REQUEST OF ALL THREE TESTS
3. PATIENT WITH HISTORY OF LUNG MALIGNANCIES OR FUNGAL INFECTIONS

Pulmonary specimens of 150 patients with suspected pulmonary tuberculosis, received retrospectively for the request of liquid AFB culture and GeneXpert from different wards , icu , and different centers to Microbiology lab and DMC Lab of Smt Kashibai Navale medical college and general hospital , pune . were reviewed from a period of January 2021 to November 2021. Pulmonary specimens included 150 BAL samples. Patient related information was collected from the Test Requisition Forms (TRF), received with the sample.

LABORATORY METHODS :

Each BAL samples received in the lab from wards and icu as per the collection and transportation policy of the laboratory were divided into 2 parts . One part was tested for gene expert and second part for AFB culture on the same day . Gene expert testing was performed according to the manufacturer's instructions. Sample reagent was added to BAL , manually agitated and kept for 10 min at room temperature , then shaken again and kept for 5 min , 2ml of the inactivated material was transferred to the test cartridge and inserted into the test platform . Only electronic results were used for comparison .

Second part was processed using the N acetyl –l cysteine – sodium hydroxide method as per the manufacturers instructions , cultured on MGIT media and incubated in MGIT BACTEC 320 liquid culture system . Sodium hydroxide is a decontaminating agent and also acts as emulsifiers and NALC acts as a mucolytic agent and also reduces the concentration of Naoh required . When the tubes were flagged positive by the system , AFB culture on 5% sheep blood agar were performed from the tube directly to see any contamination as per the manufacturers instructions .

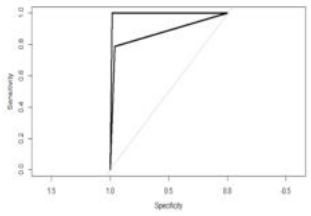
All tubes were checked for positivity till 42 days . Mycobacterium tuberculosis testing from positive cultures tubes were done by rapid immunochromatography test kit using MPT 64 antigen according to the manufacturers instructions

ANALYSIS : The data was tabulated in microsoft excel spreadsheet in a master chart and studied for correlation . Stastical analysis of the data was conducted with stastical package for the social science system version SPSS17.0. Sensitivity , specificity , ppv and npv was calculated . The sensitivity , specificity , ppv and npv for the diagnosis of pulmonary tuberculosis was calculated for AFB culture and gene expert .

Results :

A total of 150 bronchial aspirate specimens were tested, out of which 22 samples were gene expert and culture positive , 8 samples were gene expert positive , and 5 samples were only culture positive Data on categorical variables are presented as n (%) or median (interquartile range) for continuous variables. Receiver operating characteristic curves are plotted to compare AUC of gene Xpert and culture to detect MTB in bronchial aspirate samples. Youden's index was used to determine the optimal cut off point to calculate sensitivities and specificities.

p-values less than 0.05 are considered statistically significant. Analysis is done using R version 4.2.2



Test	Sensitivity [95% CI]	Specificity [95% CI]	NPV [95% CI]	PPV [95% CI]	AUC [95% CI]
Gene Xpert	0.99 [0.98 – 1.00]	0.98 [0.96 – 1.00]	0.99 [0.97 – 1.00]	0.93 [0.85 – 1.00]	0.99 [0.98 – 1.00]
Culture	0.79 [0.64 – 0.93]	0.96 [0.93 – 0.99]	0.95 [0.92 – 0.98]	0.82 [0.68 – 0.95]	0.82 [0.68 – 0.95]

DISCUSSION:

In this retrospective study , we have evaluated the diagnosis yield of gene expert to detect MTB in bronchial aspirate samples and compared it with AFB culture which was taken as gold standard . Mycobacterial cultures for detection of mycobacterium tuberculosis can be done either using solid (LJ Media) or liquid broth system (MGIT 320). Results by MGIT liquid culture medium , come earlier as compared to LJ Medium . In our study MGIT 320 culture were included . Gene xpert 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 days to come positive.

In our study overall sensitivity , specificity , PPV, and NPV of gene xpert were 99%, 98%, 99%, 93% which when compared with overall sensitivity , specificity , PPV, and NPV of afb culture were 79%, 96%, 95%, 82% suggested that overall sensitivity and specificity of gene expert is superior to that of afb culture in bronchial aspirate samples.

However gene expert 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 .

LIMITATIONS :

There were certain limitations of the study, first the study was performed retrospectively and results couldn't be correlated with radiological findings and histo pathological reports . Second , one of the important strength of the xpert assay is its ability to detect the presence of rifampicin resistance .The sensitivity and specificity of MTB/RIF assay to detect rifampicin resisitance in our study was not evaluated and not included in our objective as we didn't get the requisition for rifampicin sensitivity by the phenotypic method in all the positive samples Third , number of bronchial aspirates samples in study was less , further sudies with more number of samples need to be done .

CONCLUSION :

Gene xpert and AFB culture share almost same specificity but sensitivity of gene expert is much higher than AFB culture in bronchial aspirate samples . Although culture is considered as a gold standard method but as it takes days to come positive and simultaneous detection of rifampicin resistance is not possible with it . On the other side gene xpert can be a useful diagnostic method in patients of suspected pulmonary tuberculosis either afb smear negative or positive due to its rapidity and simultaneous detection of rifampicin resistance especially beneficial in patients with MDR and HIV associated tuberculosis . Cost effectiveness of gene expert in low income countries like india with high prevelance of tuberculosis need to be done . Positive gene expert but culture negative results need to be read cautiously and should be well correlated with clinical and treatment history of the patient

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