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An evaluation of the sensitivity, specificity and positive predictive value of CB-NAAT testing in the diagnosis of Tuberculous Pleural Effusion

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ABSTRACT:

The diagnosis of TB pleural effusion with microbiological confirmation is a challenging task it being a paucibacillary infection and most cases are diagnosed clinically and empirically started on anti tubercular drugs. We studied the diagnostic accuracy of Gene Xpert MTB/Rif assay in the diagnosis of TB pleural effusion by comparing it with clinically confirmed cases that were cured by anti tubercular drugs and also with MGIT960 liquid culture results. In our study we found the sensitivity of Gene Xpert to be 24.71% (95% CI = 15.99% to 35.25%) and specificity to be 100% (95% CI = 80.49% to 100%) by including all cases diagnosed and confirmed clinically. The positive predictive value was 100% and negative predictive value was 20.99% (95% CI = 19.04% to 23.08%) giving the accuracy at 37.25% (95% CI = 27.88% to 47.39%). However if only microbiologically confirmed cases were used to define cases for calculating the accuracy of Gene Xpert, the sensitivity was calculated to be 94.74% (95% CI 73.97% to 99.87%) and specificity was 96.51% (95% CI = 90.14% to 99.28%). The positive predictive value was 85.71%(95% CI = 66.26% to 94.83%) and the negative predictive value was 98.81% (95% CI = 92.49% to 99.82%) giving a diagnostic accuracy of 96.19%(95% CI = 90.53% to 98.95%). Also the microbiological positivity of Tuberculous pleural effusion was considerably higher in HIV positive patients (P=0.004; 95% CI =17.512% to 69.246%).To conclude Gene Xpert MTB/Rif cannot be solely relied on to make or exclude a diagnosis of Tuberculous Pleural effusion and clinical characteristics along with biochemical tests like ADA have to be taken into consideration to make a final diagnosis of pleural Tuberculosis.

Keywords: Tuberculous Pleural Effusion, Gene Xpert MTB/Rif, Sensitivity, Specificity

Introduction

India is the country with the highest burden of TB. Every year 2.2 million new cases of TB are found in India[1]. The incidence of Tuberculous pleural effusion is reported from 5% to 25%[2-3]. One study in India found the incidence of Tuberculous pleural effusion to be 23.52%[4]. It is often difficult to get a prompt etiological diagnosis of any case of chronic pleural effusion as it has a lot of differential diagnoses to be considered. Most cases of TB pleural effusion are diagnosed on the basis of the clinical features and biochemistry and cytology from pleural fluid study[5]. Often a case of Tuberculous pleural effusion is diagnosed by ruling out any other etiological cause and empirically treated with anti Tuberculous drugs.

The advent of Cartilage based Nucleic acid amplification test (CB-NAAT) has provided a

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new opportunity for rapid diagnosis of extra pulmonary Tuberculosis[6] which otherwise required a waiting period of 4-6 weeks to obtain a confirmed bacteriological evidence with culture growth of Mycobacterium Tuberculosis. Studies need to be conducted on the sensitivity and specificity of this new testing method for Extra Pulmonary Tuberculosis[7,10].

The aims and objectives of the Study were as follows:

1) To Calculate the sensitivity of Xpert MTB/Rif testing using GeneXpert system in diagnosing TB pleural effusion
2) To Calculate the specificity of Xpert MTB/Rif testing using GeneXpert system in diagnosing TB pleural effusion
3) To calculate the positive predictive value of Xpert MTB/Rif testing using GeneXpert system in TB pleural effusion
4) To calculate the negative predictive value of Xpert MTB/Rif testing using GeneXpert system in TB pleural effusion

Material and Methods

Study protocol was cleared from the Institutional Ethics Committee of the Institution. A cross sectional observational Study was done by retrospectively studying the discharge summaries and follow up details of clinically proven cases of Tuberculous Pleural Effusions because of their response to anti tubercular drugs(ATD) and no other differential diagnosis was discovered to be present as well as no other treatment was required to achieve cure. We studied the discharge summary records of all the cases of suspected TB pleural effusion admitted in the department of Respiratory Medicine in the past 2 years to find out the confirmed cases of TB pleural effusion and the criteria by which they were confirmed. All the patients included in the study had biochemical, cytological analysis done. Cases of malignant pleural effusion which were confirmed radiologically and by cytology or histopathological examination were excluded from the study as there was no need for doing a microbiological lab testing in these cases. Pleural fluid from suspected cases of Tuberculous pleural effusion were sent for testing with GeneXpert/ Xpert MTB/Rif in the Intermediate Reference laboratory (IRL) Nagpur after pleural fluid tapping with informed written consent. Another pleural fluid sample from the same patient was sent on the same day for Mycobacterial Growth Indicator Tube(MGIT) testing in IRL. MGIT960 and Gene Xpert testing was done by the IRL as recommended by RNTCP. MGIT is considered as the gold standard for the diagnosis of extra pulmonary TB pleural effusion[14].

A case of TB Pleural effusion was defined as:-

1) MGIT960 positive for MTB from pleural fluid or pleural biopsy sample.
2) Pleural Biopsy showing AFB(Acid Fast Bacilli) Positive or granuloma formation with central caseation necrosis on HPE(Histopathological Examination).
3) Sputum or pleural fluid smear positive for AFB or culture positive for MTB.
4) Lymphocytic predominant (Pleural fluid Lymphocytes > 50%)[8] exudative pleural effusion where other possible diagnoses have been ruled out and improvement after anti tubercular drug therapy.
5) Pleural fluid Adenosine deaminase (ADA) more than 40 IU/L and improvement after anti tubercular drugs.[7]
6) A pleural effusion treated as a case of Tuberculous pleural effusion with anti tubercular drugs after ruling out all other possible diagnoses and the patient showed improvement with therapy.

Microsoft Excel was used for collection, filtering and analysis of Data. Statistical analysis using MedCalc Stastical software was used to calculate sensitivity, specificity and positive predictive value and negative predictive value of Gene Xpert in the diagnosis of TB pleural effusion. For calculation of Sensitivity and Specificity cases will be determined by clinical criteria and also by the Gold Standard of culture positivity by MGIT culture.

Results and Discussion

A. Population Demographics of the Study Population:-

A total of 102 patients were included in the study group after implementing the exclusion criteria. Of the cases included in the study group 89(87%) were male and 13(13%) were female**(Figure 1a)**. Amongst those included in the study group 2(1.96%) were less than 18 yrs of age, 50(49.01%) were in the age group of 18-35 yrs old, 39(38.23%) were in the age group of 36-60 yrs and 11 were older than 60 yrs**(Figure 1b)**.

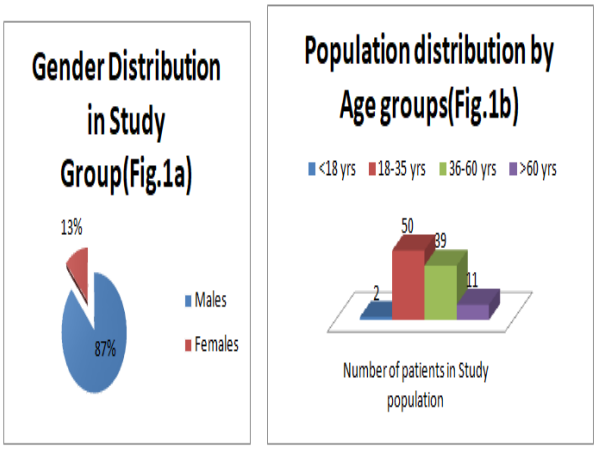

Fig.1: Population demographics of Study Population

B. Population Demographics and Clinical profile of Patients with TB pleural effusion:-

Of the 85 cases with Confirmed TB pleural effusion(TBPE) 73(85.88%) were male and 12(14.12%) were female. Among the clinically confirmed cases of TB pleural effusion 2(2.35%) were below the age of 16 years and one (50%) of them was HIV(Human Immunodeficiency Virus) positive. 44(51.76%) were between the age group of 18-35 yrs and 6(13.33%) of them were HIV positive**(Figure 2)**. Of the age group 36-60 yrs 20(23.52%) were diagnosed with TB Pleural effusion and of the age group of patients more than 60 yrs of age 7(8.23%) were having TBPE. This showed that TBPE was more common in the younger economically productive age group of 18-35 yrs as also TB in general is more common in the same age group as observed by earlier studies[9].

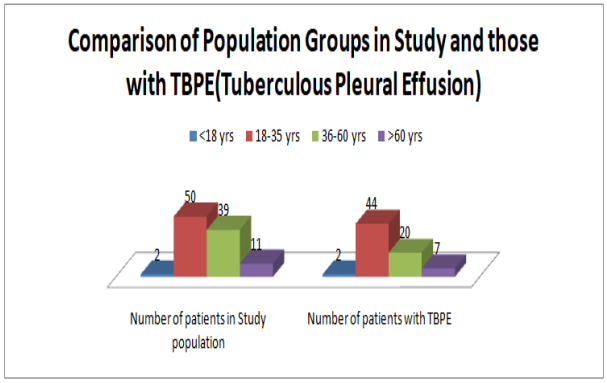

Fig.2: Comparison of different population groups included in the study and those diagnosed with Tuberculous Pleural Effusion

Cough of more than 2 weeks (76.47%) and the presence of fever (83.52%) along with pleuritic chest pain (56.47%)

were the main presenting symptoms in most of the cases. Hydropneumothorax was present in 8(9.41%) patients with TBPE and Bronchopleural fistula(BPF) was present in 1(1.17%) of the patients and the same patient also had Pleural fluid positive for AFB smear indicating a high bacillary load. The details of the clinical characteristics of the patients with and without TB pleural effusion are described in **Table 1**. Pyopneumothorax was seen in 7(8.23%) of patients with pleural TB and empyema in 8(9.41%). ADA values of >/= 40 IU were seen in 59(69.41%) of TBPE cases and ADA <40 IU was seen in 26(30.58%) of patients with TBPE. 72(84.70%) of the patients with TBPE showed a lymphocytic predominant pleural effusion and only 13(15.29%) of the patients had neutrophilic predominance in the pleural fluid.

Table 1: Clinical characteristics of Patients with TBPE and those with non Tuberculous Pleural effusions

Characteristics	TBPE n=85	non TB Pleural effusion n=17	P value
Males	73(85.88%)	16(94.11%)	0.3554
Females	12(14.12%)	1(5.88%)	0.3548
<18 yrs age	2(2.35%)	0	0.5585
18-35 yrs age	44(51.78%)	6(35.29%)	0.0033
36-60 yrs age	20(23.52%)	8(47.05%)	0.2421
>60 yrs age	7(8.23%)	3(17.64%)	0.5385
Cough > 2 weeks	65(76.47%)	6(35.29%)	<0.0001
Fever	71(83.52%)	13(76.47%)	<0.0001
Chest Pain	48(56.47%)	10(58.82%)	0.0039
Hydropneumothorax	8(9.41%)	0	0.2297
Empyema	8(9.41%)	2(11.76%)	0.3785
Pyopneumothorax	7(8.23%)	0	0.2633
Broncho Pleural Fistula	1(1.17%)	0	0.6808
Pleural Fluid AFB smear +ve	2(2.35%)	0	0.5585
MGIT Positive	19(22.35%)	0	0.0513
Xpert MTB/Rif Positive	21(24.70%)	0	0.0444
HIV POSITIVE	11(12.94%)	0	0.1534
ADA >= 40 IU	59(69.41%)	3(17.64%)	<0.0001
ADA < 40 IU	26(30.58%)	14(82.35%)	0.2926
Lymphocytic Predominant	72(84.70%)	0	<0.0001
Neutrophilic	13(15.29%)	17(100%)	0.6627

Abbreviations: ADA = Adenosine Deaminase; TBPE = Tuberculous Pleural effusion; N = sample size

Of the 11 HIV positive cases with Tuberculous Pleural effusion one(9.09%) was pleural fluid AFB smear positive along with MGIT and Xpert MTB/Rif positivity for MTB and a total of 7(63.63%) **(Figure 3)** had MTB detected by MGIT and Xpert MTB/Rif testing as opposed to 15(16.48%) out of 91 HIV negative cases of TBPE getting microbiologically confirmed. This shows the Multibacillary nature of TBPE associated with HIV significantly increasing the chances of microbiological confirmation(P=0.004; 95% CI =17.512% to 69.246%). Of the 21 cases of Gene Xpert detected MTB one specimen was also found to be resistant to Rifampicin

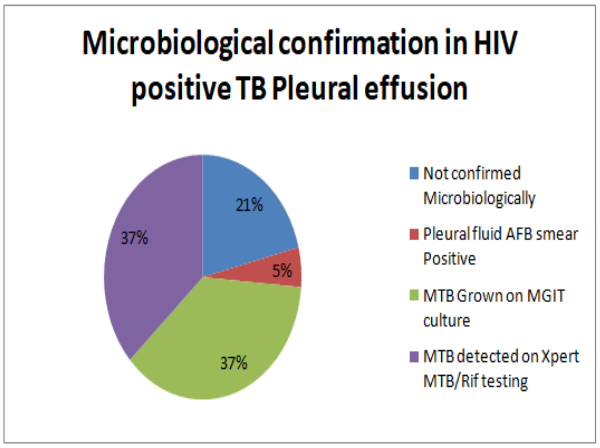

Fig.3: Microbiological confirmation in TB pleural effusion associated with HIV infection

C. Testing of Diagnostic Accuracy of Gene Xpert in detecting cases of TB Pleural Effusion:-

A total of 102 cases of suspected TBPE were included in the study. Of these 85 were determined to be confirmed cases of Tuberculous Pleural Effusion based on clinical and biochemical criteria as described earlier. IF only the clinical criteria was used to determine the total number of cases as 85/102 and Gene Xpert was able to detect 21/85 cases**(Table 2)** the sensitivity of Gene Xpert in detecting TBPE was calculated to be **24.71%** (95% CI = 15.99% to 35.25%) by MedCalc software. The specificity was **100%** (95% CI = 80.49% to 100%); the positive predictive value was **100%** and negative predictive value was **20.99%** (95% CI = 19.04% to 23.08%) giving the accuracy at 37.25% (95% CI = 27.88% to 47.39%).

Table 2: Of 102 cases included in the study 85 were determined to have TB pleural effusion by clinical criteria and a 2x2 table was used to calculate the Sensitivity, Specificity, Positive predictive value and Negative predictive value.

n=102	**Cases by Clinical Criteria**	**Controls by Clinical Criteria**
XpertMTB/Rif MTB detected	21(True Positive)	0(False Positive)
XpertMTB/Rif MTB not detected	64(False Negative)	17(True Negative)
Total	85	17

We also calculated the accuracy of Gene Xpert in testing Pleural fluid for Tuberculosis by using the Gold standard criteria of culture positivity by MGIT for defining cases **(Table 3)**.

Table 3: Of 102 cases included in the study 19 were determined to have TB pleural effusion by the Gold standard of Culture positivity by MGIT and Sensitivity, Specificity, Positive predictive value and Negative predictive value of Gene Xpert was calculated

n=102	**MGIT Culture Positive cases**	**MGIT Culture Negative controls**
XpertMTB/Rif MTB detected	18(True Positive)	3(False Positive)
XpertMTB/Rif MTB not detected	1(False Negative)	80(True Negative)
Total	19	83

By using the gold standard method to define cases of TBPE the sensitivity was calculated to be 94.74% (95% CI 73.97% to 99.87%) and

specificity was 96.51% (95% CI = 90.14% to 99.28%). The positive predictive value was 85.71% (95% CI = 66.26% to 94.83%) and the negative predictive value was 98.81% (95% CI = 92.49% to 99.82%) giving a diagnostic accuracy of 96.19%(95% CI = 90.53% to 98.95%). These finding were matching with another study on Gene Xpert sensitivity and specificity in detecting Tuberculous Pleural effusion done by Sven O. Friedrich et al[10] in Tygerberg Hospital, South Africa in 2011 found a sensitivity of 25% and specificity of 100%. They concluded that Gene Xpert study on pleural fluid samples for TB was feasible but had a low sensitivity and was associated with culture positivity of pleural fluid. Porcel JM et al[11] found out that in 67 patients with pleural effusions, of whom half had tuberculous pleuritis, Xpert yielded 15% sensitivity and 100% specificity in the detection of tuberculosis (TB). They also found out that positive Xpert results tended to be more common in patients with microbiologically confirmed TB. A study by Lusiba JK et al found a sensitivity and specificity of Xpert MTB/Rif test as 28.7% and 96.6% respectively while the positive and negative predictive values were 96.1% and 31.1% respectively.[12] To determine the accuracy of Xpert MTB/Rif test on pleural fluid in the diagnosis of pleural TB, pleural tissue MTB culture and/or histopathology was used as the reference standard. In this study, out of the 29 cases that had the reference standard negative for pleural TB one (3.4%) was Xpert MTB/Rif test positive. Similarly in our study of the 83 cases that were gold standard (MGIT culture) negative, 3(3.67%) were Xpert MTB/Rif test positive. This could be because of some technical errors in conducting the sample collecting and processing of the MGIT culture test. In another study of Twenty-six of 94 (27.7%) culture-confirmed pulmonary TB the Xpert MTB/RIF assay was found to have a sensitivity of 80.8% (95% CI = 60.0 to 92.7%) compared against MGIT culture.[13] This also shows the higher positivity of Xpert MTB/Rif assay in microbiologically confirmed cases. Another study has found a low sensitivity of Gene Xpert assay in pleural fluid[14] of up to 33.3%.

D. Limitations of our study

The study did not utilize pleural biopsy tissue samples which considerably increase the yield of MGIT960 liquid culture and could have given more MGIT960 culture positive reports. Gold standard of Culture positive cases from tissue biopsy sampling could not be utilized for higher number of cases as most cases were diagnosed clinically and treated empirically.

Conclusion

The diagnosis of Tuberculosis in a pleural effusion being a paucibacillary infection is a challenging task as microbiological confirmation can be attained in a small percentage of cases only and most of the cases are diagnosed clinically. The advent of Gene Xpert MTB/Rif assay has provided a method of rapid molecular diagnosis and confirmation of TB pleural effusion along with detection of resistance to rifampicin. The sensitivity of Gene Xpert MTB/Rif assay in diagnosing Tubercular Pleural effusion is however is as low as 15-30% (24.71% in our study) which doesn't make it an ideal test for detecting TBPE. The negative predictive value ranges from 20-30%(20.99%) in our study and a negative test cannot be utilized to rule out the diagnosis of TBPE. If only the Gold Standard of culture confirmed cases by MGIT is used to define cases for calculation of sensitivity and specificity of Gene Xpert assay the sensitivity may be falsely high as the sensitivity of MGIT960 in detecting MTB in pleural fluid itself is as low as 45%[15] and Gene Xpert assay has a higher positivity in microbiologically confirmed cases as these have a higher bacillary load.

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