



To Study the Comparative yield of Zn Staining v/s CBNAAT (Gene Xpert) in Clinically Diagnosed cases of Tubercular Pleural Effusion

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Abstract

Background: India accounts for a quarter of global TB burden in which 15-20% are extra-pulmonary TB. Tubercular pleural effusion is the most common extra pulmonary TB after Tubercular lymphadenitis. Tubercular effusion remains mostly undiagnosed due to pauci-bacillary nature. Early detection of Tuberculosis and Drug Resistance is the priority in diagnosis for early disease management and reduce morbidity. Molecular biological tests specially CBNAAT is one of the important method for early and rapid diagnosis of mycobacterium tuberculosis in specimen/tissue having pauci-bacillary nature with good sensitivity and specificity.

Materials and Methods: This is a retrospective study conducted in Department of Respiratory Medicine, MGMCH Jaipur in 150 patients of clinically diagnosed cases of tubercular pleural effusion between August 2016 to July 2018. In addition to detailed clinical history/examination and radio imagine Pleural fluid for routine Biochemical examination reports evaluated for protein, sugar ADA and cell cytology, suspecting tubercular in nature were taken in our study. In all these cases AFB by ZN staining and CBNAAT done and comparative reports evaluated.

Results: A total of 150 cases of clinically diagnosed tubercular pleural effusion, MTB was Detected by ZN staining in 11(7.33%) and CBNAAT in 38(25.33%) cases. CBNAAT yield was significantly more as compare with ZN Staining. Among 38 cases who were positive for CBNAAT only 11 cases were positive for ZN Staining showing poor yield of ZN Staining.

Conclusion: CBNAAT is newly emerging and highly sensitive and specific technique for early and rapid diagnosis of Tubercular Pleural effusion including resistant pattern of Rifampicin in view of MDR as compared to ZN staining.

Keywords: MTB, CBNAAT, ZN staining, MDR, ADA.

Background

TB is a major health problem worldwide caused by the bacillus *Mycobacterium tuberculosis* infection. It commonly affects the lungs (pulmonary TB) but can also affect any other organ (extra-pulmonary TB).¹ Overall, a relatively small proportion (5–15%) of the estimated 1.7 billion people infected with *M. tuberculosis* will develop TB disease during their lifetime. However, the probability of developing TB disease is much higher among people infected with HIV, and also higher among people affected by risk factors such as under-nutrition, diabetes, smoking and alcohol consumption. As per the Global TB report 2017 the estimated incidence of TB in India was approximately 2.8 million accounting for about a quarter of the world TB cases.²

Pleural TB is the second most common extra-pulmonary manifestation after lymph node TB worldwide.³ The clinical presentation of extra-pulmonary tuberculosis is atypical because of pauci-bacillary nature of extra-pulmonary specimen and often remain either undiagnosed or misdiagnosed due to lack of diagnostic tools.⁴ Diagnosis of pleural TB relies on the examination of pleural fluid and/or biopsy specimens using acid-fast microscopic examination, culture, polymerase chain reaction, evaluation of pleural fluid characteristics, and/or histo-pathological examination.⁵ The World Health Organization (WHO) has endorsed the implementation of GeneXpert MTB/RIF assay for the national tuberculosis programs in developing countries in 2013.⁶

Gene Xpert MTB/RIF is an automated, user friendly and rapid based on nested real-time PCR assay and molecular beacon technology for MTB detection and RIF resistance. The results were obtained within a short period of 2 hours. It is a highly specific test as it uses 3 specific primer and 5 unique molecular probes to target RpoB gene of *Mycobacterium tuberculosis* (MTB). This technique is not prone to cross contamination,

requires minimal Biosafety facilities and has a high sensitivity in smear negative pleural TB.⁷

This study was aimed to evaluate the value of GeneXpert MTB/RIF Assay as an rapid and accurate diagnosis of clinically diagnosed pleural tuberculosis cases and also compare its value with ZN staining for Acid fast bacilli in pleural fluid.

Materials and Methods

Our hospital is located at Jaipur in Rajasthan state which cater both urban and rural population from all over state even other adjoining states. It is also a referral center for higher medical services. This is a retrospective study conducted in Department of Respiratory Medicine, MGMCH Jaipur in 150 clinically diagnosed cases of tubercular pleural effusion between August 2016 to July 2018. In addition to detailed clinical history/examination and radio imagine Pleural fluid for routine Biochemical examination reports evaluated for protein, sugar ADA and cell cytology, suspecting tubercular in nature were taken in our study. In all these cases AFB by ZN staining and CBNAAT done and comparative reports evaluated.

Inclusion criteria

- Patient having clinical feature and radio-imagine suggestive of Pleural Effusion
- **Exudative nature of pleural fluid**
 - Lymphocytic predominance
 - ADA > 40 unit/Liter
 - Protein >3 gm/dl
- Patient age > 15 year age
- Patient/relative given informed written consent for study

Exclusion criteria

- Transudative Pleural Effusion
- Patients with Heart & Renal failure, Nephrotic syndrome, Liver cirrhosis
- Contraindication of thoracocentesis like serious condition, uncooperative, bleeding diathesis & patient on anticoagulant therapy
- Patient age < 15 Year
- Patient/relative not giving informed written consent

Results

Over the mentioned period of time duration, total no of 150 cases who met the inclusion criteria were evaluated carefully. Out of 150 cases 117(78%) were males & 33(22%) were females (Table no. 1). Majority of cases 88(58.67%) belong to less than 45 years of age (Table no. 2). Most of the cases belonged to rural background 101 (67.33%) and 49 (32.67%) from urban (Table no. 3).

Fever is the most common presenting symptom followed by chest pain, dyspnea Cough & weight loss (Table no. 4). Most of the pleural effusions are unilateral (94.67%) in which right side involvement 94(62.67%) cases and left side 48(32%) cases while bilateral in 8(5.33%) cases (Table no. 5).

ZN Staining for Acid Fast Bacilli & CBNAAT for Mycobacterium Tuberculosis done for in all 150 cases in which 11(7.33%) cases shows ZN Staining positivity & 38(25.33%) cases shows CBNAAT positivity (Table no. 6 & 7). All 11 cases who were ZN Staining positive shows CBNAAT for MTB Positivity (Table no. 8). Out of 38 CBNAAT positive cases Rifampicin Resistance were detected in only 6(15.79%) cases (Table no. 9).

Table 1 Gender profile

Gender	No. of cases	Percentage
Male	117	78
Female	33	22
Total	150(100%)	100

Table 2 Age profile

Age group	No. of cases	Percentage
18-29	45	30
30-44	43	28.67
45-59	34	22.67
>60	28	18.66
Total	150	100

Table 3 Distribution of cases according to residence (Urban/Rural)

Residence	No. of cases	Percentage
Rural	101	67.33
Urban	49	32.67
Total	150	100

Table 4 Symptoms profile

Symptoms	Percentage
Fever	91.33
Chest pain	82.67
Dyspnea	56.67
Cough	52.00
Weight loss	48.67

Table 5 Radiological involved of chest

Side	No. of cases	Percentage
Right	94	62.67
Left	48	32.00
Bilateral	8	5.33
Total	150	100

Table 6. Yield of AFB by ZN staining Technique

ZN Staining	No. of cases	Percentage
Positive	11	7.33
Negative	139	92.67
Total	150	100

Table 7 Yield of MTB by Gene XPERT Assay

GeneXpert	No. of cases	Percentage
Positive	38	25.33
Negative	112	74.67
Total	150	100

Table 8. Comparative Result of ZN Staining & Gene XPERT

Gene Xpert	ZN Staining			Sensitivity	Specificity
	Positive	Negative	Total		
Positive	11	27	38	100%	86%
Negative	-	112	112		
Total	11	139	150		

Table 9 Profile of Rifampicin resistance by Gene Xpert

Rifampicin resistant	No. of cases	Percentage
Detected	6	15.79
Not detected	32	84.21
Total	38	100

Discussion

Demonstration of tubercular bacilli as well as caseating granuloma are the gold standard for the diagnosis of tuberculosis.⁸ In spite of all the efforts most of the time it is negative in case of tubercular pleural effusion due to its pauci-bacillary nature and etiology of pleural fluid remain undiagnosed or misdiagnosed. Pleural fluid analysis by cytology, biochemistry and ADA is not always diagnostic, similar picture may be present in some

other diseases thus, creating diagnostic confusion.⁹

Our study was planned to know the role of Gene Xpert MTB/RIF in the diagnosis of tubercular pleural effusion and rifampicin resistance as not many studies are available in the literature.

Out of 150 cases more than 3/4th cases were males similar male predominance were reported by various studies done by Parikh et al¹⁰ who reported that 68% were male, Arya et al¹¹ reported 70% males. Approx. 3/5th cases were belongs to less than 45 years of age similar age group reported by Parikh et al¹⁰, Arya et al¹¹ Ferreiro L et al.¹² Most of studied cases belonged to rural background 2/3rd followed by 1/3rd were from urban background.

Most common symptom fever (91%) followed by chest pain (87%), dyspnea (67%) cough (52%) & weight loss (49%) similar to study J. Ferrer.¹³ Most of the Pleural effusions are Unilateral in which Right side involvement 62.67% cases followed by left side 32% cases and bilateral in 5.33% cases similar to study reported by Valdés L et al.¹⁴

In present study low Yield 7.33% cases shows ZN Staining positivity comparable with study of Mittal & Kumar¹⁵ (14%). Gene Xpert positive yield is 25.33% cases in our study compared with other studies of Ahmed et al¹⁶ (15.8%), Shukla A et al¹⁷ (20.58%), Mittal & Kumar¹⁵ (35%), Pravin & Chourasia¹⁸ (32%). All 11 cases who were ZN Staining positive shows GeneXpert Assay for MTB Positivity. We also evaluated sensitivity and specificity of Gene Xpert in smear positive and smear negative cases which was 100% sensitive and 86% specific. Rifampicin resistance were detected in only 15.79% cases out of 38 GeneXpert Assay positive cases while Pravin & Chourasia¹⁸ reported 19% and Shukla A et al¹⁷ reported 21%.

Conclusion

Tubercular Pleural Effusion important cause of morbidity & mortality, high prevalence in India, so need of rapid diagnostic method for early treatment & cure to reduce the TB burden. The

results of present study show that Gene Xpert MTB/RIF assay could play a significant role as routine diagnostic investigation in Clinico-Radiological & Biochemical suspected cases of tubercular pleural effusion and advantage of detection of Rifampicin drug resistance tuberculosis, where resources of pleural fluid culture are limited, case burden is high and is time consuming, whereas results of Gene Xpert MTB/RIF are available in same day with acceptable sensitivity and specificity.

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