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Original Article

Histopathological/Cytological Correlation of Cervical Tubercular Lymphadenitis cases with GeneXpert and MGIT TB Culture

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Abstract

Introduction: Tuberculosis (TB) is one of the major health problems in India. India is the second most populous country in the world behind China but India has the maximum number of TB cases worldwide accounting for one fourth of the global TB cases.

Materials and Methods: A hospital-based observational study was undertaken to analyze the sensitivity, specificity, positive predictive value and NPV of nucleic acid amplification assay (GeneXpert) using samples in 86 patients with suspected EPTB and compare with MGIT and histopathology/cytology.

Results: A hospital-based observational study was undertaken to analyze the sensitivity, specificity, positive predictive value, and NPV of Nucleic acid amplification assay (GeneXpert) using samples in 86 patients with suspected EPTB and compare with MGIT and histopathology/cytology. All results are depicted in form of tables in main manuscript.

Conclusion: Rapid TB tests may be the key to worldwide TB control strategies. The high sensitivity and specificity, coupled with its speed and simplicity, make the GeneXpert MTB the most useful tool in the rapid diagnosis of TB. This rapid TB diagnostic test may complement usual methods (conventional microscopy, culture, and histopathology).

Key words: Cervical lymph nodes, Drug culture, Tuberculosis

INTRODUCTION

Tuberculosis (TB) is a disease caused by mycobacterium tuberculosis (MTB) which is a gram positive, aerobic, acid, and alcohol fast bacillus. TB is one of the major health problems in India. India is the second most populous country in the world behind China but India has the maximum number of TB cases worldwide accounting for one fourth of the global TB cases. In 2013, out of the estimated global annual incidence of 9 million TB cases, 2.1 million were estimated to have occurred in India.[1] During recent years, there has been emergence of resistance to multiple drugs in TB bacilli which has become a great threat to public health. When TB bacilli become resistant to both isoniazid and rifampicin or only mono-resistant to rifampicin it is called multidrug-resistant TB (MDR TB).[2] With additional emergence of resistance to 2nd line drugs, that is, to any fluoroquinolone, and to at least one of the three injectable second-line drugs (amikacin, kanamycin, and capreomycin) it becomes Extensive Drug-Resistant TB (XDR TB).[2] According to the World Health Organization (WHO), MDR-TB update 2015 about 5% (in comparison to 3.7% in 2013) of new TB patients in the world have MDR-TB and 9.7% (in comparison to 9% in 2013) of MDR-TB cases also have resistance to two other classes of drugs or extensively drug resistant TB (XDR-TB).[3] Resistance in new TB cases is defined as primary drug resistance, the presence of resistant strains of MTB in patients who have never received anti- TB drugs or who have been treated for <1 month. Resistance in previously treated cases is defined as secondary drug resistance, the presence of a resistant strain of MTB in patients who have received anti-TB drugs in the past or who have been treated for more than 1 month.[4] Drug resistant TB (DR-

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TB) is a manmade problem[5] because of inadequate use of drug, inappropriate prescription and poor adherence or compliance to treatment. These factors permit the selective growth and multiplication of drug resistant strains. The emergence of DR-TB strains is a global problem which poses a serious threat to the best of efforts of prevention and TB control.[6] The WHO reported that DR-TB is increasing in various parts of world as well as in India.[7] TB commonly involves the lungs also known as pulmonary TB but extra-pulmonary sites are also commonly involved. Extra pulmonary TB (EPTB) is a paucibacillary disease and the affected patients are non-infectious. EPTB include[4] lymph node TB, tuberculous pleural effusion, central nervous system TB, spinal TB, abdominal TB, genitourinary TB, pericardial TB, and Skin TB. Extrapulmonary sites could be involved due to any of the following mechanisms: (a) Spread by hematogenous route is the most common mode of disease at extrapulmonary site, (b) reactivation of a pre-existing focus of TB infection (post primary TB), (c) spread due to contiguity like in pleural TB, pericardial TB, gastrointestinal TB, and (d) direct inoculation as seen in skin TB. EPTB and EPTB with MDR TB/XDR TB are a significant health problem in both developing and developed countries.[8] Diagnosis of EPTB in its different clinical presentations remains a true major challenge due to different sites of involvement and paucibacillary nature of the disease. There is scarcity of data regarding EPTB MDR patients and most of the available studies focus on total MDR patients. However details of EPTB MDR subset of patients of total MDR patients and their demographic profile is not clearly studied in the literature. More so drug resistance reported in EPTB is a challenge to diagnosis and management. Due to this fact our study is unique, that is focusing on EPTB MDR patients, their prevalence, demographic details, and associated comorbidities.

TB has been a major global public health problem from times immemorial. WHO estimates shows that globally there are 8.6 million incident cases of TB of which 80% are in 22 countries, with India ranked as the highest burden country.[9]

The diagnosis of pulmonary TB can be obtained from microscopy and culture of a number of different sources including regular sputum, induced sputum, gastric washings, and bronchoscopy. The sensitivity, specificity and diagnostic yield of each of these tests vary widely between studies.[10-16] Sputum induction with hypertonic saline requires additional resource allocation and manpower training, but has been shown to increase the diagnostic yield of sputum examination in several studies.[17-19]

Microbiological diagnosis is the main stay for the effective treatment of pulmonary TB for obtaining the correct sputum sample, patient education is imperative. However, even if the correct sample is expectorated, the bacillary population has to be at least 10000 per milliliter, to get the smear positive for acid fast bacilli (AFB).[20] Moreover, it depends on the previous treatment, default behavior, and effective cough. Difficulties arise when a patient who is suspected of active TB, both clinically and radiologically, does not produce sputum. Harris *et al.* found that 40–60% of patient with active pulmonary TB suspected clinically or radiologically may fail to produce sputum, or when it is available AFB may be negative.[21]

Diagnosis of extrapulmonary TB (EPTB) remains especially challenging since the number of MTB bacilli present in tissues at sites of disease is often low and clinical specimens from deep-seated organs may be difficult to obtain. Histology is time-consuming to undertake and establishing a diagnosis of TB with high specificity remains difficult. Tissue microscopy after special staining is often negative and when mycobacteria are seen, it is impossible to distinguish MTB from nontuberculous mycobacterial disease.

In recent times, attention has been devoted to new nucleic acid amplification diagnostic technologies, due to their rapidity, sensitivity, and specificity. One of the latest systems, the GeneXpert MTB/RIF (Xpert) assay, based on nested real-time PCR and molecular beacon technology, has been shown to be rapid, with a result for TB and RIF drug resistance under 2 h.[22]

Nucleic acid amplification tests for rapid TB diagnosis are increasingly being used. The US CDC recommends that nucleic acid amplification tests be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB.[23] However, no recommendation exists for their use in the investigation of patients suspected of having EPTB as the evidence base is limited.

The Xpert® MTB/RIF assay (Cepheid Inc., CA, USA) marks an important development in the field of rapid molecular TB diagnostics.[24,25] This multifunctional diagnostic platform is an automated, closed system that performs real-time PCR and can be used by operators with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 h. Sputum samples can be analyzed with very minimal processing, yielding positive diagnoses in 99–100% of patients with smear-positive pulmonary TB and 57–83% of patients with smear-negative pulmonary TB in clinical evaluation studies.[24] The Xpert MTB/RIF assay was rapidly endorsed by the WHO in December 2010 as a replacement for sputum smear microscopy,

particularly in settings with high rates of HIV-associated TB and multidrug-resistant TB.

Since Xpert MTB/RIF was specifically developed and optimized for testing sputum samples and initial large-scale evaluations were in patients with pulmonary TB, the WHO endorsement specifically applied to the investigation of pulmonary TB. More recently; however, evaluations of the assay have extended to a variety of non-respiratory clinical samples from patients with EPTB. The evidence base for use in the investigation of EPTB remains comparatively weak; however, many more studies assessing a variety of clinical samples other than sputum are therefore needed.

Aims and Objectives

The objectives are as follows:

- To analyze the sensitivity, specificity, positive predictive value and negative predictive value (NPV) of nucleic acid amplification assay (GeneXpert) using samples in patients with suspected EPTB
- To compare with MGIT and histopathology/cytology in suspected EPTB patients.

MATERIAL AND METHODS

A hospital-based observational study was undertaken to analyze the sensitivity, specificity, positive predictive value and NPV of nucleic acid amplification assay (GeneXpert) using samples in 86 patients with suspected EPTB and compare with MGIT and histopathology/cytology.

Study Site

A tertiary health-care institute in a metro city.

Study Population

Patients with cervical lymphadenopathy visiting the outpatient department of surgery and pulmonary medicine.

Study Design

This was a hospital-based observational study.

Study Duration

18 months.

Sample Size

86 patients.

The customized excel sheet for sample size calculation prepared from standard references (Patrikar)[33] was used to calculate sample size for present study. With reference to the study of Hillemann *et al.*,[34] the sensitivity and specificity of GeneXpert in diagnosing EPTB (prevalence was 22% in similar institute) published in article were 77% and 98%, respectively. At 20% precision the estimated sample sizes are 78. Assuming non response rate of 10% the corrected sample size is 78 + 7.8 = 86. The sample size was selected using simple random sample.

Inclusion Criteria

The following criteria were included in the study:

1. Adults >18 years male/female
2. Patients giving consent.

Exclusion Criteria

The following criteria were excluded from the study:

1. Pregnant lady
2. Children
3. Patient not giving consent.

Methodology

All patients with cervical lymphadenopathy visiting the outpatient department of surgery and pulmonary medicine. After obtaining informed written consent their evaluation included detailed history and clinical examination performed with investigations including Gene Expert, MGIT and histopathology, cytology/fnac, and Mantoux test.

Suspected case of EPTB

Clinical, Radiological (XRAY/USG/CT), Mantoux test

Biopsy done-

1. Histopathology/cytology
2. MGIT
3. GeneXpert

Laboratory Methods

Each sputum and BAL samples received in the lab from the centers as per the collection and transportation policy of the laboratory were divided into three parts; one part was immediately tested using GeneXpert, second part used for ZN smear microscopy and third part for MGIT BACTEC 320 liquid culture and performed on same day. Only one sample either BAL or sputum from a single patient was divided and processed. For liquid culture as much as sample was taken after sending for GeneXpert and ZN stain but it should be checked that volume remaining should not be <2 mL for processing.

GeneXpert testing was performed according to the manufacturer's instructions. Sample reagent was added to untreated sputum and BAL at a ratio of 2:1, manually agitated and kept for 10 min at room temperature, then shaken again and kept for 5 min; 2 mL of the inactivated material was transferred to the test cartridge and inserted into the test platform. Only electronic results were used for comparison. Direct Smear microscopy was performed to investigate presence of AFB with the second part of

the specimen using conventional ZN staining method. Slides showing red colored AFB were taken as positive and negative slides were those without any AFB.

Third part was processed using the N-acetyl-L cysteine-sodium hydroxide method (NaOH) as per the manufacturer's instructions, cultured on MGIT media and incubated in MGIT BACTEC 320 liquid culture system. NaOH is a decontaminating agent and also acts as emulsifier and NALC acts as a mucolytic agent and also reduces the concentration of NaOH required. When the tubes were flagged positive by the system, ZN staining and culture on 5% sheep blood agar were performed from the tube directly to see any contamination as per the manufacturer's instructions. All tubes were checked for positivity till 42 days. Mycobacterium other than TB (MOTT) and MTB testing from positive culture tubes were done by rapid immunochromatography test kit using MPT 64 antigen according to the manufacturer's instructions.

Statistical Analysis

Quantitative data are presented with the help of Mean and Standard deviation. Comparison among the study groups is done with the help of unpaired *t*-test as per results of normality test. Qualitative data are presented with the help of frequency and percentage table. Association among the study groups is assessed with the help of Fisher test, student "*t*" test and Chi-square test. $P < 0.05$ is taken as significant.

Pearson's Chi-squared test

$$X^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

Where X2 = Pearson's cumulative test statistic.

Oi = an observed frequency;

Ei = an expected frequency, asserted by the null hypothesis;

n = the number of cells in the table.

Results were graphically represented where deemed necessary.

Appropriate statistical software, including but not restricted to MS Excel, SPSS ver. 20 will be used for statistical analysis. Graphical representation will be done in MS Excel 2010. Sensitivity and specificity were estimated using standard formula.

OBSESRVATIONS AND RESULTS

A hospital-based observational study was undertaken to analyze the sensitivity, specificity, positive predictive value, and NPV of Nucleic acid amplification assay (GeneXpert) using samples in 86 patients with suspected EPTB and compare with MGIT and histopathology/cytology.

Distribution of Patients According to Age

Majority of the patients (42.1%) were from the age group of 31–40 years followed by 25.6% from the age group of 41–50 years, 13.8% from the age group of 51–60 years, 11.6% from the age group of 21–30 years, and 6.9% from the age group of >60 years.

Distribution of Patients According to Gender

There was male preponderance (56.9%) while female patients constituted 43.1% of the study group.

Distribution of Patients According to Symptoms

About 88.4% and 70.9% patients presented with cough and fever, respectively. The other symptoms were breathlessness (51.2%), loss of appetite (44.22%), chest pain (40.7%), hemoptysis (33.7%), and weight loss (16.3%).

Distribution of Patients According to Histopathological Findings

Histopathological findings noted that 19 (22.1%) patients had EPTB while 67 (77.9%) patients showed negative results.

Distribution of Patients According to Cytology Findings

Cytology findings noted that 20 (23.2%) patients had EPTB while 66 (76.8%) patients showed negative results.

Distribution of Patients According to Gene Xpert Findings

Gene Xpert findings noted that 21 (24.4%) patients had EPTB while 65 (75.6%) patients showed negative results.

Distribution of Patients According to MGIT Findings

MGIT findings noted that 22 (25.6%) patients had EPTB while 64 (74.4%) patients showed negative results.

Comparison of Gene X-pert Findings with Histopathological Findings

The sensitivity and specificity of Gene Xpert were calculated at 84.21% and 92.54% respectively. The positive predictive value of Gene Xpert is 76.19% and the NPV is 95.38%.

Comparison of MGIT Findings with Histopathological Findings

The sensitivity and specificity of MGIT were calculated at 78.95% and 89.55%, respectively. The positive predictive value of MGIT is 68.18% and the NPV is 93.75%.

Comparison of Cytology with Histopathological Findings

The sensitivity and specificity of cytology were calculated at 73.68% and 91.04%, respectively. The positive predictive value of MGIT is 70% and the NPV is 92.42%.

Table 1: Distribution of patients according to age

Age (years)	*n*	%
21–30 years	10	11.6
31–40 years	36	42.1
41–50 years	22	25.6
51–60 years	12	13.8
>60 years	6	6.9
Total	86	100

Table 2: Distribution of patients according to gender

Gender	*n*	%
Male	49	56.9
Female	37	43.1
Total	86	100

Table 3: Distribution of patients according to symptoms

Symptoms	*n*	%
Cough	76	88.4
Fever	61	70.9
Breathlessness	44	51.2
Loss of appetite	38	44.2
Chest pain	35	40.7
Hemoptysis	29	33.7
Weight loss	14	16.3

Table 4: Distribution of patients according to Histopathological findings

Histopathological findings	*n*	%
Positive	19	22.1
Negative	67	77.9
Total	86	100

Table 5: Distribution of patients according to Cytology findings

Cytology findings	*n*	%
Positive	20	23.2
Negative	66	76.8
Total	86	100

Table 6: Distribution of patients according to Gene Xpert findings

Gene Xpert findings	*n*	%
Positive	21	24.4
Negative	65	75.6
Total	86	100

Comparison of Various Diagnostic Methods for Diagnosing EPTB

Gene Xpert had highest sensitivity at 84.21% specificity at 92.54%, positive predictive value (PPV) of 76.19% and a NPV of 95.38%. [Tables 1-11].

Table 7: Distribution of patients according to MGIT findings

MGIT findings	*n*	%
Positive	22	25.6
Negative	64	74.4
Total	86	100

Table 8: Comparison of Gene Xpert findings with Histopathological Findings

Gene Xpert	Histopathological	
	Positive (%)	Negative (%)
Positive	16 (18.6)	5 (7.5)
Negative	3 (3.5)	62 (70.4)
Total	19 (22.1)	67 (77.9)

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Gene Xpert	84.21	92.54	76.19	95.38

NPV: Negative predictive value, PPV: Positive predictive value

Table 9: Comparison of MGIT findings with histopathological findings

MGIT	Histopathological	
	Positive (%)	Negative (%)
Positive	15 (17.4)	7 (8.1)
Negative	4 (4.7)	60 (69.8)
Total	19 (22.1)	67 (77.9)

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
MGIT	78.95	89.55	68.18	93.75

NPV: Negative predictive value, PPV: Positive predictive value

Table 10: Comparison of Cytology with Histopathological Findings

Cytology	Histopathological	
	Positive (%)	Negative (%)
Positive	14 (16.3)	6 (7.1)
Negative	5 (5.8)	61 (70.8)
Total	19 (22.1)	67 (77.9)

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Cytology	73.68	91.04	70	92.42

NPV: Negative predictive value, PPV: Positive predictive value

DISCUSSION

A hospital-based observational study was undertaken to analyze the sensitivity, specificity, positive predictive value and NPV of nucleic acid amplification assay (GeneXpert) using samples in 86 patients with suspected EPTB and compare with MGIT and histopathology/cytology.

Table 11: Comparison of various diagnostic methods for diagnosing Extrapulmonary Tuberculosis

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Gene Xpert	84.21	92.54	76.19	95.38
MGIT	78.95	89.55	68.18	93.75
Cytology	73.68	91.04	70	92.42

NPV: Negative predictive value, PPV: Positive predictive value

Diagnosis of EPTB remains a challenge due to a lack of sensitive conventional laboratory techniques. Therefore, nucleic acid amplification techniques play an important role in rapid and accurate diagnosis.

The Xpert assay has brought about a major change in the speed, simplicity, and accuracy of not only diagnosis of TB but also drug resistance to RIF in TB, which is accepted as a surrogate for MDR-TB. The rapidity and robustness of diagnosis in-turn breaks the chain of transmission in addition to early institution of treatment and improved chances for cure. The utility of Xpert assay in diagnosis of pauci-bacillary TB is the most important contribution of the test. WHO policy document 2013 adopted a GRADE system approach to arrive at recommendations[35] on the diagnostic value of the assay in pulmonary and EPTB.

In the present study, majority of the patients (42.1%) were from the age group of 31–40 years followed by 25.6% from the age group of 41–50 years, 13.8% from the age group of 51–60 years, 11.6% from the age group of 21–30 years, and 6.9% from the age group of >60 years. There was male preponderance (56.9%) while female patients constituted 43.1% of the study group. This is similar to the studies of Ghariani *et al.*,[28] Singh *et al.*,[29] and Sarfaraza *et al.*[31]

Ghariani *et al.*[28] study evaluating the performance of the GeneXpert MTB/RIF test for the detection of MTB observed male-to-female ratio was 0.47 (56/118) in a total of 174 patients. The median age of the patients was 32.3 years.

Singh *et al.*[29] prospective study assessing the performance of GeneXpert in 761 extra-pulmonary and 384 pulmonary specimens from patients clinically suspected ofTB found male: female ratio of 1.06. There were more males in the 15–30 years age group [ratio 1.15] and almost equal in number in 31–60 years age group [1.02].

Sarfaraza *et al.*[31] prospective cohort study determining the association between histopathological and microbiological findings in patients clinically suspected TBLA observed TBLA and malignancy affected young patients (median age 23 years and 22.5 years, respectively), whereas reactive nodes were found in the older age group (median age 47 years; $P < 0.0001$). The majority of TBLA and malignancy patients were female (79.2% and 68.4%, respectively), whereas a higher proportion of patients found to have reactive nodes were male (55%; $P = 0.002$).

In our study, 88.4% and 70.9% patients presented with cough and fever respectively. The other symptoms were breathlessness (51.2%), loss of appetite (44.22%), chest pain (40.7%), hemoptysis (33.7%), and weight loss (16.3%). This is consistent with the study of Sarfaraza *et al.*[31]

Sarfaraza *et al.*[31] prospective cohort study determining the association between histopathological and microbiological findings in patients clinically suspected TBLA observed chronic cough in 83 (27.9%) patients, but only six (2.7%) had concomitant PTB.

Histopathological findings in the present study noted that 19 (22.1%) patients had EPTB while 67 (77.9%) patients showed negative results. This is concordant to the studies of Ghariani *et al.*[28] and Sarfaraza *et al.*[31]

Ghariani *et al.*[28] study evaluating the performance of the GeneXpert MTB/RIF test for the detection of MTB reported histopathology was positive for 121 (69.5%) specimens showing the presence of caseation and epithelioid granulomas.

Sarfaraza *et al.*[31] prospective cohort study determining the association between histopathological and microbiological findings in patients clinically suspected TBLA reported presumed TBLA was diagnosed on the basis of suggestive histopathology in 198 (89.6%) patients.

Cytology findings in our study noted that 20 (23.2%) patients had EPTB while 66 (76.8%) patients showed negative results. This is comparable to the studies of Singh *et al.*,[29] Suzana *et al.*,[36] Ghariani *et al.*,[28] and Bagdia *et al.*[32]

Singh *et al.*[29] prospective study assessing the performance of GeneXpert in 761 extra-pulmonary and 384 pulmonary specimens from patients clinically suspected of TB reported 72 pulmonary and 35 extra-pulmonary samples were culture positive.

Suzana *et al.*[36] study evaluating the use of Xpert MTB/Rif assay in a routine diagnostic mycobacteriology laboratory for the diagnosis of EPTB reported of 494 samples analyzed against culture, 101 were smear positive and 393 were smear negative.

Ghariani *et al.*[28] study evaluating the performance of the GeneXpert MTB/RIF test for the detection of MTB

reported AFB smears were positive for 41 cases (23.6%). Scanty AFB (<10 AFB) were observed in 75.6% of smear-positive specimens. 79 (45.4%) of the 174 specimens tested were culture positive. MTBC was isolated on MGIT and LJ medium in, respectively, 78 (98.7%) and 40 (50.6%) culture-positive samples. Among the 174 samples tested, the Xpert detected the DNA of MTBC in 134 samples (77%). 79 specimens (45.4%) were culture positive 55 (31.6%) being smear negative and 24 (13.8%) being smear positive); 22 (12.6%) were "probable TB" cases; 43 (24.7%) were only histologically/cytologically positive showing necrosis, caseation, or epithelioid granuloma suggestive of "possible TB" cases; and 30 (17.2%) patients had no evidence of TB and were "not TB" cases.

Bagdia *et al.*[32] study comparing various diagnostic methods for EPTB found out of 97 total cases, cytology detected 87 as positive, while ZN stain detected only 9 as positive for EPTB, culture detected 20 as positive for EPTB.

Gene Xpert findings in our study noted that 21 (24.4%) patients had EPTB while 65 (75.6%) patients showed negative results. These findings were consistent with the studies of Singh *et al.*,[29] Ghariani *et al.*,[28] Bagdia *et al.*,[32] and Sarfaraza *et al.*[31]

Singh *et al.*[29] prospective study assessing the performance of GeneXpert in 761 extra-pulmonary and 384 pulmonary specimens from patients clinically suspected of TB reported in pulmonary group Gene Xpert detected TB in 72 culture positive and 114 culture negative patients, while in extra-pulmonary group it detected TB in 35 culture positive and 181 culture negative patients.

Ghariani *et al.*[28] study evaluating the performance of the Gene Xpert MTB/RIF test for the detection of MTB observed Xpert detected MTBC DNA in 75/79 of culture-positive specimens.

Bagdia *et al.*[32] study comparing various diagnostic methods for EPTB observed out of the 83 cases on Gene Xpert 58 cases were positive for EPTB.

Sarfaraza *et al.*[31] prospective cohort study determining the association between histopathological and microbiological findings in patients clinically suspected TBLA found of these six patients with PTB, three were infectious with sputum smear or GeneXpert positivity and the rest were diagnosed on clinical and radiological grounds.

MGIT findings in the present study noted that 22 (25.6%) patients had EPTB while 64 (74.4%) patients showed negative results. This finding was consistent with the study of Agrawal *et al.*[30]

Agrawal *et al.*[30] retrospective study evaluating the sensitivity of Nucleic acid amplification assay (GeneXpert) and comparing with AFB smear microscopy and AFB culture reported among the 21 Sputum samples, 11 samples were culture and GeneXpert positive, 1 sample was GeneXpert positive. 38 (22%) specimens were culture positive for AFB; 35 (20%) isolates were found to belong to MTB (11 were from sputum specimens, 24 were from BAL specimen), while the remaining 3 (1.7%) strains from BAL samples were identified as mycobacterium other than TB MOTT species. Out of 170 samples, only 14 samples (6 BAL and 8 sputum samples) were found AFB smear positive. All these AFB smear positive samples were culture and GeneXpert positive.

It was observed in the present study that the sensitivity and specificity of Gene Xpert were calculated at 84.21% and 92.54%, respectively. The positive predictive value of Gene Xpert is 76.19% and the NPV is 95.38%. This is in concordance to the studies of Bagdia *et al.*,[32] Suzana *et al.*,[36] Agrawal *et al.*,[30] and Ghariani *et al.*[28]

Bagdia *et al.*[32] study comparing various diagnostic methods for EPTB reported cytology was compared with Gene Xpert out of 56 cases, cytology could detect 53 cases and Gene Xpert could detect 6. Gene Xpert detected 3 cases, which were negative by cytology. Out of these 3 cases, 2 were also negative by culture, ZN stain and LED microscopy.

Suzana *et al.*[36] study evaluating the use of Xpert MTB/Rif assay in a routine diagnostic mycobacteriology laboratory for the diagnosis of EPTB reported of 46 smear-positive, culture-positive samples, and Xpert/Rif detected 45 of 46. Of 55 smear-positive, culture-negative samples, and Xpert MTB/Rif detected 43 of 55. All 43 were diagnosed as TB by the CGS. Of 54 smear-negative, culture-positive samples, 44 of 54 were detected by Xpert MTB/Rif. All 44 were diagnosed as TB by the CGS. Of 339 smear-negative, culture-negative samples, Xpert MTB/Rif detected 59. In total, 58 of 59 were diagnosed as TB by the CGS. Xpert MTB/Rif had a sensitivity of 87% (95% CI 0.79–0.93) and specificity of 73% (95% CI 0.69–0.78). In total, 102 cases detected by the Xpert MTB/Rif assay, whereas mycobacterial culture remained negative. Of these, 101 patients had either clinically or histologically proven TB or a clinical response when treated with ATT Xpert MTB/Rif had a pooled sensitivity of 89% and specificity of 74%.

Agrawal *et al.*[30] retrospective study evaluating the sensitivity of Nucleic acid amplification assay (GeneXpert) and comparing with AFB smear microscopy and AFB culture reported overall sensitivity, specificity, PPV, and NPV of Gene Xpert were 86.8%, 93.1%, 78.5%, and 96%, respectively.

Ghariani *et al.*[28] study evaluating the performance of the GeneXpert MTB/RIF test for the detection of MTB reported sensitivity and specificity of the Xpert assay were 94.9% and 37.9%, respectively, when compared with culture. The sensitivity of the molecular test in smear- positive or -negative and culture-positive samples was, respectively, 100% and 92.7%. The Xpert test detected TB in 77.6% (45/58) of patients with negative cultures and positive histology. Furthermore, the Xpert assay showed 8 positive results in "not TB" cases.

It was observed in our study that the sensitivity and specificity of MGIT were calculated at 78.95% and 89.55%, respectively. The positive predictive value of MGIT is 68.18% and the NPV is 93.75%. Bagdia *et al.*[32] noted similar observations in their study.

Bagdia *et al.*[32] study comparing various diagnostic methods for EPTB observed histopathology detected 10 as positive, while ZN stain detected 5 as positive for EPTB.

It was observed in the present study that the sensitivity and specificity of Cytology were calculated at 73.68% and 91.04%, respectively. The positive predictive value of MGIT is 70% and the NPV is 92.42%. Similar observations were noted in the studies of Bagdia *et al.*[32] and Agrawal *et al.*[30]

Bagdia *et al.*[32] study comparing various diagnostic methods for EPTB found out of 97 total cases, cytology detected 87 as positive, while ZN stain detected only 9 as positive for EPTB, culture detected 20 as positive for EPTB. Culture detected 3 as positive for EPTB.

Agrawal *et al.*[30] retrospective study reported among 156 AFB smear microscopy negative samples, 123 samples were negative for all three methods. In rest 33 AFB smear negative samples, 19 samples were culture and Gene Xpert positive, 9 samples were Gene Xpert positive and culture negative, and 5 samples were culture positive and Gene Xpert negative. In comparison with culture used as gold standard, sensitivity, specificity, PPV, and NPV for Smear microscopy for BAL sample were recorded as 22.2%, 100%, 100%, and 85.3%, respectively.

In our study, Gene Xpert had highest sensitivity at 84.21% specificity at 92.54%, positive predictive value (PPV) of 76.19% and a NPV of 95.38%. This is similar to the studies of Bagdia *et al.*,[32] Agrawal *et al.*,[30] Ghariani *et al.*,[28] Sarfaraza *et al.*,[31] Suzana *et al.*,[36] Meldau R *et al.*,[27] and Vadwai V *et al.*[26]

Bagdia *et al.*[32] study comparing various diagnostic methods for EPTB reported Gene Xpert had highest sensitivity at 85.71% and a NPV of 98.67%, while LED-FM had the highest specificity at 98% (same as of ZN stain) and highest positive predictive value (PPV) of 86.36%. Positivity rate of histopathology was 90.90% while of LED-FM and ZN was 45.45% and of culture was 27.27%. Sensitivity and specificity of histopathology were 66.67% and 33.33%, respectively.

Agrawal *et al.*[30] retrospective study evaluating the sensitivity of Nucleic acid amplification assay (GeneXpert) and comparing with AFB smear microscopy and AFB culture reported of the 170 specimens, 14 samples were positive and 123 specimens were negative by all three methods used. Among 170 samples, 42 samples (24.7%) were GeneXpert TB positive. Among the 149 BAL samples, 22 samples were culture and GeneXpert positive, 8 samples were GeneXpert positive, and 5 samples were only culture positive. GeneXpert assay had an overall sensitivity of 86.8% and for BAL sample 81.4% for PTB, which is superior to that of smear microscopy (overall 36.8% and for BAL 22.2%). Overall Specificities of GeneXpert and smear microscopy were 93.1% and 100%, respectively. For smear negative samples, sensitivity and specificity of GeneXpert was 79.1% and 93.1%, respectively. For smear positive cases, sensitivity was 100%.

Ghariani *et al.*[28] study evaluating the performance of the GeneXpert MTB/RIF test for the detection of MTB reported sensitivity and specificity of Xpert assay when compared with smear microscopy, culture results and histological findings were 87.5% and 73.3%, respectively. Positive predictive value (PPV) was 94%, whereas the NPV was 55%.

Sarfaraza *et al.*[31] prospective cohort study determining the association between histopathological and microbiological findings in patients clinically suspected TBLA reported microbiological evidence was positive in a minority with Gene Xpert, mycobacterial culture, and AFB smear positivity, and was seen in 90 (32.6%), 72 (26.6%), and 34 (12.5%), respectively. The sensitivity of smear, culture, and Gene Xpert was found to be 12.7%, 30.7%, and 33.2%, respectively, when compared with histopathology suggestive of TB. Gene Xpert was positive for MTB in 44 (65.7%) culture-positive cases and 38 (19.6%) culture-negative cases. 16 Gene Xpert-positive, 11 culture-positive, and six AFB smear-positive patients had a reactive cytology.

Suzana *et al.*[36] study evaluating the use of Xpert MTB/Rif assay in a routine diagnostic mycobacteriology laboratory for the diagnosis of EPTB reported compared to culture, pooled sensitivity and specificity of Xpert MTB/Rif were 89% and 74%, respectively. When Xpert MTB/Rif was compared to the CGS, pooled sensitivity and specificity were 62% and 100%, respectively, for fluids. Xpert MTB/

Rif specificity was 95% for CSF, 83% for tissue, 27% for pus, 59% for LN and 90% for fluids.

Meldau R *et al*.[27] prospective cohort study evaluating the performance of the Xpert MTB/RIF assay, and other diagnostic biomarkers, with suspected pleural TB reported Xpert MTB/RIF sensitivity and specificity (95% CI) was 22.5% (12.4–37.6) and 98% (89.2–99.7), respectively, and centrifugation did not improve sensitivity (23.7%).

Vadwai V *et al*.[86] study on diagnostic accuracy assessments of smear and culture results and clinical, radiological, and histological findings reported sensitivity of the Xpert assay was 81% (228/283 specimens) (64% [89/138] for smear-negative cases and 96% [139/145] for smear-positive cases), with a specificity of 99.6%. The sensitivity was found to be high for the majority of specimen types (63–100%) except for cerebrospinal fluid, the sensitivity of which was 29% (2/7 specimens). The Xpert test correctly identified 98% of phenotypic rifampin (RIF)-resistant cases and 94% of phenotypic RIF-susceptible cases. Sequencing of the 6 discrepant samples resolved 3 of them, resulting in an increased specificity of 98%.

CONCLUSION

Rapid TB tests may be the key to worldwide TB control strategies. The high sensitivity and specificity, coupled with its speed and simplicity, make the GeneXpert MTB the most useful tool in the rapid diagnosis of TB. This rapid TB diagnostic test may complement usual methods (conventional microscopy, culture, and histopathology). Diagnosis of EPTB is challenging due to the paucibacillary nature as well as atypical clinical presentations. Its diagnosis should hence be made by considering more than one diagnostic methods.

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