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Original Article

Utility of CBNAAT (GeneXpert MTB/RIF assay) in rapid diagnosis of extrapulmonary tuberculosis in a hepatobiliary tertiary center

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ABSTRACT

Objectives: Newer diagnostic techniques like cartridge-based nucleic acid amplification techniques (CB NAAT) need to be evaluated for extrapulmonary tuberculosis (EPTB), as being a paucibacillary condition, it is often underdiagnosed with conventional methods. We conducted this study to assess the utility of CB NAAT (GeneXpert MTB/RIF assay) in rapid diagnosis of extrapulmonary tuberculosis.

Material and Methods: Liver disease patients admitted from June 2019 to June 2020 were investigated for EPTB based on clinical and radiological suspicion. EPTB was diagnosed based on one of the following: (i) histological evidence of caseating granulomas; (ii) smear positivity for acid-fast bacilli; (iii) CB NAAT (GeneXpert MTB/RIF assay, Cepheid, USA).

Results: A total of 290 EPTB specimens received in the laboratory were included. The extrapulmonary samples that were received included body fluids (n = 143) which included pleural fluid, ascitic fluid, drain fluids, and pus aspirates, followed by biopsies (n = 82), lymph nodes (n = 43), urine (n = 19), and CSF (n = 3). GeneXpert MTB/RIF assay was positive in 10.3% (n = 30) samples, whereas negative in 89.7% (n = 260) samples. The overall sensitivity of GeneXpert MTB/RIF assay was 61.36% (95% CI 46.62%–74.28%), specificity 89.29% (95% CI 72.8%–96.29%), positive predictive value (PPV) 90% (95% CI 74.38%–96.54%), and negative predictive value (NPV) 59.52% (95% CI 44.49%–72.96%).

Conclusion: The GeneXpert MTB/RIF assay is a valuable tool for extrapulmonary tuberculosis. In addition to other tests like smear, culture GeneXpert MTB/RIF assay helps in the confirmation of diagnosis. Rapid diagnosis of tuberculosis with overall good sensitivity and specificity makes it a beneficial test.

Keywords: Extrapulmonary Tuberculosis, GeneXpert, Histopathology, Paucibacillary, Radiology

INTRODUCTION

Diagnosing extrapulmonary tuberculosis (EPTB), an entity distinct from pulmonary TB is a challenge. EPTB has a high burden comprising 1/5th of all TB cases, which consists of 15%–20% of all TB cases in HIV negative patients, and 40%–50% of new TB cases which are HIV positive.[1] A wide spectrum of disease with commonly involved sites, which include lymph node, urogenital tract, central nervous system, bone and joints, gastrointestinal tract, and cardiovascular system.[2] Being a paucibacillary condition there needs to be a strong diagnostic armamentarium. While most studies have been performed on respiratory samples, we lack literature on extrapulmonary samples. With the advent of recent techniques, nucleic acid amplification techniques (NAATs) play a definitive role in diagnosis of TB.[3] Literature states the use of cartridge-based nucleic acid amplification techniques (CB NAAT) (GeneXpert MTB/RIF assay, Cepheid, USA) in the diagnosis of pulmonary tuberculosis, but there is lesser documentation of its use in EPTB.[4] As compared to smear microscopy and culture, GeneXpert Mycobacterium tuberculosis complex/resistance to Rifampin (MTB/RIF) assay has a low detection limit of 131 CFU/mL, making it beneficial for conditions like paucibacillary EPTB.[5] In a previous study from our center, the prevalence of EPTB was found to be 15.65% among liver disease patients.[6] Thus with this in mind, the study was conducted to assess the role of GeneXpert MTB/RIF assay as a rapid diagnostic tool in EPTB.

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MATERIAL AND METHODS

Statement of Institutional Review Board Approval

Research of the following retrospective study has been approved and recommended by the Institutional Ethics Committee (IEC/2020/56/MA05).

Study population

A total of 290 consecutive patients admitted to our tertiary care center for hepatobiliary disease from June 2019 to June 2020 were investigated. A retrospective analysis of extrapulmonary samples of suspected EPTB patients was done. Patients were investigated for EPTB based on clinical and radiological suspicion. Extrapulmonary samples included lymph node samples, tissue biopsy, urine, Cerebrospinal fluid (CSF), and body fluids such as ascitic fluid, pleural fluid, pus, and drain fluids.

Processing of samples

Samples were processed and subjected to Ziehl–Neelsen (ZN) staining and molecular assay CB NAAT (GeneXpert MTB/RIF assay, Cepheid, USA). Simultaneously samples were sent for cytologic analysis and histopathology. EPTB was diagnosed based on one of the following: (i) histological evidence of caseating granulomas; (ii) smear positivity for acid-fast bacilli; (iii) CB NAAT (GeneXpert MTB/RIF assay, Cepheid, USA).

Statistical data analyses were performed using SPSS Statistics 19 (SPSS Inc., Chicago, IL, USA). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of GeneXpert MTB/RIF assay were calculated using histopathology and response to therapy as the gold standard.

RESULTS

Based on clinical suspicion, patients were sent for radiological examination and patient samples for histopathological, cytological, and microbiological examination. A total of 290 EPTB specimens received in the laboratory within the one year as categorized in Table 1 were included. Analyses were done on a per-sample basis and not on a per-patient basis. Gender distribution included males (n = 206) and females (n = 84). Table 2 shows the results of radiological and histopathological examination along with GenXpert results.

Table1: Description of routine EPTB specimens received and tested using GeneXpert MTB/RIF assay.

Specimen type	Samples received in laboratory, n (%)
Body fluids	143 (49.3%)
Biopsies	82 (28.3%)
Fine-needle aspirates [lymph node]	43 (14.8%)
Urine	19 (6.6%)
CSF	3 (1%)

EPTB: Exptrapulmonary tuberculosis, MTB/RIF: Mycobacterium tuberculosis complex/resistance to Rifampin, CSF: Cerebrospinal fluid

Table 2: Results of radiological, histopathological, and GeneXpert examination.

Examination	TB Suggestive/ detected	Not suggestive/ not detected
Radiology	64 (22.1%)	226 (77.9%)
Histopathology	32 (11%)	258 (89%)
GeneXpert	30 (10.3%)	260 (89.7%)

Results of microbiological examination of samples mainly included ZN staining and GeneXpert MTB/RIF assay. There were few samples received for culture hence combined histopathology and response to therapy were considered the gold standard. GeneXpert MTB/RIF assay was positive in 10.3% (n = 30) samples, whereas negative in 89.7% (n = 260) samples. Histopathology was suggestive of tubercular etiology in 11% (N = 32) samples. Seventy two percent patients received antituberculosis treatment (ATT) based on the results of laboratory or radiological examination or clinical diagnosis in case of negative reports. 44 (61.11%) patients responded to therapy with clinical improvement. To evaluate the performance of the GeneXpert test for lymph node samples we compared the results by combining histopathology and response to therapy. For body fluid samples, response to therapy was considered the gold standard. Table 3 shows the GeneXpert MTB/RIF assay-positive samples. The results were recorded according to the critical threshold values which included very low, low, medium, and high as described in Table 4.

Table 3: Results of GeneXpert MTB/RIF assay positive samples.

Samples	GeneXpert MTB/RIF assay Positive
Lymph node	15/43
Biopsy	2/82
Body fluids	13/143
Urine	0/19
CSF	0/3

MTB/RIF: Mycobacterium tuberculosis complex/resistance to Rifampin, CSF: Cerebrospinal fluid

Table 4: Interpretation of GeneXpert MTB/RIF assay results.

GeneXpert MTB/RIF assay result	%
Very Low	1.7%
Low	5.2%
Medium	2.8%
High	0.7%

MTB/RIF: Mycobacterium tuberculosis complex/resistance to Rifampin

Table 5: Sensitivity, specificity, PPV, and NPV of GeneXpert MTB/RIF assay in EPTB samples.

Sample	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV% (95% CI)
GeneXpert compared to histopathology and response to therapy	61.36 (46.62–74.28)	89.29 (72.8–96.29)	90 (74.38–96.54)	59.52 (44.49–72.96)

EPTB: extrapulmonary tuberculosis, PPV: positive predictive value, NPV: negative predictive value, CI: confidence interval, MTB/RIF: Mycobacterium tuberculosis complex/resistance to Rifampin.

In our study no rifampicin resistance was detected. Using histopathology and response to therapy as the gold standard, the sensitivity, specificity, PPV, and NPV for GeneXpert MTB/RIF assay are given in Table 5.

DISCUSSION

Extrapulmonary TB (EPTB), a paucibacillary disease and a more neglected diagnosis needs to be investigated with as much concern as pulmonary TB. The causes responsible for a difficult diagnosis of EPTB include a neglected clinical suspicion, problems in sample collection, paucibacillary condition, and lack of data on EPTB.[1] Laboratory diagnostic techniques such as microscopy and culture techniques have less sensitivity. The scanty literature on the utility of GeneXpert MTB/RIF assay for extrapulmonary samples makes this study a very important one. Histopathological examination is the gold standard for the diagnosis of EPTB from tissue samples, however, the sampling is often difficult, laboratory set up, is time consuming and needs expertise for diagnosis.[7] The study was conducted in a hepatobiliary tertiary care center, which even further makes the diagnosis important as liver cirrhosis has been implicated as a risk factor for extrapulmonary TB.[8] From our previous study, out of a total of 816 samples which included 260 pulmonary and 556 extrapulmonary samples, the positivity rate by Mycobacterial Growth Indicator Tube culture (MGIT) or MTB qPCR (Cobas TaqMan MTB assay) included pulmonary 31/260 (11.92%) and extrapulmonary 87/556 (15.65%) samples.[6] Thus, at our institute, there is a higher prevalence of EPTB, as opposed to pulmonary TB mandating the need for the establishment of a proper diagnostic protocol. The chances of recovery or detection of organisms from the extrapulmonary samples are less because of the lower load of bacilli present, as opposed to pulmonary samples like sputum in pulmonary TB.[9] Also an invasive sampling technique, lesser quantity of sample mounts difficulties in diagnosis. The diagnostic techniques available are smear microscopy (ZN staining), culture, GeneXpert MTB/RIF assay, and histopathology.[10] The diagnostic value of ZN staining is 0%–40%. In our previous study, in extrapulmonary disease smear positivity (14.9%) was much lower than the pulmonary disease (61.2%).[6] Mycobacterial culture sensitivity varies from 30% to 80%, but because it is a time-consuming method (2–6 weeks) it is not useful in the initiation of an early therapy.[6] Thus it becomes important to have a more rapid test for diagnosis.

Nucleic acid amplification techniques (NAATs) are a substantial aid in the diagnosis. [11] GeneXpert MTB/RIF assay, a real-time PCR is a robust assay with a shortened turnaround time (2 h) with a lower bacterial load (131 Colony forming unit/mL (CFU/mL)) required for detection. An automated method wholly integrated with an individual cartridge reduces the hands-on time and chances of cross-over contamination.[12] In various studies done, sensitivity of GeneXpert MTB/RIF assay ranged from 25% to 95%, with most of the studies exceeding 50%.[13] In our study, the overall sensitivity of GeneXpert MTB/RIF assay was 61.36% (95% CI 46.62%–74.28%), specificity 89.29% (95% CI 72.8%–96.29%), positive predictive value (PPV) 90% (95% CI 74.38%–96.54%), and negative predictive value (NPV) 59.52% (95% CI 44.49%–72.96%). The sensitivity is higher as compared to other diagnostic modalities like ZN stain and radiology. The recent Index TB guidelines for the diagnosis of EPTB categorize its use in various presentations, including lymph node TB, TB meningitis, abdominal TB, and TB pericarditis, and showed a pooled sensitivity and specificity of 83.1% and 93.6%, respectively.[14] Thus along with cytology, smear, and culture, GeneXpert MTB/RIF assay helps in confirmation of the diagnosis of EPTB. Also being a rapid test in comparison with other diagnostic modalities, a quicker diagnosis helps in the early initiation of therapy. The guidelines state that GeneXpert should not be used to diagnose pleural TB because of the low sensitivity of 46.4%, which is like our study where only 1/47 pleural fluid samples were positive.[14] Whereas, in other samples like urine and CSF none of the samples were positive. Amongst the GeneXpert MTB/RIF assay positive samples, being a paucibacillary condition the load of organisms was low (5.2%) followed by medium (2.8%), very low (1.7%), and high (0.7%) based on the critical threshold values. Thus, this study reinforces the fact that GeneXpert MTB/RIF assay is a test which is of great value in EPTB.

A strong clinical suspicion and a better and more rapid diagnostic armamentarium substantiate a quicker and confirmative diagnosis of EPTB. Thus, we tried to analyze different rapid diagnostic modalities to construct a well-formed protocol. In a patient with a clinical suspicion of EPTB histopathology and GeneXpert MTB/RIF assay

remain the two most important diagnostic tests for a rapid diagnosis. A recent modification of GeneXpert MTB/RIF assay is the GeneXpert Ultra which has a lower limit of detection of 10 CFU/mL and thus appears to be extremely useful in such paucibacillary samples.[15] Based on the cycle threshold (CT) value, GeneXpert Ultra has an additional result interpretation called Mycobacterium tuberculosis complex (MTB) trace, further strengthening the diagnostic modality. WHO recommends the use of Xpert Ultra as the initial diagnostic test for suspected tuberculous meningitis with a higher sensitivity of 95%, as compared to either Xpert/culture both having a sensitivity of 45%.[16] The limitation of this study is that very few samples were received for culture, so comparison with culture could not be done.

CONCLUSION

The CBNAAT assay (GeneXpert MTB/RIF)is a valuable tool for rapid diagnosis of extrapulmonary tuberculosis. It is an important tool for diagnosis and can be supplementary to other tests like smear microscopy, mycobacterial culture and histopathology. The test shows comparable sensitivity and specificity and can be used for diagnosis of various extrapulmonary tuberculosis samples.

Author's contribution

All authors have substantive intellectual contributions to this study.

Ethical approval

The authors declare that they have taken the Institutional Ethics Committee approval and the approval number is IEC/2020/56/MA05.

Declaration of patient consent

Patient's consent not required as patients identity is not disclosed or compromised.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

REFERENCES

1. Sharma SK, Mohan A. Extrapulmonary tuberculosis. Indian J Med Res 2004;120:316–53.
2. Lee JY. Diagnosis and treatment of extrapulmonary tuberculosis. Tuberc Respir Dis (Seoul) 2015;78:47–55.
3. Blakemore R, Story E, Helb D, Kop J, Banada P, Owens MR, *et al*. Evaluation of the analytical performance of the Xpert MTB/RIF assay. J Clin Microbiol 2010;48:2495–501.
4. Agrawal M, Bajaj A, Bhatia V, Dutt S. Comparative study of GeneXpert with ZN stain and culture in samples of suspected pulmonary tuberculosis. J Clin Diagn Res 2016;10:DC09–DC12.
5. Pang Y, Shang Y, Lu J, Liang Q, Dong L, Li Y, *et al*. GeneXpert MTB/RIF assay in the diagnosis of urinary tuberculosis from urine specimens. Sci Rep 2017;7:6181.
6. Kale P, Khillan V, Gupta P, Sarin SK. The prevalence and profile of mycobacterial infections in liver diseases from tertiary care hepatobiliary centre. J Clin Diagn Res 2018;12:DC07–DC10.
7. Purohit M, Mustafa T. Laboratory diagnosis of extra-pulmonary tuberculosis (EPTB) in resource-constrained setting: State of the art, challenges and the need. J Clin Diagn Res 2015;9:EE01–EE06.
8. Cho YZ, Lee SM, Yoo CG, Kim YW, Han SK, Shim YS, *et al*. Clinical characteristics of tuberculosis in patients with liver cirrhosis. Respirology 2007;12:401–5.
9. Dam T, Bose M. Paucibacillary tuberculosis – A retrospective study. J Indian Med Assoc 2002;100:231–3.
10. Chakravorty S, Sen MK, Tyagi JS. Diagnosis of extrapulmonary tuberculosis by smear, culture, and PCR using universal sample processing technology. J Clin Microbiol 2005;43:4357–62.
11. Nurwidya F, Handayani D, Burhan E, Yunus F. Molecular diagnosis of tuberculosis. Chonnam Med J 2018;54:1–9.
12. Hillemann D, Rüsch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. J Clin Microbiol 2011;49:1202–5.
13. Scott LE, Beylis N, Nicol M, Nkuna G, Molapo S, Berrie L, *et al*. Diagnostic accuracy of Xpert MTB/RIF for extrapulmonary tuberculosis specimens: Establishing a laboratory testing algorithm for South Africa. J Clin Microbiol 2014;52:1818–23.
14. Sharma SK, Ryan H, Khaparde S, Sachdeva KS, Singh AD, Mohan A, *et al*. Index-TB guidelines: Guidelines on extrapulmonary tuberculosis for India. Indian J Med Res 2017;145:448–63.
15. Dorman SE, Schumacher SG, Alland D, Nabeta P, Armstrong DT, King B, *et al*. Xpert MTB/RIF Ultra for detection of mycobacterium tuberculosis and rifampicin resistance: A prospective multicentre diagnostic accuracy study. Lancet Infect Dis 2018;18:76–84.
16. Bahr NC, Nuwagira E, Evans EE, Cresswell FV, Bystrom PV, Byamukama A, *et al*. Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIV-infected adults: A prospective cohort study. Lancet Infect Dis 2018;18:68–75.

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