Clinico-radiological profile and diagnostic yield of various procedures in analysis of tubercular pleural effusion

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Abstract

Background: Tuberculosis is the leading cause of pleural effusion in developing countries. Early diagnosis and treatment of this condition is imperative as it is associated with high morbidity and mortality. Pleural biopsy has been considered the gold standard in diagnosis of TPE but it is invasive, so that pleural fluid markers of TPE have been extensively evaluated as an alternative to pleural biopsy.

Methods: Prospective study included 100 cases with Detailed clinical history, Radiological examination and NAATs i.e. MTB DNA PCR with other conventional diagnostic techniques like pleural fluid biochemistry, ADA, cytology and culture for mycobacterium tuberculosis was carried out in all patients.

Results: Out of total 100 cases, 09% cases were sputum positive for AFB, 3% pleural fluid positive for AFB, 28% were culture positive, 74% were DNA PCR positive, and 85% cases had ADA >40 units/liter, 87% cases had a LN Ratio greater than 0.75. Sensitivity, specificity, PPV & NPV of PCR for MTB was observed 92.86%, 33.33%, 35.13% & 92.30% respectively ($p<0.01$). ATT response was observed in 78% cases in 2 weeks, 98% cases in 4 weeks and 100% cases at the end of 6 weeks.

Conclusions: Delay in diagnosis of TPE leads to sequelae as well as spread of infection to other organs. More than one diagnostic procedure is often needed for confirmation of TPE. PCR targeting IS6110 is the simple, rapid and highly sensitive test used in the early diagnosis of TPE.

Keywords: ADA; MTB DNA PCR; Tuberculous pleural effusion

Introduction

Pleural effusion is an abnormal collection of fluid in the pleural cavity[1]. Malignancy and tuberculosis (TB) are major causes of pleural effusion which are usually exudative[2]. Tuberculosis is a global health problem, as it is the second most common cause of death from infectious diseases[3]. One third of the world's population is estimated to be infected with Mycobacterium tuberculosis and eight million active cases occur annually[4,5]. According to W.H.O. in 2013, 9 million people became ill with TB and 1.5 million died[6], most of which occurred in developing countries[7] like India. Usually tuberculous pleural effusion (TPE) can resolve spontaneously, if untreated 65% of cases can develop active TB[8]. Hence rapid and accurate diagnosis with early treatment is necessary. Conventional methods for diagnosing TB were found to have low sensitivity and specificity[5].

The diagnosis of tuberculous pleuritis depends on the confirmation of tubercle bacilli in the sputum, pleural fluid, or pleural biopsy specimen, or the demonstration of granulomas in the pleura. 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 studies[9]. The role of nucleic acid amplification tests i.e. MTB DNA PCR in the diagnosis of TPE has been evaluated extensively as an alternative diagnostic tool and has yielded variable results, with sensitivities ranging between 42 and 100% and specificities ranging between 85 and 100% using various PCR targets such as IS6110, 65kDa, TRC4, GCRS, etc[10]. Low sensitivity values can be explained by the low bacillary load and the presence of substances that inhibit amplification in pleural fluid. There is possibility of a false positive PCR finding due to the presence of old healed tuberculosis infection in a patient having non tubercular effusion due to other diseases[11].

Materials and Methods

This is a prospective study, conducted in department of Pulmonary Medicine, Dr. S.N. Medical College, Jodhpur, India during Jan 2014 to Sept. 2015. The Institute Ethics Committee approved the protocol and written informed consent was obtained from all patients. Detailed clinical history, Radiological examination and NAATs i.e. MTB DNA PCR with other conventional diagnostic techniques like pleural fluid biochemistry, ADA (adenosine deaminase level), cytology and culture for mycobacterium tuberculosis was carried out in all patients. Other investigations like complete blood counts, sputum for AFB (acid fast bacilli), tuberculin testing were also done.

The effusion were classified as exudative and transudative according to Lights criteria[12]. ADA estimation*:* ADA activity was measured by the method of Guisti et al[13].

Inclusion criteria

1. Patients of age of more than 12 years.

2. Pleural effusion by clinical examination, chest X ray and ultrasonography
3. Exudative pleural effusion by Light's criteria.

Exclusion criteria

1. Patient's refusal for pleural fluid aspiration.
2. Cases with empyema thoracis.
3. Transudative pleural effusion.
4. Hemothorax
5. Contraindication to thoracocentesis like patient on mechanical ventilation, uncooperative patients, bleeding diathesis, patient on anticoagulation therapy
6. Patients on antitubercular chemotherapy.

Statistical analysis

Statistical analysis was done mainly by student t-test to compare two variables and Chi square test when comparing many variables. P value was considered significant if it was below 0.05 and highly significant in case <0.001.

Result

100 patients enrolled in the study (males were 77 and 33 were females). The mean age of presentation was 45±17.83 years. Majority of the patients were in the age group 21-50 years (52%), minimum age of 13 years and the maximum of 82 years. 7% had past history of tuberculosis and 14% had family history of being treated for tuberculosis. Most common symptom was chest pain (98%) followed by cough (93%), fever (72%), dyspnea (71%), expectoration (37%). Commonest side involved was right (61%), in 4% of the cases the effusion was bilateral whereas 96% had a unilateral pleural effusion. 9% of the patients had a sputum smear positive for tubercular bacilli. 59 patients (59%) of the patients had a positive Mantoux test. Pleural fluid examination shows 3% were positive for AFB, 28% were culture positive, 74% were DNA PCR positive, and 85% cases had pleural fluid ADA >40 units/liter, 87% cases had a LN Ratio greater than 0.75. Combined yield of pleural fluid culture, ADA>40 units/liter, DNA PCR and LN ratio >0.75 gave a positive diagnostic yield in 98% of cases, 2% with diagnostic dilemma were diagnosed by pleural biopsy (Table 3).

Table 1: Various parameters of the population studied (N=100)

Parameter	Mean
Age	45.20±17.83
Gender(M/F)	77/23
Residence(U/R)	52/48
Complete blood counts	
HB	10.92±1.75
ESR	60.7±30.34
TLC	10819±6018
Neutrophil (%)	75.35±9.02
Lymphocyte (%)	20.17±8.23
Others (%)	4.63±3.52
Pleural fluid	
PH	7.31±0.09
Sugar	64.14±13.99
Protein	4.70±1.00
ADA	70.69±48.29
TLC	1565±1591
Neutrophil (%)	18.89±21.62
Lymphocyte (%)	74.75±22.09
Others (%)	6.36±5.61
LN Ratio	11.63±11.59
ZN Stain	3
DNA PCR(MTuberculosis)	74
L J Culture	28

Table 2: Comparing MTBPCR (NAAT) results with pleural fluid MTB Culture

	Culture +ve	Culture -ve	Total
PCR +VE	26	61	87
PCR –VE	2	11	13
	28	72	100
(chi square 7.18,DF=1, p value<0.01) Sensitivity=92.86% Specificity=33.33% Positive Predictive Value=35.13% Negative Predictive Value=92.30%			

Table 3: Yield of various diagnostic procedures

Parameters	Yield (N)	Percentage
Pleural fluid AFB smear	03	03%
Sputum for AFB	09	09%
Pleural fluid culture	28	28%
PCR	74	74%
Pleural fluid ADA >40 units/liter	85	85%
Pleural fluid lymphocytes >50%	87	87%
LN Ratio 0.75	91	91%
Pleural fluid Fluid lymphocyte >50% +LN CulturE+PCR+ ADA+Pleural ratio>0.75	98	98%
Pleural fluid culture+PCR+ ADA+LN Ratio+Pleural biopsy	100	100%

Discussion

Role of pleural fluid DNA PCR: Sensitivity of PCR in our study was 92.85% and Specificity of 33.33%, Positive predictive value of 28.57%, Negative Predictive Value of 77.77%, this low specificity can be explained by cross contamination during the procedure which is a common problem in laboratories using in house protocol.

Reechaipichitkul et al.[17] mentioned a sensitivity of 50% and specificity of 61% and had PCR positive in 100% of culture positive TB effusion and only in 30-60% of culture negative pleural fluid. Chakravarthy et al.[18] found 40 PCR positive cases out of 53 patients studied (75.47%), with a sensitivity of 75.5% and specificity of 93.8% PPV 97.6% and NPV 53.6.

In this study, 2 cases with culture positive report were found to have MTB PCR negative (false negative) this can be caused by technical error in PCR processing, presence of inhibition enzyme DNA polymerases, destruction of DNA in extraction process and sample containing no specific or low amount of DNA and false positive due to dead bacilli, bacilli not having active metabolism (dormant) or contamination and inflammation reaction to part of mycobacterium TB.

In our study 87 patients had PCR positive and 28 patients had culture positive, is the result of high sensitivity of PCR compared with the culture which needs 50-100 bacilli/ml sample, dead bacilli, bacilli not having active metabolism (dormant) or contamination and inflammation reaction to part of mycobacterium TB another possible reason was uneven distribution/ mycobacterium forms a group at the infective site.

Role of Pleural Fluid ADA in our Study: 85% of the patients had ADA levels > 40 units /liter and the mean ADA was of the patients studied was 70.69±48.29. Among them 25 (25%) were culture positive and 60 (60%) were culture negative and the mean ADA in culture positive cases and negative cases were 64.82±34.11 and 72.96±52.82 units/liter with no significant difference between the values. All 9 sputum ZN positive patients had an ADA >40 units/litre, 63 of the 85 cases with ADA > 40 units/liter had positive PCR DNA for mycobacterium tuberculosis. 76 of the 85 patients with ADA >40 units/liter had a LN ratio >0.75.75 of the 85 patients with ADA >40 units/liter had a protein level >3.5 g/dl in pleural fluid. Sensitivity of ADA in our study was 89.28%.

Patel et al.[19] observed mean ADA was greater than 40 units/liter in their patients. Swamy et al.[20] reported a mean ADA of 100±19.48.

Conclusions

Delay in diagnosis of TPE leads to sequelae as well as spread of infection to other organs. More than one diagnostic procedure is often needed for confirmation of TPE. PCR targeting IS6110 is the simple, rapid and highly sensitive test used in the early diagnosis of TPE. In cases with exudative pleural effusion with Lymphocyte in pleural fluid >50% and L/N ratio>0.75 with ADA <40 units, MTB DNA PCR (NAATs) will be very useful in confirming tuberculosis as a cause for pleural effusion. This can be applied when there is strong clinical suspicion, especially when the conventional techniques are negative.

Conflicts of interest: None declared

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