Section: Microbiology

Original Research Article

DIAGNOSTIC PERFORMANCE OF GENE XPERT MTB/RIF ASSAY IN CSF: AN OBSERVATIONAL STUDY AT TERTIARY CARE CENTER

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ABSTRACT

Background: Tuberculosis (TB) caused by Mycobacterium tuberculosis is a major cause of death and has now been a global public health problem due to increasing number of drug resistance. Tuberculosis burden in south east Asian region is 4.3 million and 3.14 lac in Uttar Pradesh in 2021. Incidence was 19,33,381 according to Global TB report. Late diagnosis and delay in treatment causes increase in mortality and morbidity. A reliable and rapid diagnostic tool is the priority for TB control. In December 2010 WHO has approved a new automated diagnostic molecular test "Gene Xpert MTB/RIF" a Cartridge Based Nucleic Acid Amplification Test (CBNAAT) which is not only sensitive to detects 131 CFU/ml of tubercular bacilli but also a rapid test as results are available in 2 hours. **Aim & Objective:** To evaluate the diagnostic performance of Gene Xpert MTB/RIF assay in CSF.

Materials and Methods: A Cross-Sectional Observational study was done in Department of Microbiology, MLN Medical College, Prayagraj from January 2023 to December 2023. GeneXpert MTB/RIF was performed on cerebrospinal fluid which detected Mycobacterium tuberculosis along with Rifampicin resistance.

Results: This study revealed the mean age of CSF sample patients to be 20.6 years. GeneXpert MTB positivity was observed in 30% of CSF samples. The sensitivity was 88.24%, specificity 100%, PPV 100%, NPV 94.29%, and overall accuracy was found to be 96.0%.

Conclusion: Xpert MTB/RIF assay demonstrated high sensitivity and specificity in detecting Mycobacterium tuberculosis in CSF.

Keywords: MTB (Mycobacterium Tuberculosis), Gene Xpert, RIF (Rifampicin), CBNAAT (cartridge based nucleic acid amplification test).

INTRODUCTION

Tuberculosis (TB), caused by Mycobacterium tuberculosis complex (MTBC), is a global public health concern, with 10 million new cases and 1.4 million fatalities in 2019.[1] According to the Global TB Report 2021, the incidence of tuberculosis in India in 2020 was 188 per 100,000 people (129-257 per 100,000 population). The overall number of incident TB patients (new and relapse) registered in 2021 was 19,33,3812.

There are five categories of drug-resistant TB used by the national health programmes at present: isoniazid (INH)-resistant TB, RRTB and MDR-TB (RR and INH resistant), plus pre-extensively drug-resistant TB (pre-XDRTB) and XDR-TB. Pre-XDR-TB is TB that is resistant to rifampicin (MDR/RR-TB) and any fluoroquinolone (a class of second-line anti-TB drug). XDR-TB is TB that is resistant to rifampicin

(MDR/RR-TB), plus any fluoroquinolone, plus at least one of the drugs, Bedaquiline, Delamanid and Linezolid.[2]

For public health purposes, tuberculosis (TB) is divided into two types: pulmonary TB (PTB), which affects the lung parenchyma and is infectious, and extra-pulmonary TB (EPTB), which affects organs other than the lungs and is estimated to account for 16%-20% of all TB cases. It has a worse result in people who are also afflicted with HIV. The clinical, radiological, histological, and bacteriological identification (smear examination, culture and molecular biology) are used to make the diagnosis of tuberculosis. However, culture is considered as gold standard for confirmation of tuberculosis. Bacterial growth is also essential for drug susceptibility testing (DST). Unfortunately, because of slow growth and sluggish development pattern, results usually take several weeks. Late diagnosis and delay in susceptibility testing result in increased morbidity, death, and disease spread. Among the issues in TB control is the development of a speedy and reliable diagnostic test for the diagnosis of MTB and its resistance to first-line medications.[3,4]

As a result, the World Health Organisation (WHO) has supported the use of quick molecular laboratory technologies for PTB diagnosis, such as the GeneXpert MTB/RIF (Xpert) (Cepheid, Sunnyvale, CA, USA) since Dec 2010.[5,6] The Gene Xpert MTB/RIF assay (Cartridge-Based Nucleic Acid Amplification Test) is a new automated diagnostic molecular test. This assay is based on nested real time PCR and molecular beacon technology, and it has a sensitivity of detecting 131 cfu/ml of Mycobacterium tuberculosis in sputum or 4.5 genomes per reaction, as well as simultaneous detection of rifampicin resistance mutations (81 bp region of the rpo B gene) within two hours and a sample preparation time of about 15 minutes. MDR-TB is diagnosed using the rpo B gene as a surrogate marker.[7,8]

There is limited literature available in India, related to the utility of the Xpert MTB/RIF assay for the extra-pulmonary tuberculosis diagnosis. Hence this study was undertaken to evaluate the diagnostic performance of Xpert MTB/RIF assay in CSF.

Aim & Objectives

Aim: To evaluate the diagnostic performance of Xpert MTB/RIF assay in CSF.

Objectives:

1. To detect the diagnostic performance of Xpert MTB/RIF assay in CSF.
2. To detect the resistance against Rifampicin (RIF).

MATERIALS AND METHODS

A Cross-Sectional Observational study was done from January 2023 to September 2023. The patients were selected from OPD & IPD of department of Neurology, Swaroop Rani Nehru Hospital and Paediatric Department of Sarojini Naidu Children Hospital, Prayagraj. From all the patients clinically suspected of tubercular meningitis, cerebrospinal fluid samples were collected and processed in the Department of Microbiology, Moti Lal Nehru Medical College. Patients with Bacterial meningitis, viral meningitis, and patients not willing to be a part of study were not included in the study.

Methodology: The study obtained the institutional ethics clearance prior to recruitment of patients. After obtaining the informed consent from patients, the detailed history of the patient was obtained including prior treatment history and presence of any co-morbid condition. The patient's samples like CSF (2ml) collected aseptically by lumber puncture and were prepared according to the manufacturer's user instructions. Sample reagent was added directly into the falcon tube. The cap was placed and the tube was vortexed 10-20 times and incubated at room temperature. After 10 minutes, the specimen was vortexed again for 10-20 times. Sample ID. was labelled on the Cartridge and 2ml of processed specimen was transferred with the help of sterile disposable transferring pipette into it. Cartridge barcode was scanned the sample ID was entered in the system, the cartridge was positioned on the Xpert machine and test was started. Two components were noted in the result, first the detection of MTB and the second component was identification of RIF resistance. Xpert performs and incorporates the steps of bacterial lysis, DNA extraction, amplification and amplicon detection using a disposable plastic cartridge. Results are available within two hours.

RESULTS

Total sample of cerebrospinal fluid received from January 2023 to December 2023 were 46. The age-wise distribution revealed distinct demographic trends across different age groups. The majority of cases were observed in the 1–20 years age group, comprising 50.0% of females and 53.5% of males. A smaller proportion of CSF cases were in the 21–40 years group, accounting for 13.6% of females and 21.5% of males. Interestingly, a small fraction of CSF cases was found in infants under one year, with 18.2% of females and 3.6% of males affected. In the 41–60 years group, both males and females had an equal proportion (14.2% and 18.2%, respectively). Notably, only 7.2% of male CSF cases were reported in the 61–80 years age bracket, and no cases were seen beyond 80 years in either gender.

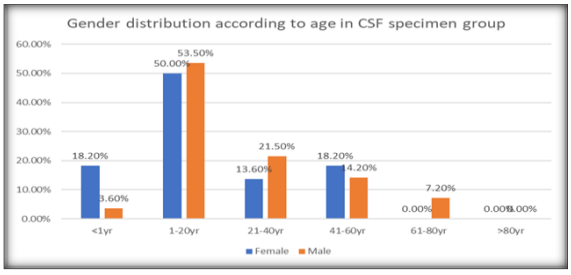

Figure 1: Gender distribution according to age in CSF specimen group

FINDINGS OF GENE XPERT:

The Gene Xpert MTB result among patients showed (30%) of CSF samples were positive, while 70% were negative.

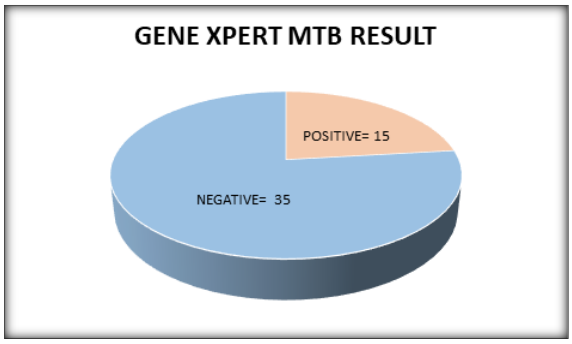

Figure 2: Showing the Gene Xpert among the patients

FINDINGS OF RIF

Majority (70.0%) of cases being negative for MTB, RIF was not performed (NP), resistance (R) to RIF was observed in 4.0% of cases and sensitivity (S) to RIF was 26.0%.

FINDINGS OF BIOCHEMICALS

The biochemical test cerebrospinal fluid highlights the distinct physiological and pathological roles. Protein concentration was notably higher (101.2 ± 68.8 g/dl), reflecting its crucial role in brain homeostasis and potential alterations in neurological disorders. Glucose levels were significantly low (30.7 ± 41.2 mg/dl), suggesting possible infections or inflammatory conditions affecting the central nervous system. Adenosine deaminase (ADA) values in CSF were (9.19 ± 30.03 U/L). Additionally, lactate dehydrogenase (LDH) levels were (54.9 ± 45.5 U/L). These findings underscore the importance of fluid analysis in distinguishing between neurological, aiding in accurate diagnosis and targeted treatment strategies.

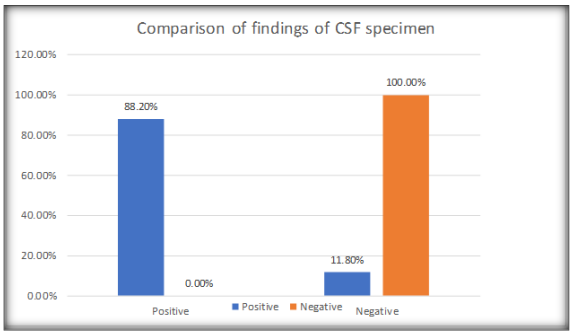

Figure 3: Comparison of findings of CSF specimen

Table 1: Gender distribution across different age groups

AGE WISE DISTRIBUTION	FEMALE N=22 (100.0%)	MALE N=28 (100.0%)
<1yr	4 (18.2%)	1 (3.6%)
1-20yr	11 (50.0%)	15 (53.5%)
21-40yr	3 (13.6%)	6 (21.5%)
41-60yr	4 (18.2%)	4 (14.2%)
61-80yr	0 (0.0%)	2 (7.2%)
>80yr	0 (0.0%)	0 (0.0%)

Table 2: Showing the Gene Xpert results among the patients

		CSF N=50	
		Count	N %
GENE XPERT MTB	Negative	35	70.0%
	Positive	15	30.0%

Table 3: Showing RIF in CSF sample

		CSF	
		Count=50	N %
RIF	I	0	0.0%
	NP	35	70.0%
	R	2	4.0%
	S	13	26.0%

Table 4: Showing mean level of biochemical parameters

	CSF	
	Mean	SD
Protein gm/dl	101.2	68.8
Glucose mg/dl	30.7	41.2
ADA U/L	9.19	30.03
LDH IU/L	54.9	45.5

Table 5: Comparison of findings of CSF specimen

CSF GENE XPERT MTB	FINAL DIAGNOSIS		
	Positive	Negative	Total
Positive	15 (88.2%)	0 (0.0%)	15
Negative	2 (11.8%)	33 (100.0%)	35
Total	17	33	

Table 6: Diagnostic characteristics of CSF GeneXpert MTB

Statistic	Value	95% CI
Sensitivity	88.24%	63.56% to 98.34%
Specificity	100.0%	89.42% to 100.0%
Positive Predictive Value	100.0%	62.52% to 100.0%
Negative Predictive Value	94.29%	81.91% to 98.36%
Accuracy	96.0%	86.29% to 99.51%

DISCUSSION

The GeneXpert MTB test for cerebrospinal fluid analysis demonstrated strong diagnostic performance in detecting Mycobacterium tuberculosis. The sensitivity was 88.24% (95% CI: 63.56%–98.34%), indicating a high ability to correctly identify true positive cases, though some cases were missed. The specificity was 100.0% (95% CI: 89.42%–100.0%), reflecting a low rate of false positives. The Positive Predictive Value (PPV) was 100.0% (95% CI: 62.52%–100.0%), meaning that most positive results were accurate. The Negative Predictive Value (NPV) was 94.29% (95% CI: 81.91%–98.36%), ensuring reliability in ruling out tuberculosis in negative cases. Overall, the test achieved an accuracy of 96.0% (95% CI: 86.29%–99.51%), making it a valuable tool for diagnosing tuberculous meningitis. However, given the potential for false negatives, additional diagnostic methods, including clinical evaluation and other laboratory tests, should be considered for comprehensive assessment. Present study, the mean age between the group was significantly different, with mean age of CSF sample as 20.6yrs and pleural fluid specimen was 42.0yrs.

In study by Rai A et al., 55 children (less than and equal to 18 years) with tubercular meningitis. 14 (25.4%) children were less than 5 years of age, 28 (50.9%) of 5-10 years of age; and 13 (23.7%) were 10-18 years age. In study by Raj A et al., documented with out of total 831 samples included in the study, 507 (61.01%) were males, 324 (38.99%) were females. The mean age of patients was 52.01.[11] Gupta H et al., the mean age of the study group was 38.01 yrs. and the sex ratio was 2.63:1.[12] The mean age of patients in both the group was 42±19.41 years in study by Chopra V et al.,[13] The mean age was 36.86 years in study by Aricha SA et al.,[14]

In CSF sample 30% were positive on GeneXpert MTB and 26% were RIF sensitive. Biochemical parameters such as protein, Glucose, ADA and LDH were measured in CSF showed significant findings. Bahr NC et al., documented that only 39% of the cases were positive by culture as well as Xpert on centrifuged CSF. CSF centrifugation improves Xpert's diagnostic efficacy in the diagnosis of TBM. The most instances were found using a combination of culture and Xpert.[8]

In study by Rice JP et al., found that Xpert MTB/RIF assay demonstrated an overall sensitivity of 89.6% for detecting MTBC, with 97.7% sensitivity in smear-positive cases and 74.5% in smear-negative cases, along with a specificity of 97.2%. In comparison, the acid-fast bacilli (AFB) smear test showed a sensitivity of 64.9% and a specificity of 77.8%. Xpert MTB/RIF successfully identified 35 out of 47 smear-negative, culture-positive specimens and ruled out 124 of 137 smear-positive, culture-negative specimens.[15]

CONCLUSION

Tubercular Meningitis (TBM) often presents with nonspecific symptoms, making its diagnosis challenging and can lead to severe neurological complications and mortality if left untreated. Early and accurate detection of TB in CSF is crucial. Biochemical markers such as adenosine deaminase (ADA), lactate dehydrogenase (LDH), and protein levels in CSF provide supportive diagnostic information, but their specificity varies. Therefore, a reliable molecular test like Xpert MTB/RIF is essential to improve diagnostic efficiency and patient outcomes. The advantages of Gene Xpert MTB/RIF are minimum turn-around time, low complexity, high sensitivity and specificity, minimum safety concerns and detects MTB along with Rifampicin resistance.

Results from this study helps to bridge the gap in TB diagnosis, reporting and early and prompt treatment of positive patients.

Conflicts of Interest

Authur states that there is no conflict of Interest.

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