

## ORIGINAL ARTICLE

## Clinical utility of ADA / Gene Xpert for the Diagnosis of Tubercular Pleural Effusion in a Tertiary Care Centre

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### ABSTRACT

**BACKGROUND AND OBJECTIVES:** Tuberculosis is one of the oldest and most prevalent diseases in our country. The greatest burden of Tuberculosis incidence & mortality in India is in adults which include the most productive members of the society. Advances in Sputum microscopy and radiological methods improved the diagnostic yield in pulmonary tuberculosis, but the diagnosis of Extrapulmonary tuberculosis still remains a difficult job due to the paucibacillary nature of the disease. The main objective of our study was to evaluate the combined utility of Genexpert/PCR with ADA levels in diagnosis of EPTB in Pleural Effusions. **METHODS:** Our study was a cross sectional comparative study. A total of 44 cases who met the inclusion and exclusion criteria were included in the study, after taking ethical committee approval and proper consent from patients. Every diagnosis was recorded, tabulated and compiled to yield the Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value. **RESULTS:** The statistical analysis showed that ADA compared to Genexpert had a sensitivity of 94.11%, specificity of 3.70%, Positive predictive value of 38.09% and a Negative predictive value of 50%. in Pleural Fluid and Gene expert showed a sensitivity of 38.10%, specificity of 50%, Positive predictive value of 94.12% and a Negative predictive value of 3.70%. **CONCLUSION:** The authors concluded that collective use of ADA and Genexpert can significantly improve the diagnostic yield of Tubercular Pleural Effusions

**Keywords:** Tuberculosis, Pleural effusion, ADA, Genexpert

### INTRODUCTION

Tuberculosis is one of the oldest and most prevalent diseases in our country. Tuberculosis is a barrier to socio-economic development. The greatest burden of Tuberculosis incidence & mortality in India is in adults which include the most productive members of the society. TB has been a major global public health problem from times immemorial.

World Health Organization (WHO) estimates shows that globally there are 8.6 million incident cases of TB of which 80% are in 22 countries, with India ranked as the highest burden country.<sup>1</sup> EPTB constitutes about 15 to 20% of all cases of TB. The annual global incidence of EPTB has been increasing in the last decade due to the changing TB control practices, spread of HIV (human immunodeficiency virus), the population growth and the cure of infectious cases of TB might have resulted in a relative rise of annual EPTB case detection. During the last decade, remarkable progress has been made in the diagnostics of pulmonary tuberculosis; however, diagnostic challenges in extrapulmonary tuberculosis (EPTB) remain to be addressed. Diagnosis of EPTB is difficult due to the pauci-bacillary nature of disease, the variable clinical presentation, and need for invasive

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procedures to secure appropriate sample, and lack of laboratory facilities in the resource-limited settings. A more accurate test to diagnose various forms of EPTB, which can easily be incorporated in the routine TB control programme, would contribute significantly towards improving EPTB case-detection and thus reducing the morbidity and mortality. In this overview, we describe the status of combined clinical utility of ADA and PCR/Genexpert for laboratory diagnosis of pleural and peritoneal extra pulmonary TB. Aim of the study is to evaluate the combined utility of PCR with ADA levels in diagnosis of EPTB in pleural fluids. To Study about the clinical significance of routine PCR/GENE XPRT & ADA of Pleural and Peritoneal Fluids. To Compare the Sensitivity, Specificity and Positive Predictive Value of PCR/GENE EXPERT and ADA for the diagnosis of Tuberculosis.

## MATERIALS AND METHODS

The inclusion and Exclusion criteria of our study were as follows;

### Inclusion criteria

- Samples from Patients admitted to Department Of Pulmonary Medicine and Internal Medicine department with clinical Diagnosis Of Pleural TB.
- Patients aged > 12 years

### Exclusion criteria

- Samples from Patients with co-morbidities like Malignancy, CHF or Liver diseases and other definite causes of pleural effusion
- HIV positive patients
- Age < 12 years

### Study Design

- Cross sectional
- Comparative

### Sample Size

44 samples were included in this study. Every diagnosis was recorded, tabulated and compiled to yield the Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value Informed consent from all cases were taken.

**Sample Collection:** All the appropriate samples of Pleural fluid from patients admitted to Pulmonary Medicine and Internal Medicine Departments of Index Medical College, Hospital & Research Centre, Indore from date of approval from ethical committee to august 2018 was included in the study.

- A total of 44 samples from clinically diagnosed patients admitted to the Pulmonary Medicine, Internal Medicine and Surgery Department without any comorbidities as mentioned in the exclusion criteria were collected for ADA & GENE EXPERT Evaluation.
- **ADA:** Adenosine deaminase activity was assayed on the same day as collection of samples. ADA activity was measured by a spectrophotometric method described by Guisti and Galanti, using ADA- MTB(R) kit from Microexpress a division of *Erba diagnostics*.

**Principle:** The assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) by xanthine oxidase (XOD). H<sub>2</sub>O<sub>2</sub> is further reacted with N-Ethyl-N-3-Methylaniline (EHSPT) and 4-Aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored at 546nm. One unit of ADA is defined as amount of ADA that generates one micro mole of inosine from adenosine per minute at 37°C. Pleural fluid should be collected in a sterile or heparinised tube and processed within 2 hours at room temperature or 2 to 8°C or -20°C for 2 days. Ready to use reagent 1 and reagent 2 are used.

Reagent 1:

Tris.HCL pH 8.0-50ml

4-AA-2mM

PNP -0.1U/ml

XOD -0.2U/ml peroxidase - 0.6U/mL

Reagent 2:

Tris.HCL, pH 4.0 -50mM

Adenosine -10mM

EHSTP - 2mM

Normal Value Pleural and peritoneal fluid : 0-30 U/L

An ADA value of >40 in pleural and >30 in ascitic fluid is taken as suggestive of tuberculosis.

**GENE XPRT/PCR**

**Process**

**Step One:** Preparing the pleural fluid sediments:

Using a sterile transfer pipette, transfer at least 0.5 mL of re-suspended pellet to a conical, screw-capped tube. Add 1.5 mL of Xpert M.tb/RIF Sample Reagent (SR) to the 0.5 mL of re-suspended sediment using a sterile transfer pipette and shake vigorously 10 – 20 times, (a single shake is one back-and-forth movement). Incubate the specimen for 15 minutes at room temperature. Between 5 and 10 minutes of incubation, shake the specimen vigorously again 10 – 20 times. Samples should be liquefied with no visible clumps . Particulate matter may exist that is not part of the sample.

**Step Two:** Preparing the Cartridge:

Label each Xpert M.tb/RIF cartridge with the lab accession number by writing on the sides of the cartridge.Using the sterile transfer pipette provided with the kit, aspirate the liquefied sample into the transfer pipette until the meniscus is above the minimum mark. Do not process the sample further if there is insufficient volume.Open the cartridge lid and transfer sample into the open port of the Xpert M.tb/RIF cartridge. Dispense sample slowly to minimize risk of aerosol formation. Close the cartridge lid and make sure the lid snaps firmly into place. Remaining liquefied sample may be kept for up to 12 hours at 2 – 8°C should repeat testing be required.

**RESULT AND DISCUSSION**

The present study was conducted in Index Medical College Hospital and Research Centre, Nemawar Road as per the above laid out Protocol and following observations were recorded. The hospital covers local population and the population of Indore and the adjoining districts. All the appropriate samples of Pleural fluid from patients admitted to Department of Pulmonary Medicine and Medicine Departments of Index Medical College, Hospital & Research Centre, Indore from date of approval from ethical committee to august 2018 was included in this study. A total of 44 samples from patients admitted to the Department Of Pulmonary Medicine and Medicine Department who fulfilled all inclusion criteria and without any co-morbidities as mentioned in the exclusion criteria were collected for ADA & PCR/GENE EXPERT Evaluation.

**Table 1: Statistical Study of ADA against Gene Xpert in Pleural Fluid**

Table 1: ADA against Genexpert in Pleural Fluid								
		Gene Xpert			Sensitivity	Specificity	PPV	NPV
		MTB Detected	MTB Not Detected	Total Samples				
ADA	Negative	1	1	2	94.11%	3.7%	38.09%	50.00%
	Positive	16	26	42				
	Total Samples	17	27	44				

The comparative study of ADA against Genexpert in Pleural fluid, out of 42 ADA positive cases in 16 cases Genexpert for Mycobacterium TB was detected, meanwhile in 26 cases Genexpert for Mycobacterium TB was not detected.Two cases turned out to be ADA negative in which 1 sample was detected with Genexpert for Mycobacterium TB and the other sample showed a negative study.On statistical analysis, ADA compared to Genexpert in Pleural fluid has a sensitivity of 94.11% , specificity of 3.70%, Positive predictive value of 38.09% and a Negative predictive value of 50%.

**Table 2: Statistical Study of GeneXpert against ADA in Pleural Fluid**

Table 2: GeneXpert against ADA in Pleural Fluid					Sensitivity	Specificity	PPV	NPV
		ADA						
		Negative	Positive	Total Samples				
GeneXpert	Detected	1	16	17	38.10%	50.00%	94.12%	3.70%
	Not Detected	1	26	27				
	Total Samples	2	42	44				

The comparative study of Gene Xpert against ADA in Pleural Fluid, among the 44 samples of Pleural Fluid, Genexpert for Mycobacterium TB was detected in only 17 and ADA positive cases were 16 out of the 17 Mycobacterium TB detected cases. Remaining 27 Pleural Fluid samples, Genexpert for Mycobacterium TB was not detected, and in that 26 cases turned out to be ADA positive and the other sample showed a negative study. On statistical analysis, Gene Xpert compared to ADA in Pleural fluid has a sensitivity of 38.10%, specificity of 50%, Positive predictive value of 94.12% and a Negative predictive value of 3.70%. Tuberculosis still remains as one of the main public health problems, despite all the advances made in its treatment and management, particularly in developing countries like India which accounts for nearly 25 percent of the global Tuberculosis burden<sup>2</sup>, about 13 million people are affected & 2.5 million cases are added every year<sup>3</sup>. Extra pulmonary tuberculosis is a major public health issue existing for centuries, accounting for almost 20% of tuberculosis in India.<sup>1</sup> The aim of this study was to evaluate the clinical utility of Gene Xpert/PCR with ADA levels in diagnosis of EPTB in pleural and peritoneal fluids, with the objectives, to Study about the clinical significance of routine PCR/GENE XPERT & ADA of Pleural fluids and to Compare the Sensitivity, Specificity, Positive Predictive Value and Negative predictive value of PCR/GENE XPERT and ADA for the diagnosis of Extra Pulmonary Tuberculosis. A total of 44

samples from patients admitted to the Department Of Pulmonary Medicine and Internal Medicine Department who fulfilled all inclusion criteria and without any co-morbidities as mentioned in the exclusion criteria were collected for ADA & PCR/GENE EXPERT Evaluation.

Tubercular pleural effusion is the second most common form of extrapulmonary<sup>4</sup> tuberculosis and the common cause of pleural effusions in India. Pleural TB usually presents with pleural effusion caused by the immune system's response to the presence of mycobacterial antigens in the pleural space, generating inflammation and causing fluid to accumulate. Pleural TB accounts for 3–25% of patients with TB.<sup>5</sup> The utility of ADA measurement in pleural fluid, since then numerous studies have evaluated the diagnostic performance of ADA in tuberculous pleurisy.<sup>6</sup> In this study we selected 44 Clinically diagnosed cases of Extra pulmonary tuberculosis (Peritoneal and Pleural) which were analysed to compare the efficacy of 2 easily available and widely used non-invasive tests, ADA and Gene Xpert/PCR to determine the sensitivity, specificity, positive predictive value and negative predictive of both for the diagnosis of extra pulmonary tuberculosis. We performed both ADA and Gene Xpert in all the fluids collected. The cut off value for pleural fluid ADA was taken as 40 U/L, as suggested in standard textbooks of the field and are similar to the cutoff values in many studies conducted before<sup>12</sup> In our comparative study of ADA against Genexpert in Pleural fluid, ADA compared to Genexpert had a sensitivity of 94.11%, specificity of 3.70%, Positive predictive value of 38.09% and a Negative predictive value of 50%. During review of literature we noticed that the ADA has a high sensitivity and specificity in earlier studies, while Culture was taken as the Gold standard. **Liang et al**<sup>7</sup> and **Greco S et al**<sup>8</sup> showed that ADA have sensitivity and speciality above 90% in case of Pleural fluid. In Pleural Fluid, Gene expert

showed a sensitivity of 38.10% , specificity of 50%, Positive predictive value of 94.12% and a Negative predictive value of 3.70%. These values were bit lower compared to the literatures and previous studies we reviewed . Studies by **Lima et al**<sup>9</sup> , **Sehgal IS et al**<sup>10</sup> and **J Du et al**<sup>11</sup> showed the sensitivity for Gene xpert for diagnosis tubercular pleural effusion similar to our study. Among all the studies and literatures we reviewed the systemic review by Indrapaul Singh et al (90) was the most recent one, in these systematic reviews they investigated the role of Xpert MTB/RIF in the diagnosis of tuberculous pleural effusion (TPE) , The pooled sensitivities and specificities of Xpert MTB/RIF were 51.4% and 98.6%, respectively, with culture used as a reference standard and 22.7% and 99.8%, respectively, with a composite reference standard (CRS) used as the benchmark. Xpert MTB/RIF has low sensitivity but excellent specificity in the diagnosis of TPE. The results of this meta-analysis suggest that Xpert MTB/RIF can detect TPE in 22.7% and 51.4% of patients, using a CRS and a pleural fluid culture as the reference standard, respectively. The true sensitivity of Xpert MTB/RIF is expected to lie somewhere between the two estimates when an ideal reference standard such as thoracoscopic biopsy specimens with histopathology is used. The specificity of Xpert MTB/RIF was very high with either reference standard, making it an excellent “rule-in” test. Compared to the previous meta-analysis on TPE, in this study the reviewers identified 14 additional studies and almost twice as many patients, they also performed a subgroup analysis to determine factors affecting the yield of GeneXpert. Further, in this study they used the bivariate random effect model for pooling estimates. Thus, the validity of the results were significantly higher than that of the previous meta-analysis. And the results of my study was similar to the one observed in the systemic review by Sehgal IS et al<sup>10</sup> proving its validity. The high

positive predictive value of Gene expert makes it useful for the confirmation of the tubercular aetiology. As clearly evident from the statistical analysis and the bar diagrams of our results, the collective use of ADA and GeneXpert can significantly improve the the diagnosis of Extrapulmonary tuberculosis, in our study we were able to establish tuberculosis as aetiology in 42 samples out of the 44 samples, with a sensitivity of 90.48% and Specificity of 50%. Both the tests are noninvasive and easily available with a very less turnover time, making them ideal to use as routine investigations in all cases of pleural and ascitic fluids. The added benefit of detecting Rifampicin resistant cases by Gene Xpert is really beneficial in present scenario of rise in MDR cases of tuberculosis. Early diagnosis and treatment will aid in fast recovery, better quality of life and improve in the mortality rate of patients. There were many limitations in our study, since our study period was for a shorter duration our study group itself was relatively small , we included only pleural effusion , we didn't include the HIV positive cases, we didn't do any followup of the patients to asses the outcome. To asses the combined efficiency of ADA/Gene expert in extra pulmonary tuberculosis, all types of extrapulmonary samples from multiple centres should be collected and evaluated in a much larger scale with histopathological confirmation as the Gold standard. The pauci-bacillary nature of the Extrapulmonary tuberculosis makes it difficult to use Culture as the Gold standard, rather the method used by Sehgal IS et al<sup>10</sup> for their study was excellent with reliable standardisation and can even can be adopted for a large group of patients.

## CONCLUSION

The study was concluded as ADA should be done in all cases of Pleural effusion to rule out the aetiology as mycobacterium tuberculosis. Its inexpensive, easily available and has got reliable sensitivity and negative predictive value. ADA alone should not be used for the diagnosis

aetiology as Mycobacterium Tuberculosis. Gene Xpert shouldn't be used routinely for the diagnosis of extra pulmonary tuberculosis as the sensitivity is less and should be used in those cases where other investigations are not giving a conclusive diagnosis as the specificity and positive predictive values are high. Although we should keep in mind the added benefit of detecting Rifampicin resistant cases through Gene expert. A combined use of ADA and Gene Xpert is better than using individually for the diagnosis of Mycobacterium tuberculosis aetiology in pleural and ascitic fluids.

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