

Original Research Article

GeneXpert versus histopathological examination and culture as a diagnostic tool for spinal tuberculosis: a comparative analysis

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ABSTRACT

Background: Spinal tuberculosis, a common type of osteoarticular tuberculosis accounts for a significant proportion of extrapulmonary tuberculosis cases. This study compares the sensitivity, specificity of GeneXpert with histopathology and acid-fast bacilli (AFB) culture.

Methods: This study conducted at a tertiary health care and teaching institution from October 2018 to October 2023 included 60 patients with suspected spinal tuberculosis. Samples including vertebral tissue and pus, obtained by open biopsy, fluoroscopy or computed tomography (CT) guided percutaneous vertebral body biopsy were collected in sterile containers with 2-3 ml of saline and immediately sent for testing with GeneXpert and AFB culture. Histopathological samples were placed in 10% formalin prior to examination.

Results: When comparing GeneXpert with AFB culture, Sensitivity was found to be 82.2%, specificity 40.0%, positive predictive value (PPV) 80.4%, negative predictive value (NPV) 42.9% and accuracy was 71.7%. When comparing GeneXpert with histopathological examination (HPE), sensitivity was found to be 84.8%, specificity 50.0%, PPV 84.8%, NPV 50.0% and accuracy was 76.7%.

Conclusions: Despite longer diagnostic time as compared to GeneXpert, AFB culture is still the gold standard diagnostic modality.

Keywords: GeneXpert, Xpert MTB/RIF, Spinal tuberculosis, Spinal TB, AFB culture, Histopathological examination

INTRODUCTION

For the efficacious and prompt treatment of musculoskeletal tuberculosis (TB), an accurate diagnosis is most necessary. Easy availability of results is crucial for initiation of timely anti tubercular treatment (ATT). Delays in diagnosis, especially in spinal TB, may cause disastrous neurological complications and lasting disability.¹ The paucity of a rapid and accurate diagnostic test for mycobacterium tuberculosis is a significant impediment to manage spinal TB. Skeletal involvement occurs in about 10% of extrapulmonary TB cases. Spinal TB constitutes 50% of skeletal TB cases. Computed tomography (CT) guided needle or tissue biopsy from the

involved site provides samples for microbiologic and histopathologic diagnosis.²

GeneXpert (Cepheid, Sunnyvale, California), which can simultaneously detect TB bacilli and rifampicin resistance is an automated semi-nested polymerase chain reaction (PCR) diagnostic test. It is a rapid, cartridge based, nucleic acid amplification molecular diagnostic modality. The World Health Organization (WHO) recommended it as the first line investigation for patients suspected to have pulmonary TB.³ It has been shown to have similar sensitivity and specificity to traditional methods of investigation like TB culture. GeneXpert simultaneously detects TB and rifampicin resistance in a few hours. Its use

as a replacement test for testing non-respiratory samples from patients suspected to have extrapulmonary TB received conditional recommendation of the WHO.

Spinal TB usually occurs as secondary infection to an active or latent extraspinal source (primary focus), a pulmonary or genitourinary infection which disseminates through hematogenous route, reaching the dense vasculature of vertebral cancellous bone. The region of the spinal column most affected is the thoracolumbar junction, followed by the lumbar and cervical spine.⁴ The following may be seen in spinal TB: progressive bony destruction which leads to vertebral collapse, kyphosis, formation of cold abscess (by spread of infection into adjacent ligaments and soft tissues), spinal cord compression and neurologic deficits which result from spinal canal narrowing due to abscesses, granulation tissue or direct dural invasion.⁵

No isolated diagnostic modality can definitively diagnose every case of spinal TB. Owing to the low yield of *Mycobacterium tuberculosis* from bone, clinical examination, imaging diagnosis, acid fast bacilli (AFB) smear, mycobacterial culture, molecular methods, findings from histology and/or cytology and response to therapy are relied upon.^{6,7} The single definitive diagnostic investigation for spinal TB, regarded as gold standard modality, is growth of *Mycobacteria* on culture specimens taken from infected tissue. However, histopathological examination which demonstrates classical granulomas and staining of smears for the identification of AFB, owing to their very poor sensitivity are considered as reference standards. Besides serological markers detecting inflammation indirectly, immunological tests also employed yielded diverse outcomes. Molecular diagnostic tests are commonly performed, owing to their rapidity and reliability.⁴ Although imaging may suggest the diagnosis, laboratory confirmation is necessary for definitive tissue diagnosis.⁸

GeneXpert MTB/RIF (Cepheid) identifies DNA from mycobacteria and resistance to rifampicin within 2 hours (earliest by 90 minutes). A single GeneXpert cartridge integrates sample processing, nucleic acid amplification test (NAAT) and detection. As compared to the 10,000 bacilli/ml detection limit by microscopic examination, its limit is "5 genome copies of purified DNA reaction or 131 CFU/ml".⁷ It also assigns a semiquantitative estimate (very low, low, medium and high) of the sample's concentration of TB bacilli based on the cycle threshold.⁹

Around 60% of spinal TB patients are diagnosed by histopathology. Classic hallmarks include caseating necrosis, epithelioid cell granuloma, lymphocytic infiltrate and Langhans giant cells. A positive histopathological diagnosis merely suggests a probable diagnosis of mycobacterial infection.¹⁰ Owing to the limited specificity of histopathological examination diagnosis and treatment are doubtful, despite it providing accurate information about tissue and sample material.¹¹ This study aimed to

determine the role of GeneXpert as a diagnostic modality for spinal tuberculosis in comparison to histopathology and AFB culture.

METHODS

This cross-sectional observational study was undertaken in the Department of Orthopaedics at Bharati Vidyapeeth (Deemed to be) University Medical College, Pune, a tertiary health care and teaching institution in Western India over a period of 60 months from October 2018 to October 2023.

After obtaining the institutional ethics committee's approval, 60 consecutive patients older than 18 years with suspected spinal tuberculosis, who presented with back pain, constitutional symptoms, radiological evidence of infection on X-rays and magnetic resonance imaging (MRI) scans were included in the study. Patients with osteoporotic fractures, secondaries in the spine, traumatic and degenerative spine conditions were excluded from the study.

Microbiological samples including vertebral tissue and pus, obtained by open biopsy, fluoroscopy or CT guided percutaneous vertebral body biopsy were collected in sterile containers with 2-3 ml of saline and immediately sent for testing. Histopathological samples were placed in 10% formalin.

GeneXpert MTB/RIF test was performed using a modified Helb method. Vertebral bone specimens containing blood, pus and tissues were crushed and centrifuged for 10 minutes. Supernatant from the samples were subsequently added to a buffer solution in a 1:2 ratio. The sample and buffer mixtures were vortexed and then incubated at room temperature for 15 minutes. Two millilitres of the resultant were inserted into the cartridge, which was then put into the GeneXpert MTB/RIF device and run for two hours, after which results were automatically read.

The data on categorical and continuous variables was presented as "n" (% of cases), Mean and standard deviation (SD) respectively. Intergroup distribution of categorical variables was compared using Chi-square test or Fisher's exact probability test, if more than 20% cells had expected frequency less than 5.

Statistical agreement between two diagnostic methods was assessed by the Cohen-kappa technique. Diagnostic efficacy indices including sensitivity, specificity, positive predictive (PPV), negative predictive value (NPV) and accuracy were calculated with respect to appropriate gold standard.

P values less than 0.05 were considered statistically significant and all hypotheses were formulated using two tailed alternatives against each null hypothesis. Collected data was statistically analysed using statistical package for

social sciences (SPSS) version 22.0, IBM Corporation, USA for Microsoft Windows.

RESULTS

Of 60 cases studied, 37 (61.7%) were female while 23 (38.3%) were male. Male to female sex ratio was 0.62:1. The gender distribution is presented in Table 1. Age distribution of 60 studied cases (Table 2) was as follows, 4 (6.7%) between 18 and 20 years, 21 (35.0%) between 21 and 30 years, 9 (15.0%) between 31 and 40 years, 8 (13.3%) between 41 and 50 years, 10 (16.7%) between 51 and 60 years, 6 (10.0%) between 61 and 70 years, 2 (3.3%) between 71 and 80 years. Mean $\pm$ SD for age of studied cases was 39.67 $\pm$ 16.39 years and ages ranged from 18 to 75 years.

Table 1: Gender distribution of cases studied.

Sex	No. of cases	% of cases
Male	23	38.3
Female	37	61.7
Total	60	100.0

Table 2: Age distribution of cases studied.

Age group (years)	No. of cases	% of cases
18–20	4	6.7
21–30	21	35.0
31–40	9	15.0
41–50	8	13.3
51–60	10	16.7
61–70	6	10.0
71–80	2	3.3
Total	60	100.0

Involvement of the lumbar, dorsal, lumbosacral, cervical, sacral, sacroiliac, dorsolumbar spine was seen in 18 (30.0%), 21 (35.0%), 5 (8.0%), 10 (16.7%) 2 (3.3%), 2 (3.3%) and 2 (3.3%) patients respectively. 49 (81.7%) of the 60 cases underwent an open procedure, while 11 (18.3%) had a closed procedure for collection of samples.

GeneXpert was found to be positive in 46 (76.7%) and negative in 14 (23.3%) of the 60 cases studied. Strength of mycobacterium tuberculosis (MTB) detection by

GeneXpert was found to be very low in 9 (15.0%), low in 25 (41.7%) and medium in 12 (20.0%). Rifampicin resistance (RIF) was detected in 5 (8.3%) cases. Histopathological examination of the 60 cases studied, was positive in 46 (76.7%) and negative in 14 (23.3%). AFB culture was positive in 45 (75.0%) cases and negative in 15 (25.0%).

Of 46 cases with positive GeneXpert, 37 (80.4%) had positive AFB culture and 9 (19.6%) had negative AFB culture. Of 14 cases with negative GeneXpert, 8 (57.1%) had positive AFB culture and 6 (42.9%) had negative AFB culture. No significant association was found between diagnosis by AFB culture and GeneXpert (P-value >0.05; 0.078) and Cohen Kappa value of 0.227 was very low, suggesting fair statistically non-significant agreement between diagnosis by GeneXpert and AFB culture in the study group (Table 3). When comparing GeneXpert with AFB culture, sensitivity was found to be 82.2%, specificity 40.0%, PPV 80.4%, NPV 42.9% and accuracy was 71.7%.

Of 46 cases with positive GeneXpert, 39 (84.8%) had positive HPE and 7 (15.2%) had negative HPE. Of 14 cases with negative GeneXpert, 7 (50.0%) had positive HPE and 7 (50.0%) had negative HPE. Significant association was found between diagnosis by HPE culture and GeneXpert (p value <0.05; 0.007) with low Cohen Kappa value of 0.348, suggesting fair agreement between diagnosis by GeneXpert and HPE in the study group (Table 4). When comparing GeneXpert with HPE, sensitivity was found to be 84.8%, specificity 50.0%, PPV 84.8%, NPV 50.0% and accuracy was 76.7%.

Of 46 cases with positive HPE diagnosis, AFB culture was positive in 40 (87.0%) and negative in 6 (13.0%) cases. 5 (35.7%) of the 14 cases with negative HPE had positive AFB culture while 9 (64.3%) had negative AFB culture. Significant association was found between diagnosis by HPE and AFB culture (p value <0.05; 0.001) with moderate Cohen Kappa value of 0.500 suggesting moderate agreement between diagnosis by HPE and AFB culture in the study group (Table 5).

When comparing HPE with AFB culture for diagnosis, sensitivity was found to be 88.9%, specificity 60.0%, PPV 86.9%, NPV 64.3% and accuracy was 81.7%.

Table 3: Distribution of diagnosis by GeneXpert according to AFB culture in the study group.

GeneXpert	AFB culture						Cohen-Kappa	P value
	Positive	Negative	Total					
	N	%	N	%	N	%		
Positive	37	80.4	9	19.6	46	100.0	0.227	0.078 ^{NS}
Negative	8	57.1	6	42.9	14	100.0		
Total	45	75.0	15	25.0	60	100.0		

P value by Chi-square test, Cohen-Kappa for statistical agreement between two modalities, p value <0.05 is considered to be statistically significant, NS—statistically non-significant

Table 4: Distribution of diagnosis by GeneXpert according to histopathological examination diagnosis in the study group.

GeneXpert	HPE diagnosis						Cohen-Kappa	P value
	Positive		Negative		Total			
	N	%	N	%	N	%		
Positive	39	84.8	7	15.2	46	100.0	0.348	0.007**
Negative	7	50.0	7	50.0	14	100.0		
Total	46	76.7	14	23.3	60	100.0		

P value by Chi-square test, Cohen-Kappa for statistical agreement between two modalities, p value <0.05 is considered to be statistically significant, **p value <0.01

Table 5: Distribution of diagnosis by histopathological examination according to AFB culture in the study group.

HPE	AFB culture						Cohen-Kappa	P value
	Positive		Negative		Total			
	N	%	N	%	N	%		
Positive	40	87.0	6	13.0	46	100.0	0.500	0.001***
Negative	5	35.7	9	64.3	14	100.0		
Total	45	75.0	15	25.0	60	100.0		

P value by Chi-square test, Cohen-Kappa for statistical agreement between two modalities, p value <0.05 is considered to be statistically significant, ***p value <0.001

DISCUSSION

Among the most common infectious diseases affecting the spine, TB also is the 10th leading cause of worldwide mortality. Since 2007 it is the main cause of death from a single infectious agent.¹² The initial latent phase of infection often involves pulmonary (PTB) as well as extrapulmonary (EPTB) sites. 10-42% of TB cases are extrapulmonary, of which 10-25% are musculoskeletal TB. Vertebral affection, with or without neurological deficits and deformities, occur in nearly 50% of musculoskeletal TB cases.¹³

The commonest type of musculoskeletal tuberculosis is tubercular spondylodiscitis. Several techniques exist to identify the organism. Culture on Lowenstein Jensen medium is widely accepted as the 'gold standard' modality for diagnosis. Culture yields a result between 6 to 8 weeks.¹⁴

Xpert MTB/RIF (GeneXpert), recommended by the WHO for detection of *Mycobacterium tuberculosis* complex and screening of rifampicin (RIF) resistance is a rapid molecular diagnostic test. The Mycobacterial genome identified from captured bacteria, deoxyribonucleic acid (DNA) is amplified by polymerase chain reaction. The relevant 81 base pair (bp) fragment, from rpoB gene of *Mycobacterium tuberculosis* (M. tb) is identified with fluorescent probes known as molecular beacons. GeneXpert employs PCR amplification of the rpo gene to detect M. tb while rifampicin resistance is determined by probing of this region for mutations, associated with rifampicin resistance. The test's turnaround time is 90 minutes.⁹

GeneXpert was validated and recommended by the WHO for diagnosing pulmonary TB, requires around 130 bacilli per ml (of sputum) to yield a positive result. Since the test is specific for *M. tb* complex, it can be used to differentiate *Mycobacterium tuberculosis* from other mycobacteria. It is carried out for each specimen, in a closed system (cartridge), reducing cross contamination and human error.⁹

In the 2014 study by Held et al of 69 patients Xpert MTB/RIF was found to be positive in 97.2% of samples analysed, results of the drug sensitivity tests (DST) were available in 48 hours. Sensitivity and specificity were 95.6% and 96.2% respectively, detection limit was 139 CFU/ml of bacilli. Rifampicin resistance was detected in 4 patients (5.8%). Thus, the possibility of MDR TB was suspected and subsequently confirmed in 3 patients by testing sensitivity for rifampicin and isoniazid after culture results were obtained. In 25% of samples, GeneXpert demonstrated rifampicin resistance, despite negative TB culture. MDR-TB would have been missed without GeneXpert.³

Gu et al study evaluating the Xpert MTB/RIF assay in diagnosing BJTB (bone and joint TB), reported sensitivities of smear, culture and Xpert as 26%, 48%, 82% respectively, while specificities of all tests were 100%. Xpert was found to be 100% concordant with MGIT 960 based drug susceptibility testing (DST) for detecting resistance to rifampicin.¹⁵

Arockiaraj et al study, assessing accuracy of Xpert MTB/RIF diagnosis and identification of resistance to rifampicin in patients with infective spondylodiscitis, found sensitivity and specificity of Xpert MTB/RIF in comparison to culture was 88.4 and 63.7%. In comparison

to culture and histopathological examination, it was 80.9 and 80.6%. Sensitivity and specificity of assay in comparison to CRS was respectively 71.2 and 100%. Sensitivity of identification of rifampicin resistance by assay was 100%. Rifampicin resistance prevalence was 5.1%.¹⁴

Massi et al study of 70 suspects of tubercular spondylitis, showed that MGIT 960 liquid culture was positive in 31.42%, while GeneXpert MTB/RIF was positive in 88.57%. GeneXpert MTB/RIF showed sensitivity and specificity of 100% and 16.6% respectively, PPV of 35.48% and NPV of 100%. Rifampicin resistance was detected by GeneXpert in 6.45% of positive samples tested.¹⁶

Wen et al meta-analysis evaluating accuracy of Xpert MTB/RIF to diagnose musculoskeletal TB, identified 12 studies with consolidated sensitivity and specificity of 0.81 (95% confidence interval (CI) 0.78–0.83) and 0.83 (95% CI 0.80–0.86) respectively. Xpert was highly sensitive (0.89, 95% CI 0.79–0.95) and specific (0.96, 95% CI 0.92–0.98) in identifying rifampicin resistance. Area under the curve (AUC) >0.9 found suggested comparatively high diagnostic accuracy overall of Xpert in detection of rifampicin resistance and musculoskeletal TB.¹⁷

Wang et al found study of 319 patients with spinal TB, according to composite reference standard (CRS), Xpert demonstrated higher sensitivity (85.27%) than either histopathology (73.04%) or culture (51.72%). The specificities of Xpert, histopathology and culture were 100%, 93.94% and 100% respectively. The study also considered the merit of concomitant use of various tests, with incremental sensitivity being demonstrated by Xpert with culture (85.89%), Xpert with histopathology (91.22%) and Xpert with culture with histopathology (91.54%).¹⁸

Shen et al meta-analysis, to evaluate accuracy of Xpert MTB/RIF for diagnosing osteoarticular TB, included 19 independent studies comparing Xpert MTB/RIF to CRS and 14 studies comparing it to culture. Consolidated sensitivity and specificity of Xpert MTB/RIF were respectively 81% (95% CI, 77–84) and 99% (95% CI, 97–100) compared to the CRS and 96% (95% CI, 90–98), 85% (95% CI, 57–96) respectively to culture.¹⁹

Solanki et al prospective study, found sensitivity and specificity of GeneXpert to be 91.18% and 100% respectively, positive and negative predictive values were 100% and 93.88% respectively. Of all the cases of spinal TB, GeneXpert was positive in 91.18% but only 54.69% were AFB culture positive, while 88.33% were histopathologically conclusive of TB. GeneXpert was also found positive in 100% cases of spinal TB for which histopathology was inconclusive and in 86.21% cases where AFB culture was negative.²⁰

Patel et al study assessing efficacy of Xpert MTB/RIF for diagnosing spinal TB and resistance to rifampicin among 360 patients, found sensitivity and specificity to be 86.3% and 85.3% respectively, against culture for diagnosis of spinal TB and showed sensitivity, specificity of 75.86% and 96.12% respectively for RIF resistance in comparison to DST.¹³

Kanade et al study found sensitivity, specificity, positive and negative predictive value of the Xpert assay were 97.01%, 99.25%, 98.48% and 98.52% respectively, in comparison to culture.²¹

Patel et al study assessing if GeneXpert MTB/RIF could replace AFB smear and histopathology to diagnose spinal TB, found that the assay showed sensitivity and specificity of 97.62% and 96.38% respectively, PPV of 89.13%, NPV of 99.25% and diagnostic accuracy of 96.67%, in comparison to AFB smear and showed sensitivity and specificity of 95.75% and 96.04% respectively, PPV of 98.41%, NPV of 89.81% and diagnostic accuracy of 95.83% in comparison to histopathology for diagnosis of spinal tuberculosis.²²

Jain et al review of current concepts for tuberculosis of the spine, proposed a pyramid representing a hierarchy for investigative modalities used in the diagnosis of TB spine based on current evidence.⁷ Mycobacterial culture remains the gold standard, followed by molecular methods (including GeneXpert) and then histopathological examination.

In our study, of the 60 cases studied, 46 (76.7%) had positive, while 14 (23.3%) had negative GeneXpert result. Sensitivity, specificity, PPV, NPV and accuracy of GeneXpert compared to AFB culture in the detection of M. tb were 82.2%, 40.0%, 80.4%, 42.9% and 71.7% respectively. Sensitivity, specificity, PPV, NPV and accuracy of GeneXpert compared to HPE for diagnosis was 84.8%, 50.0%, 84.8%, 50.0% and 76.7% respectively. Of 60 cases studied, 55 (91.7%) did not have rifampicin resistance (RIF), while in 5 (8.3%) cases, rifampicin resistance (RIF) was detected. Sensitivity, specificity, PPV, NPV and accuracy of HPE compared to AFB culture for detecting M. tb was 88.9%, 60.0%, 86.9%, 64.3% and 81.7% respectively.

Limitations

Limitations of our study were that drug sensitivity testing following positive AFB culture could not be performed to assess multi drug resistance. Also, the study was performed on a smaller sample size as compared to other larger studies, which might be responsible for results in this study that differ from those previously published.

CONCLUSION

GeneXpert can be widely used as a rapid diagnostic test for spinal tuberculosis yielding results within two hours

compared to AFB culture which yields results up to 8 weeks. Despite this, AFB culture continues to remain the gold standard diagnostic modality. Sampling methods could be responsible for the lower sensitivity & specificity values found in our study compared to other published reports.

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