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A PROSPECTIVE STUDY ON EVALUATION OF DIAGNOSTIC PERFORMANCE OF GENEXPERT MTB IN CSF FOR EARLY DIAGNOSIS OF TUBERCULAR MENINGITIS IN A TERTIARY CARE HOSPITAL ODISHA.

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Abstract

Aim

The study aims to establish the diagnostic accuracy of GeneXpert MTB/RIF in CSF for early diagnosis of tubercular meningitis and to compare the efficacy of CSF GeneXpert MTB/RIF with CSF culture for mycobacterium.

Methodology

This was a prospective, cross-sectional study conducted in the Department of Medicine, Neurology, S.C.B Medical College, Cuttack. All patients of age >18 with clinical features suggestive of tubercular meningitis were included in the study. All routine blood tests were performed along with malaria, leptospira, chest radiograph, CT scan or MRI (selected patients) and CSF study to exclude other causes of meningitis. CSF sample subjected to biochemistry, cytology, ZN stain, MGIT culture, and Xpert MTB/RIF. The positive results for each test (ZN stain, MGIT culture, and Xpert MTB/RIF) were compared using Pearson's chi-squared test. All statistical analyses were done using the SPSS 21.0 version.

Results

Out of 100 suspected TBM patients 40 were finally classified as definite TBM, 6 were probable TBM, 2 were possible TBM, and 52 were not having TBM. Tubercular meningitis occurred more commonly in the population 21-40 years and in males. The overall sensitivity of CSF GeneXpert MTB/RIF, Zn stain, and MGIT culture was 62.5%, 29.16%, and 66.5% respectively, and specificity of 100% for each in diagnosing TBM. Rifampicin resistance was detected only in two cases.

Conclusion

GeneXpert MTB/RIF test can rapidly confirm a diagnosis of TBM with 62.5% sensitivity and 100% specificity, along with rifampicin resistance. It can be a useful diagnostic method in patients of suspected TBM either AFB smear-negative or positive due to its rapidity and simultaneous detection of rifampicin resistance.

Recommendation

Positive GeneXpert results are to be read cautiously and should be well correlated with the clinical and treatment history of the patient.

Keywords: GeneXpert, Tuberculous Meningitis, Diagnostic Accuracy
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Introduction

Tuberculous meningitis is a common infectious disease of the CNS that results in high fatality and disability rates among severe infectious diseases and is also a major health problem in developing countries like India. It is the most severe type of extrapulmonary tuberculosis, accounting for 1-5% of all new cases annually [1]. Tuberculous meningitis also poses a major health concern

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even in the developed world due to the increasing number of individuals infected with HIV [2, 3].

The diagnosis and treatment of different meningitis pose a challenge for the primary care physician. Routine CSF analysis and radiological investigations are often insufficient in identifying the cause of meningitis. Gram stain and Zn stain of CSF are rapid methods of detection of organisms, but with reduced sensitivity [4]. Apart from routine CSF analysis, other additional tests like latex agglutination, counter-immunoelectrophoresis, radio-immunoassay, and CSF lactate measurement have been applied with distinct degrees of validation for the diagnosis of bacterial meningitis. The culture of CSF gives a definite diagnosis but takes a longer time [5]. Tuberculous meningitis is the most severe form of tuberculosis, accounting for approximately half of the death or disability of those it affects [6]. Delayed diagnosis of tuberculous meningitis is strongly linked to poor treated outcomes, a condition aggravated by conventional diagnostics modalities. There needs a fast and dependable test for rapid bedside diagnosis. Adenosine deaminase (ADA) assay has been successfully evaluated and may be considered as an aid in the diagnosis; on the other hand, its expanse is unable to make distinct of TB meningitis from other forms of bacterial meningitis [7, 8].

GeneXpert MTB/RIF test is a closed-cartridge-based system that can be operated by minimally trained staff and produce results in a lapse of around 24 hours [9]. Several studies have flourishing outcomes in the application of the Xpert MTB/RIF test on extrapulmonary samples, with overall sensitivities above 80% and specificity reaching cent percent [10, 11]. "Meta-analyses of diagnostic performance of Xpert for tuberculous meningitis showed pooled sensitivities of 79·5–80·5% compared with mycobacterial culture and specificities of 98·6–98·8% for *M tuberculosis* detection in CSF" [12, 13]. Nevertheless, the sensitivity of diagnosis modalities was affected by the volume of CSF tested, and whether CSF centrifugation was done before testing [14].

Due to the urgency of diagnosis in suspected TBM cases because of a rapid reduction of survival chances with the increase of severity; rapid, accurate diagnostic tests like GeneXpert MTB/RIF which also can identify rifampicin resistance could have a significant impact on survival of tubercular meningitis patients and further capable to prevent disabilities.

Aims and Objectives

The study aims to establish the diagnostic accuracy of GeneXpert MTB/RIF in CSF for early diagnosis of tubercular meningitis and to compare the efficacy of CSF GeneXpert MTB/RIF with CSF culture for mycobacterium.

Materials and Methods

Study Design: This was a prospective cross-sectional study conducted in the Department of Medicine and Neurology, SCB Medical College, Cuttack.

Inclusion criteria

All patients >18 years of age with clinical features suggestive of tuberculous meningitis either visiting or admitted to the Department of Medicine and Neurology were included in the study.

Exclusion criteria

Patients with fever of less than 7 days, without neurological symptoms, and also patients in whom spinal tap is contraindicated were excluded.

Methodology

All patients satisfying the above criteria were included in the study. All routine blood investigations were performed along with chest radiographs, CT scans, or MRI (selected cases), A CSF study was done to exclude other causes of meningitis. CSF biochemistry, Indian ink stain for fungi, gram stain, culture, viral PCR (for herpes simplex virus, varicella-zoster virus, and IgM and IgG serology for Japanese encephalitis were also performed to exclude other causes of meningitis when there was strong clinical suspicion.

"Clinical entry criteria include symptoms and signs of meningitis including one or more of the following: headache, irritability, vomiting, fever, neck stiffness, convulsions, focal neurological deficits, altered consciousness, or lethargy. Patients diagnosed with TBM were classified as having definite, probable, or possible TBM using the standardized case definition" [15].

The diagnosis of TBM was established based on CSF ZN Stain, CBNAAT, mycobacterial culture, biochemistry, cytology, clinical findings, and neuroimaging. Approximately 8 ml of CSF sample was collected for a better microbiological confirmation rate [16]. Approximately two ml sample was sent to microbiology and biochemistry laboratories and the remainder was sent to TB laboratory. In the TB laboratory, CSF samples were centrifuged for 15 minutes. The supernatant was removed to leave a 0.5-ml deposit, which was then used for Ziehl-Neelsen smear preparation (100 µl), inoculation of MGIT culture (100 µl), and GeneXpert testing (200 µl).

Bias: There was a chance that bias would arise when the study first started, but we avoided it by giving all participants identical information and hiding the group allocation from the nurses who collected the data.

Ethical considerations

The ethical aspects of the research were carefully thought out to preserve patient privacy and confidentiality.

Statistical Analysis

The results were expressed as the mean ± standard deviation and percentages. The positive results for each test (ZN stain, MGIT culture, and Xpert MTB/RIF) were compared using Pearson's chi-square test. The sensitivity of Xpert MTB/RIF stratified by CSF volume was also analyzed. All statistical analyses were performed using SPSS version 21.0. A p-value of less than 0.05 was considered significant.

Results

A total hundred suspected TB Meningitis patients enrolled during the study span. At the initial stage, several 200 patients were examined for eligibility, however, 100 patients were excluded from this study due to not being eligible. These hundred enrolled patients were further classified into forty as definite, six patients with probable, two patients as possible TBM, and fifty-two patients placed in a non-TBM category. In a total of fifty-two non-TBM categories, thirty-one patients were diagnosed with viral meningoencephalitis, twenty patients with bacterial meningitis, and one patient was found to be dengue positive. The percentages of tubercular, viral, and pyogenic meningitis were 48, 20%, and 32% respectively.

Tuberculous meningitis occurred more in the population 21-40 years. Viral meningitis and bacterial meningitis were seen in all age groups but a higher percentage in the 21 -40 age group. The association among different types of meningitis with different age groups was statistically not significant (Table 1). Most males were affected in each meningitis group, the highest in pyogenic meningitis where 65% of males were affected. In tubercular meningitis 64.5% of males and in viral meningitis 56.25% of males were affected (Table 2).

Table - 1: Age Distribution of Meningitis

Age group	TBM	PM	VM	
18-20	4(8.33%)	1(5%)	6(8.75%)	
21-40	24(50%)	15(75%)	14(43.75)%	**x^2=7.691**
41-60	15(21.25%)	2(10%)	9(28.13%)	**df=6**
>60	5(10.42%)	2(10%)	3(9.37%)	**p=.262**
Total	48	20	32	

Table- 2: Gender Distribution of Meningitis

Meningitis	Female	Male	Total	
TBM	17(35.5%)	31(64.5%)	48	
PM	7(35%)	13(65%)	20	**x^2=.661**
VM	14(43.75%)	18 (56.25) %	32	**df=2**
Total	38	62	100	**p=.718**

Table- 3: Clinical Presentation of Meningitis

Clinical feature	TBM	PM	VM	TOTAL	Percentage
Fever	48	20	32	100	100%
Headache	30	22	24	76	76%
Vomiting	42	18	30	90	90%
Seizure	39	18	30	87	87%
Focal Deficits	12	0	0	0	12%
Irritable	22	14	19	55	55%
Stupor	24	4	13	41	41%
Comatose	2	0	0	2	2%

TBM-Tubercular meningitis, PM-Pyogenic meningitis, VM-Viral meningitis

Almost all the patients of meningitis had fever (100%). 90% of patients had vomiting, 76% of patients had headaches, 87% of patients had seizures and the majority of them had tubercular meningitis. Seizure associated with tubercular meningitis was mostly generalized. 12 patients had focal deficits and two patients were comatose all of them belong to tubercular meningitis category (Table-3). TBM group consists of a total forty-eight case, in which thirty cases were Genexpert positive, thirty-two cases found MGIT positive, and only fourteen cases had Zn stain positive result. All the results of above diagnostic modalities among fifty-two non TBM groups were negative. Overall sensitivity of Xpert MTB/RIF was 62.5% and specificity was 100%. Positive predictive value and negative predictive value was 100% and 74.28% respectively. The sensitivity and specificity of ZN stain relative to final clinical diagnosis was 29.16% and 100%. The PPV and NPV relative to final clinical diagnosis was 100% and 60.46% respectively. Overall sensitivity of MGIT culture was 66.6% and specificity was 100%. The PPV and NPV relative to final clinical diagnosis was 100% and 76.47% respectively (Table-4).

In table-5 diagnostic performance of gene Xpert MTB was compared to ZN stain. Sensitivity of Zn stain and Xpert MTB of CSF sample was 29.16% and 62.5% respectively. The difference was statistically significant with P =0.001. In the table-6 sensitivity of MGIT culture (66.66%) was compared with sensitivity of Zn stain (29.16%). The difference was also found to be statistically significant. P =0.0001.

Table- 4: Results of Zn stain, MGIT culture, and XPERT MTB/RIF testing by Final Diagnosis

Test result	No. (%)			Total
		TBM	Not TBM	
Xpert MTB/RIF	Positive	30 (62.5)	0	30
	Negative	18 (37.5)	52(100)	70
	Total	48 (100)	52 (100)	100
Ziehl-Neelsen stain	Positive	14 (29.16)	0	14
	Negative	34 (70.83)	52 (100)	86
	Total	48 (100)	52 (100)	100
MGIT culture	Positive	32(66.66)	0	32
	Negative	16(33.33)	52(100)	68
	Total	48 (100)	52(100)	100

Table -5: Comparison of Diagnostic accuracy among Xpert MTB, Zn Stain and MGIT in detecting TBM in Meningitis cases

Test	Sensitivity	Specificity	PPV	NPV
XPERT MTB	62.5%	100%	100%	74.28%
Zn stain	29.16%	100%	100%	60.46%
MGIT	66.66%	100%	100%	76.47%

Table- 6: Comparison of diagnostic performance between Xpert MTB and Zn Stain in TBM group

TEST	SENSITIVITY	SPECIFICITY	Z value P value
XPERT MTB	62.5%	100%	Z=4.72 p=0.001
Zn stain	29.16 %	100%	

Hundred CSF sample was received for analysis. Volumes of each sample were measured and according to volume of sample they were further divided into low, medium and high volume sample. High volume measures more than five ml accounts for twenty-two percent, sixty percent sample were medium volume (2-5 ml) and seventeen percent sample were low volume (approximately 2 ml). The sensitivities of Xpert MTB/RIF for low-volume samples, medium-volume samples, and high-volume samples were 55.5%, 84.6%, and 76.4% respectively (Table-7 and 8). Although the sensitivities for medium- and high-volume samples were better than those for low-volume samples, this difference did not reach statistical significance (P = 0.341). Rifampicin resistance was detected in 2 patients. In both cases, the result was confirmed by line probe assay performed on DNA extracted from a positive MGIT culture. The sensitivity of Xpert for diagnosis of MDR TBM is not evaluated due to the low prevalence of MDR TBM in our study.

Table- 7: Comparison of diagnostic performance between MGIT culture and Zn stain in TBM Group

Test	Sensitivity	Specificity	Z value P value
MGIT culture	66.66%	100%	Z =4.576 p=0.0001
Zn Stain	29.16%	100%	

Table 8: Diagnostic accuracy of Xpert MTB/RIF by CSF volume

CSF volume	No of cases	Definite TBM	Xpert MTB Positive	Xpert MTB sensitivity
Low Volume 2ml	18	18	10	55.56%
Medium volume 2.1-5ml	59	13	11	84.62%
High Volume >5ml	23	17	13	76.48%

Discussion

Almost half of the suspected meningitis patients were TB meningitis followed by pyogenic and viral meningitis. Among tb meningitis and other categories of meningitis male patients were more commonly affected and most belonged to the age group 21-40 years. This is by north Indian study by Harsimran Kaur et al. and LUO et al. study where tb meningitis is more common in this age group with male preponderance [17, 18].

Almost all the patients presented with fever followed by vomiting, seizure, and headache. Seizure associated with TB meningitis patients was generalized, twelve patients with TB meningitis had focal deficits and two patients were comatose among TB meningitis patients. This suggests that TB meningitis patients have severe illness. In contrast LUO et al. study, where headache most common presenting symptom followed by fever neurological complications percentage was lesser than in our study. Probably our patients presented late.

The overall sensitivity of Zn stain, Gene Xpert, and MGIT culture was 29.16%, 62.5%, and 66.6% respectively. The specificity of all three diagnostic modalities was 100%. The sensitivity of Zn stain depends upon careful examination of slides by experienced technicians. Since Xpert MTB/RIF is less dependent on time and technician skills, will improve the diagnostic affirmation of TBM in high-volume laboratories. Though smear microscopy is substantially cheaper than the Xpert MTB/RIF test the hands-on time required to achieve high sensitivity in smear testing is greater. Furthermore, Xpert detected rifampicin resistance of less than three hours.

Though the specificity of both MGIT culture and GeneXpert were the same but overall sensitivity of MGIT culture was higher than that of GeneXpert (66.6% vs 62.5%). These findings were not by Chen et al. [19]. MGIT culture requires a larger volume of CSF and it requires more time for a positive result though it has greater sensitivity as compared to Genexpert. This limits the usefulness of MGIT culture in deciding to treat TBM over Gene Xpert.

The GeneXpert sensitivities for high, medium, and low CSF volume samples were not statistically significant. Data on MDR TB meningitis are limited. In a South African study out of 350 patients with TB meningitis 30(8.6%) patients were multi-drug resistant [20]. The prevalence of MDR TB meningitis in this study was less than 5 percent. So, it is not possible to draw robust conclusions about the sensitivity of Xpert MTB for the diagnosis of MDR TBM due to the low prevalence of MDR TBM in our study.

Conclusion

Although culture is considered the gold standard method, it takes a longer period to give positive results and also concurrent detection of rifampicin resistance is not possible. On the other hand, Gene Xpert is a useful diagnostic technique in suspected TBM patients irrespective of AFB smear status, because of its rapidity and concurrent identification of rifampicin resistance.

The Xpert MTB/RIF test confirms the diagnosis of TB Meningitis with a sensitivity of 62.5 percent and specificity of 100 percent. GeneXpert will help in the rapid and precise diagnosis of tubercular meningitis and will have a significant impact on patient survival. However, culture-negative but GeneXpert-positive results need to be read vigilantly and should be well correlated with the patient's clinical history.

Limitations

The number of cases was limited because the work had to be completed in a desired frame of time. In low-income countries like India with a high burden of tuberculosis cost effectiveness of GeneXpert was not evaluated. The sensitivity of GeneXpert for diagnosis of MDR TBM was not evaluated. The pediatric population was not included in our study although TBM was more prevalent in these groups.

Recommendation

Positive GeneXpert results are to be read cautiously and should be well correlated with the clinical and treatment history of the patient.

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List of Abbreviations

MTB- Mycobacterium tuberculosis
CNS- central nervous system
HIV- Human Immunodeficiency Virus
CSF- Cerebrospinal fluid
ADA- Adenosine deaminase
RIF- rifampicin
TB- Tuberculosis
TBM- Tuberculous meningitis
CT- Computed tomography
MRI- Magnetic Resonance Imaging
PCR- polymerase chain reaction
IgM- Immunoglobulin M
IgG- Immunoglobulin G
CBNAAT- Cartridge-Based Nucleic Acid Amplification Test
MGIT- Mycobacteria Growth Indicator Tube
PM- Pyogenic meningitis
VM- Viral meningitis
PPV- positive predictive value
NPV- negative predictive value
DNA- Deoxyribonucleic acid
MDR- multiple drug resistance
AFB- Acid- Fast Bacilli

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Conflict of Interest

None declared.

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