Neurology Asia 2023; 28(2) : 291 – 296

Gene Xpert MTB/RIF assay in patients with clinical suspicion of tuberculous meningitis

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Abstract

Early diagnosis and treatment of tubercular meningitis (TBM) is one of the best predictors of survival. The smear testing of the cerebrospinal fluid (CSF) has a low sensitivity and cultures, though having higher sensitivity, take a long time in TBM diagnosis. The present study was done to evaluate the proportion of positive Gene Xpert test, a rapid nucleic acid amplification test, in clinically suspected patients with TBM. The results of Gene Xpert tests were then compared with the culture results. One hundred and fifty prospective patients with a clinical suspicion of TBM were classified as probable or possible TBM and underwent CSF examination for culture and Gene Xpert assay. Out of 124 patients available for statistical evaluation, 52 were in the probable and 72 in possible TBM group. The proportion of patients with Gene Xpert positivity were significantly more in probable compared to possible TBM (63.15% vs 36.85%, p<0.001). Twenty-eight patients had a positive culture result. The sensitivity of Gene Xpert relative to culture testing was 68.42%, specificity of 97.61%, a positive predictive value of 92.85% and a negative predictive value of 87.23%. Gene Xpert testing provided a rapid diagnosis in patients suspected with TBM. The high sensitivity and specificity of this test relative to culture testing is a strong indication that it should be included as one of the gold standard tests in patients with suspected TBM.

Keywords: Tubercular meningitis, early diagnosis, Gene Xpert testing, culture testing

INTRODUCTION

Tuberculous meningitis (TBM) is the most devastating consequence of infection with *Mycobacterium tuberculosis* with approximately a third of patients dying during hospital stay and majority having severe neurological sequelae.[1,2] Early diagnosis and treatment for TBM have been shown in numerous studies to be the best predictor of survival.[3] However, there is often a delay in diagnosis because initial signs are non-specific, and more importantly, rapid and sensitive diagnostic tests are lacking. Ziehl-Neelsen (ZN) microscopy staining of cerebrospinal fluid (CSF) is the most widely applied rapid diagnostic technique with a low sensitivity of 20% for TBM.[4,5] Liquid culture techniques, including the mycobacterial growth indicator tube (MGIT; Bactec) and the mycobacterial observation drug susceptibility assay (MODS) culture offer improved sensitivity of almost 60%.[6] The clinical value of culture techniques is limited as it takes 1 to 4 weeks to return a positive result, and negative results cannot be used to confirm an exclusion of TBM.[6]

Amongst the wide array of nucleic acid amplification techniques (NAAT), the GeneXpert MTB/RIF test (Cepheid), a closed-cartridge based system was approved by the WHO in 2010 for the diagnosis of pulmonary tuberculosis.[7,8] The test is easy to operate by minimally trained staff and gives results in approximately two hours.[8] The test is based on a real-time hemi-nested PCR test which detects the presence of *M. tuberculosis* complex bacilli.[9] Additionally, it also determines susceptibility to rifampin, which can be used as a surrogate marker for multidrug resistance (MDR).[9] The test has shown sensitivity above 90% for culture positive tuberculosis, with high specificity in sputum samples.[9,10] Several studies with a small number of CSF samples examined, have reported

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Date of Submission: 25 October 2022; Date of Acceptance: 28 February 2023

https://doi.org/10.54029/2023ktz

successful use of the Xpert MTB/ RIF test on extra pulmonary samples, with overall sensitivities of over 80% and specificity reaching 100%.[11,12] As early therapeutic intervention prevents morbidity and mortality in TBM, the need of the hour is a test that aids in an early TBM diagnosis.[12]

The present study was thus aimed at evaluating the proportion of Gene Xpert MTB/RIF test positivity in a large patient cohort with a clinical suspicion of TBM. We also compared Gene Xpert with MGIT, the gold standard diagnostic culture test for diagnosis of tubercular meningitis.

METHODS

The study was conducted in the Department of Neurology at Dr Ram Manohar Lohia Institute of Medical Sciences, a tertiary care teaching institute in Lucknow, India. One hundred and fifty prospective patients with a clinical suspicion of TBM seen from November 2016 till October 2018 were recruited in this study. The clinical diagnosis of TBM was made according to the standard case definition for tubercular meningitis by the working committee consensus 2010 (Table 1).[13] Thus, patients were classified as probable or possible TBM based on the clinical entry criteria and the scoring system.[13] Cases where an established cause of meningoencephalitis was proven to be due to other infective or inflammatory etiologies after spinal fluid examination were excluded. Patients with end stage renal and hepatic disease, severe sepsis, pregnant females, and children less than five years of age and those in whom lumbar puncture was a contraindication were also excluded from the study.

A detailed clinical examination was followed by routine blood investigations that included a complete blood count, tests for renal and liver functions, blood sugars, enzyme linked immunosorbent assay (ELISA) for hepatitis B, hepatitis C and human immunodeficiency virus (HIV) and tests for thyroid function in all patients. Radiological investigations included a chest radiogram, contrast enhanced computed tomography (CT scan) brain in all patients and magnetic resonance imaging (MRI) of the brain, only if required when clinically indicated. All patients underwent a spinal fluid examination with 8 mL of cerebrospinal fluid (CSF) collected through a lumbar puncture. CSF analysis included gram stain, culture, biochemistry, adenosine deaminase (ADA), cell count and India ink stain for fungi. A part of the CSF was used for Ziehl-Neelsen smear preparation, inoculation of MGIT culture, and Gene Xpert testing. Based on the CSF results, the proportion of cases with Gene Xpert positivity in the probable and possible groups was evaluated and also the sensitivity of Gene Xpert relative to MGIT was determined. The study was approved by Institutional ethics committee (IEC 27/16).

Statistical analysis

Any significant difference between the clinical features in probable and possible TBM was measured using Pearson Chi-Square test. CSF protein, sugar and cell counts in the two groups were compared using non parametric Wilcoxon Rank Sum test. The gene Xpert and MGIT status in the two groups was measured and compared using Chi Square test.

RESULTS

Out of the 150 patients clinically suspected with TBM, 26 were excluded with established other etiologies for their meningoencephalitis and 124 patients were included for evaluation. Patients that fell in the Non-TBM group included: Cryptococcal meningitis (7), Japanese Encephalitis (8), herpes simplex virus encephalitis (4), carcinomatous meningitis (3), dengue encephalitis (2), scrub typhus encephalitis (1) and varicella zoster encephalitis (1). Fifty-two patients were classified as probable and seventy two as possible TBM group prior to the CSF culture/ Gene Xpert or smear results. The demographic profile of these patients is listed in Table 2. Five patients were found to have HIV positive status and 4 had hepatitis B infection. Patients with possible TBM were slightly older than those in the probable group. On clinical examination, systemic symptoms of TB were significantly more in the probable group (Table 2). Chest X-ray findings were abnormal in 13 patients (upper zone infiltrates in 2, pleural effusion in 5, military mottling in 5, hydropneumothorax in 1 patient). CSF examination showed significantly higher proteins, sugars and cell counts in probable TBM compared to the possible TBM group (Table 2). CSF gene Xpert testing showed a significant difference in probable and possible TBM groups with the testing being negative in 67.45% possible TBM group and positive in 63.15% probable group (P – 0.001) (Table 3). Five Gene Xpert positive patients had rifampicin resistance, four in probable and one in possible TBM group. Twenty-eight patients (22.58%) had a positive MGIT test result. A significant association was

Table 1: Clinical criteria for definite, probable and possible TBM as per Marais *et al*.[13]

Clinical Criteria		**Score**
	Symptom duration of more than 5 days	4
	Systemic symptoms suggestive of tuberculosis (one or more of the following): weight loss (or poor weight gain in children),night sweats, or persistent cough for more than 2 weeks	2
	History of recent (within past year) close contact with an individual with pulmonary tuberculosis or a positive TST or IGRA (only in children <10 years of age)	2
	Focal neurological deficit (excluding cranial nerve palsies)	1
	Cranial nerve palsy	1
	Altered consciousness	1
CSF Criteria		
	Clear appearance	1
	Cells: 10–500 per μ1	1
	Lymphocytic predominance (>50%)	1
	Protein concentration greater than 1 g/L	1
	CSF to plasma glucose ratio of less than 50% or an absolute CSF glucose concentration less than 2•2mmol/L	1
Imaging Criteria		
	Hydrocephalus	1
	Basal meningeal enhancement	2
	Tuberculoma	2
	Infarct	1
	Pre-contrast basal hyperdensity	2
Evidence of TB Elsewhere		
	Chest radiograph suggestive of active tuberculosis: signs of tuberculosis=2; miliary tuberculosis=4	2/4
	CT/ MRI/ ultrasound evidence for tuberculosis outside the CNS	2
	AFB identified or *Mycobacterium tuberculosis* cultured from another source—i.e., sputum, lymph node, gastric washing, urine,blood culture	4
	Positive commercial *M tuberculosis* NAAT from extra-neural specimen	4
Exclusion of other causes		

TST=tuberculin skin test. IGRA=interferon-gamma release assay. NAAT=nucleic acid amplification test. AFB=acid-fast bacilli. The individual points for each criterion (one, two, or four points) were determined by consensus and by considering their quantified diagnostic value as defined in studies

seen in MGIT test positive status and probable TBM ($p<0.003$) (Table 3). The sensitivity of Gene Xpert relative to MGIT, the gold standard culture test was 68.42%, specificity of 97.61%, a positive predictive value of 92.85% and a negative predictive value of 87.23% (Table 4).

DISCUSSION

Extra pulmonary TB accounts for about 25% of all TB and an even higher percentage in children and immunocompromised patients. Diagnosis of extrapulmonary TB is often challenging, requiring the clinicians to obtain specimens that either have a low number of bacilli or are deep seated and not

Table 2: Demographic, clinical profile and CSF analysis in patients with Probable and possible TBM.

	Probable TBM (52)	Possible TBM (72)	P value
Sex Ratio (M:F)	25:27	39:33	NA
Clinical Features	**N (%)**	**N (%)**	**Chi- Square test**
Headache	47 (43.12%)	62 (56.88%)	0.151
Vomiting	44 48.88%)	46 (51.12%)	0.833
Irritability	11 (42.30%)	15 (57.70%)	0.433
Fever	50 (42.38%)	68 (57.62%)	0.097
Neck stiffness	21 (44.68%)	26 (55.32%)	0.465
Seizures	1 (33.34%)	2 (66.66%)	NA
Altered sensorium	7 (36.84%)	12 (63.15%)	0.252
Cranial nerve involvement (Convulsion)	13 (44.82%)	16 (55.18%)	0.577
Systemic symptoms	21 (67.44%)	10 (32.26%)	**0.048***
	Mean ± SD	**Mean ± SD**	**Wilcoxon Rank Sum test**
Age (Years)	32.29 ± 18.00	37.75 ± 18.39	**0.048***
CSF Examination			
Proteins (mg %)	200.2 ± 135	138.9 ± 119.5	**0.0006***
Sugars (mg %)	50.7 ± 49.2	59.1 ± 34.9	**0.0128***
Cell count (All lymphocytes)	153.1 ± 140.9	122.1 ± 175.9	**0.0034***

always amenable to biopsy.[14] Culture results take a long time and unnecessary delays in diagnosis compromise patient management.[15] Xpert MTB/RIF assay (Cepheid Inc., CA, USA) is a rapid molecular nucleic acid amplification test for TB diagnosis.[7,16] Recent recommendations from the WHO suggest that the Gene Xpert be used as the initial diagnostic test in adults suspected of having MDR-TB or HIV-associated TB and for CSF specimens from patients suspected of having TB meningitis over conventional microscopy and culture.[17,18] However, as these recommendations come as a result of very low-quality evidence, additional studies have been recommended.

In the present study, we determined Xpert MTB/RIF positivity in patients with a clinical suspicion of TBM. The patients were divided into probable and possible TBM group as per the clinical criterion by Marais *et al*.[13] This distinction is primarily based on a clinical scoring system and in the absence of a gold standard pathological diagnosis of TBM; patients are classified as probable or possible TBM based on clinical and imaging criteria and CSF biochemistry and cell count estimation. The proportion of Gene Xpert/RIF positivity in probable TBM was significantly higher compared to possible TBM. These patients with Gene Xpert positive status were confirmed as having Definite TBM.[13] Thus, a simple CSF Gene Xpert testing provided strong objective evidence and conferred a diagnosis of definite TBM, more so in the probable group (63% vs. 34 %). This is to our knowledge, the largest series of patients with TBM from India where Gene Xpert assay was assessed and also compared with MGIT, the culture gold standard test for

Table 3: Cross tabulation of Gene Xpert and MGIT with probable TBM and possible TBM. (* p<0.05)

Gene Xpert test /Group	Probable TBM	Possible TBM	Total	p-value
Negative	28 (32.55%)	58 (67.45%)	86	**0.001***
Positive	24 (63.15%)	14 (36.85%)	38	
Total	52	72	124	
MGIT /Group				
No Growth	62 (65.58%)	34 (35.42%)	96	**0.006**
Positive	10 (35.71%)	18 (64.29%)	28	
Positive	10 (35.71%)	18 (64.29%)	28	

Table 4: Cross tabulation of Gene Xpert and MGIT group

	Gene Xpert Positive	Gene Xpert Negative	Total	Chi Square P value
MGIT Positive	26(92.85%)	2 (7.14%)	28	<0.001
MGIT Negative	12 (12.76%)	82 (87.23%)	94	
Total	38	84	122	

Sensitivity: 68.42% (51%-82%)
Specificity: 97.61% (92%-100%)
PPV: 92.85% (76%-99%)
NPV: 87.23% (79%-93%)

TBM diagnosis. In our patient group, Gene Xpert when compared to gold standard culture testing had a high specificity and moderate sensitivity. The sensitivity of CSF Gene Xpert testing was similar to an earlier study done on extra pulmonary samples of a large cohort of Indian patents with extra pulmonary tuberculosis (68%).[19] Nhu and colleagues estimated the sensitivity of Gene Xpert to be 85%, much higher than our value.[20] A similar high sensitivity was reported by Metcalf *et al*., in their patient cohort of 37 patients.[21] The difference can be explained by a large number of HIV co-infection in their cohorts compared to our patient group. The small number of HIV positive patients in our cohort is probably related to our referral system where all patients with HIV co-infection are admitted and managed under general medicine and infectious diseases unit. HIV infected patients have a high bacterial load in the CSF, and thus, it is understandable that in HIV co-infected individuals, the sensitivity of Gene Xpert would be high.[22] Unlike Nhu *et al*., we did not do a prevalence study of gene Xpert in our patient group as clinical suspicion is a poor gold standard and hence, the sensitivity and specificity of Gene Xpert relative to clinical suspicion cannot be relied upon. An Indian study done by Rai *et al*., in 55 pediatric patients showed a low sensitivity and high specificity of Gene Xpert compared to culture results (40% and 92% respectively).[23] A similar low sensitivity (29%) was reported in an earlier study by Vadvai *et al*. and the authors attributed the same to a low volume of CSF sampled for Gene Xpert testing (2mL) and a lack of preprocessing the CSF by centrifuging it at a high speed and using the pellet for processing.[24] The preprocessing followed by our microbiologists could have attributed to the increased sensitivity in a paucibacillary CSF sample in our study group. The results of our study are particularly important from an Indian perspective where tuberculosis still remains the most common infectious disease and tubercular meningitis still has a high mortality of 1.5 per 100,000 population.[25] The rapid results obtained within 2 hours and an additional knowledge on rifampicin resistance are extremely helpful in timely management of patients suspected with TBM, especially when culture takes about 4-6 weeks and ZN smear has a very low sensitivity.[26,27] The advantages of Gene Xpert testing and its positivity confirming a diagnosis of definite TBM in a short time is a strong evidence that it should be included as a gold standard test in TBM work up, along with culture and smear examination. However, with the variable sensitivities compared to culture tests, the members of the international research consortium have cautioned against Xpert as the sole diagnostic test for TBM. They also suggest on using large volumes of CSF and its preprocessing to increase the sensitivity of Gene Xpert testing.[28]

In conclusion, the significant proportion of probable TBM cases with Gene Xpert MTB/RIF test positivity and a high sensitivity and specificity of this test relative to culture testing is a strong indication that it should be included as one of the gold standard tests in patients with suspected TBM. Gene Xpert testing establishes not only an early diagnosis but also detects rifampicin resistance thereby allowing for a faster and more effective treatment with a potential for an improved quality of life and decreased complications of Tubercular meningitis.

ACKNOWLEDGEMENTS

We thank Mr. Anurag Pathak, Biostatistician, Dr Ram Manohar Lohia Institute of Medical Sciences, for helping us with the statistical analysis of our patient data.

DISCLOSURE

Financial support: None

Conflict of interest: None

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