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Short communication

Tissue PCR Diagnosis of Patients Suspicious for Tuberculous Pleurisy

*[1]Mahnaz Amini, [2]Davood Attaran, [3]Kiarash Ghazvini, [4] Habibollah Esmaily, [4]Mahmood Bagheri

Abstract

Objective(s)
This study planned to assess the value of PCR IS6110 assay in tissue specimens of needle pleural biopsy in patients suspicious to pleural tuberculosis.
Materials and Methods
Sixty eight patients with lymphocytic exudative pleural effusion underwent pleural biopsy. Tissue samples were sent for pathologic examination and PCR IS6110 assay. The results of PCR reported as positive/ negative and assessed according to the current gold standard pathologic diagnosis**.**
Results
Twenty nine patients had tuberculous and 12 had malignant pleural involvement, respectively. The remaining 27 samples were reported as non-specific pleurisy. Results of PCR were positive in 35 out of 68 total subjects and in 19 out of 29 TB patients. Sensitivity and specificity of PCR were calculated as 67.9% and 62.5%, respectively.
Conclusion
An acceptable sensitivity and specificity for PCR examination of pleural tissue can serves it as a useful adjunct in undergoing needle pleural biopsy for possibility of tuberculosis.

Keywords: DNA Primers, *Mycobacterium tuberculosis*, Pleural, Polymerase chain reaction

1- Department of Internal Medicine , Imam Reza Hospital, Mashhad University of Medical Science (MUMS), Mashhad, Iran
* Corresponding author: Tel: +98-511-8598818; Fax: +98-511-8598818; email: aminim@mums.ac.ir
2- Department of Internal Medicine, Ghaem Hospital, Lung and Tuberculosis Research Center, MUMS, Mashhad, Iran
3- Department of Mycobacteriology, Ghaem Hospital, MUMS, Mashhad, Iran
4- School of Medicine, MUMS, Mashhad, Iran

Introduction

Tuberculosis (TB) is a major health problem especially in developing countries. The most effective approach for controlling the disease is accurate diagnosis and treatment of active TB cases (1). Tuberculous pleural effusion occurs in up to 30% of patients with pulmonary TB (2) and constitutes the major portion of the extrapulmonary TB morbidity (3). Diagnosis of tuberculous pleural effusion depends on the demonstration of the presence of tubercle bacilli in the sputum, pleural fluid, or pleural biopsy specimen, or the demonstration of granuloma in the pleura by histo-pathologic examination (4). In many cases, the findings of the histo-pathologic examination of the pleural biopsy specimen may be negative or nonspecific (4). Microbiological methods for diagnosing tuberculous pleural effusion include acid-fast bacilli (AFB) stain and laboratory culture of the causative organism *Mycobacterium tuberculosis.* The detection of mycobacterium in pleural fluid by microscopy has low sensitivity and cannot be used to distinguish between the various members of the mycobacterium genus (5). On the other hand, laboratory culture of *M. tuberculosis* is sensitive, but it takes up to 8 weeks to yield a positive result (6, 7). Diagnostic assays based on nucleic acid amplification methods such as polymerase chain reaction (PCR) dramatically decrease the time required to identify an organism in clinical specimens (8). PCR also has been used to detect *M. tuberculosis* in pleural fluid samples, with highly variable sensitivity (11 to 81%) in different studies (9-11). Comparatively little work has focused on the utility of PCR in detecting *M. tuberculosis* in pleural biopsy specimens.

Chen (12) examined 212 pleural fluid specimens suspected to be possibly associated with tuberculosis with negative acid-fast smears to test for the presence of *M. tuberculosis* DNA, using nested PCR. The target for the amplification was a segment of IS6110 in the genome of *M. tuberculosis*. The final diagnosis of TB pleurisy was based on combining clinical judgment with radiologic findings, microbiologic tests, and the histo-pathologic findings. Forty-nine patients were excluded due to incomplete or inconsistent clinical information. Of 163 patients enrolled, PCR was positive in 23 (43.4%) of 53 patients with TB pleurisy and 5 (4.5%) of 110 patients with non-TB pleurisy, with a sensitivity and specificity of 43.4% and 95.5%, respectively. Positive culture of pleural fluid was found in 15 (28.3%) of the TB pleurisy group and none in the non-TB group. Fifteen patients (55.6%) of 27 with pleural biopsy demonstrated chronic granulomatous inflammation with or without caseous necrosis. Of these 27 patients, PCR was positive in 12 (44.4%).

Liu *et al* (13) carried out a cross-sectional, observational study on 74 patients with pleural effusions of varying etiology. Soluble TREM-1 (sTREM-1) was measured in pleural fluid samples. Concentrations of sTREM-1 were significantly higher in infectious and neoplastic pleural effusions (189.1±36.7 and 69.9±22.8 ng/ml, mean±SEM) than in transudates (10.1±5.3 ng/ml, $P<0.001$). Among infectious effusions, the sTREM-1 levels were significantly higher in para-pneumonic than in tuberculous effusions (301.8±49.8 vs. 38.9±17.3 ng/ml; $P<0.001$).

In this study, an attempt was made to find the value of a PCR assay (IS6110) specific for *M. tuberculosis* to validate its results according to pathologic findings.

Materials and Methods

The present study comprised patients with lymphocytic exudative pleural effusion, admitted to Ghaem Hospital, Thoracic Medicine Department, Mashhad University of Medical Sciences, Mashhad, Iran, from Aug 2004 to Dec 2007. Informed consent was received from each patient involved in the study. Patients with other types of pleural effusion, such as transudates, exudates with dominancy of poly-morphonuclear cells, empyema, hemithorax and chylothorax were excluded from this study. The study was performed prospectively in a blinded manner in which the clinical diagnosis was not available to the laboratory personnel. Each patient underwent the following procedures:

Monteux tuberculin skin test, with indurations of ≥ 10 mm considered to be positive; chest radiographs in the posterior-anterior (PA) and lateral views; and

ultrasound of the pleural space, if needed, sputum examination, including AFB staining, and culture; and diagnostic thoracentesis and pleural biopsy, using the Abram pleural biopsy needle.

Twenty milliliters of the pleural fluid was aspirated and sent to the Microbiology Department to be subjected to decontamination and concentrated by centrifugation for 20 min at 10,000 g and at 4 °C. The sediments were re-suspended in sterile normal saline solution, and used for AFB smear and culture. The primer set (up-stream 5'-CCT GCG AGC GTA GGC GTC GG-3', downstream 5'-CTC GTC CAG CGC CGC TTC GG'-3') were designed to amplify a 123 bp sequence of IS6110 (specific for *M. tuberculosis* complex). IS6110 is an insertion sequence present in multiple copies within the chromosome of the *M. tuberculosis* complex, thus accounting for its high sensitivity. The pleural biopsy was performed, using Abram needles, and 3 to 6 punches of parietal pleura were divided equally into two parts, one part fixed in formalin for histo-pathologic examination and AFB smear and the other part put into sterile distilled water, homogenized and used for PCR study by IS6110 (CinaGen, Iran). In this reaction, PCR mixture contained 2.5 µl 10× buffers, 2.5 µl deoxynucleoside triphosphates, and 1 µl of each primers, to which 0.3 µl Taq DNA polymerase, 7.7 µl distilled water, 10 µl target DNA extracted from each pleural biopsy sample were added. The thermal cycle for this reaction was programmed as initial denaturation at 94 °C for 5 min and 30 cycles of denaturation at 94 °C for 2 min, annealing at 68 °C for 2 min, and extension at 72 °C for 2 min. The amplified products were detected by gel electrophoresis, using 1.5% agarose gel (sigma) with ethidium bromide, and the presence of 123 bp amplified band indicated the presence of *M. tuberculosis*. Precautions to avoid cross-contamination and false-positive results were taken in every assay. Each set of the PCR reaction contained a positive control containing DNA extracted from TB bacilli.

The results of clinical evaluation and diagnostic tests were analyzed, using computer software (SPSS, version 10). The results of PCR were compared to the final diagnosis of TB, with individual patient used as the unit of analysis. Continuous data were shown as mean±SEM. The categorical variables were compared, using the χ^2 test, with a *P*-value of <0.05 considered to be significant.

Results

The most common final diagnosis of our patients was pleural tuberculosis, with 29 out of 68 patients (42%) diagnosed as pleural tuberculosis. Twelve patients had malignant infiltration of pleural tissue and the remainder (27 patients) had chronic non-specific pleurisy. Of 29 TB patients, 24 were men and 5 were women, with age of 51.9±20.8 years (mean±SEM). Among those 12 patients with malignant infiltration of the pleura, 7 were men with the age range of 67.1±14.4 years (mean±SEM). Using logistic regression concerning both gender and malignancy it was found that female gender are more susceptible to pleural TB than malignancy.

Results of PCR IS6110 were positive in 34 out of 68 total patients, and in 19 out of 28 TB patients. The remainder 15 positive PCR IS6110 results belonged to patients with non-specific biopsy reports, with just 1 patient in 12 malignant patients who had positive PCR (Figure 1).

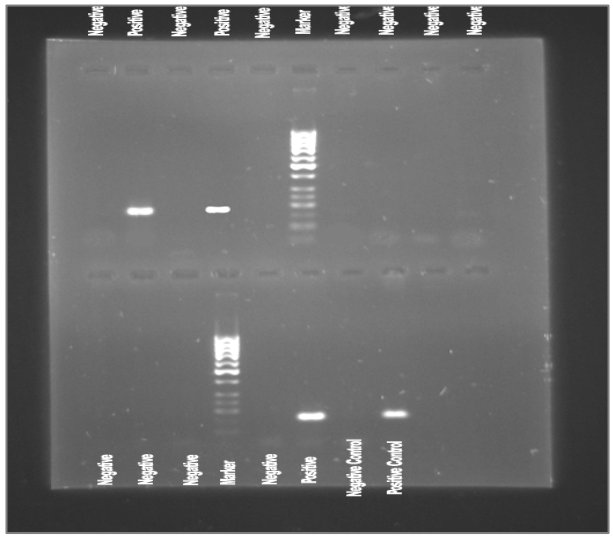

Figure 1. PCR IS6110 report: Two columns of pleural tissue extract samples (right and left) - The 3rd sample from bottom left is positive, as this has the same chromatographic 123 bp amplified band of the positive control. The other samples were reported as negative, with chromatographic patterns different from positive control. Negative and positive controls are seen at the bottom of the left panel.

Available chest radiographs of TB patients were also reviewed. Most of them (11 out of 17) had right sided effusion, 5 patients with left-sided effusion and 1 patient showed bilateral pleural effusion (with right side predominant). Fluid occupied 1/3 of hemithorax in 10 patients, half of hemithorax in 6 and more than 2/3 in 1 patient. It was observed that lung parenchymal infiltrates was concurrent with pleural effusion in 6 out of 17 patients. By using contiguity table (Table l), the sensitivity and specificity of PCR IS6110 were calculated as 67.9% and 62.5%, respectively. Negative and positive predictive values of 73.5% and 55.9%, were also observed respectively for PCR IS6110.

Table 1. Contiguity table results for IS6110 in cases of pleural TB.

		Tuberculosis		
		Negative (%)	**Positive (%)**	**Total (%)**
PCR IS6110	**Negative No. (%)**	25 (62.5%)	9 (32.1%)	34 (50%)
	Positive No. (%)	15 (37.5%)	19 (67.9%)	34 (50%)
	Total (%)	40 (100%)	28 (100%)	68 (100%)

Discussion

Differential diagnoses of exudative pleural effusion are broad and rapid diagnosis of its most common diagnosis (tuberculous pleurisy) would greatly facilitate the management of the patients. Rapid diagnosis and treatment of tuberculous pleural effusion is crucial to reduce its morbidity and mortality from untreated TB (1).

Pleural TB is commonly in its pauci-bacilliary form (few bacilli enter the pleural space from a small adjacent focus of inflammation in lung parenchyma caused by primary *M. tuberculosis* infection), so the results of pleural fluid smear are frequently dismissing. At present, the most reliable method for the diagnosis of tuberculous pleural effusion is the identification of *M. tuberculosis* in the pleural specimens (14).

The goal in this study was to improve the diagnosis of tuberculous pleural effusion, by using PCR IS6110; and to determine sensitivity and specificity of this rapid diagnostic method.

A reasonable sensitivity of 67.9% for PCR IS6110 was shown in this study. Most of the studies have shown a sensitivity of 11 to 81% (9, 11). Specificity of PCR IS6110 in this study was 62.5%. The other studies have shown higher specificities for PCR pleural fluid assays (up to 95.5%). As the malignant group of pleural involvement was not supposed to have tuberculosis, specificities of the test were analyzed again in these 12 patients and specificity of 91.7% ensued. It has been considered that the single result of positive PCR among 12 malignant pleural biopsies may be a false positive result. Although that subject did not have any personal or family history of TB and had negative tuberculin skin test, there can be two other explanations for this positive result. Multiple studies have shown a higher incidence of tuberculosis in malignancy (14, 15) and the possibility of cross contamination of biopsy needle with DNA from a previous sample containing *M. tuberculosis*.

Conclusion

Rapid access to PCR results can help the physician with making the right decision to treat which patients empirically. The use of PCR (IS6110 primer) can be a time and cost saving tool in diagnosis of tuberculous pleurisy by its potential for rapid detection of the Mycobacterial DNA, and it can prevent the spread of disease in those forms concomitant with parenchymal disease. Tissue PCR assay in every patient planned for closed pleural biopsy by needle is recommended. Treatment of those who show positive results in appropriate clinical setting seems advisable.

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