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Correlation of GeneXpert and cerebrospinal fluid culture in patients of tubercular meningitis

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ABSTRACT

Background: *Mycobacterium tuberculosis* (MTB) remains one of the most common causes of mortality and morbidity in India. Therefore, an apart from an early diagnosis and detection of rifampin (RIF) resistance is also very essential for management. A novel integrated diagnostic device GeneXpert MTB/RIF assay has revolutionized the rapid diagnosis of tuberculosis and along with RIF resistance in patients. However, its use is not been explored in extrapulmonary specimens.

Objective: We determined the performance of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis from cerebrospinal fluid (CSF) specimens obtained from possible tubercular meningitis (TBM) cases.

Materials and Methods: A cross-sectional study was conducted where CSF sample of suspected TBM patients was evaluated for TB by GeneXpert and liquid culture (mycobacteria growth indicator tube) and compared.

Results: Of 53 patients, who were included in the study period, 5 patients were culture positive and 48 were culture negative for *M. tuberculosis*. GeneXpert MTB/RIF test showed a sensitivity of 60% and specificity is 97.9% for diagnosing TBM, the negative predictive value was 95.9% and positive predictive value was 75%.

Conclusion: GeneXpert MTB/RIF test is highly specific and can be used for diagnosis of TBM adjunct to clinical finding, biochemical and radiological features. Its role as sole diagnostic test needs to be studied in larger sample.

Key words: GeneXpert, meningitis, tuberculosis

INTRODUCTION

Tuberculous meningitis (TBM) is one of the types of extrapulmonary tuberculosis (EPTB) associated with high morbidity and mortality. Globally, EPTB constitutes 25% of all TB cases and even higher percentages in HIV-infected individuals and children.[1-3] The classical triad of fever, headache, and neck rigidity (signs of meningism) may not be present in all the TBM patients.[4] Children present with altered sensorium more than adults as presenting feature.[5] Signs of meningism may be diminished or absent in elderly. However, seizures tend to occur more frequently.[6] HIV coinfected patients present differently with less signs of meningism and more in altered behavior.[7,8] The World Health Organization recommends the use of GeneXpert *Mycobacterium tuberculosis* (MTB)/rifampin (RIF) as initial test for diagnosis of TBM in comparison to microscopy and culture.[9] The spread of multidrug-resistant TB (MDR-TB), the detrimental convergence with HIV infection, and the unavailability of rapid diagnostic tools have contributed to the failure of global TB control.[10-12] Coinfection with HIV alters the course of disease and makes diagnosis difficult leading to increase in morbidity and hence mortality associated with disease.[13,14] With emergence of MDR-TB, early diagnosis of TB and resistance is essential. Although the "gold standard" is culture, it takes 2–8 weeks for results. Poor sensitivity and a low positive predictive value (PPV) limit the use of smear microscopy for acid-fast bacilli (AFB). Nucleic acid amplification techniques have emerged as an effective diagnostic tool for rapid identification, early treatment, and its associated with improved patient outcomes. The GeneXpert MTB/RIF test is a cartridge bases test which performs processing of sample with real-time polymerase chain reaction (PCR) not only for TB diagnosis but also it detects of RIF resistance.[15,16]

The aim of this study was to evaluate the diagnostic validity of laboratory cerebrospinal fluid (CSF) parameters using GeneXpert and liquid culture. The results obtained by the MTB/RIF assay were compared with the results obtained by liquid culture (mycobacteria growth indicator tube [MGIT]).

MATERIALS AND METHODS

A cross-sectional study was carried out in the Department of General Medicine and Department of Microbiology of Institute of Medical Sciences, BHU, Varanasi, from January 2016 to January 2017. The records of all the patients of TBMs in our unit were studied and details were noted. Clinical assessment of all cases was carried out and details were recorded in the per forma and blood samples and CSF samples were obtained and analyzed.

All cases presenting with symptoms of meningitis of more than 18 years of age were included in the study. A clinical suspicion of

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meningitis was defined as having any combination of the following symptoms: Headache, irritability, vomiting, fever more than 2 weeks, neck stiffness, convulsions, focal neurological deficit, and altered consciousness or lethargy with no other general conditions explaining them.

Selection of patients with clinical suspicion of meningitis by clinical details (history and clinical examination) and relevant investigations were done. Lumbar puncture was performed after ruling out raised intracranial tension. All the specimens were received from Medicine unit, BHU, Varanasi, for bacteriological evaluation of TBM. For this study, all demographic details, pathological details, and related investigations were done and entered into Excel sheet. Patient informed consent was taken before including the patient for the study. At least 1 mL CSF was collected in sterile container and was transported immediately to TB culture drug sensitivity test laboratory for the GeneXpert and liquid culture, and the results of the two were correlated for the diagnosis of tubercular meningitis (TBM) using appropriate statistical methods.

RESULTS

In our study period, a total of 53 patients were studied. About 20.8% (n = 11) subjects were aged between 11 and 20 years, 47.1% (n = 25) were aged between 21 and 40 years, and 15.1% (n = 8) aged between 41 and 50 years, 17% (n = 9) were aged >50 years. Among these, 60.4% were male and 39.6% were female. The mean age of the patients was 35.13 ± 15.34 years and 9.4% of patients were HIV positive. Demographic and biochemical profile of patient is shown in Table 1.

Table 1: Demographic and biochemical profile of patients (n=53)

Variables	Mean±SD
Age (years)	35.13±15.342
Duration of illness (weeks)	10.045±10.6288
Hb (g/dL)	11.747±1.8342
TLC (mm^3)	1.05E4±5581.035
N (mm^3)	74.274±13.5853
L (mm^3)	18.285±13.8158
PLT (mm^3)	2.22E5±109008.212
MCV (fL)	86.857±7.9465
RBS (mg/dL)	118.460±50.0186
Urea (mg/dL)	39.821±29.1315
Cr (mg/dL)	0.892±0.5151
Na (mmol/L)	136.604±12.4968
K (mmol/L)	4.195±0.8189
SGOT (aspartate transaminase U/L)	55.81±55.241
SGPT (alanine transaminase U/L)	51.57±60.909
TB (mg/dL)	0.747±0.4818
DB (mg/dL)	0.353±0.3625
CSF_Sugar	55.047±23.8556
CSF_Protein	134.953±77.7458
CSF_Total_count	218.36±652.336
CSF_N	27.34±26.994
CSF_L	68.68±29.676

Hb: Hemoglobin, TLC: Total leukocyte count, N: Neutrophils, L: Lymphocytes, PLT: Platelet, MCV: Mean corpuscular volume, Cr: Creatinine, RBS: Random blood sugar, Na: Sodium, K: Potassium, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic-pyruvic transaminase, TB: Total bilirubin, DB: Direct bilirubin

52 patients (98.1%) had complaints of fever, 36 patients (67.9%) had headache, 22 patients (41.5%) had vomiting, and 11 patients (20.8%) presented with seizures at the time of admission in the medicine ward, while 31 patients (58.5%) presented with altered sensorium. Fever was one of the most prominent symptoms followed by headache.

Of 53 patients suspected of TBM clinically, four patients were Xpert positive and five patients were MGIT culture positive. The details of positivity in Xpert and culture and sensitivity, specificity, PPV, and negative predictive value (NPV) of test are shown in Tables 2a and b.

DISCUSSION

TBM occurs in Indian population across all ages. In our study, the disease was found more common in males as compared to females and majority of patients were in the age group of 20–40 years. Similar prevalence was seen in the study conducted by Sarkar *et al.*, where 53.3% were aged between 20 and 39 years with 56.7% being male and 43.3% were female.[17] In another study by Po Chang Hsu *et al.*, 71 (65.7%) were male and the mean age of the patients was 54.9 ± 18.6 years.[18]

Among symptoms, the frequency of fever in TBM has been reported to vary between 60% and 95%.[19] A comparative data of

Table 2a: CSF MTB GeneXpert performance compared to CSF MTB cultures

Single table analysis

	Positive	Negative	Total
Positive	3 (75)	1 (25)	4 (100)
Negative	2 (4.1)	47 (95.9)	49 (100)
Total	5 (9.4)	48 (90.6)	53 (100)
Parameter	**Estimate**	**Lower-upper 95% CIs**	**Method**
Sensitivity	60%	(23.07, 88.24[1])	Wilson score
Specificity	97.92%	(89.1, 99.63[1])	Wilson score
PPV	75%	(30.06, 95.44[1])	Wilson score
NPV	95.92%	(86.29, 98.87[1])	Wilson score
Diagnostic accuracy	94.34%	(84.63, 98.06[1])	Wilson score

PPV: Positive predictive value, NPV: Negative predictive value

Table 2b: CSF MTB PCR performance compared to CSF MTB cultures in HIV patients

Single table analysis

	Positive	Negative	Total
Positive	1 (100)	0 (0)	1 (100)
Negative	1 (25)	3 (75)	4 (100)
Total	2 (40)	3 (60)	5 (100)
Parameter	**Estimate (%)**	**Lower-upper 95% CIs**	**Method**
Sensitivity	50	(9.453, 90.55[1])	Wilson score
Specificity	100	(43.85, 100[1])	Wilson score
PPV	100	(20.65, 100[1])	Wilson score
NPC	75	(30.06, 95.44[1])	Wilson score
Diagnostic accuracy	80	(37.55, 96.38[1])	Wilson score

PPV: Positive predictive value, NPV: Negative predictive value, CSF: Cerebrospinal fluid, MTB: *Mycobacterium tuberculosis*, PCR: Polymerase chain reaction

Table 3: Studies showing presenting features in TBM

Symptoms	Sarkar *et al.*, 2013[17]	Hsu *et al.*, 2010[18]	Cagatay *et al.*, 2004[20]	Verdon *et al.*, 1996[21]	Hosoglu *et al.*, 1998[22]
Fever (%)	91.7	81.5	90.5	65	91.1
Headache (%)	70	60.2	97.6	-	96
Vomiting (%)	43.3	20.3	35.7	-	81.2
Convulsions (%)		13.5	9.5	17	6
Altered sensorium (%)	45	63		25	72.3

TBM: Tubercular meningitis

Table 4: Sensitivity and specificity of GeneXpert in various studies

GeneXpert	Nhu *et al*, 2014[25]	Pai *et al.*, 2003[26]	Arzu *et al.*, 2011[24]	Our study
Sensitivity (%)	59	56	70	60
Specificity (%)	99	98	100	97.92
NPV (%)	72.5	44	90.6	95.92
PPV (%)	99.1	35	100	75

PPV: Positive predictive value, NPV: Negative predictive value

symptoms in various studies are shown in Table 3. Seizures have been reported to occur in TBM with variable incidence ranging from 17% to 93%.[23]

In a study by Sarkar *et al.*, 2013, in TBM patients, CSF sugar (mmol/L) was low 2.6 ± 1.2 (0.8–6.5), protein (mg/dL) was high 212.3 ± 275 (137–1440), total leukocytes count (/cmm) was high 484.7 ± 1317 (5–6400), and lymphocytic pleocytosis 72.08 ± 31.2/cmm (10–100).[17] In our study, mean CSF sugar was 55.047 ± 23.85 mmol/L (4–98), protein was 134.9 ± 77.7 mg/ dL (15.4– 385), total leukocytes count was 218.36 ± 652.33/cmm (0– 4800), and lymphocytic pleocytosis 68.68 ± 29.67/cmm (0–100).

By Verdon *et al.*, 1996, during admission to the intensive care unit, a lymphocytic predominance was observed in 72% of the patient.[21] In our study, during admission, lymphocytic predominance was observed in 77% of the patients.

In our study, in 53 patients diagnosed clinically, the sensitivity, specificity, NPV, and PPV of the Xpert test were found to be 60%, 97.9%, 95.9%, and 75%, respectively. Sensitivity, specificity, NPV, and PPV of Xpert in various studies are shown in Table 4.

In our study, 5.6% of TBM cases were positive both by culture and Xpert testing. In the study by Bahr *et al.*, 2015, only 39% of TBM cases being positive both by culture and Xpert testing.[27] In our study, in HIV-positive patients, sensitivity of the Xpert MTB/RIF assay for culture-positive TB was 50%, the specificity was 100%, the PPV was 100%, and NPV was 75%. The overall sensitivity of the Xpert MTB/RIF assay for culture-positive TB was 73.3% (specificity, 99.2%) compared to 28.0% (specificity, 100%) using smear microscopy in HIV-positive patients[10] and by Nyugen *et al.* among HIV patients, sensitivity was 78.8%, while it was 47.9% in non-HIV-infected patients.[25]

In a meta-analysis by Denkinger *et al.*,[28] studies evaluated Xpert in CSF against culture. Sensitivity varied widely (51–100%) with pooled sensitivity of 80.5% (95% CI 59.0–92.2%) and pooled specificity of 97.8% (95% CI 95.2–99.0%). The prevalence of HIV and the condition of the specimen did not have an effect on Xpert sensitivity and specificity in CSF. However, a concentration step in the processing of the sample appeared to enhance the sensitivity and specificity of Xpert as compared to unconcentrated samples.[28]

In the study by José *et al.*, 2008, a total of 148 patients of TBM were included in the study. Positive CSF cultures and PCR for MTB were found in 4.0% and 3.3%, respectively, of the patients. Of six patients with positive CSF cultures for MTB, three had a negative CSF PCR for MTB. In addition, two of five patients with positive CSF PCR for MTB had negative CSF cultures for MTB.[29] In our study, CSF culture and PCR for MTB were found in 9.4% and 7.5% of patients, respectively, of the patients. Two of the five patients with positive CSF cultures for MTB had a negative CSF PCR for MTB. In addition, one of four patients with positive CSF PCR for MTB had negative CSF cultures for MTB.

CONCLUSION

GeneXpert is highly specific diagnostic test for diagnosis of TBM, but the use of this test as sole diagnostic test will lose large number of patients with TBM as negative test does not rule out TBM. The diagnosis is still made in combination clinical findings and other biochemical and radiological investigations.

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