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Evaluation of Direct Detection of *Mycobacterium tuberculosis* Complex in Tissue Specimens Using XPERT MTB/RIF Assay

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Abstract: India has the World's largest burden of tuberculosis and approximately 15-20% of these cases have extrapulmonary disease (EPTB). The diagnosis of tuberculosis from tissue specimens is usually made by histopathological examination (HPE). However, histopathology does not always give specific findings and needs more than one week for final reporting. In 2013, WHO endorsed Xpert MTB/RIF assay for the rapid diagnosis of EPTB. In the present study the efficacy of this assay to diagnose tuberculosis from tissue specimens was assessed, taking composite reference standard (CRS) as reference standard. A total of 63 consecutive specimens of EPTB (June 2016 to May 2017), one showed the growth of non-tubercular mycobacterium on culture and was excluded from the study. Among the remaining 62 specimens, the most common were female genitourinary specimens (endometrial biopsies)- 30.64%, followed by vertebral tissue 29.03% and bone tissue 9.68%. The positivity shown by smear microscopy and HPE was 12.9% each, culture 14.52% and Xpert MTB/RIF assay 32.26%. The sensitivity and specificity of Xpert MTB/RIF assay in comparison to culture were 100% and 79.25% respectively. Whereas, CRS which includes smear microscopy, culture, histopathological, clinical and radiological findings, as reference standard, Xpert MTB/RIF assay showed sensitivity 64.52%, specificity 100%. This study suggests that Xpert MTB/RIF assay has good diagnostic potential for the rapid diagnosis of tuberculosis in tissue specimens, which could help in the timely initiation of antitubercular treatment and prevention of progression to irreversible changes.

Keywords: *Mycobacterium tuberculosis*, tuberculosis, extrapulmonary tuberculosis, vertebral tissues, endometrial biopsies, Xpert MTB/RIF assay, histopathological examination, culture, CRS, rifampicin.

INTRODUCTION

India accounts for one fourth of the global burden of tuberculosis (TB) [1]. In 2016, World health organisation (WHO) gave an estimated incidence of 2.79 million cases of TB in India [1, 2]. Approximately, 15-20% of these cases have extrapulmonary disease [3]. Diagnosis of extrapulmonary tuberculosis (EPTB) is especially challenging since the number of bacilli present in the tissue at the site of disease is often low and the clinical specimens from deep seated organs are difficult to procure. The traditional diagnosis of TB from tissue specimens is usually made by histopathological examination (HPE) which depends upon the presence of granulomatous inflammation and caseous necrosis [4]. However, the process is time consuming and usually takes more than a week for final reporting. High expertise is also needed to establish the diagnosis of TB with specificity. Tissue microscopy (acid fast staining) is often negative in these specimens as it requires 10^4-10^6 bacilli/ml of tissue to give a positive result [5, 6]. Culture for mycobacterium is more sensitive but it still needs 10^1-10^2 bacilli/ml of the sample for the diagnostic yield, this often leads to considerable delay, compromising patient care and outcome [4]. Xpert MTB/RIF assay which was endorsed by WHO in 2011 for the detection of pulmonary TB represent a major advance in the diagnosis of tuberculosis [7]. It is an automated, closed system assay that performs real time PCR enabling diagnosis of TB and simultaneous assessment of rifampicin resistance. The results are obtained within 2 hours. WHO endorsed this technique for the diagnosis of EPTB in 2013 [8]. Since there is paucity of such studies from India, the present study was undertaken to assess the efficacy of Xpert MTB/RIF assay to diagnose TB from tissue specimens [3, 9]. Composite reference standard (CRS) which included smear microscopy, culture, clinical findings, radiological findings (site

specific computerised tomography, scan/ magnetic resonance imaging and Xrays) and follow up at 3 months was taken up as reference standard [10].

EXPERIMENTAL SECTION

The present retrospective study was conducted on 63 consecutive tissue specimens obtained for Xpert MTB/RIF assay from clinically suspected patients of EPTB between June 2016 to May 2017. Each specimen was subjected to smear microscopy (Ziehl-Neelsen staining), culture for *Mycobacterium tuberculosis* (MTB) and Xpert MTB/RIF assay. Xpert MTB/RIF assay was done by processing the tissue specimens according to standard operative procedure [11]. The processed specimen (2ml) was pipetted into the cartridge and the cartridge was inserted in the machine to start the test. Results were available within 2 hours. Histopathological study of the specimen collected in formalin was conducted in the pathology department of our hospital.

The relevant clinical findings based on the site of involvement female genitourinary specimen (endometrial biopsy)- pelvic pain, mass, irregular menstrual cycle, infertility, recurrent abortions; vertebral tissue- back pain, abscess, night cries, low grade fever, night sweats, weight loss, anorexia and malaise; lymph node biopsy- enlarged lymph nodes and mass formation; skin biopsy- visible lesions and tender nodules; soft tissue biopsy- soft tissue pain and soft tissue swelling; intestinal biopsy- abdominal pain and diarrhoea; CNS tissue biopsy- hydrocephalus, headache, fever and focal neurological deficit) and radiological findings which includes consolidation, infiltration, nodules, mediastinal lymphadenopathy, granuloma, calcification and pleural effusion etc. were recorded. On the basis of followup of 3 months, a specimen was considered as positive if the patient was on anti-tubercular therapy (ATT) and negative if the patient responded to non ATT.[10] Finally, CRS score was calculated and the patients from whom the specimens were obtained were classified into confirmed TB, probable TB, possible TB and no TB [10].

The study was approved by authorisation committee of our hospital and was carried out in accordance with previously reported recommendations on the design and conduct of diagnostic accuracy assessments [12].

RESULTS AND DISCUSSION

Of the 63 tissue specimens included in the study, one showed the growth of NTM (non-tubercular mycobacterium) on culture and was excluded from the study. The details of the remaining 62 specimens were as follows; female genitourinary (endometrial biopsy) specimens 19/62 (30.64%), vertebral tissue 18/62 (29.03%), bone tissue 6/62 (9.68%), lymph nodes and bone marrow tissue 5/62 (8.06%) each, skin biopsy 4/62 (6.45%) and soft tissue 3/62 (4.84%). There was one (1.61%) specimen each of synovial/articular tissue and CNS tissue was obtained.

Of the various modalities used to diagnose TB in the present study, Xpert MTB/RIF assay showed maximum positivity (32.26%), followed by radiological findings (19.35%). Histopathological findings and smear microscopy were positive in 12.9% specimens each. Culture for MTB which is considered as traditional gold standard test for diagnosis of TB, showed positivity of 14.52% and there was statistically significant difference between the positivities shown by Xpert MTB/RIF assay and culture (p value = 0.0000). The sensitivity and specificity of Xpert MTB/RIF assay in comparison to culture were 100% (95% CI 66.37-100%) and 79.25% (95% CI 65.89-89.16%) respectively (Table-1).

Table-1: Positivity of tuberculosis by various diagnostic techniques (n=62)

Technique	Number of positive samples	Percentage of positive samples
Smear microscopy	8	12.9%
Culture	9	14.52%[a]
Radiological findings	12	19.35%
Histopathological examination	8	12.9%
Xpert MTB/RIF assay	20	32.26%[b]
CRS score	31 (confirmed TB cases 9, probable TB cases 16 and possible TB cases 6)	50% (confirmed TB cases 14.5%, probable TB cases 25.81% and possible TB cases 9.67%)

p value between a and b = 0.0000 (statistically significant)

Comparison of Xpert MTB/RIF assay to culture showed:- Sensitivity of 100% (95% CI 66.37-100%); Specificity of 79.25% (95% CI 65.89-89.16%)

CRS which was used as reference standard in the present study, categorised the patients (whose specimens were included in the present study) as confirmed TB cases (if culture was found to be positive) 14.5% (9/62); probable TB cases (clinically, radiologically, and /or histologically positive) 25.81% (16/62); possible TB cases (clinically positive and responded to ATT) 9.67% (6/62). Thirty one of 62 (50%) cases had evidence of TB (no TB cases) (Table-2).

Table-2: Algorithm for patient categorization into different categories on the basis of composite reference standard [3] (n=62)

CRS Category	Smear	culture	Symptoms/ Signs	Radiology	Histopathology Examination	Follow-up at 3months
CONFIRMED TB (n=9)	+/-	+	+	+/-	+/-	+
PROBABLE TB (n=16)	+/-	-	+	+	+	+
	+/-	-	+	+	-	+
	+/-	-	+	-	+	+
POSSIBLE TB (n=6)	+/-	-	+	-	-	+
NO TB (n=31)	-	-	+	-	-	-

Sensitivity and specificity of Xpert MTB/RIF assay results were assessed in comparison to a CRS. It was observed that of the 62 specimens studied, 20 (32%) were positive and 31 (50%) were negative in both Xpert MTB/RIF assay and CRS. On the other hand, there were 11 specimens in which CRS score showed that they were from cases of TB (confirmed, probable, possible), while the specimens were negative for MTB on Xpert MTB/RIF assay. No specimen was there, which was positive in Xpert MTB/RIF assay but whose CRS score was negative. Thus, the sensitivity, specificity, PPV, NPV of Xpert MTB/RIF assay in comparison to CRS was 64.52% (95% CI 45.37-80.77%), 100% (95% CI 88.78-100%), 100% (95% CI 83.16-100%), 73.81% (95% CI 63.68-81.92%) respectively (Table-3).

Table-3: Showing sensitivities and specificities of Xpert MTB/RIF assay in comparison to composite reference standard (CRS)

		CRS SCORE		
		POSITIVE	NEGATIVE	TOTAL
XPERT MTB/RIF assay	POSITIVE	20 (32%)	0 (0%)	20 (32%)
	NEGATIVE	11 (17.74%)	31 (50%)	42(67.7%)
	TOTAL	31 (50%)	31 (50%)	62(100%)

Sensitivity = true positive/ true positive + false negative
= 20 / 20+11 = 64.52%
[95% CI 45.37-80.77%]

Specificity= true negative/ true negative + false positive
= 31/31+0 = 100%
[95% CI 88.78-100%]

PPV= true positive/ true positive + false positive
= 20/20+0 =100%
[95% CI 83.16-100%]

NPV= true negative/ true negative+ false negative
=31/31+11 = 73.81%
[95% CI 63.68-81.92%]

Out of 20 specimens which were positive for MTB on Xpert MTB/RIF assay, 2 (3.2%) were found to be rifampicin resistant. Both the resistant specimens were of female genital tract (endometrial biopsies) from females diagnosed with infertility.

EPTB constitute about 15-20% of all cases of TB in immunocompetent patients and more than 50% in HIV positive individuals [3]. Although, EPTB can involve any organ system in the body, different studies have reported variable localisation of EPTB [13, 14]. In the present study, skeletal tissue (vertebral tissue and bone tissue), lymph nodes and female genitourinary specimen were the most common organs/sites involved with maximum number of tissue specimens from female genitourinary specimens (endometrial biopsy- 30.64%). This could be because female genital TB is an important cause of infertility and because of social stigma attached to it, females approach tertiary care centres for seeking treatment essentially for this problem.

The result of the various conventional means of diagnosis of EPTB of the present study showed that they have poor diagnostic yield- 12.9% for histopathology and smear microscopy each and 14.52% of culture (Table-1). A study conducted by Jing *et al.*, reported sensitivity of 5.2% for smear microscopy and 23.8% for culture. Their study did not include cytological/histopathological examination [15]. Chawla *et al*., who compared the result of HPE with that of PCR in tissue samples, obtained 25.96% positivity by HPE. This shows that many cases of EPTB could be missed and remains untreated when only conventional means are used for diagnosis of EPTB. Keeping the priority of early detection and multidrug resistance of TB in mind, WHO laid down guidelines for EPTB diagnosis by Xpert MTB/RIF assay in 2013 (Xpert MTB/RIF assay was endorsed by WHO for rapid detection of pulmonary TB in India in 2011 [8, 7]. In the present study, Xpert MTB/RIF assay detected MTB in 32.26% tissue specimens and there was statistically significant difference between the positivities shown by Xpert

MTB/RIF assay and culture (p value= 0.0000). It was also observed that in comparison to culture, the sensitivity and specificity of Xpert MTB/RIF assay was 100% (95% CI 66.37-100%) and 79.25% (95% CI 65.89-89.16%) respectively (Table 1). This is consistent with the findings of other studies which have also shown improvement in the diagnosis of EPTB with the use of Xpert MTB/RIF assay [16, 17].

Acknowledging the fact that not all disease (TB) could be culture confirmed, culture has become a suboptimal reference standard for the diagnosis of EPTB [10]. Therefore, we compared Xpert MTB/RIF assay with CRS to evaluate its true diagnostic potential for EPTB and observed that it has sensitivity of 64.52% (95% CI 45.37-80.77%) and specificity of 100% (95% CI 88.78-100%). There are not many studies which have taken CRS as reference standard [10, 18]. In 2011, a study was conducted by *Vadwai et al* on 284 tissue specimens in Mumbai. Taking CRS as reference standard, it showed sensitivity of Xpert MTB/RIF assay as 81% (95% CI 75.5-85%) and specificity 99.6% (95% CI 97.8-100%) [10]. Tortoli *et al.*, reported sensitivity and specificity of Xpert MTB/RIF assay in comparison to CRS as 81.3% and 99.8% respectively [18]. The difference in the reported sensitivity and specificity of Xpert MTB/RIF assay could be because of variation in the nature, number and composition of extrapulmonary specimens included in various studies.

CONCLUSION

Thus, our study suggests that Xpert MTB/RIF assay has good potential in the rapid diagnosis of tuberculosis in tissue specimens. Its regular use would help in the timely detection and initiation of treatment which could further prevent progression to irreversible changes and improves patient outcome.

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