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Evaluation of diagnostic performance of Xpert® MTB/RIF Ultra assay in suspected tuberculous meningitis cases in a tertiary care setting

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ABSTRACT

Objectives: Tuberculous meningitis is the most lethal manifestation of tuberculosis. Xpert® MTB/RIF Ultra is a nucleic acid amplification test which gives result in 80 minutes and simultaneously provides information on rifampicin resistance. This study was carried out to evaluate the diagnostic performance of Xpert Ultra assay.

Materials & Methods: After obtaining ethics permission, cerebrospinal fluid (CSF) of 50 adult cases of clinically suspected tuberculous meningitis were tested by liquid culture, Xpert® MTB/RIF assay and Xpert® Ultra assay**.**

Statistical analysis: Of the 50 cases, MTB was detected in 16 cases. Of these, three were positive by Xpert®, 11by Ultra and 12 by culture. Of the six cases that gave trace positive result by ultra, four were negative by Xpert® and culture. One strain was rifampicin (RIF) resistant by all three methods.

Results: Sensitivity, Specificity, Positive predictive value (PPV) and Negative predictive value (NPV) of Xpert® Ultra was 58.33%, 89.47% ,63.63% and 87.17% in comparison with liquid culture.

Conclusion: Xpert® MTB/RIF ultra-assay showed better sensitivity and high negative predictive value when compared to Xpert® MTB/RIF assay. Hence can be used in suspected cases of TBM to prevent complications like death and serious neurological disability. Trace results do not provide information on rifampicin (RIF) resistance.

Keywords*: Xpert MTB/RIF Ultra, Tuberculous meningitis, Xpert assay.*

INTRODUCTION

Tuberculous meningitis (TBM) is the most lethal manifestation of tuberculosis and requires a rapid diagnosis and initiation of appropriate treatment to prevent mortality and serious neurological disability [1]. In the developed world, where there is a lower prevalence of TB in the population, estimates are that TBM accounts for 6% of all causes of meningitis. In locations with a higher prevalence of Mycobacterium tuberculosis (MTB) in the population, estimates are that TBM accounts for up to one-third to one-half of all bacterial meningitis [2]. The disease accounts for 5-10 % of all cases of extra-pulmonary TB [3] .In India, 16 % of cases are extrapulmonary tuberculosis(EPTB) in nature of which 5-10 % of cases are reported as TB meningitis.

Cerebrospinal fluid (CSF) is paucibacillay in natures, therefore a highly sensitive test is required for early diagnosis. The conventional diagnostic techniques such as microscopy and culture perform poorly on CSF. A definitive diagnosis of TBM is provided by examination of CSF for causative agent of Mycobacterium tuberculous complex.

In 2013, WHO endorsed the Xpert MTB/RIF assay (Xpert Mycobacterium Tuberculosis(MTB) and Rifampicin (RIF) resistance assay) as the preferred initial test to investigate tuberculous meningitis[4,5,6]. Xpert MTB/RIF assay is a rapid,

highly sensitive nucleic acid amplification test which simultaneously detects Mycobacterium tuberculosis complex (MTBC) and rifampicin resistance. It is based on hemi-nested real-time polymerase chain reaction (PCR) technique which detects and amplifies an M tuberculosis specific rpoB gene in bacterial genome. Positive results by Xpert assay confirm the diagnosis of tuberculous meningitis but negative results do not exclude tuberculous meningitis. Meta-analyses of the diagnostic performance of Xpert for tuberculous meningitis showed pooled sensitivity of 79·5–80·5% and specificities of 98·6–98·8% for M tuberculosis detection in CSF in comparison with gold standard liquid culture [7,8]. Sensitivity of any test can be improved if greater volume of the sample is used or greater number of molecular targets are detected. An improved version of Xpert assay is Xpert MTB/RIF Ultra (Xpert Ultra) which aims to improve the sensitivity of tuberculosis diagnosis and enhance rifampicin resistance detection [9]. By using larger amount of specimen for amplification and detection of deoxyribonucleic acid (DNA) and two additional amplifying molecular targets to detect TB. These modifications have resulted in a limit of detection that lower from 131 cfu/ml to 16cfu/ml providing and improved sensitivity on a clinical specimen [9].

Hence this study was undertaken in clinical setting to evaluate diagnostic performance of Xpert Ultra assay against currently recommended Xpert assay and culture.

METHODOLOGY

This prospective observational study was conducted in The Department of Microbiology at a tertiary care teaching hospital after ethics committee approval. Xpert MTB/RIF assay (Xpert assay) is the initial diagnostic test performed on all CSF's received from clinically suspected cases of TBM. Sample of CSF whose volume was 2ml or more were included for further testing with ultra and culture after obtaining informed consent from patient. Patients already initiated on antituberculosis treatment were excluded. Relevant demographic and clinical details were obtained from their case files. CSF was first processed by fluorescent microscopy for detection of acid-fast bacilli .500 µl was inoculated in MGIT tube and incubated in MGIT 960 system till growth was detected or for 42 days whichever was earlier. Remaining sample was used for testing by Xpert Assay as well as Xpert Ultra assay. Both the tests were performed as per manufacturer's instructions [10,11]. Consecutive CSF samples fulfilling inclusion criteria were processed.

RESULTS

Result of Xpert assay was available after 1 hour 50 min as "MTB DETECTED" or "MTB NOT DETCTED". The bacterial load reported was "Very low, Low, Medium and High" along with RIF resistance DETECTED / NOT DETECTED/ INDETERMINATE.

Result of Xpert Ultra was available after 80 min as "MTB DETECTED" or "MTB NOT DETCTED" along with detection of one additional bacterial load as "Trace". RIF resistance report is same as Xpert assay.

All the 50 CSF specimens included in the study were tested by all 4 test modalities viz. fluorescent microscopy, Xpert assay, Xpert ultra-assay and MGIT liquid culture. Out of the 50 cases, MTB was detected in total of 16 cases, of these, 3 by flouroscent microscopy, 3 by Xpert MTB/RIF assay, 11 by Xpert Ultra and 12 by liquid culture. 3 were positive by all the 4 tests as shown in Table.1

All samples positive by Xpert MTB/RIF were also positive by Xpert Ultra. Discordance was observed in detection by Xpert Ultra vs MGIT in 9 samples of these, 5 were culture positive and Ultra negative while 4 were Ultra positive and culture negative.

Xpert Ultra detected MTB in 8 additional CSF samples compared to Xpert MTB/RIF assay. The sensitivity and specificity of Xpert Ultra in comparison with Xpert assay was 100% and 82.97% respectively.

CSF Xpert Assay	CSF Xpert Assay	CSF Culture	Total
POSITIVE	**POSITIVE**	**POSITIVE**	3
NEGATIVE	**POSITIVE**	NEGATIVE	4
NEGATIVE	**POSITIVE**	**POSITIVE**	4
NEGATIVE	NEGATIVE	**POSITIVE**	5
NEGATIVE	NEGATIVE	NEGATIVE	34
Total			50

Table 1. Comparison of the results of different diagnostic modalities (n=50)

(Note: - CSF=Cerebrospinal fluid)

The lower specificity can be due to the improved sensitivity of ultra. In these 8 cases, bacterial load reported was trace in 6, very low in 1 and low in 1 specimen. In the present study, no specimen had high or medium bacterial load either by Xpert assay or Xpert ultra assay may be due to paucibacillary nature. Sensitivity, Specificity, PPV and NPV of Xpert® Ultra

was 58.33%, 89.47% ,63.63% and 87.17% in comparison with liquid culture. The sensitivity, specificity, PPV and NPV of CSF Xpert Ultra assay as compared to Xpert assay was 100%, 82.97%, 27.27%,100% respectively. The combined yield using Xpert Ultra and MGIT culture was more than two test when performed individually(16v11v12) RR was observed in only one CSF sample by all three methods**.**

DISCUSSION

Rapid, early and accurate case detection is the cornerstone of patient management and tuberculosis control. Microbiological diagnosis provides etiological confirmation as well as gives information about the drug susceptibility or resistance. The use of molecular tests can provide a rapid and sensitive means to detect TB and detect mutations known to confer drug resistance. CSF is a very precious sample and it cannot be collected repeatedly or in larger quantity. Age old technique of fluorescent or Ziehl–Neelsen (ZN) microscopy is highly specific with very poor sensitivity. Culture has long been considered as the gold standard but takes 2-8 weeks to give final results. WHO has recently recommended the use of molecular platform such as Xpert MTB/RIF Ultra as be the initial diagnostic test in tuberculous meningitis (TBM)[12]. In the present study, 50 CSF samples of clinically suspected cases of tuberculous meningitis were tested by Xpert MTB/RIF assay, Xpert MTB/RIF Ultra and the results were compared with culture as the gold standard.

In the present study, the sensitivity of Xpert Ultra assay on cerebrospinal fluid (CSF) was found to be 58.33% It is worth noting that the sensitivity of the Xpert Ultra assay in CSF in different studies, ranges from 44.00% to 95.45%[13,14,15,16].Notably, these studies often utilize a Composite Reference Standard (CRS) for comparison rather than relying solely on culture, as was done in the present study.

As regards specificity, in the present study, the Xpert Ultra assay demonstrated a specificity of 89.47% when compared to culture. The lower specificity compared to culture indicates either a superior performance of the molecular platform or its ability to detect nucleic acid from dead bacilli or false positivity. Literature reports a specificity of 100% when using culture as the reference standard. It's important to consider that some other studies, such as those by Bahr et al. (2018) [13], Cresswell et al. (2020) [16], and Donovan et al. (2020) [17], tested the Xpert Ultra assay for TBM diagnosis on larger volumes of centrifuged CSF (5-6 ml), which can potentially increase sensitivity. In contrast, the present study utilized smaller CSF volumes (2-3 ml) for the various tests, which may have contributed to the lower sensitivity of the Xpert Ultra assay. It's important to note that CSF is inherently a sample with low bacillary load and less quantity can impact test performance.

In the present study, Xpert Ultra assay identified Mycobacterium tuberculosis (MTB) in an additional 8 specimens, representing 16% more positive results compared to the Xpert assay. Similar findings have been reported by Zhang et al, where 6 (28.57%) additional positive specimens were detected by the Xpert Ultra assay [15]. Xpert Ultra gives result within 80 min as compared to 1 hour 50 minutes by Xpert assay [18]. The improved performance of the Xpert Ultra assay can be attributed to several factors, including the incorporation of two different multicopy amplification targets (IS6110 and IS1081), enhanced assay chemistry, and a larger reaction chamber (50µl). These improvements have led to an improved limit of detection (LOD) for M. tuberculosis of 16 bacterial colony forming units per ml (CFU/ml), in contrast to the Xpert assay's LOD of 131 CFU/ml. The LOD of culture is 1-10 viable bacilli / ml [19]. These results demonstrate that, even when using a 2ml volume of cerebrospinal fluid (CSF), the Xpert Ultra assay can outperform the Xpert assay for the diagnosis of tuberculous meningitis (TBM).

Xpert Ultra assay detects one additional bacterial load as “trace”. The trace result identifies samples that are M. *tuberculosis* positive due to the presence of the IS*6110* and/or IS*1081* molecular signals (*CT* > 37) in the absence of a signal from the *rpoB* SMBs sloppy molecular beacons [20]. The “trace” category was designed to identify samples with the lowest number of *M. tuberculosis* targets. Detection of rifampicin resistance in Xpert Ultra is based on interpretation of the melting curves of sloppy molecular probes. In Xpert assay, Ct values are used to detect rifampicin resistance, which may cause false-positive results.

Xpert Ultra detected MTB (trace results) in four additional cases compared to culture, whereas culture detected five additional cases which were ultra-negative giving a total of nine discordant results. The limitation of this test is it gives trace positive results (n=50) which cannot be used for initiating treatment. The combination both ultra and culture provided a better yield. Trace results must be confirmed by culture. It is always advisable to perform dual testing (Phenotypic and Genotypic) for paucibacillary samples like CSF as culture also gives information about drug susceptibility to most of the available anti-TB drugs.

The limitation of the present study was the small sample size due to cost of the test. As per the statistical calculation, sample size is 72, but as the cost of the test is high (Rs.75,000/- for 50 tests) and considering availability of fund, study was conducted only on 50 samples. A study with larger sample size would be desirable for giving more realistic results which can be applied in the community for early diagnosis of TB meningitis and appropriate treatment for the same.

Xpert MTB/RIF Ultra assay performed on CSF Samples of clinically suspected cases of tuberculosis meningitis can help in early diagnosis of TBM and detection of drug resistance, thereby preventing death and serious neurological disability. High sensitivity of Xpert Ultra will definitely help National Tuberculosis elimination Program to reduce the burden of tuberculosis by early detection of extrapulmonary tuberculosis.

CONCLUSION

To conclude, Xpert MTB/RIF ultra assay showed better sensitivity and high negative predictive value when compared to Xpert MTB/RIF assay. This test is easy to perform, has a turnaround time of 80 minutes and can also detect rifampicin resistance, which is surrogate marker for multidrug resistant tuberculosis. Hence Xpert MTB/RIF ultra assay can be used as a point of care test for early detection of tuberculosis and rifampicin resistance in suspected cases of TBM to prevent complications like death and serious neurological disability in cases of TB meningitis. Trace results do not provide information on RIF resistance.

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