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Original Article

Challenges in the Diagnosis of Extrapulmonary Tuberculosis: Role of Gene Xpert Mycobacterium Tuberculosis/Rifampicin Assay

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Abstract

Introduction: Extrapulmonary tuberculosis (EPTB) accounts for about 25% of all cases of active TB. Difficulty in sampling from the extrapulmonary sites and the paucibacillary nature of the specimens make EPTB a diagnostic challenge. Xpert mycobacterium TB (MTB) /rifampicin (RIF) is a fully automated diagnostic test which simultaneously detects TB and RIF drug resistance within few hours.

Aim: The aim is to study the diagnostic role of gene Xpert MTB/RIF in cases of EPTB.

Materials and Methods: All the specimens from patients diagnosed to have EPTB with a composite reference standard (CRS) were subjected to Xpert MTB/RIF. The CRS included parameters such as smear, culture, histology, and cytology reports (for biopsy samples and aspirates, respectively), biochemical tests such as adenosine deaminase levels (for pleural fluid, ascitic fluid, and cerebrospinal fluid [CSF]), and response to treatment during follow-up visits.

Results: Of 108 EPTB cases, maximum 51 (47.2%) were cases of lymph node TB, followed by TB pleural effusion 38 (35.1%), abdominal Koch's 12 (11.1%), and TB meningitis 7 (6.4%). The sensitivity of Xpert MTB/RIF for lymph node specimens was observed to be 62.7%, for pleural fluid 31.5%, and for ascitic fluid 41.6%. None of the CSF samples was reported MTB positive, of 7 by Xpert MTB/RIF.

Conclusion: Xpert MTB/RIF results may not be fully satisfactory from the clinical point of view. However, test should still be done because of its simplicity, reliability, and rapid results. Not only MTB detection but also rapidly determining the patient's multidrug-resistant tuberculosis status in such cases is of prime importance. However, the result should be adjunct to the results of other prevalent techniques of diagnosis of EPTB.

Key words: Extrapulmonary tuberculosis, Multidrug-resistant tuberculosis, Xpert mycobacterium tuberculosis/rifampicin

INTRODUCTION

Tuberculosis (TB) affects one-third of the global population in developing countries, with annual estimates of 9.0 million new cases and 1.5 million deaths. While pulmonary TB (PTB) is the most common presentation, extra PTB (EPTB) is also an important clinical condition.[1]

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Worldwide, EPTB accounts for approximately 25% of all TB cases and even higher percentages in HIV-infected individuals and children.[2]

In Indian scenario, the most common extrapulmonary manifestation of TB is peripheral lymphatic TB, always an accurate clinical diagnosis if the clinician has experience and retains a high index of clinical suspicion. The next common manifestation is TB pleural effusion diagnosis of which remains as difficult as it has always been. For the lethal forms of EPTB like meningeal and disseminated, unfortunately, the same goes true, and the clinician remains pretty much left to his or her own devices of making a clinical diagnosis on not much more than clinical presentation and careful history taking.

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In all these manifestations of EPTB other than clinical judgement, the diagnosis relies on additional laboratory support with histopathology, chemical and cell analysis of fluid, and response to empirical anti-TB therapy.[3] However, the collection of extrapulmonary material often requires invasive procedures, expertise, and it is not easy to obtain additional samples.[4] The culture on Lowenstein- Jensen medium takes up to 8 weeks and 6 weeks on liquid medium to get the final result. Given the limitations of procedures for confirming a diagnosis of EPTB, patients are often started on anti-TB therapy and its response then noted.[5]

In recent times, attention has been devoted to nucleic acid amplification diagnostic technologies with ease of use and promising results.[3] One of the latest systems Xpert mycobacterium TB (MTB)/Rifampicin (RIF) (Xpert) (Cepheid, Sunnyvale, CA, USA), a fully automated real-time heminested PCR system implementing molecular beacon technology for the diagnosis of PTB infection,[6] has been recently endorsed by the Scientific and Technical Advisory Board of the WHO as the most sensitive fast test for TB diagnosis in paucibacillary respiratory samples.[7] The characteristic also makes it a potentially attractive tool for extrapulmonary specimens. A series of meta-analyses has shown that nucleic acid amplification tests (NAATs) have high specificity and positive predictive value with highly variable sensitivity, especially in cases of EPTB.[8]

In various studies, Xpert has usually been compared to culture, which is known to be a very suboptimal reference standard for EPTB. In this study, we have evaluated the performance of the Xpert system on number of different extrapulmonary specimens and evaluated its diagnostic potential by performing Xpert tests in cases diagnosed with a smear microscopy, (Lowenstein-Jensen) LJ culture, clinical findings, histology/cytology, site-specific computerized tomography scan/ultrasonography, and response to anti-TB therapy which formed a composite reference standard (CRS).[8]

MATERIALS AND METHODS

This study was conducted in the Department of Respiratory Medicine, where extrapulmonary samples obtained during the clinical routine between May 2016 and March 2017 were investigated. All the patients were treated for TB on the basis of diagnostic criteria that included smear, (Lowenstein-Jensen) LJ culture, histology, biochemical testing results, clinical presentation of signs, symptoms, site-specific computerized tomography scan/ ultrasonography, and response to treatment with anti-TB therapy which formed a CRS. The final diagnosis of EPTB for the patients was established by the clinician.[9] Follow-up after every 2 weeks from the date of enrolment for anti-TB therapy was done to adjudge the response in intensive phase of treatment. Patients who had received anti-TB treatment within the past 2 years were not included in the study.[9]

TB Pleural/Ascitic/Cerebrospinal Fluid (CSF)

A diagnosis of TB was established when any of the following criteria was met:

1. Identification of bacilli in fluid, sputum, or pleural biopsy specimen by ZN staining or (Lowenstein-Jensen) LJ culture.
2. Presence of granuloma in pleural biopsy tissue; or
3. Lymphocytic exudates with adenosine deaminase (ADA) levels >40 U/l in the absence of any other obvious cause of pleural and ascitic fluid.
4. Lymphocytic exudates with ADA levels >10 U/l in CSF in absence of any other obvious cause of meningitis.
5. Clinical response to anti-TB therapy.

Other causes of pleural effusions were defined using well-established clinical criteria.[10]

Xpert MTB/RIF Assay

TB detection was done by Xpert MTB/RIF assay, made by Cepheid-Sunnyvale-USA. Extrapulmonary specimens were processed according to the GeneXpert system operator manual given by Central TB division, Government of India, Guidance document for the use of cartridge-based nucleic acid amplification test (CB-NAAT) under RNTCP. Our machine contains 4 cartridges, so 4 samples were processed for each run. According to standard operating procedure, the sampling reagent (containing NAOH and isopropanol) was added at 2:1 ratio to the sample and kept for 15 min at room temperature with intermittent shaking. 3 ml of this treated sample was transferred to the cartridge, and the cartridge was inserted in the module of CB-NAAT machine. An automatic process completed the remaining assay steps, and the results were displayed on the monitor of Gene Xpert after 1 h and 50 min. Xpert MTB/RIF cartridge is a disposable, single self-enclosed test unit in which all steps of NAAT, i.e., Sample processing, PCR amplification, and detection are automated and integrated. The manual steps involved in the assay are adding reagent and sample loading. The test procedure is made biosafe by tuberculocidal property of the assay's sample reagent.[11,12]

TB Lymphadenitis

Fine-needle aspiration cytology (FNAC) specimens were collected from consenting patients by aspirating two passes of a 23- or 25-gauge needle attached to a 10 ml syringe. Two smears were prepared from each aspirate, for histocytology and ZN staining. Smears were evaluated for adequacy and a morphological diagnosis, and cases were excluded from

the analysis if either one or both the passes had inadequate cellular materials on smear.

A diagnosis of TB lymphadenitis was established if any of the criteria was met; direct detection of mycobacteria by ZN staining or (Lowenstein-Jensen) LJ culture, cytomorphological features of TB were seen, clinical response to anti-TB therapy was seen.[13]

Xpert MTB/RIF Assay

The residual material from the remaining aspirate was rinsed into 0.7 ml sterile phosphate-buffered saline homogenized sample preparation buffer, was then added to the vial in a 2:1 ratio, incubated at room temperature, and subsequently processed for Xpert MTB/RIF testing as previously described.[11,12]

Statistical Analysis

All the data required for this study were collected and analyzed statistically to determine the sensitivity and positive predictive value of different parameters using the commercially available statistical software MedCalc version 14.8.1 and Microsoft Office 10.

RESULTS

A total of 108 extrapulmonary specimens were obtained from 108 EPTB patients (median age 47.5 ± 22.2 years; 78 males and 30 females) and were included in the study. 22 (20.3%) of the 108 patients were culture positive. The remaining 86 (79.6%) EPTB patients were culture negative, but their clinical history and other investigation evidence were sufficiently indicative of TB (CRS criteria).

According to the results for 108 EPTB patients, the sensitivity of the MTB/RIF assay was 45.3% (49/108). With positive culture results, the sensitivity of MTB/RIF assay was 72.7% (16/22) (Table 1).

Of 108 EPTB cases, maximum 51 (47.2%) were cases of lymph node TB (LNTB), followed by TB pleural effusion 38 (35.1%), abdominal Koch's 12 (11.1%), and TB meningitis 7 (6.4%). Sensitivity of Xpert MTB/RIF for lymph node specimens was observed to be 62.7%, for pleural fluid 31.5%, and for ascitic fluid 41.6%. None of the CSF samples was reported MTB positive, of 7 by Xpert MTB/RIF (Table 2).

Xpert detected 5 (4.6%) rifampicin-resistant and 103 (95.3%) RIF susceptible specimens in EPTB patients.

Xpert test also provided a semi-quantitative report of the number of DNA copies detected in the sample; it was "very low" or "low" in the (70.5%) of the samples reported MTB positive.

DISCUSSION

The conventional methods of culture on solid and liquid media are gold standard for diagnosis of EPTB. However, in pleural TB which is the second most common site in EPTB after peripheral lymphatic TB, the sensitivities of pleural fluid microscopy and culture are about 10% and 20%, respectively.[13-15] In this study, the performance of the Xpert MTB/RIF assay with pleural fluid samples diagnosed with a CRS was investigated. The previous studies have reported much lower sensitivities between 15% and 48%.[1,5,10] In this study also, pleural fluid sensitivity was found to be low at 31.5% comparable to that of other studies. Rufai *et al.* showed that the Xpert MTB/RIF assay test has very low diagnostic sensitivity of 14.2% in pleural fluid, even in culture proven cases.[1] Results of meta-analysis suggest that Xpert MTB/RIF can detect TB pleural effusion in 22.7% of patients using a CRS and also concluded poor sensitivity of Xpert for the diagnosis of TB pleural effusion.[1,16] Porcel *et al.* concluded that the Xpert MTB/RIF assay has a limited diagnostic capacity for pleural fluid samples of TB origin.[10] This study found the sensitivity of Xpert MTB/RIF in pleural fluid specimens to be low, with more than half of all pleural TB patients being missed by this test. Guidelines on EPTB for India recommend that Xpert MTB/RIF should not be used to diagnose pleural TB exclusively.[17]

This study found the Xpert detection in lymph node samples to be higher than that in the any other specimen type (pleural fluid, ascetic fluid, and CSF) as reported by other studies[1,2,4,8] and the sensitivity to be 62.7%. Similar studies that assessed Xpert in lymph node samples against a CRS showed a sensitivity range of 72-87%.[8,9,13,18] We

Table 1: Xpert MTB/RIF assay in culture-positive and culture-negative specimens

Diagnosis of EPTB	*n*	Xpert assay		Sensitivity (%)	Positive predictive value (%)
		Positive	Negative		
Culture positive	22	16	6	72.7	100
Culture negative	86	27	59	31.3	100
CRS*	108	49	59	45.3	100

*CRS: Composite reference standard. MTB: Mycobacterium tuberculosis, RIF: Rifampicin, EPTB: Extrapulmonary tuberculosis

Table 2: Xpert MTB/RIF assay in EPTB Specimens diagnosed with CRS

Specimen type	*n*	Xpert assay		Sensitivity (%)	RIF resistance
		Positive	Negative		
Lymph node	51	32	19	62.7	3
Pleural fluid	38	12	26	31.5	1
Ascitic fluid	12	5	7	41.6	1
CSF	7	0	7	0	0
Total	108	49	59	45.3	5

CSF: Cerebrospinal fluid, MTB: Mycobacterium tuberculosis, RIF: Rifampicin, EPTB: Extrapulmonary tuberculosis

found that Xpert MTB/RIF can be useful in confirming a diagnosis in patients suspected of LNTB when considered alongside the results of FNAC, but a negative Xpert MTB/RIF test does not rule out lymph node TB. Guidelines on EPTB for India also recommend that Xpert MTB/RIF should be used as an additional test to conventional smear microscopy, culture, and cytology in FNAC specimens.[17]

Stakes are particularly high in the diagnosis of TB meningitis due to the high mortality associated with this disease, especially when the diagnosis is delayed.[17] Studies that assessed Xpert in CSF samples against a CRS found a variable sensitivity of 20-86%.[2,6,19,20] Xpert did not detect MTB in any of the seven samples in this study, suggesting that a negative Xpert result does not rule out TB meningitis. Guidelines on EPTB for India recommend that Xpert may be used as an adjunctive test for TB meningitis, and decision to give ATT should be based on clinical features and CSF profile.[17] For ascitic fluid, we found a sensitivity of 41.6% similar to the range of 8-50% reported by Sharma *et al.*[21]

The overall low sensitivity of the Xpert test in this study (45.3%) probably reflects the low mycobacterial loads, and consequently, DNA in EPTB samples. Furthermore, number of DNA copies detected were in the "very low" or "low" range in the large majority (70.5%) of the samples that scored positive. As expected, the higher the bacterial load the greater the likelihood of obtaining positive Xpert result.[10] False positivity due to contamination is less likely because the technology uses closed reaction chamber and surfaces where specimens are processed and were extensively cleaned.[4]

Not only MTB detection but also rapidly determining the patient's multidrug-resistant tuberculosis (MDR-TB) status in such cases is of prime importance in bringing to an end the spread of MDR-TB and decreasing mortality. Treatment under guidelines on programmatic management of drug-resistant TB (PMDT) could be started in five (4.6%) patients with RIF resistance detected by Xpert RIF/MTB in the present study.

Thus, Xpert results may not be fully satisfactory from the clinical point of view, but the Xpert MTB/RIF for the identification of MTB in EPTB samples should still be done because of its simplicity, reliability, and rapid results. However, the result should be adjunct to the results of other prevalent techniques of the diagnosis of EPTB.[10,13] Xpert must be regularly used in resource-limited settings or decentralized laboratory settings. The high cost of this sophisticated technology is offset to an extent by the rapid turnaround time, similar to that of smear microscopy (2 h), with less biohazard risk and only minimal training needed.[8]

Limitation

The limitation of this study is the small sample size for each of the different specimen types. Definitive interpretation of the results for each category of specimens should be done with great care.

CONCLUSION

The Gene Xpert MTB/RIF performance varies with the EPTB sample type. Although it has limited sensitivity, it detects RIF resistance and can be used in Indian health-care setting only as an additional tool for the diagnosis of EPTB.

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