Gene X-Pert in diagnosing extrapulmonary TB

Original Article

GENE XPERT A PROMISING TOOL IN DIAGNOSIS OF EXTRAPULMONARY TB IN DEVELOPING COUNTRIES

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ABSTRACT

Introduction: Although Tuberculosis mostly affects lungs in about 85% cases, but can cause lesion almost in every part of the body. Extrapulmonary TB (EPTB) accounts for 15 to 20% which involves other parts of the body beside the lungs. There are several methods that can diagnose Pulmonary TB (PTB) conclusively, but extrapulmonary TB is very difficult to diagnose till now especially in resource limited settings. Though it is not communicable but diagnostic delay has made it significant cause of morbidity and mortality. The study was aimed to find out the Gene Xpert as one of the diagnostic tool for EPTB.

Methods: A laboratory based descriptive cross sectional study was conducted over a period of 17 months from January 2017 to May 2018 to ascertain the performance of Gene Xpert technique as a diagnostic tool for EPTB. Data were collected through checklist and a total of 77 clinical samples were collected purposively with prior informed consent from suspected EPTB patients following ethical issues. Laboratory investigations were performed at Rhodolphe Merieux Laboratory, Chittagong, Bangladesh with Gene Xpert MTB/Rif assay, conventional culture (LJ media) and Microscopy (ZN stain) for the presence of Mycobacterium tuberculosis (MTB).

Results: Among the 77 samples from suspected cases, seven(9.09%) from CSF, one(1.29%) from pus and one(1.29%) from lymphnode specimens were positive by Gene Xpert MTB/Rif assay.Only one(1.29%) CSF specimen was found to be culture and microscopy positive which was Gene Xpert positive also. Except one specimen from pus that is both Gene Xpert and microscopy positive but culture negative, no other specimens from EPTB cases were culture and microscopy positive.

Conclusion: Diagnosis of EPTB is challenging in worldwide. As it is paucibacillary, routine diagnostic test in detecting MTB is difficult. Gene X pert showed promising outcome in early detection of life threatening EPTB cases like TB meningitis which is common in developing countries.

Key words: EPTB, Gene Xpert, diagnosis

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INTRODUCTION

TB is the ninth leading cause of death worldwide from a single infectious agent, ranking above HIV/AIDS. In 2017, there were an estimated 10.4 million people fell ill with TB and 1.3 million TB deaths among HIV-negative people (down from 1.7 million in 2000) and an additional 374 000 deaths among HIV-positive people. According to WHO Global TB Report 2016, Bangladesh is one of the world’s 30 high TB burden countries with annual occurrence of 362,000 new Tuberculosis cases. About 73,000 people die annually due to Tuberculosis.1 TB remains a key challenge to global public health and our ability to tackle this disease has been severely hampered by inadequate diagnostic assays. Now there are several methods that can diagnose Pulmonary TB (PTB) conclusively, but extrapulmonary TB is very difficult to diagnose till now especially in resource limited settings. EPTB constitutes about 15–20% of TB cases and can constitute up to 50% of TB cases in HIV-infected individuals.2,3,4 Diagnosis of extrapulmonary TB (EPTB) remains especially challenging since the number of Mycobacterium tuberculosis (MTB) bacilli present in tissues at sites of disease is often low and clinical specimens from deep-seated organs may be difficult to obtain.
Various methods are employed for the diagnosis of EPTB such as smear microscopy, culture identification, histopathology, tuberculin skin test (TST), serological assays, interferon-gamma release assays (IGRAs) and nucleic acid amplification (NAA) tests. Smear microscopy is widely used in the diagnosis of EPTB but has drawbacks owing to low and variable sensitivity values (0–40%) and could not differentiate between MTB and non-tuberculous mycobacteria. A negative smear for acid-fast bacilli, lack of granulomas on histopathology and failure to culture Mycobacterium tuberculosis do not exclude the diagnosis of EPTB.

The Xpert® MTB/RIF assay (Cepheid Inc., CA, USA) marks an important development in the field of rapid molecular TB diagnostics. The Xpert MTB/RIF assay was rapidly endorsed by the WHO in December 2010 as a replacement for sputum smear microscopy, particularly in settings with high rates of HIV-associated TB and multidrug-resistant TB. However, no recommendation exists for their use in the investigation of patients suspected of having EPTB as the evidence base is limited. Novel diagnostic modalities such as Gene Xpert can be useful in varied forms of EPTB. Though EPTB is not communicable but diagnostic delay has made it significant cause of morbidity and mortality. So, a study was conducted to ascertain the performance of Gene Xpert technique as a diagnostic tool for EPTB.

RESULTS

A total of 77 clinical specimens were tested for presence of MTB by Gene Xpert. Of them 48 cerebrospinal fluid (CSF) from suspected meningitis patients, 4 pus from infected wound, 2 lymph node biopsy specimen, 16 plural fluid, 4 ascitic fluid, 1 sinovial fluid and 2 urine samples.

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrospinal fluid</td>
<td>48</td>
<td>62.33</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>16</td>
<td>20.77</td>
</tr>
<tr>
<td>Fine needle aspirate</td>
<td>02</td>
<td>2.59</td>
</tr>
<tr>
<td>Pus</td>
<td>04</td>
<td>5.19</td>
</tr>
<tr>
<td>Ascitic fluid</td>
<td>04</td>
<td>5.19</td>
</tr>
<tr>
<td>Urine</td>
<td>02</td>
<td>2.59</td>
</tr>
<tr>
<td>Sinovial fluid</td>
<td>01</td>
<td>1.29</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>77</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Out of 48 CSF samples seven were positive for gene Xpert. Besides CSF, one from pus and one from lymph node specimens were positive by Gene Xpert MTB/RIF assay. Only one CSF specimen was found to be culture and microscopy positive. Except one specimen from pus that is both Gene Xpert and microscopy positive.
but culture negative, no other specimens from EPTB cases were culture and microscopy positive.

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Gene Xpert Frequency (%)</th>
<th>Microscopy (Florescence) Frequency (%)</th>
<th>Culture Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrospinal fluid</td>
<td>07(9.09)</td>
<td>01(1.29)</td>
<td>01(1.29)</td>
</tr>
<tr>
<td>Fine-needle aspirate (mostly lymphnode)</td>
<td>01(1.29)</td>
<td>00</td>
<td>0</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>00</td>
<td>00</td>
<td>0</td>
</tr>
<tr>
<td>Pus</td>
<td>01(1.29)</td>
<td>01(1.29)</td>
<td>00</td>
</tr>
<tr>
<td>Ascitic fluid</td>
<td>00</td>
<td>00</td>
<td>0</td>
</tr>
<tr>
<td>urine</td>
<td>00</td>
<td>00</td>
<td>0</td>
</tr>
<tr>
<td>Sinovial fluid</td>
<td>00</td>
<td>00</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>09(11.68)</strong></td>
<td><strong>02(2.59)</strong></td>
<td><strong>01(1.29)</strong></td>
</tr>
</tbody>
</table>

Multiple responses. Total positive specimen was 7 out 48.

**DISCUSSION**

Diagnosis of Extra pulmonary TB in resource limited setting is very challenging. For the diagnosis of tuberculosis molecular techniques is confirmatory and highly sensitive than the conventional laboratory techniques like direct microscopy and culture that are far from being sensitive. Molecular techniques have substantially changed the field of tuberculosis diagnosis and have been proven to yield rapid results. Numerous PCR assays employing a number of different M. tuberculosis targets have recently been described. Though Gene Xpert is introduced for diagnosis of pulmonary TB, we evaluated this technology for rapid and accurate diagnosis of EPTB from different clinical sample in addition of other diagnostic method used to diagnose EPTB.

In total, 09 were detected by the Xpert MTB/Rif assay from EPTB cases, whereas only two were detected by microscopy and only one by mycobacterial culture. We found that the Xpert MTB/Rif had a pooled sensitivity of 11.68%. The observed sensitivity of Xpert MTB/RIF for EPTB is not entirely consistent with seven other published studies in which reported sensitivities ranged from 25.0 to 95.1%.

These findings are dissimilar from those of other studies from Spain, with 81% (95% CI, 76% to 86%) for CSF and for tissue biopsy specimens, FNA, pleural fluid, gastric aspirates, pus, urine, and peritoneal and synovial/pericardial fluids, 58% sensitivity (95% CI, 49% to 68%) for pleural fluid, lymph node, abscess aspirates, and tissues from India, with 81% (95% CI, 76% to 85%) for tissue biopsy specimens, pus, and body fluids from Italy. In their study the sensitivity is more than this study. Due to very small sample size it is very difficult to draw any conclusion and the heterogeneity between studies may reflect differences between patient populations, patient selection, type of EPTB, the quality of the specimens, differences in sample processing and the diagnostic gold standard used.

EPTB diagnosis from tissue samples is usually made by histopathological examination that depends on the presence of granulomatous inflammation and caseous necrosis. However, histology does not distinguish between EPTB and infections from other granulomatous diseases such as NTM, sarcoidosis, leprosy and systemic lupus erythematosus (except for the presence of acid-fast bacilli; AFB).

In the present study, the sensitivity is very poor for other specimen except CSF. There were no positive tests findings from tissue samples or plural fluid. Possibly this is due to the small sample number of the study and also due to specimen collection, storage, and preparation techniques or to reduced numbers (below
the 131 CFU/ml threshold) 24, 25 of M. tuberculosis in the specimen, Taking into account that Xpert MTB/RIF is less affected by contaminating bacteria, its use for diagnosing EPTB could significantly reduce labor in the laboratory needed for culture and reduce the diagnostic delay.

Diagnosis of EPTB, in particular, is difficult owing to paucibacillary nature of the specimens, lack of adequate clinical sample volumes and non-uniform distribution of bacteria in those specimens as well as the disease localized in sites that are difficult to access. Many forms of extrapulmonary TB require invasive diagnostic sampling, and gathering adequate specimens can pose a risk of harm to the patient and be costly.

Culture identification for M. tuberculosis also has variable sensitivities (0–80%) in different extrapulmonary specimens. In some cases, the Xpert assay result was positive but the culture remained negative. Here in this study only 1.2% EPTB sample was found to be culture positive. Acknowledging the fact that culture is still the gold standard for diagnosis, the finding is due to paucibacillary nature of the specimen. Nevertheless, culture takes several weeks, requires a highly-equipped laboratory, and has reduced sensitivity in paucibacillary disease. Seemingly histology relies on highly trained operators and characteristic morphology is shared with other diseases. In resource-limited settings, skilled personnel and laboratory settings required for mycobacterial culture and histological examination are not widely available. As a result of these difficulties, diagnosis of extrapulmonary TB is often made on the grounds of clinical suspicion alone, and many people receive the wrong diagnosis leading to unnecessary TB treatment or poor outcomes from untreated extrapulmonary TB.

So this study will provide local data to support the introduction of TB screening of EPTB specimens with GeneXpert technology and similarly follows the pulmonary Xpert MTB/RIF algorithm with confirmation by DST and its use in the context of clinical suspicion.

The limitation of this study is the small sample size and purposeful selection of the different specimen types that can hardly make an inference of the performance of Gene Xpert. Even though Xpert assay can be applied to diagnose extrapulmonary TB, for its rapid detection and simplicity.

CONCLUSION

Effective control of TB requires early diagnosis and immediate treatment initiation for better management and to limit further transmission. Delay in diagnosis and treatment results more advancement of the disease along with morbidity and mortality. So for faster and reliable technique for EPTB diagnosis, Gene Xpert is the suitable and inevitable method in resource-limited settings.

REFERENCES


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