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To compare gene Xpert and cytochemical analysis of pleural fluid in diagnosis of tuberculosis in exudative pleural effusion in Bikaner district

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Abstract

Background: Tuberculosis is a bacterial disease spread from one person to another principally by airborne transmission. The causal agent is Mycobacterium tuberculosis (the tubercle bacillus). Tuberculosis can affect any organ in the body. Pulmonary tuberculosis is the most frequent site of involvement; extrapulmonary tuberculosis is less frequent. Only pulmonary tuberculosis is infectious.

Methods: This hospital based cross-sectional observ ational study conducted at Department of Respiratory medicine, S.P. Medical College and P.B.M Hospital, Bikaner on 100 Patients with high suspicion of exudative tuberculous pleural effusion based on clinical signs and symptoms of tuberculosis confirmed with Light's criteria in addition to radiological proof of a pleural effusion considered large enough for a pleural biopsy, also highly positive tuberculin skin test and absence of other causes of pleural effusion.

Results: 32.00% patients CBNAAT was positive. Mean ADA level was 81.32±20.02 IU/L. Mean total leucocytes level was 2734±1421 per cu mm, mean lymphocyte level was 82.10±8.01% and mean polymorphs level was 17.02±8.5%. Mean sugar level was 59.20±21.02 mg/dl, mean protein level was 4.12±0.78 mg/dl.

Conclusion: Regarding the current results, assessment of pleural fluid by GeneXpert usually gave negative data, even in TB-assured cases, and could not be used in case of availability of other cheap and simple methods. The definite diagnosis of pleural TB is by pleural biopsy as tissue is the issue. The utility of medical thoracoscopy to diagnose pleural TB has been approved previously. Actually, medical thoracoscopy is less invasive as it is done under local anesthesia.

Keywords: ADA, TLC, TB, CBNAAT

Introduction

Tuberculosis is a bacterial disease spread from one person to another principally by airborne transmission. The causal agent is Mycobacterium tuberculosis (the tubercle bacillus). Tuberculosis can affect any organ in the body. Pulmonary tuberculosis is the most frequent

site of involvement; extrapulmonary tuberculosis is less frequent. Only pulmonary tuberculosis is infectious.[1]

The Xpert MTB/Rif test is a cartridge-based fully automated NAAT (nucleic acid amplification test) currently recommended by WHO [2] and adopted by revised national tuberculosis control programme run by government of India for detection of tuberculosis case and rifampicin resistance. The underlying principle of Xpert assay being detection MTB and rifampicin resistance by polymerase chain reaction-based amplification of the 81-bp rpoB gene segment and probing for the mutations that are related to rifampicin resistance. .[3]

The test is highly specific and does not give cross reactions with any other bacterial species including a comprehensive panel of mycobacteria thereby excluding non-tubercular mycobacteria. Although molecular amplification is already a proven technology in TB diagnosis, other existing tests are too complex for routine and widespread use in field conditions at peripheral level. GeneXpert, the test device platform, was launched by Cepheid in 2004 and simplifies molecular testing by fully integrating and automating the three processes (sample preparation, amplification and detection) required for real-time PCR-based molecular testing.[4]

The Xpert MTB/RIF test uses a cartridge containing all elements necessary for the reaction, including lyophilized reagents, liquid buffers and wash solutions. With observing aseptic technique, sample was collected in a falcon tube. The sample was loaded into cartridge and analyzed for presence of mycobacteria and rifampicin resistance in GX4 System (with 4 modules).

Materials and methods

Study design

Hospital based Cross-sectional observational study.

Study duration

From the approval of thesis to till achievement of the sample size, whichever is earlier.

Study place

Department of Respiratory medicine, S.P. Medical College and P.B.M Hospital, Bikaner

Sample size

A sample size calculation show that 100 patients was required as per reference article Mohammad Fawzi Al-Sokrom et al.

Sampling Method

Systematic random sampling

Study Group

A total of 100 laboratory diagnosed multi drug resistant tuberculosis patients of both sex, who have consented to participate in the study during treatment was selected.

Inclusion criteria

Patients with high suspicion of exudative tuberculous pleural effusion based on clinical signs and symptoms of tuberculosis confirmed with Light's criteria in addition to radiological proof of a pleural effusion considered large enough for a pleural biopsy, also highly positive tuberculin skin test and absence of other causes of pleural effusion.

Exclusion criteria

Any patient received anti-TB treatment within two months prior to the study or any patient with positive test for acid fast bacilli in sputum or patient with any contraindications to pleural biopsy procedure and patients with any chronic disease rather than tuberculosis

Data Collection

This study was undertaken with the approval of the Institutional Ethics Committee of S.P. Medical College and PBM Hospital, Bikaner, and written informed consent was obtained from each participant prior to participation in the study and the sample collection process.

A total of 100 patients were enrolled in the study and underwent thoracoscopy under local anaesthesia to obtain samples of pleural tissue. Pleural biopsies was stored in 4% formalin for his to pathology and saline solution for TB culture and Xpert assay. The Bactec method and solid culture medium was used for the detection of M. tuber culosis. Pleural tissue processing for the Xpert assay and culture was performed according to World Health Organization recommendations. The Xpert MTB/ RIF cartridge was used for detecting *M.* tuberculosis and rifampicin resistance.

Data Analysis

To collect required information from eligible patients a pre-structured pre-tested proforma was used. Data was collected and was analysed by required statistical test.

Results

Table 1: General characteristics

Mean age	51.23±6.38 Years
Male: Female	73:27
Site involved (left: Right)	54:46
CBNAAT present	32
ADA	81.32±20.02 IU/L
TLC (per cu mm)	2734±1421 per cu mm
Lymphocyte (%)	82.10±8.01%
Polymorphs (%)	17.02±8.5%
Sugar (mg/dl)	59.20±21.02
Protein (mg/dl)	4.12±0.78

32.00% patients CBNAAT was positive. Mean ADA level was 81.32 ± 20.02 IU/L. Mean total leucocytes level was 2734±1421 per cu mm, mean lymphocyte level was 82.10±8.01% and mean polymorphs level was 17.02±8.5%. Mean sugar level was 59.20±21.02 mg/dl, mean protein level was 4.12±0.78 mg/dl.

Discussion

ADA was elevated in the current study, with mean of 81.32±20.02 IU//L. Different studies like Tayand Tee [5] have explained that pleural fluid ADA in TPE is >40 IU/L, also it clarifies that there is correlation between pleural fluid ADA and age and pleural fluid protein as pleural fluid ADA decrease with older population and when pleural fluid protein are low.

Liang et al. [6] stated during interpretation of ADA levels that the clinicians should additionally be aware of situations which may increase the likelihood of both false-negative and false-positive ADA results, no specific pleural fluid biomarkers including ADA can give definite diagnosis of TPE, so ADA results should be interpreted in parallel with clinical findings and the results of con ventional tests, including microbiological examination and pleural biopsy.

In present study, mean total leucocytes level was 2734 ± 1421 per cu mm, mean lymphocyte level was 82. 10 ± 8.01% and mean polymorphs level was 17.02±8.5%

Pleural fluid cytology showed excess lymphocytes (82.01%) and neutrophil (17.02%), and other inflammatory cells like macrophages (18.31%). This agrees with Bays and Pierson ,[7]but with variable percentages. This also agrees with San José et al. [8].

In the current study, the medical thoracoscopic picture showed that more than half of patients showed thickened parietal pleura with simple adhesion and diffuse micronodules (59.15%), whereas 32.39% of studied patients presented with thick pleura only, and 8.45% of them had extensive capsular fibrin deposition, and this agrees with another study[9]

In the present study regarding assessment of GeneXpert in pleural fluid, it gave positive data in 32 (32.00%) cases of studied patient. Sajed et al. [7] reported that GeneXpert was positive in 15.8% of total studied patients. Moreover, Shukla et al. [11] found GeneXpert detected MTB in 20.58% pleural effusion cases

Conclusion

Regarding the current results, assessment of pleural fluid by GeneXpert usually gave negative data, even in TB-assured cases, and could not be used in case of availability of other cheap and simple methods. The definite diagnosis of pleural TB is by pleural biopsy as tissue is the issue. The utility of medical thoracoscopy to diagnose pleural TB has been approved previously. Actually, medical thoracoscopy is less invasive as it is done under local anesthesia.

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