“ROLE OF TB PCR IN CLINICALLY DIAGNOSED AND TREATED CASES OF TUBERCULAR PLEURAL EFFUSION”.

Medicine

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ABSTRACT

Background: Tuberculosis is still a major health problem worldwide. It is estimated that about one-third of the world's population is infected with mycobacterium tuberculosis. While pulmonary tuberculosis is most common presentation; pleural effusion is one of the common complications of primary tuberculosis or in conjunction with pulmonary infiltrate typical of post primary tuberculosis. Diagnosis of pleural tuberculosis (TB) remains a challenge due to its nonspecific clinical presentation and paucibacillary nature. TB PCR is a self-automated method from Roche for the detection of mycobacterium tuberculosis. This test allows for detection and identification of MTB from direct specimens within 24 – 48 hours of the receipt in the laboratory. We determined the role of TB PCR in diagnosis of tubercular etiology in pleural effusion in our study.

Materials and methods: Prospective and analytical study in a tertiary care centre of the study area was conducted after Ethics Committee permission. The study period was from June 2018 to December 2019. Data of 36 tubercular pleural effusion patients was retrieved. Role of TB PCR in the diagnosis of tubercular etiology was assessed.

Results: Pleural fluid samples collected were sent for various investigations like Routine microscopy, cytology, Pleural fluid pH, protein, LDH and TB PCR. Serum protein and serum LDH were also done. The diagnosis of Tubercular Pleural Effusion was made by clinical examination, radiological presentation, results of pleural fluid investigations and response to Anti Tubercular treatment collectively. Out of the 36 patients diagnosed to have tubercular pleural effusion, 8(22%) patient's pleural fluid TB PCR report was positive.

Conclusion: TB PCR is not a useful test in the diagnosis of tubercular etiology in pleural effusion.

KEYWORDS

Tuberculosis, extrapulmonary tuberculosis, pleural effusion, tubercular pleural effusion, TB PCR, polymerase chain reaction.

INTRODUCTION:

Tuberculosis is still a major health problem worldwide. It is estimated that 8–9 million new cases develop every year¹. India is the highest tuberculosis burden country. The recent increase in tuberculosis in the world and the emergence of multidrug-resistant strains have prompted more rapid and sensitive methods in the laboratory diagnosis than previous conventional diagnostic techniques²⁻⁴. The diagnosis of tuberculosis at an earlier stage would be advantageous, because it is less contagious and is associated with a lower morbidity and mortality during the early stage.

Tuberculosis can potentially involve any system or organ of the body. Tuberculosis may involve pulmonary as well as extrapulmonary sites (EPTB); former constituting the bulk of the burden. Pulmonary tuberculosis is most common presentation; extrapulmonary tuberculosis is also an important clinical problem^{3,4}. Diagnosis of pulmonary tuberculosis poses lesser difficulties when compared to extrapulmonary tuberculosis. Pleural effusion is one of the common complications of primary tuberculosis or in conjunction with pulmonary infiltrate typical of post primary tuberculosis.

The obvious explanation for the development of the tuberculous pleural effusion is that the delayed hypersensitivity reaction increases the permeability of the pleural capillaries to protein, intense inflammatory reaction in the parietal pleura impedes the lymphatic drainage from the pleural space and leads to the accumulation of pleural fluid⁵. The diagnosis of tuberculous pleuritis depends on the demonstration of tubercle bacilli in the sputum, pleural fluid, or pleural biopsy specimen. Pleural fluid smear for AFB is positive in less than 10 percent instances in most reports, while mycobacteria can be cultured from pleural fluid in 10-70 percent cases in various studies⁶.

The tubercular pleural fluid is serous or hemorrhagic with formation of coagulum. Pleural fluid is an exudate with proteins frequently more than 5 g/dl and lymphocytic predominance. Pleural fluid glucose may be reduced but is usually similar to the serum glucose level. The pH is

usually above 7.3 but may be reduced in some cases. The lactate dehydrogenase level in the pleural fluid is usually higher than that of serum. Presence of eosinophils or mesothelial cells is unlikely. Intense lymphocytic infiltration covers both pleural surfaces and prevents the mesothelial cells from entering the pleural space. Various studies have confirmed that the pleural fluid from patients with tuberculosis rarely contains more than 5% mesothelial cells^{7,8}.

Diagnosis of pleural tuberculosis (TB) remains a challenge due to its nonspecific clinical presentation and paucibacillary nature. Several tests have been proposed for improving the diagnostic yield in pleural tuberculosis, including the measurement of inflammatory markers involved in the immune response. Adenosine deaminase (ADA) is a marker of lymphocyte activation and has been shown to be a useful diagnostic test⁹. ADA activity is usually measured with a colorimetric method, which is fast, inexpensive, reproducible and easy to perform¹⁰. However, the predictive value of ADA activity depends on the local prevalence of TB¹¹. Moreover, ADA activity can be increased in other diseases that present with pleural effusion, such as lymphoma, collagen vascular diseases and bacterial empyema, and it must be used with care in countries with a low incidence of TB¹².

For several decades smear microscopy and conventional culture techniques have been the mainstay of diagnostic testing for tuberculosis. While smear microscopy has poor sensitivity and issues related to quality control¹³, conventional solid culture techniques have the limitation of long turnaround time of several weeks. Liquid culture techniques were developed for early detection of MTB growth, but mean turnaround time of 21 days is still long for a diagnostic test to be effective in curbing transmission¹⁴. Such delays in diagnosis increase morbidity and mortality, predispose to secondary resistance and cause transmission of resistant strains.

More specific molecular-based tests are necessary in this setting. DNA amplification by polymerase chain reaction (PCR) has changed the entire approach in the identification of pathogens. This has enabled

early detection of mycobacterial DNA directly from clinical specimens, especially in effusions. TB PCR is a self-automated method from Roche for the detection of mycobacterium tuberculosis. This test allows for detection and identification of MTB from direct specimens within 24 – 48 hours of the receipt in the laboratory.

Aim:

To assess the utility of TB PCR test in the diagnosis of tubercular etiology in pleural effusion.

Objectives:

To find out the reliability of TB PCR as a single test in the detection of tubercular pleural effusion.

INCLUSION CRITERIA:

- Patients clinically diagnosed as tubercular pleural effusion.
- Chest X-ray / CT Chest suggestive of pleural effusion.
- Pleural fluid cytology suggestive of exudative fluid, lymphocytic predominance and absence of malignant cells

EXCLUSION CRITERIA:

- Patients not willing for the study.
- Patients who did not respond to six months of standard anti tubercular treatment.

METHODOLOGY:

The study is a prospective and analytical study conducted at MGM Hospital Aurangabad after obtaining permission of the institutional ethical committee.

The study used data from June 2018 to December 2019.

Patients who fulfill the inclusion criteria and give consent were included in the study.

After clinical examination, chest X-ray/CT chest and USG chest was done. Thoracentesis of the patients was done after taking written and informed consent. Pleural fluid samples were sent for various investigations like Routine microscopy, cytology, Pleural fluid pH, protein, LDH and TB PCR. Serum protein and serum LDH were also done.

The diagnosis of tubercular pleural effusion was made by clinical examination, radiological presentation, results of pleural fluid investigations and response to Anti Tubercular treatment upon completion of the course collectively. The results of TB PCR in patients diagnosed with tubercular pleural effusion were observed.

MATERIALS:

- Chest X-ray / CT Chest
- USG chest
- Pleural fluid cytology
- Serum protein and serum LDH
- TB PCR

RESULTS:

Total of 36 patients were included in the present study. Patients were selected in accordance with the inclusion and exclusion criteria. These were the patients who first visited the Respiratory medicine OPD of the tertiary care centre with various symptoms like fever, cough, chest pain, loss of appetite and loss of weight. After thorough clinical examination of the patients, they were subjected to chest X-ray or CT chest. The presence of pleural effusion was confirmed through USG chest. Subsequently all the patients were subjected to thoracentesis after taking written and informed consent. Pleural fluid samples were sent for various investigations like Routine microscopy, cytology, Pleural fluid pH, protein, LDH and TB PCR. Serum protein and serum LDH were also done.

The diagnosis of tubercular pleural effusion was made by clinical examination, radiological presentation, results of pleural fluid investigations and response to Anti Tubercular treatment upon completion of the course collectively.

Patients who did not respond to six months of standard anti tubercular treatment were excluded from the study.

Of the 36 patients with pleural effusion who were diagnosed to have tubercular etiology based on clinical examination, radiological

examination, pleural fluid reports and response to anti tubercular treatment, 8(22%) patient's pleural fluid TB PCR report was positive.

The above comparison of the results of clinical examination and various investigations which helped in the diagnosis of tubercular etiology of pleural effusion with that of TB PCR results point towards the fact that TB PCR is not a very useful or reliable test in the diagnosis of tubercular etiology in pleural effusion.

DISCUSSION:

Extrapulmonary tuberculosis contributes to a significant burden of morbidity and mortality due to its complex and subclinical presentations, leading to a delay in diagnosis. Diagnosis of pleural tuberculosis remains a challenge due to its nonspecific clinical presentation and paucibacillary nature. The conventional methods such as culture DST are time consuming and require trained laboratory personnel. Hence, there is a need for a more rapid method of examining pleural fluid for tubercular etiology.

TB PCR is a self-automated method from Roche for the detection of mycobacterium tuberculosis. This test allows for detection and identification of MTB from direct specimens within 24-48 hours of the receipt in the laboratory.

In a study conducted on Pleural fluid ADA, IgA-ELISA and PCR sensitivities for the diagnosis of pleural tuberculosis by A. TRAJMAN1, C. KAISERMANN2, R. R. LUIZ3, R. D. SPERHACKE4, M. L. ROSSETTI4,5, M. H. FERES SAAD6, I. G. SARDELLA6, N. SPECTOR2 & A. L. KRITSKI2, the sensitivities of pleural fluid culture and AFB smear were 42% and 1%, respectively. Sensitivities of ADA and PCR were 91% and 82%, while specificities were 93% and 85%, respectively. They concluded that the routine use of ADA activity measurement in pleural fluid can obviate the need for a pleural biopsy in the initial diagnostic approach to pleural effusions, while PCR technique, potentially more specific test, needs further refinement to improve its accuracy.

In another study conducted on Role of Nucleic Acid Amplification Tests (NAATs) in Tuberculous Pleural Effusion by Patil Shital, Halkanche Gajanan and Ayachit Rujuta, they subjected all the pleural fluid samples for DNA PCR and observed positive in 74 (74%) of the cases, among which 26 (26%) were culture positive, 50 were MT positive, 8 were sputum positive for AFB, 63 (63%) had an ADA greater than 40 units/liter. 64 of the PCR positive patients had a lymphocyte count greater than 50% in their pleural fluid. 65 patients had protein greater than 3.5 gm/dl. Sensitivity of PCR in the study was 92.85%.

In yet another study conducted on Rapid diagnosis of tuberculous pleural effusion using polymerase chain reaction by Reechaipichitkul W, Lulitanond V, Sungkeeree S, Patjanasoon B, The sensitivity of AFB-staining was 6%; while cultures on LJ media was 17%. The sensitivity of the PCR-assay was 50%. They concluded that PCR assay was more sensitive than AFB staining and mycobacteria culture for diagnosis of tuberculous pleural effusion.

While TB PCR has been said to be a useful tool in diagnosing pulmonary as well as extrapulmonary tuberculosis in various studies, our study (although does not include pulmonary and extrapulmonary tuberculosis cases other than tubercular pleural effusion) suggests that it is not much useful test in detection of tubercular etiology in pleural effusion.

CONCLUSION:

Various methods for rapid diagnosis of tubercular etiology in pleural effusion are being utilized so as to overcome the challenge of early

diagnosis which has been made difficult by the nonspecific clinical presentation and paucibacillary nature of the disease. TB PCR is one such method which has been claimed to be useful in various studies which is in contrast to our study. In our study, we conclude that TB PCR is not a very useful or reliable test in the diagnosis of tubercular etiology in pleural effusion.

Declarations:

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Conflict of interest: None declared

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