Original Article

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

Jabin Akhter[1] Prof MA Hassan Chowdhury[2]

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 perfomed at Rhodolphe Merieux Laboratory, Chittagong, Bangladesh with Gene Xpert MTB/Rif assay, conventional culture (LJ media) and Microscopy (ZN stain) for the presence of Mycobacteium 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.

JOPSOM 2020; 39(1): 26-30
https://doi.org/10.3329/jopsom.v39i1.51859

Key words*:* EPTB, Gene Xpert, diagnosis

1. *Associate Professor, Dept of Microbiology, NIPSOM, Dhaka.*
2. *Director, Bangladesh Institute of Tropical and Infectious Diseases, Fouzderhat, Chittagong*

Correspondence:
Dr. Jabin Akhter
Associate Professor & Head
Department of Microbiology, NIPSOM
e-mail: jabinakhter140872@gmail.com

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.[5,6] 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.[7,8,9] 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.[10, 11] 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.[12, 13] 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.

METHODS

A descriptive cross sectional study was performed over a period of 17 months from January 2017 to May 2018 in the Rhodolphe Merieux Laboratory, Chittagong, Bangladesh, which provides a routine diagnostic service to public sector hospitals and clinics. The study was primarily laboratory-based and no patient demographics were recorded other than those provided by the requesting clinician such as age, gender, and location. Data were collected using a checklist through reviewing medical and laboratory records of the respective participants. The majority of EPTB specimens were obtained purposively from hospitalized patients of Chittagong Medical College Hospital and Private hospitals from Chittagong city with prior informed consent. A total 77 clinical samples were collected which comprised cerebrospinal fluid (CSF) from suspected meningitis patients, pus from infected wound, lymph node biopsy specimen, plural fluid, ascitic fluid, sinovial fluid and urine samples. Specimens were subjected to Gene Xpert MTB/Rif assay, conventional culture (LJ media) and Microscopy (ZN stain) for the presence of MTB.

Sample processing differed according to specimen type. Non sterile samples were subjected to standard N-acetylcysteine sodium hydroxide decontamination and concentration by centrifugation, whereas sterile samples only underwent simple mechanical homogenization (if required). Large-volume specimens were centrifuged, tissue biopsy specimens were homogenized. All samples underwent fluorescence microscopy for acid-fast bacilli and culture on solid (Lowenstein–Jensen) media. Xpert MTB/RIF assay follows manufacturer's protocol. Ethical clearance was obtained from the Institutional ethical committee. All collected data was kept anonymous. The design and delivery of data collection for this study was ensured enrolled populations' rights to privacy, confidentiality, informed consent, freedom of movement. Access to information are safeguarded and any key populations are free from discrimination, involuntary treatment, isolation, detention and incarceration. Collected data were analyzed with the help of Excel software. Data were presented in frequency tables to identify distribution and laboratory findings.

RESULTS

A total of 77 clinical specimens were tested for presence of MTB by Gene X pert. 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 1: Type of EPTB specimens

Specimen type	Frequency	Percentage
Cerebrospinal fluid	48	62.33
Pleural fluid	16	20.77
Fine needle aspirate	02	2.59
Pus	04	5.19
Ascitic fluid	04	5.19
Urine	02	2.59
Sinovial fluid	01	1.29
Total	**77**	**100**

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 2: Methods of diagnosis of positive EPTB specimens

Specimen type	Positive sample		
	Gene Xpert	Microscopy (Florescence)	Culture
	Frequency (%)	Frequency (%)	Frequency (%)
Cerebrospinal fluid	07(9.09)	01(1.29)	01(1.29)
Fine-needle aspriate (mostly lymphnode)	01(1.29)	0	0
Pleural fluid	0	0	0
Pus	01(1.29)	01(1.29)	0
Ascitic fluid	0	0	0
urine	0	0	0
Sinovial fluid	0	0	0
Total	**09(11.68)**	**02(2.59)**	**01(1.29)**

Mutiple responses. Total positive specimen ws 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.[14] Numerous PCR assays employing a number of different M. tuberculosis targets have recently been described.[15, 16] 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%.[17]. These findings are dissimilar from those of other studies from Spain[18], 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 [19] , with 81% (95% CI, 76% to 85%) for tissue biopsy specimens, pus, and body fluids from Italy[20]. 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.7, [21] 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).[22, 23]

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[21] 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.[26] 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. [26] In some cases, the Xpert assay result was positive but the culture remained negative. Here in this study only 1.29% 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 provides 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

1. WHO Global Tuberculosis Report 2017. (Available from:http://www.who.int/tb/publications/global_report/gtbr14_main_text.pdf).
2. Noussair L, Bert F, Leflon-Guibout V, Gayet N & Nicolas- Chanoine MH (2009) Early diagnosis of extrapulmonary tuberculosis by a new procedure combining broth culture and PCR. J Clin Microbiol 47: 1452–1457.
3. Cortez MV, Oliveira CM, Monte RL, Arau´ jo JR, Braga BB, Reis DZ, Ferreira LC, Moraes MO & Talhari S (2011) HIVassociated tuberculous lymphadenitis: the importance of polymerase chain reaction (PCR) as a complementary tool for the diagnosis of tuberculosis – a study of 104 patients. An Bras Dermatol 86: 925–931.
4. Cutrufello NJ, Karakousis PC, Fishler Peto HM, Pratt RH, Harrington TA, LoBue PA & Armstrong LR (2009) Epidemiology of extrapulmonary tuberculosis in the United States, 1993-2006. Clin Infect Dis 49: 1350–1357
5. Katoch VM (2004) Newer diagnostic techniques for tuberculosis. Indian J Med Res 120: 418–428.
6. Lange C & Mori T (2010) Advances in the diagnosis of tuberculosis. Respirology 15: 220–240.
7. Liu KT, Su WJ & Perng RP (2007) Clinical utility of polymerase chain reaction for diagnosis of smear-negative pleural tuberculosis. J Chin Med Assoc 70: 148–151.
8. Haldar S, Bose M, Chakrabarti P et al. (2011) Improved laboratory diagnosis of tuberculosis – the Indian experience.Tuberculosis 91: 414–426.
9. Derese Y, Hailu E, Assefa T, Bekele Y, Mihret A, Aseffa A, Hussien J, Ali I & Abebe M (2012) Comparison of PCR with standard culture of fine needle aspiration samples in the diagnosis of tuberculosis lymphadenitis. J Infect DevCtries 6: 53–57.
10. Lawn SD, Nicol MP. Xpert(R) MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis

and rifampicin resistance. Future Microbiol. 2011; 6:1067–1082.

11. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N. Engl. J. Med. 2010; 363:1005–1015.
12. Greco, S., M. Rulli, E. Girardi, C. Piersimoni, and C. Saltini. 2009. Diagnostic accuracy of in-house PCR for pulmonary tuberculosis in smear-positive patients: meta-analysis and metaregression. J. Clin. Microbiol. 47:569– 576.
13. Ling, D. I., L. L. Flores, L. W. Riley, and M. Pai. 2008. Commercial nucleicacid amplification tests for diagnosis of pulmonary tuberculosis in respiratory specimens: meta-analysis and meta-regression. PLoS One 3:e1536.
14. Ligthelm LJ, Nicol MP, Hoek KG, et al. Xpert MTB/RIF for rapid diagnosis of tuberculous lymphadenitis from fine-needle-aspiration biopsy specimens. J. Clin. Microbiol. 2011; 49:3967–3970.
15. Moure R, Munoz L, Torres M, Santin M, Martin R, Alcaide F. Rapid detection of Mycobacterium tuberculosis complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. J. Clin. Microbiol. 2011; 49:1137–1139.
16. Lawn SD, Zumla AI. Diagnosis of extrapulmonary tuberculosis using the Xpert MTB/RIF assay. Expert Rev Anti Infect Ther 2012: 10: 631–635.
17. Moure R, Martin R, Alcaide F. 2012. Effectiveness of an integrated real-time PCR method for detection of the Mycobacterium tuberculosis complex in smear-negative extrapulmonary samples in an area of low tuberculosis prevalence. J. Clin. Microbiol. 50:513–515.
18. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. 2011. Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis. J. Clin. Microbiol. 49: 2540 –2545.
19. Tortoli E, Russo C, Piersimoni C et al. Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. Eur Respir J 2012: 40: 442–447.
20. Almadi MA, Ghosh S & Aljebreen AM (2009) Differentiating intestinal tuberculosis from Crohn's disease: a diagnostic challenge. Am J Gastroenterol 104: 1003–1012
21. Bravo FG & Gotuzzo E (2007) Cutaneous tuberculosis. Clin Dermatol 25: 173–180.
22. Chawla K, Gupta S, Mukhopadhyay C, Rao PS & Bhat SS (2009) PCR for M. tuberculosis in tissue samples. J Infect Dev Ctries 3: 83–87.
23. Friedrich SO, von Groote-Bidlingmaier F, Diacon AH. 2011. Xpert MTB/RIF assay for diagnosis of pleural tuberculosis. J. Clin. Microbiol. 49:4341–4342.
24. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, Kop J, Owens MR, Rodgers R, Banada P, Safi H, Blakemore R, Lan NT, Jones-Lopez EC, Levi M, Burday M, Ayakaka I, Mugerwa RD, McMillan B, Winn- Deen E, Christel L, Dailey P, Perkins MD, Persing DH, Alland D. 2010. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J. Clin. Microbiol. 48: 229–237
25. Blakemore R, Story E, Helb D, Kop J, Banada P, Owens MR, Chakravorty S, Jones M, Alland D. 2010. Evaluation of the analytical performance of the Xpert MTB/RIF assay. J. Clin. Microbiol. 48:2495–2501.
26. Mehta PK, Raj A, Singh A, Khuller GK. Diagnosis of extrapulmonary tuberculosis by PCR. 2012. Immunol Med Microbiol 66 (2012) 20–36