

# Gene Xpert MTB/RIF – Diagnostic Role in Tuberculous Pleurisy

Muhammad Khalid Ch,<sup>1</sup> Muhammad Saqib Saeed,<sup>2</sup> Rizwan Iqbal,<sup>3</sup> Akhtar Ali<sup>4</sup>

## Abstract

**Background:** Tuberculous pleurisy is a common extrapulmonary manifestation and is second to tuberculous lymphadenitis. Due to its paucibacillary in origin, its diagnosis is a challenge. Furthermore, it is also complicated by the emergence of MDR. Gene Xpert MTB/RIF is a new rapid promising innovation for the detection of mycobacterium tuberculosis and its resistance to Rifampicin, giving result within two hours.

**Objective:** To evaluate the diagnostic role of Gene Xpert MTB/RIF in suspected cases of tuberculous pleurisy.

**Study Design:** Cross sectional study.

**Study Setting:** Institute of Chest Medicine Mayo Hospital – a Tertiary Care Hospital affiliated with King Edward Medical University, Lahore.

**Methodology:** The study group, one hundred (M-60

and F-40) cases having exudative, predominantly lymphocytic pleural effusion with strong suspicion of tuberculous in origin were enrolled randomly for Gene Xpert MTB / RIF.

**Results:** Among one hundred study cases, Gene Xpert detected mycobacterium tuberculosis in 12(12%) cases [M-8 (13.3%) and F-4 (10%)]. Resistance to Rifampicin was detected in 6 (6%) cases [M-2 (3.3%) and F-4 (10%)].

**Conclusion:** Gene Xpert MTB/RIF is a useful new rapid promising technique to diagnose tuberculous pleurisy. Although at present it has limited availability and utility.

**Key Words:** Gene Xpert. MTB – Mycobacterium tuberculosis. RIF – Rifampicin. TB – Tuberculosis. MDR – Multidrug resistance.

Conflict of Interest: No

Funding Source: No

Ch M.K.<sup>1</sup>

Associate Professor, Institute of TB & Chest Medicine  
King Edward Medical University / Mayo Hospital, Lahore

Saeed M.S.<sup>2</sup>

Chairman, Institute of TB & Chest Medicine  
King Edward Medical University / Mayo Hospital, Lahore

Iqbal R.<sup>3</sup>

Director, PMRC, King Edward Medical University, Lahore

Ali A.<sup>4</sup>

Institute of TB & Chest Medicine  
King Edward Medical University / Mayo Hospital, Lahore

## Introduction

Worldwide, Tuberculosis (TB) is an important leading cause of morbidity and mortality. It is a common public health disease with one third of world's population infected with it.<sup>1</sup> Developing countries have 90% of the burden of TB.<sup>2</sup> TB manifests itself with involvement of pleura or lymph nodes in 25% of TB patients.<sup>3</sup> Tuberculous pleural effusion has been reported as the most common form of extra pulmonary tuberculosis in certain areas of India but worldwide pleural TB is the second commonest manifestation of extra pulmonary TB.<sup>4</sup> Pleural disease in TB patient's is higher in developing countries constituting 30%, as compared to European countries where it is 3 – 5%.<sup>5,6</sup> Pleural TB was detected in 82% of all pleural effusions in Rawanda study<sup>7</sup> and 91% of exudative pleural effusions in a study conducted in Uganda.<sup>8</sup>

Diagnosis of tuberculous pleural effusion is a challenge due to poor sensitivity of conventional dia-

gnostic methods and increasing drug resistance. Pleural tissue culture of MTB or histopathology – gold standard for diagnosis has variable sensitivity ranging from 40 – 80% for pleural tissue culture and 50 – 97% for histopathology.<sup>9</sup> Pleural biopsy either by Abraham’s needle or by Thoracoscopy is an invasive procedure that needs skill and equipment which are not available in many health centres. The tissue culture on Lowenstein Johnson (L.J) solid medium takes up to 8 weeks and six weeks on liquid medium to get final result.<sup>10</sup>

To reduce morbidity and mortality, rapid diagnosis of Pleural TB is needed. The newer serological tests like interferon gamma release assays do not differentiate between latent and active TB infection.<sup>11</sup> More rapid tests such as nucleic acid amplification provides timely and definitive diagnosis of pleural tuberculosis. Gene Xpert MTB/RIF is an integrated fully automated specimen processing real time nucleic acid amplification test which detects Mycobacteria tuberculosis (MTB) and rifampicin resistance within two hours.<sup>12,13</sup> Gene Xpert MTB / RIF is a promising innovation having high sensitivity, specificity and rapid result.<sup>14</sup> At present there are a few studies with a relatively small number of pleural TB cases which have sensitivity between 15 – 25% and specificity of 100% of Xpert MTB / RIF on pleural fluid for pleural TB diagnosis.<sup>15-17</sup>

This study was undertaken to evaluate the possible utility of Xpert MTB / RIF as an option for an accurate and timely diagnosis of Pleural TB in a tertiary health care setting.

**Objective**

To evaluate the diagnostic role of Gene Xpert MTB / RIF in the diagnosis of tuberculous pleurisy.

**Study Design:** Cross sectional study.

**Study Setting:** Institute of Chest Medicine, Mayo Hospital. A Tertiary Care hospital affiliated with King Edward Medical University Lahore.

**Subjects and Methods**

Randomized one hundred patients of exudative, predominantly lymphocytic pleural effusion who have strong suspicion of tuberculosis etiology were included in the study. Only adult without any gender discrimination patients were enrolled in the study. 10cc pleural fluid aspirated with patient consent and sent to

PMRC TB Research Centre (Pakistan Medical TB Research Centre) affiliated with Mayo Hospital, for Gene Xpert MTB / RIF.

**Laboratory Procedure**

First of all, the fluid was centrifuged at 3000 RPM for 15 minutes. The supernatant was discarded and the sediment was processed for Gene Xpert MTB / RIF test. Lysing agent was added to the sediment in a ratio of 2:1 and kept for 15 minutes. During this period, the sample was shaken vigorously twice. Later on 2ml sediment was transferred to Gene Xpert cartridge and cartridge was placed in Gene Xpert instrument which gave result within 2 hours. Patients having expectoration also examined for AFB smear and culture to document the associated parenchymal disease.

**Results**

One hundred cases (male 60, female 40) of exudative predominantly lymphocytic pleural effusion who have strong suspicion of tuberculous etiology were included in the study. Males aged between 22 – 73 years and females between 14 – 64 years (Table 1). Bioche-

**Table 1:** Study Patients (Gender and Demographic Characteristics).

Total	Male	Female	Male Age	Female Age
100	60	40	22-73 years	14-64 years

**Table 2:** Pleural Fluid Chemistry.

Protein	3.5 – 8.6gm/dl
LDH	300 – 800 U/L

**Table 3:** Lymphocyte Count.

Lymphocyte count	Cases
50 – 59%	1%
60 – 69%	3%
70 – 79%	4%
80 – 89%	31%
90 – 100%	61%

mistry and lymphocyte counts were described in Table 2 and 3. Two cases have AFB smear positive and culture was also positive in 1% (Table 4). Chest radiology represented right sided pleural effusion in 73%, left sided pleural effusion in 25% and bilateral effusion in 2% cases (Table 5). Gene Xpert detected MTB in 12% cases and Rifampacin resistance in 6% cases (Table 6).

## Discussion

Pleural effusion is second to lymphatic involvement in extrapulmonary tuberculous manifestations. It may occur either as primary or as a reactivation disease.<sup>18,19</sup> Tuberculous pleurisy which was thought to result due to pure delayed hyper sensitivity reaction is now believed to be the direct infection of pleura with Mycobacterium tuberculosis resulting in lymphocytic driven immunological response. Detection of Mycobacterium tuberculosis in the pleural fluid, pleural biopsy tissue either by microscopy or culture and demonstration of granulomatous lesion with caseation, is the gold standard for the diagnosis of tuberculous pleurisy.<sup>20,21</sup> Gene Xpert MTB/RIF is a new technique to detect mycobacterium tuberculosis and its resistance to Rifampicin, which gives results within two hours.

Our study results showed MTB detection in 12 (12%) cases (Male 8 (13.13%) and female 4 (10%). Out of 12 (12%) MTB detected patients, 6 (6%) cases (Male 2 (3.3%) and Female 4 (10%) have Rifampicin resistance. So this testing not only confirmed the diagnosis of tuberculous pleurisy but also lead to detection of drug resistance. So it was possible to start drug resistance treatment at an early stage. 2% of the study cases also have positive microscopy for AFB and 1% has also culture positive for AFB which confirmed the associated parenchymal disease. Parenchymal disease co-exists with pleural effusion in 20% chest X-rays and 80% on CT Chest.<sup>22</sup> All smear positive and culture positive cases have positive Gene Xpert MTB. Randomized registered study cases showed that right sided pleural effusion (73%) was more frequent as compared to left sided effusion (25%). Bilateral pleurisy was a rare manifestation.

Study by Sahn SA et al concluded that one third of pleural effusions can be diagnosed by pleural fluid

**Table 4:** AFB Smear and Culture.

	Male	Female	Total	% age
AFB Smear	1 (1.7%)	1 (2.5%)	2	2%
AFB Culture	0	1 (2.5%)	1	1%

**Table 5:** Chest Radiology (CXR).

Right Sided Effusion	Left Sided Effusion	Bilateral Effusion
73%	25%	2%

Table 6: Gene Xpert MTB / RIF.

Gene Xpert Report	Total	Male	Female
MTB Detected	12 (12%)	8 (13.3%)	4 (10%)
RIF Resistance	6 (6%)	2 (3.3%)	4 (10%)

analysis alone where no other technique / method is used for confirmation of tuberculous pleurisy.<sup>23</sup>

Comparing Gene Xpert results with a local study by Javed N et al, where it was 8% positivity for MTB detection.<sup>24</sup> This study also had pleural biopsy which was positive in 14% cases representing granulomatous lesions with caseous necrosis. Other international studies like Vadwair Vet al,<sup>25</sup> Friedrich SOI et al,<sup>26</sup> Christopher DJ et al<sup>27</sup> and John K et al<sup>28</sup> showed sensitivity and specificity on pleural fluid as 63% and 100%, 25% and 100%. 16% and 100% and 28.7% and 96.6% specificity respectively. So different studies have different sensitivity results but specificity is the same i.e. 100%.

Analyzing the above studies results which have sensitivity between 8% to 63% and specificity between 96.6% to 100%, this rapid technique of MTB/RIF. detection can be used to diagnosis TB pleurisy. This rapid non invasive technique not only helped to confirm the diagnosis but also lead to detect drug resistance at an early stage to start treatment of MDR TB.

## Conclusion

Xpert MTB / RIF is a useful rapid technique to diagnose tuberculous pleurisy. Although at present, it has limited availability and utility.

## References

1. World Health organization. Global tuberculosis report 2013. World health organization.
2. Corbett EL, Whatt CJ, Walker N, Maher D, William BG, et al (2003). The growing burden of Tuberculosis, global trends and interactions with the HIV epidemic. *Arch int med*.163: 1009 – 1021 Doi, 10, 1001/archinte.163.9, 1009.
3. Gopi A. Madhavan SM, Sharma SK, Sahn SD, Diagnosis and treatment of Tuberculous Pleural Effusion in 2006. *Chest* 2007; 131: 880 – 89.
4. Sharma SK, Mohan A. Extra Pulmonary Tuberculosis, *Indian J Med Res* 2004; 120: 316 – 53.
5. Wolfgang Frank. Tuberculous Pleural Effusion, Tuberculosis – current issues in diagnosis and management, Edn 2013, IS BN; 978 – 953 – 51-1049-1.
6. Light RW. Pleural diseases, 5<sup>th</sup> edn. Lipponcot, Williams and Wilkins, Baltimor, MD 2007.
7. Batungwanayo J, Taelman H, Allen S, Bogaerts J, Kagome A, et al (1993), pleural effusion, tuberculosis and HIV – I infection in Kigali, Rawanda. *Aids* 7: 73 – 79, Doi: 10-1097/00002030-199301000-00011.
8. Luzze H, Elliott AM, Joloka ML, Odide M, Oweka-Onyce J, et al. Evaluation of suspected tuberculous pleurisy; clinical and diagnostic findings in HIV-1-positive and negative adults in Uganda, *Int J Tuberc Lung disease*, 2001; 5: 746-53.
9. Aggarwal AN, Gupta D, Jindal SK. Diagnosis of tuberculous pleural effusion. *Indian J Chest Dis Allied Sci*. 1999; 41: 89-100.
10. Kumar S, Sesnadri MS, Kashi G, John TJ (1981). Diagnosing tuberculous pleural effusion. Comparative sensitivity of mycobacterial culture and histopathology. *Br Med J (Clin Res Ed)*, 283:20. Doi. 10.1136/lung.283.6283.20.
11. Pal M, Minion J, Sohn H, Zwerling A, Perkins MD. Novel and improved Technologies for tuberculosis diagnosis: Prognosis and challenges. *Clin Chest Med*. 2009; 30: 701-16.
12. Steingart KR, Sohn H, Sbiller I, Kloda LA, Boehme CC, et al (2013). Xpert (R) MTB / Rif assay for pulmonary tuberculosis and rifampicin resistance in adults. Doi: 10.1003/14651858.cd 009 5 9 3, Pub<sup>2</sup>.
13. Boehme CC, Nabete P, Hillemann D, Nichol MP, Shgenai S et al. Rapid molecular detection of tuberculosis and rifampicin resistance, *N Eng J Med*. 2010; 363: 1005 – 15.
14. Van Rie A, Page – Ship L, Scott L, Sanne I, Steven W (2010). Xpert(R) MTB/Rif for point of care diagnosis of TB in high HIV burden, resource limited countries expert *Rev Mol Diagn* 10: 937 – 946, Doi: 10. 15861 cm.10.67.
15. Friedrich SO, Von Groote – Bidlingmair F, Diacon AH (211). XpertMTB/Rif assay for diagnosis of pleural tuberculosis. *J clin Microbiol* 49: 4 341-4342. Doi: 10.1128/Jcm – 05454-11.
16. Meldau R, Peter J, Theron G, Calligro G, All Wood B, et al (2014). comparison of same day diagnostic tools including Gene Xpert and unstimulated IFN – N. gamma for the evaluation of pleural tuberculosis. A prospective cohort study. *BMC pulm Med* 14: 58. Doi: 10, 186/1471 – 2466 – 14 – 58.
17. Porcel JM, Palma R – Valdes L, Bicsa S – San – Jose \_ E, et al (2013). Xpert(R) MTB/Rif in pleural fluid for the diagnosis of tuberculosis. *Int.J-Tuberc Lung Dis* 17: 1217-1219, Doi: 10/5588/ijtld -13.0178.
18. Kian H J, Lee HJ, Kwon Sy, et al. The prevalence of pulmonary parenchymal tuberculosis in the United State: incidence and drug resistance. *Chest*, 2007; 131: 1125 – 32.
19. Torgerson J, Dormen SE, Barnch N et al. Molecular epidemiology of pleural and other extra pulmonary tuberculosis: a Maryland state review. *Clin Infect Dis*. 2006; 42: 1375 – 82.
20. Light RW. Update on tuberculous pleural effusion. *Respirology*, 2010; 15: 451 – 8.
21. Koegelenberg CF, Bolliger CT, Theron J et al. Direct comparison of the diagnostic yield of ultrasound assisted Abram and Tru – cut needle biopsies for pleural tuberculosis. *Thorax*, 2010; 65: 857 – 62.
22. Kim HJ, Lee HJ, Kwon SY et al. The prevalence of pulmonary parenchymal tuberculosis in patients with tuberculous pleuritis. *Chest*, 2006; 129: 1253-8.
23. Sahn SA, Huggins JI, San Jose ME, Alvarez–Dabano JM, Valdes L. Can tuberculous effusions be diagnosed by pleural fluid analysis alone? *Int J Tuberc Lung Dis*. 2013; 17 (6): 787-93.
24. Javed N, Aslam M, Mushtaq AM, Khan J, Sheen ZM et al; Role of Gene Xpert in the diagnosis of tuberculous pleural effusion comparison with pleural biopsy *ERJ Sept*. 2014. <http://erj.ersjournals.com/content/44/Suppl-58/P2655>.
25. Vad Wair V, Boehme c, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert – MTB / RIF, A new pillar in the diagnosis of extra pulmonary tuberculosis?. *J Clin Microbiol*. 2011; 49: 2540 – 45.
26. Friedrich SO 1, Von Groote – Bidlingmaier F, Diacon AH. Xpert MTB/RIF Assay for diagnosis of pleural tuberculosis. *J Clin Microbiol*. 2011: 4341-42.
27. Christopher DJ, Shumacher SG, Michal JS. Performance of Xpert MTB / RIF on pleural tissue for the diagnosis of pleural tuberculosis. *Eur Respir J*. 2013; 42 (5): 1427 – 29.
28. John K, Lusiba, Lydia Nakiyingi, Brue J, Kirengaetal July 22, 2014. Doi: 10, 137/Journal. *Pone*. 0102702.