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COMPARATIVE STUDY OF SMEAR MICROSCOPY AND GENEXPERT WITH MGIT CULTURE IN CLINICAL SAMPLES OF SUSPECTED EXTRAPULMONARY TUBERCULOSIS[EPTB]

Clinical Microbiology

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ABSTRACT

Introduction: EPTB comprises a wide spectrum of disease affecting all parts of the body excluding the lungs. Challenges faced in diagnosis of EPTB is difficulty in sample collection from deep.seated tissue and a good number of specimens are smear negative. It is thus easy to misdiagnose. There is an urgent need for rapid and accurate laboratory diagnosis of EPTB. **Materials And Methods:** study, conducted over a period of 1 year. Total Samples (n=680) from suspected EPTB patients (age >18 years) were tested for smear microscopy, GeneXpert and AFB culture. **Observation And Results:** A total of 680 samples were tested. Out of which, 62 samples were positive and 485 samples were negative by all three methods. Among 680, 102 samples were culture and GeneXpert positive, 18 samples were only GeneXpert positive and 13 samples were only culture positive.Out of 680 samples, 62 samples were found to be AFB smear positive. All these AFB smear positive samples were culture and GeneXpert positive. **Discussion And Conclusion:** GeneXpert has a higher sensitivity than AFB smear microscopy .GeneXpert can be a useful tool for early diagnosis of patients with high clinical suspicion of EPTB. GeneXpert is found to be a rapid test for the diagnosis of TB and rifampicin resistance when compared to smear microscopy and culture.

KEYWORDS

EPTB, GENEXPERT, ZN STAIN, MGIT.

INTRODUCTION

In India, tuberculosis (TB) is a community health problem. According to WHO Tuberculosis Report (2017), 24% of the global TB burden is seen in India.[1] It is worth noting that 27% of TB cases were extra-pulmonary infections. Of the 5.4 million new tuberculosis cases notified to WHO in 2013, 0.8 million were extrapulmonary disease [2]. Extrapulmonary tuberculosis (EPTB) comprises a wide spectrum of disease affecting all parts of the body excluding the lungs. Commonly affected sites include lymph nodes, pleura, urogenital tract, bones and joints, meninges, central nervous system (CNS), bowel and/or peritoneum, pericardium and skin. Challenges faced in diagnosis of EPTB is the difficulty in sample collection from deep.seated tissue and a good number of specimens are paucibacillary or smear negative [3,4]. It is, thus, easy to misdiagnose. There is an urgent need for rapid and accurate laboratory diagnosis of EPTB. By detecting active Extrapulmonary TB early, an appropriate treatment can be initiated, and disease rate can be decreased. Diagnosis of extrapulmonary TB (EPTB) remains especially challenging since the number of *Mycobacterium tuberculosis* (MTB) bacilli present in tissues at sites of disease is often low and clinical specimens from deep-seated organs may be difficult to obtain. Early diagnosis is imperative for early patient management and successful patient outcomes. Ziehl-Neelsen (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 [5,6,7]. More recently, the WHO endorsed the GeneXpert (Xpert® MTB/Rif assay) for the diagnosis of TB [8]. The GeneXpert utilizes a DNA-PCR technique for simultaneous detection of *MTB* 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 [9,10]. Patients with high risk of tuberculosis like presumptive HIV-associated TB patients and extra pulmonary cases in whom AFB smear is usually negative, are the most likely to be benefited from GeneXpert. [10,11].

AIM:

To evaluate the Sensitivity, Specificity, PPV and NPV of ZN stain and GeneXpert assay with smear positive and smear negative samples of suspected EPTB and compared with AFB Liquid culture(MGIT).

MATERIALAND METHODS:

This is a prospective study, conducted over a period of 1 year august 2018 to july 2019, at the Department of Microbiology, Tertiary care hospital, Telangana, India. Total Samples (n=680) from suspected EPTB patients (age >18 years) were subjected for ZN staining , GeneXpert MTB/RIF assay and AFB culture. Various samples included were FNAC, lymph node biopsy, transbronchial needle aspiration, abscess , pleural fluid ,pleural biopsy ,CSF and urine. Patients who were on ATT, relapse cases and swabs, stool and blood samples were excluded from the study.Non-sterile clinical samples were pre-treated by conventional *N*-acetyl-L-cysteine-NaOH digestion decontamination procedure. Specimens from sterile sites were centrifuged and used directly[12].Each sample was divided into three parts; one for GeneXpert, second for ZN smear microscopy and third for MGIT BACTEC 320 liquid culture.

Analysis :

The diagnostic test evaluation calculator MEDCALC easy-to-use statistical software https://www.medcalc.org/calc/diagnostic_test.php was used for statistical analysis. The sensitivity, specificity, PPV and NPV was calculated for AFB smear microscopy and the GeneXpert, using MGIT culture as gold standard. By taking culture method as reference, samples that were positive and negative in culture were considered true positive and true negative. Culture negative and GeneXpert positive samples were taken as false positive samples. GeneXpert negative and culture positive samples were considered false negative.

Observation And Results:

A total of 680 samples were tested. Out of which, 62 samples were positive and 485 samples were negative by all three methods respectively. Among 680, 102 samples were culture and GeneXpert positive, 18 samples were only GeneXpert positive and 13 samples were only culture positive

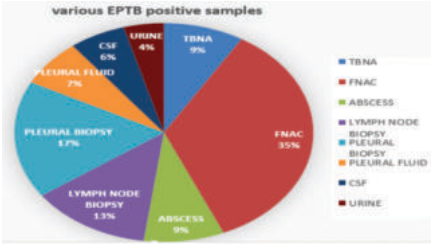

Fig-1: Distribution of Various positive EPTB samples,depicted as percentage.

Table-1: Comparison of results from GeneXpert with culture as gold standard.

Xpert TB	Culture	
	Positive	Negative
Positive	102	18
Negative	13	485

Altogether, 115 (17%) samples were culture positive for AFB; 112 isolates were found to belong to MTB, while the remaining 3 strains were identified as Mycobacterium other than tuberculosis (MOTT) species. Further speciation of these isolates was not done. Out of 680 samples, 62 samples were found AFB smear positive. All these AFB smear positive samples were culture and GeneXpert positive.

Table 2 : Comparison of results from AFB smear and culture as gold standard.

ZN stain	Culture	
	Positive	Negative
Positive	62	0
Negative	53	485

Among 618 AFB smear microscopy negative samples, 485 samples were negative for all three methods. In rest 133 AFB smear negative samples, 102 samples were culture and GeneXpert positive, 18 samples were GeneXpert positive and culture negative and 13 samples were culture positive and GeneXpert negative.

Overall sensitivity, specificity, PPV and NPV of GeneXpert when culture was taken as reference method is illustrated as:

Table- 3

Specimen type	Sensitivity	Specificity	PPV	NPV
All samples (n=680)	89.60%	96.93%	86.15%	97.76%
AFB negative (n=618)	79.37%	96.42%	73.53%	97.39%

Overall sensitivity, specificity, PPV and NPV of AFB smear microscopy when culture was taken as reference method is shown in:

Table 4

ZN stain	Sensitivity	Specificity	PPV	NPV
	69.14%	100%	100%	93.15%

DISCUSSION:

Diagnosing EPTB remains challenging because of various reasons: symptoms varying depending on the organ involved, clinical samples obtained from relatively inaccessible sites being paucibacillary; all these factors decreasing the sensitivity of diagnostic tests. Since the conventional smear microscopy has a low sensitivity, negative results cannot exclude the presence of TB. All these factors lead to a delay in diagnosis. Accurate and rapid laboratory investigations have therefore gained importance. This study assesses the diagnostic usefulness of smear microscopy and GeneXpert to detect MTB in extra pulmonarysamples. The results obtained are compared with the MGIT culture, which is taken as the gold standard.

MGIT culture showed a negative result for 5 samples which were positive by GeneXpert.,as it can amplify any DNA ,live or dead.,therefore Clear history of treatment with ATT is required to avoid false positive results [13,14]. 58 Samples were positive by GeneXpert and negative by ZN staining. AFB smear examination requires 10000 CFU/mL to give a positive result[15]. Most EPTB samples are paucibaillary making the smear negative. Moreover, these GeneXpertpositive samples had grown MTB by MGIT culture, showing that the GeneXpert result was truly positive.

Arora D et al[16] reported ZN sensitivity, specificity, PPV, NPV of 65.7, 95.7, 79.3, 91.8 respectively ,which were similar to present study. A survey on extrapulmonary samples by Habouset al[17] reported the sensitivity specificity, PPV and NPV of GeneXpert to be 82.7%, 100%, 100%, and 92.8%, respectively.[24] which were similar to present study.

CONCLUSION:

GeneXpert has a higher sensitivity than AFB smear microscopy .GeneXpert can be a useful tool for early diagnosis of patients with high clinical suspicion of EPTB. Positive GeneXpert, but culture negative results should be read cautiously and be well correlated with clinical and treatment history of the patient. GeneXpert is found to be a rapid test for the diagnosis of TB and rifampicin resistance when compared to smear microscopy and culture. 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 other side GeneXpert can be a useful diagnostic method in patients of suspected extrapulmonary tuberculosis due to its rapidity and simultaneous detection of Rifampicin resistance. GeneXpert., as it can amplify any DNA, live or dead. 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.

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