Research Article

Comparison of conventional methods with gene xpert mtb/rif assay for rapid detection of mycobacterium tuberculosis and rifampicin resistance in extra-pulmonary samples

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Abstract

Introduction: Tuberculosis (TB) is a major health problem in India. The World Health Organization has recently in 2010 endorsed the Gene Xpert MTB/RIF assay for rapid detection of smear negative and multidrug resistance tuberculosis and more recently for extra pulmonary tuberculosis. **Objectives:** Evaluation of role of Cartridge based nucleic acid amplification test (CBNAAT) in extra-pulmonary tuberculosis (EPTB) in comparison with Ziehl Neelsen (ZN) staining and evaluating rifampicin resistance with the same test. **Materials & Methods:** Extra-pulmonary samples, including pleural fluid, pus, CSF, lymph tissue & others were divided in 2 parts: one for MTB/RIF assay & other for ZN staining. Both were then compared. **Results:** A total of 300 extra pulmonary samples were processed in this study, which included 103 pleural fluids, 81 pus, 45 CSF, 35 Lymph node tissue, 20 ascitic fluids and 16 synovial fluid. Out of these 37% (111) patients were Gene Xpert MTB/RIF Assay positive and 36 % (40 out of 111) were ZN smear positive. M.tuberculosis was detected in 56.7% pus samples, 23.3% pleural fluid samples, 54.2% lymph node samples, 33.3 % CSF samples, 20% ascitic fluid samples and 18.7% synovial fluid samples. In this study, we found that Gene Xpert MTB/RIF assay is a rapid method for diagnosis of EPTB as compared to conventional methods along with advantage of detecting Rifampicin resistance. **Conclusion:** Because of its simplicity, rapidity and sensitivity, this seems to be a very novel tool for diagnosis of extra pulmonary tuberculosis from clinical samples and that it should be researched more thoroughly.

Key words: Extra pulmonary tuberculosis, Mycobacterium tuberculosis, Gene Xpert MTB/RIF Assay

Introduction

TB remains a key challenge in the face of global public health and inadequate diagnostic assays have hampered our chances to tackle this disease effectively. According to WHO there were 8.6 million new TB cases in 2012 and even 1.3 million TB deaths. Extra-pulmonary tuberculosis (EPTB) accounts for 20% (234 029 cases out of 1183 373) of total burden of tuberculosis globally [1]. It is estimated that approximately 70 million people will die from tuberculosis within the next 20 years and it is because of inadequate measures for the TB control [2].

Manuscript received: 17th Jan 2016
Reviewed: 28th Jan 2016
Author Corrected: 08th Feb 2016
Accepted for Publication: 15th Feb 2016

As the number of bacilli are very less in extra pulmonary samples and because of difficulty in obtaining tissues from deep seated organs; diagnosis is delayed in most cases. Histology is time-consuming to undertake and establishing a diagnosis of TB with high specificity remains difficult. Special stains developed for diagnosing Mycobacterium tuberculosis on tissue microscopy are cumbersome and even after that distinguishing M. tuberculosis from non-tuberculous mycobacteria is next to impossible.

Reliance on culture, the mainstay of diagnosis, often leads to considerable delays, compromising patient care and outcomes.

The Gene Xpert MTB/RIF assay marks an important development in the field of rapid molecular TB diagnostics. This assay was rapidly endorsed by the WHO (World Health Organization) in December 2010 as a replacement for sputum smear microscopy, particularly in settings with high rates of HIV-associated TB and multidrug-resistant TB developed for testing sputum samples [2].

This multifunctional diagnostic platform is an automated, closed system that performs real-time PCR and can be used by operators with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 hours [3, 5]. The test detects DNA specific for Mycobacterium tuberculosis by polymerase chain reaction [7].

More recently, however, evaluations of the assay have extended to a variety of non-respiratory clinical samples from patients with extra-pulmonary tuberculosis. A definitive diagnosis can be made by detection of *M. tuberculosis* in extra-pulmonary samples [4].

Extra-pulmonary TB is far more complex because of the diversity of clinical sample types, difficulties in obtaining adequate tissue for analysis and in the extraction of M. tuberculosis DNA (MTB DNA) from the samples.

With the improvement of Nucleic Acid Amplification techniques (NAAT) in TB detection, sensitivity of tests for TB detection has been rising [6].

As there are limited studies about the use and efficacy of the Xpert test (CBNAAT) in extra-pulmonary tuberculosis, there is a need for more and more research on this novel approach.

This study aims to assess the performance of Xpert MTB/RIF test for diagnosis of TB in a particular population.

Materials & Methods

This study is conducted to evaluate the clinical value of CBNAAT (cartridge based nucleic acid amplification test) MTB/RIF assay in patients with suspected extra pulmonary tuberculosis by comparing with ZN staining. We also evaluated for the rifampicin resistance if present, from the same test.

Inclusion criteria: Cases of pleural effusion, cold abscess, meningitis, lymphadenopathy, ascites & synovitis with the samples being pleural fluid, pus, CSF, lymph tissue, peritoneal fluid & synovial fluid respectively were included. All the cases were being suspected to be of tubercular aetiology after screening by various clinical & conventional methods.

Exclusion criteria: Cases of isolated pulmonary TB (no extra-pulmonary component), pyogenic meningitis, non-tubercular effusion (for ex. cardiogenic, traumatic, hypoproteinaemia, cirrhosis, renal, collagen vascular disorders and malignancy), malignant & inflammatory lymphadenopathy, and non-tubercular ascites (for ex. trauma, hypoproteinaemia, renal causes, hepatic causes, fungal infection & non-specific peritonitis) were excluded.

All samples were taken with informed consent and under all aseptic conditions and precautions, sent in sterile containers with no time delay.

Methods: The sample was divided equally into 2 parts: one part was used for the Xpert test (CBNAAT) & the second was tested by direct and concentrated acid fast bacillus (AFB) microscopy (Ziehl-Nielson [ZN] staining).
For the test procedure, the sample is poured into a single-use disposable cartridge that is placed in the Xpert Dx module, with the results produced in less than 2 hours. The system automatically interprets all results from measured fluorescent signals, with embedded calculation algorithms, into the following categories: invalid, if PCR inhibitors are detected with amplification failure; negative or positive. If positive, the strain was categorized as susceptible or resistant to rifampicin.

Results

Of the 300 samples taken, of which includes samples of pleural fluid, pus, CSF, ascitic fluid, synovial fluid, lymph node tissue, the following distribution was seen at our setting:-
Out of 300 cases, 40 cases were ZN smear positive and came out to be positive with Gene Xpert assay, 71 were ZN smear negative but came out to be positive with Gene Xpert assay test, 189 cases were negative for both ZN smear & Gene Xpert MTB/RIF assay.

Table 1: Distribution of Extra-pulmonary tuberculosis cases included in the study

Type of specimen	Frequency	Percent
Pleural fluid	103	34.33
Pus	81	27
CSF	45	15
Lymph node tissue	35	11.67
Ascitic fluid	20	6.67
Synovial fluid	16	5.33
Total	**300**	**100**

Table 2: Results of samples analysed by Gene Xpert MTB/RIF assays

Type of specimen	Positive	%	Negative	%
Pus	46	56.7	35	43.3
Pleural fluid	24	23.3	79	76.7
CSF	15	33.3	30	66.7
Lymph node	19	54.2	40	45.8
Ascitic fluid	4	20	16	80
Synovial fluid	3	18.7	13	81.3
Total	**111**	**37**	**189**	**63**

Table 3: Comparison of Gene Xpert MTB/RIF assay with ZN staining

Gene Xpert assay	Diagnosis on ZN stain	
Positive results on Gene Xpert	Positive	Negative
	40	71
Negative results on Gene Xpert	0	189
Total	**40**	**260**

Table 4: Results of Rifampicin resistance analysed in the same test.

Type of Specimen	MTB detected	Sensitive to Rifampicin	Resistant to Rifampicin
Pus	46	43	3
Pleural fluid	24	23	1
Lymph node	19	18	1
CSF	15	10	1
Ascitic fluid	4	4	0
Synovial fluid	3	3	0
Total	**111**	**105**	**6**

It is noted that none of the ZN smear positive gave negative results by Gene Xpert. On the other hand many of ZN negative samples came to be positive with Gene Xpert indicating Xpert MTB/RIF assay is highly sensitive and specific technique. We also found out of 111 samples which came positive with Gene Xpert assay , 6 were resistant to Rifampicin which we would have missed with ZN staining or by other conventional methods. The result of the study revealed a maximum positivity rate by Gene Xpert which indicated that it is a more sensitive technique as compared to conventional methods.

Discussion

Extra pulmonary tuberculosis constitutes 20% of burden of TB globally. EPTB being a pauci-bacillary disease, the number of bacteria are less to detect and are deep seated in the organs. Furthermore conventional methods including histology and smear microscopy are cumbersome and never diagnostic, leaving us with culture methods which are time consuming. Therefore

there is a need for newer and faster diagnostic methods and recent attention has been given to nucleic acid amplification techniques like Gene Xpert (CBNAAT).

Ahmed et al in 2014 did a similar study with a total of 100 extra pulmonary samples were processed, (60 pus, 19 pleural fluids, 16 ascitic fluids and 5 CSF). Out of these 37% patients were Gene Xpert MTB/RIF Assay positive, 17% were LJ culture positive and 12 % were Zn smear positive. MTB was detected in 31 out of 60 (51.7%) pus samples, 3 out of 19 (15.8%) pleural fluid samples, 1 out of 16 (6.3%) ascitic fluid samples and 2 out of 5 (40.0%) cerebro-spinal (CSF) samples [8].

Doris Hillemann in 2011 compared Gene Xpert MTB/RIF (Xpert) assay system with conventional liquid and solid culture methods. 521 specimens (91 urine, 30 gastric aspirate, 245 tissue, 113 pleural fluid, 19 cerebro-spinal fluid and 23 stool specimens) were submitted. The combined sensitivity and specificity of the Xpert assay were calculated to be 77.3% and 98.2%, respectively [9]. Raquel Moure et al, in 2012 conducted a study; in this research, out of 108 smear-negative extra pulmonary samples 63 (58.3%) were positive with the Xpert MTB/RIF assay (Gene Xpert) for Mycobacterium tuberculosis [10]. In a similar study by Vadwai in 2011, the sensitivity of the Xpert assay was 81% (228/283 specimens), 64% for smear-negative cases and 96% for smear-positive cases), with a specificity of 99.6% [11].

Gu Y et al, in 2015 conducted a study for bone and joint tuberculosis, in which they found sensitivity of smear to be very less (26%) as compared to Xpert test (86%) [12]. The higher detection rate in above mention studies was due to the fact that they included diagnosed cases of TB while our study was performed on TB suspects.

A similar study for sputum negative TB patients was conducted at our centre in which 72 sputum smear negative patients were included and broncho-alveolar lavage(BAL) taken by doing a bronchoscopy which was then subjected to Gene Xpert test. From these 72 patients 34 came out to be positive on Gene Xpert test further proving the efficacy of test on pulmonary samples also [13].

In the present study, M. tuberculosis was detected in 46 out of 81 (56.7%) pus samples, 24 out of 103 (23.3%) pleural fluid samples, and 15 out of 45 (33.3%) cerebro-spinal fluid samples. The study revealed that the Xpert test has true diagnostic potential with good sensitivity for specimens such as pus which is difficult to diagnose by other laboratory techniques.

Our findings supported the use of Gene Xpert test in routine for extra pulmonary TB diagnosis, especially for pus, lymph node & pleural fluid samples where a very high detection rate was observed as compared to conventional techniques.

Conclusion

Gene Xpert MTB/RIF assay is efficient and reliable technique for the rapid diagnosis of extra pulmonary TB, especially in smear negative cases. Its simplicity, sensitivity, speed and automation, make this technique a very attractive tool for diagnosis of Mycobacterium tuberculosis from extra pulmonary samples in MDR cases and smear negative cases of TB suspects.

Funding: Nil,
Conflict of interest: None.
Permission of IRB: Yes

References

1. Eurosurveillance editorial team. WHO publishes Global tuberculosis report 2013. Euro Surveill. 2013 Oct 24;18(43). pii: 20615.

2. Giri PK, Khuller GK. Is intranasal vaccination a feasible solution for tuberculosis? Expert Rev Vaccines. 2008 Nov;7(9):1341-56. doi: 10.1586/14760584.7.9.1341.

3. 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-López EC, Levi M, Burday M, Ayakaka I, Mugerwa RD, McMillan B, Winn-Deen E, Christel L, Dailey P, Perkins MD, Persing DH, Alland D. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol. 2010 Jan;48(1):229-37. doi: 10.1128/JCM.01463-09. Epub 2009 Oct 28.

4. Hillemann D, Rüsch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF

system. J Clin Microbiol. 2011 Apr;49(4):1202-5. doi: 10.1128/JCM.02268-10. Epub 2011 Jan 26.

5. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R, Rustomjee R, Milovic A, Jones M, O'Brien SM, Persing DH,Ruesch-Gerdes S, Gotuzzo E, Rodrigues C, Alland D, Perkins MD. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med. 2010 Sep 9;363(11):1005-15. doi: 10.1056/NEJMoa0907847. Epub 2010 Sep 1.

6. Agarwal SP, Dhingra S, Chauhan LS. The Role of IEC in the RNTCP. Tuberculosis control in India 2005. Directorate General of Health Services. Ministry of Health and Family Welfare, New Delhi. [Last accessed on 2005]. Available from:http://tbcindia.nic.in/pdfs/Tuberculosis%20Control%20in%20India19.pdf.

7. Van Rie A, Page-Shipp L, Scott L, Sanne I, Stevens W. Xpert(®) MTB/RIF for point-of-care diagnosis of TB in high-HIV burden, resource-limited countries: hype or hope? Expert Rev Mol Diagn. 2010 Oct;10(7):937-46. doi: 10.1586/erm.10.67.

8. Ahmad N S, Sana khan, Aneeqa Shamshad Butt. Rapid detection of Mycobacterium tuberculosis and Rifampicin Resistance in extra pulmonary samples using Gene Xpert MTB/RIF assay. IOSR Journal of Dental and Medical Sciences 11/2014; 13(11):2279-861. DOI: 10.9790/0853-131145053.

9. Hillemann D, Rüsch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. J Clin Microbiol. 2011 Apr;49(4):1202-5. doi: 10.1128/JCM.02268-10. Epub 2011 Jan 26.

10. Moure R, Muñoz L, Torres M, Santin M, Martín 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 Mar;49(3):1137-9. doi: 10.1128/JCM.01831-10. Epub 2010 Dec 29.

11. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis? J Clin Microbiol. 2011 Jul;49(7):2540-5. doi: 10.1128/JCM.02319-10. Epub 2011 May 18.

12. Gu Y, Wang G, Dong W, Li Y, Ma Y, Shang Y, Qin S, Huang H. Xpert MTB/RIF and GenoType MTBDRplus assays for the rapid diagnosis of bone and joint tuberculosis. Int J Infect Dis. 2015 Jul;36:27-30. doi: 10.1016/j.ijid.2015.05.014. Epub 2015 May 21.

13. Avashia S, Choubey S, Mishra S, et al. To study the usefulness of CBNAAT (Cartridge Based Nuclear Acid Amplification Test) in BAL (Bronchoalveolar Lavage) samples in the diagnosis of smear-negative/non-sputum producing patients with suspected tuberculosis. J Evolution Med Dent Sci 2016;5(1):55-59, DOI: 10.14260/jemds/2016/13.

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How to cite this article?

Avashia S, Bansal D, Ahuja K, Agrawal V. Comparison of conventional methods with gene xpert mtb/rif assay for rapid detection of mycobacterium tuberculosis and rifampicin resistance in extra-pulmonary samples.*Int J Med Res Rev* 2016;4(2):181-185. doi: 10.17511/ijmrr.2016.i02.010.

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