Sky Journal of Medicine and Medical Sciences Vol. 1(1), pp. 7 - 15, January, 2013
Available online http://www.skyjournals.org/SJMMS
ISSN 2315-8808 ©2013 Sky Journals

Full Length Research Paper

The diagnostic efficacy of polymerase chain reaction and adenosine deaminase in tubercular effusion

Mohd Arif Kelam[1], Bashir Ahmad Shah[1], Farooq Ahmad Ganie[2]*, Shabir Ahmad Ganie[3], Masaratul- Gani[4], Hafeezulla Ione[2]

Department of General Medicine[1], Cardiovascular and thoracic surgery[2] SKIMS, Soura, Kashmir - 190 011, kidney Hospital Srinagar[3] and J and K Health services[4].

Accepted 02 January, 2013

The aim of this study was to evaluate the efficacy of combination of adenosine deaminase and polymerase chain reaction in the diagnosis of tubercular effusions. This study was conducted in the department of General Medicine and Cardiovascular and thoracic surgery SKIMS for a period of two years between November 2008 to November 2010. A total of 57 patients presenting with pleural effusion during these two years were included in this study. Patient's criteria: In order to be included in this study, patients had to present with clinical manifestations suggestive of tuberculosis, i.e., the presence of productive cough, low-grade fever, night sweats, weight loss and chest pain, especially if these symptoms last ≥ 4 weeks. If the patients presented with less than two of these symptoms and especially if the clinical manifestations were of < 4 weeks in duration, they were excluded from the study. The combination of both ADA and PCR with a positive result by either of the two methods increased the sensitivity over both the tests. When used in combination, both ADA and PCR picked up 38 out of the 39 diagnosed pleural tuberculosis patients increasing the sensitivity to 97.4% at the cost of specificity (50%). Pleural biopsy was performed in 37 of the 57 studied patients. Out of the 39 patients with pleural tuberculosis, pleural biopsy was performed in 21 patients. Histological findings suggestive of tuberculosis were found in six patients. In our study the sensitivity of pleural biopsy was only 28.6% and a specificity of 100%. The combination of ADA and PCR improved the sensitivity to 97.4% which was much higher than pleural biopsy (28.6%), PCR (51.3%) and even ADA (89.7%) but at the cost of specificity which dropped to 50%. Comparison of sensitivity and specificity of this combination to ADA and PCR and to the "gold standard" pleural biopsy supports the feasibility of using the PCR of pleural fluid together with determination of ADA activity as the first diagnostic approach in circumstances in which pleural tuberculosis is suspected and access to procedures such as needle biopsy of pleura is limited.

Key words: Polymerase chain reaction (PCR), pleural effusion, tuberculosis, pulmonary tuberculosis (PTB), adenosine deaminase (ADA).

INTRODUCTION

Tuberculosis still remains an important cause of morbidity and mortality worldwide. Recent estimates show that 8 - 10 million new tuberculosis (TB) cases occur each year in the world and 2 - 3 million die (Singh et al., 2004). In developing countries, TB is one of the common opportunistic infections in people who are seropositive for HIV (Harries, 1990). Tuberculosis is classified as pulmonary, extra pulmonary or both (Mario et al. (2001). Pleural tuberculosis accounts for fewer than 1% of all exudative effusions in western countries, occurring in only 3 - 5% of tuberculosis patients and in developing countries like India, it is responsible for 30 - 80% of all pleural effusions encountered (Udwadia, 2010). Pleural effusions in TB usually have lymphocytic and exudative characteristics (Aggarwal et al., 1999). Exudates are due to pleural inflammation (pleurisy), with an increased permeability of the pleural surface to proteinaceous fluid and various types of cells. Lymphatic obstruction may also contribute to accumulation of pleural fluid (Sahn, 1995). Tubercular pleuritis is usually associated with primary disease and in those cases results from the rupture of sub-pleural focus, which may not be evident radiologically (Rossman et al., 1993). It may also result

*Corresponding author. Email: ganiefarooq@yahoo.com. Tel: 09469064259.

from intra-pulmonary cavity or lymphohematogenous dissemination, or from an adjacent source e.g., lymph node or spine (Rossman et al., 1993). Hypersensitivity to the tubercle bacillus also plays an important role in determining the occurrence and amount of pleural effusion. Tuberculous pleurisy is thought to be the result of a delayed hypersensitivity reaction in response to the presence of mycobacterial antigens in the pleural space (Kataria and Khurshid, 2001). This immunologic reaction causes the stimulation and differentiation of lymphocytes, which release lymphokines, which in turn activate macrophages for an enhanced bactericidal effect (Kataria and Khurshid, 2001). The difficulty in determining the cause of a pleural effusion, which is shown by the "unknown etiology" rates of up to 20% in some published case series, is largely due to the great variety of diseases that can bring about this condition. The etiology of pleural effusions depends on the geographic region, patient age and advances in the diagnosis and treatment of the underlying cause (Valdes et al., 1996). Due to the limitations of conventional tests and the delay of several weeks for mycobacterial culture results, there has been a resurgence of interest in newer rapid tests and biomarkers. New approaches for the rapid detection of mycobacterial growth have been developed with the aim to reduce the time needed for diagnosis. Measurement of adenosine deaminase (ADA) activity levels, have proven to be sensitive and specific for pTB in special circumstances, such as in regions with a high prevalence of tuberculosis (Villegas et al., 2000). The levels of adenosine deaminase (ADA), an enzyme found in most cells, are increased in tuberculous pleural effusions and this determination has acquired popularity as a diagnostic test in high-incidence areas for TPE because it is not invasive, the assay is not expensive and it is readily accessible. The polymerase chain reaction (PCR) is a new strategy used for the tuberculosis diagnosis and two kits have been approved by the US Food and Drug Administration for use on clinical samples; however, their cost is prohibitive for developing countries where tuberculosis remains an important public health problem. PCR has been used to detect mycobacterial DNA in pleural fluid, with sensitivities ranging from 20 to 80% and specificities of 78 to 100%, depending on the area of the genome that is amplified and the technique used for DNA extraction (De Wit, 2000). The high biological sensitivity and specificity of PCR for *M. tuberculosis* suggest that this method, when used in combination with measurement of ADA activity levels, could improve the efficiency of the laboratory diagnosis of PTB (Villegas et al., 2000). when used alone, PCR and ADA activity do not yield very good results, but when used in combination, they can be very useful as additional diagnostic methods for the achievement of a more rapid and precise diagnosis of pTB. When combined, sensitivity increases at the expense of specificity.

Pleural biopsy is usually considered important for the diagnosis of pleural effusions, especially for distinguishing between tuberculosis and neoplasia, even though tuberculous pleural fluid contains sensitive biochemical marker (Valdés et al, 1995). Results of needle pleural biopsy have been reported positive in 50 – 80**%** of cases of tuberculous pleurisy (Light, 1995; Danielle et al., 2003).

MATERIALS AND METHODS

Methods

The patients included in this study were subjected to a thorough clinical examination and extensive investigations to establish a diagnosis of TB effusion. Complete hemogram, chest x-ray, chemistry including s.LDH, mantoux test were done. Pleural fluid was subjected to a detailed analysis including TLC, DLC, sugar, protein, LDH, gram stain, AFB stain and, M cells. Sputum AFB staining, pleural fluid ADA and PCR was done in all. Pleural biopsy was done in all the patients after obtaining an informed consent. Patients who did not give consent/ or had any contraindication were not subjected to biopsy).

Case definition of tuberculosis

Cases of pTB were defined as those patients with clinical manifestations suggestive of tuberculosis presenting with one of the following:

i.) A positive Ziehl-Neelsen result of the pleural fluid or other biological material (sputum, BAL, lymph node aspirate).
ii.) Histopathologic findings suggestive of tuberculosis on pleural biopsy (presence of caseating granulomas was considered as tuberculosis).
iii.) Patients with clinical and radiologic findings that lack microbiological/histopathologic confirmation but responded positively to empirical therapy were also considered tuberculosis cases. A positive response to therapy was defined as the improvement of clinical and radiologic findings after 2 months of the therapy. The patients were divided into two broad categories:
a) Tuberculous (as per our broad definition of TB)
b) Non -tuberculous

Laboratory evaluation of pleural fluid

A single specimen of pleural fluid (50 - 100 ml) was submitted for cytological examition, ZN staining, ADA activity determination and PCR. Pleural fluid samples were concentrated by centrifugation for 20 min at 10,000rpm at 4°C. Concentrated sample was taken for ZN staining. ADA activity was determined in 1 ml of pleural sample using the calorimetric method described

by Giusti and Galanti, 1974). A positive result was defined as a value > 40.0 IU/L, which is based on previous studies of pleural fluid samples of patients with proven tuberculosis. Polymerase chain reaction: In this study, the target for the PCR assay was MPB64 gene which codes for an immunogenic protein.

Sample collection

Pleural fluid samples were collected in sterile plastic containers with a tight cap and was stored at -20°C till processed.

Sample processing for DNA

DNA was extracted from pleural effusion samples by centrifugation at 3000 rpm for 30 min followed by treating 200 µl of the sediment with equal volume of lysis buffer (consisting of 0.2M NaOH, 2M NaCl and 1% SDS) and 100 µg proteinase-K at 60°C for 1 h followed by 95°C for 15 min. to inactivate proteinase -K. The lysate was extracted successively with chloroform. The aqueous phase was adjusted to 0.3M sodium acetate (pH 5.2), precipitated with ethanol and dissolved in double distilled water.

Amplification reaction

DNA amplification by PCR was performed in the total reaction volume of 25 µl with 5 µl of extracted DNA, 10mM Tris-Cl (pH 8.3), 1.5mM $MgCl_2$, 50mM NaCl, gelatin 0.01% (w/v), 100µM of each dNTP, 0.5µM of each primer & 0.5 Units of Taq polymerase. Initial denaturation at 94°C for 5 min was proceeded by 30 cycles each of denaturation (94°C for 30 s), annealing (60°C for 1 min) and extension (72°C for 2 min) followed by a final extension at 72°C for 7 min.

Analysis of PCR product

The amplified product was electrophoresed into 2% agarose gels. The gels were stained with ethidium bromide and visualized in a UV-transilluminator. The presence of a 240 bp fragment indicated a positive test. Closed needle pleural biopsies were done in our patients using Abrams Needle. Histological examination was done in our pathology department as per the hospital protocol. A biopsy demonstrating either caseating or non-caseating epithelioid granulomas was considered as indicative of tuberculosis.

Data analysis

The results of clinical evaluation, diagnostic tests, ADA activity, PCR and pleural biopsies were analysed using computer software (SPSS, version 11.5). The sensitivity, specificity, PPVs and NPVs of ADA, PCR, combination of ADA and PCR (either/or) and histology were compared. The results of histological testing were used as the "gold standard".

RESULTS

A total of 57 patients with pleural effusion were enrolled in our study. The mean age of the study subjects was 41.6 ± 15.3 years with age ranging from 15 - 70 years. Out of 57 patients 42 (73.7%) were males with and 15 (26.3%) were females. Majority of males (n = 17, 40.5%) were in 4^{th} and 5^{th} decade and most of females (n = 11,53.3%) were in 3^{rd} and 4^{th} decades. The p value was insignificant suggesting that there was no age and gender bias in selecting the patients for this study. According to our broad case definition of tuberculosis, the patients were broadly divided in two groups –tubercular (39 of 57 patients; 68.4%) and non-tubercular (18 of 57 patients; 31.6%). In non-tubercular group 11 (19.2%) patients had effusion of undetermined etiology, two (3.6%) patients had empyema and five (8.8%) patients were having malignant effusion. Most of the patients diagnosed as pleural TB were in their 2^{nd}, 3^{rd} and 4^{th} decades of life. 31 out of 39 (79.4%) patients were below the age of 50 years, suggesting a higher incidence of tubercular effusions in younger age group. A statistically significant association ($p < 0.009$) was found between pleural TB and younger age. Productive cough and fever were the most common presenting symptoms of the patients of tuberculous effusion and both the symptoms in combination were present in 69% of patients of pleural TB (27 of 39). Fever was more frequently present in patients with pleural TB (31 of 39; 79.5%) than non-tubercular group (10 of 18; 55.6%) [p =0.06; OR = 3.1]. The p values for chest pain (p = 0.001) and weight loss (0.001) were statistically significant among the two groups with chest pain favouring non-tuberculous etiology and weight loss favouring tuberculosis.

All the patients of pleural tuberculosis in our study were having lymphocytic exudative effusions with mean lymphocyte differential count of 85.1 ± 9.9 %. The pleural fluid total lymphocyte count of 100-1000 cells/mm^3 was seen in 82.1 % of the patients of pTB. Elevated pleural fluid lactate dehydrogenase (LDH) of > 400 U/ was present in 87.4% of the patients of pTB. A significant association of pleural TB was seen with pleural fluid sugars < 60mg/dl (29 of 39 ; 74.3% ; p = 0.026) and pleural fluid protein > 5g/dl (25 of 39 ; 64.1% ; p=0.032) . Tuberculin test was positive in 35.9% of pleural TB patients (14 of 39; 35.9%) compared with non-tubercular effusion patients (2 0f 18; 11%) [p=0.05].

The results of 39 patients with tubercular pleural effusions diagnosed by Broad Case Definition are shown in Table 1. Z N staining of pleural fluid was negative in all the 39 patients with pleural tuberculosis; however AFB was detected in seven patients on ZN staining from

Table 1. Results of 39 patients with tubercular pleural effusions diagnosed by Broad Case Definition.

Patient no.	ZN Staining		Biopsy		ADA	PCR	Response to ATT
	pleural fluid	other material	pleural	lymph node			
1	Negative	Positive	Negative	Not done	Positive	Negative	Responsed
2	Negative	Negative	Not done	Not done	Positive	Positive	Responsed
3	Negative	Negative	Not done	Not done	Positive	Positive	Responsed
4	Negative	Negative	Suggestive	Not done	Positive	Negative	Responsed
5	Negative	Negative	Suggestive	Negative	Positive	Negative	Responsed
6	Negative	Negative	Negative	Negative	Positive	Positive	Responsed
7	Negative	Negative	Not done	Negative	Positive	Positive	Responsed
8	Negative	Positive	Not done	Not done	Positive	Positive	Responsed
9	Negative	Negative	Not done	Not done	Negative	Positive	Responsed
10	Negative	Negative	Negative	Not done	Positive	Positive	Responsed
11	Negative	Negative	Suggestive	Not done	Negative	Negative	Responsed
12	Negative	Negative	Negative	Not done	Positive	Negative	Responsed
13	Negative	Negative	Negative	Not done	Positive	Positive	Responsed
14	Negative	Negative	Negative	Not done	Positive	Negative	Responsed
15	Negative	Negative	Negative	Not done	Positive	Negative	Responsed
16	Negative	Negative	Not done	Not done	Positive	Negative	Responsed
17	Negative	Positive	Negative	Not done	Positive	Negative	Responsed
18	Negative	Positive	Negative	Negative	Positive	Positive	Responsed
19	Negative	Negative	Negative	Not done	Positive	Positive	Responsed
20	Negative	Negative	Suggestive	Not done	Positive	Negative	Responsed
21	Negative	Positive	Not done	Not done	Positive	Negative	Responsed
22	Negative	Negative	Not done	Not done	Positive	Positive	Responsed
23	Negative	Negative	Not done	Not done	Positive	Positive	Responsed
24	Negative	Negative	Not done	Not done	Positive	Positive	Responsed
25	Negative	Negative	Not done	Not done	Positive	Negative	Responsed
26	Negative	Negative	Not done	Not done	Negative	Positive	Responsed
27	Negative	Negative	Suggestive	Not done	Positive	Negative	Responsed
28	Negative	Negative	Not done	Not done	Positive	Positive	Responsed
29	Negative	Negative	Not done	Not done	Positive	Positive	Responsed
30	Negative	Positive	Negative	Not done	Positive	Negative	Responsed
31	Negative	Negative	Negative	Not done	Positive	Negative	Responsed
32	Negative	Negative	Not done	Not done	Positive	Negative	Responsed
33	Negative	Negative	Negative	Not done	Positive	Positive	Responsed
34	Negative	Negative	Negative	Not done	Negative	Positive	Responsed
35	Negative	Negative	Suggestive	Not done	Positive	Negative	Responsed
36	Negative	Negative	Negative	Positive	Positive	Negative	Responsed
37	Negative	Positive	Negative	Negative	Positive	Negative	Responsed
38	Negative	Negative	Not done	Not done	Positive	Positive	Responsed
39	Negative	Negative	Not done	Negative	Positive	Positive	Responsed

(other material includes sputum ,BAL, and lymph node aspirate)

sputum, BAL and lymph node aspirate (sputum, n=4; BAL, n=2; LN, n=1). Mycobacterium tuberculosis was detected on AFB staining of lymph node aspirate of only one patient. PCR was positive in 20 out of the 39 diagnosed pleural tuberculosis cases, out of whom two were also sputum smear positive for AFB. Seven patients were diagnosed based on the finding of granulomatous inflammation on biopsy samples ; six from pleural biopsy and one from lymph node. Seven patients (not diagnosed by any confirmatory method) were considered as tuberculosis as per our broad case definition showing response to ATT after two months. 35 of the diagnosed tuberculosis patients had pleural fluid ADA levels above 40 U/L. All the 39 patients responded to anti-tubercular therapy.

The mean ADA activity levels in all the 57 patients was 109.4 U/L .The mean ADA activity levels in pleural TB patients was 79.7 U/,while in the controls it was 64.4 U/L (p=0.381). Considering 40 U/L as the cut off, the results were positive in 35 out of 39 tuberculosis patients and 9 out of 18 controls. The sensitivity of ADA for tubercular effusions worked out to be 89.7%, with a specificity of 50% only (Figure 1). In our study, out of 39 diagnosed tubercular effusions PCR was positive for mycobacterium tuberculosis in 20 patients. Out of the controls one patient tested positive for PCR who was

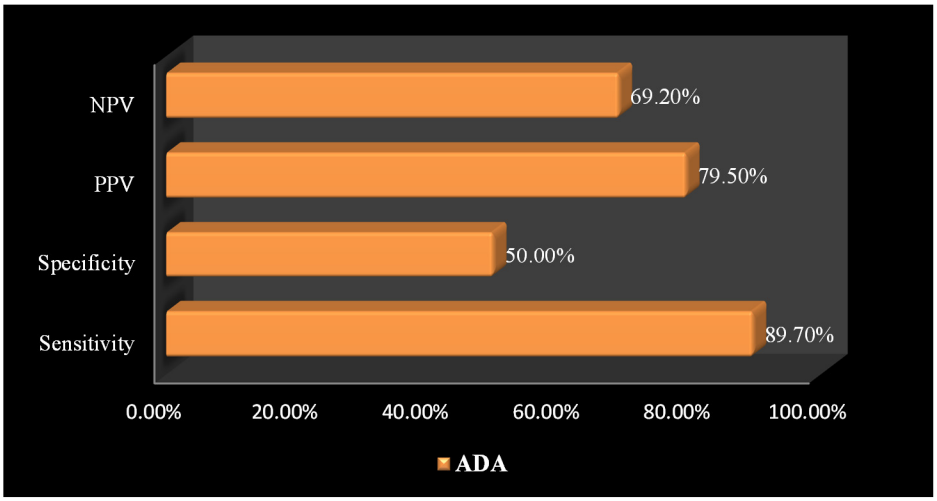

Figure 1. Sensitivity,specificity ,PPV and NPV of ADA in tuberculous effusions.

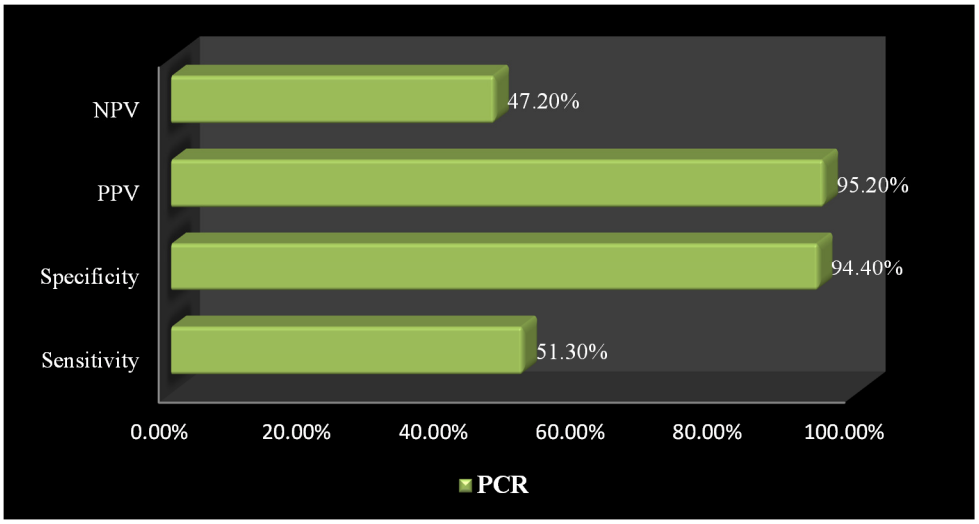

Figure 2. Sensitivity, specificity, PPV and NPV of PCR in tuberculous effusions.

diagnosed as malignancy on pleural biopsy. This patient was considered as non-tubercular as the diagnosis of tuberculosis in this patient could not be established. Thus sensitivity of PCR was 51.3% in our study, with a specificity of 94.4% and a positive predictive value of 95.2% (Figure 2).

The combination of both ADA and PCR, with a positive result by either of the two methods increased the sensitivity over both the tests (Figure 3). When used in combination both ADA and PCR picked up 38 out of the 39 diagnosed pleural tuberculosis patients increasing the sensitivity to 97.4% at the cost of specificity (50%).

Pleural biopsy was performed in 37 of the 57 studied patients. Out of the 39 patients with pleural tuberculosis, pleural biopsy was performed in 21 patients. Histological findings suggestive of tuberculosis were found in six patients. In our study the sensitivity of pleural biopsy was only 28.6% and a specificity of 100% (Figure 4).

DISCUSSION

Over the last decades tuberculosis has been one of the major causes of morbidity and mortality in the developing nations. Recent estimates are that 8 - 10 million new tuberculosis (TB) cases occur each year in the world and 2 - 3 millions die (Singh et al., (2004). Bacteriological diagnosis of pleural TB still remains a problem. It is essential to demonstrate the presence of M. tuberculosis in a clinical sample to establish the diagnosis of tuberculosis. The evaluation of the efficiency of the diagnostic tests is conducted in reference to the best

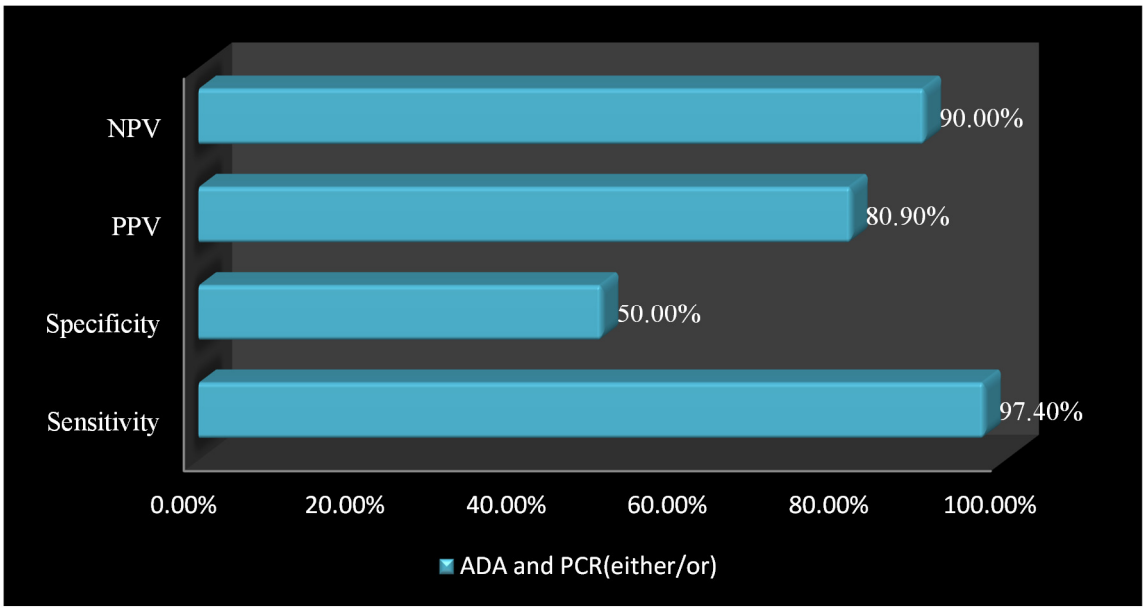

Figure 3. Sensitivity, specificity, PPV and NPV of combination of ADA and PCR (either/or) in tuberculous effusions.

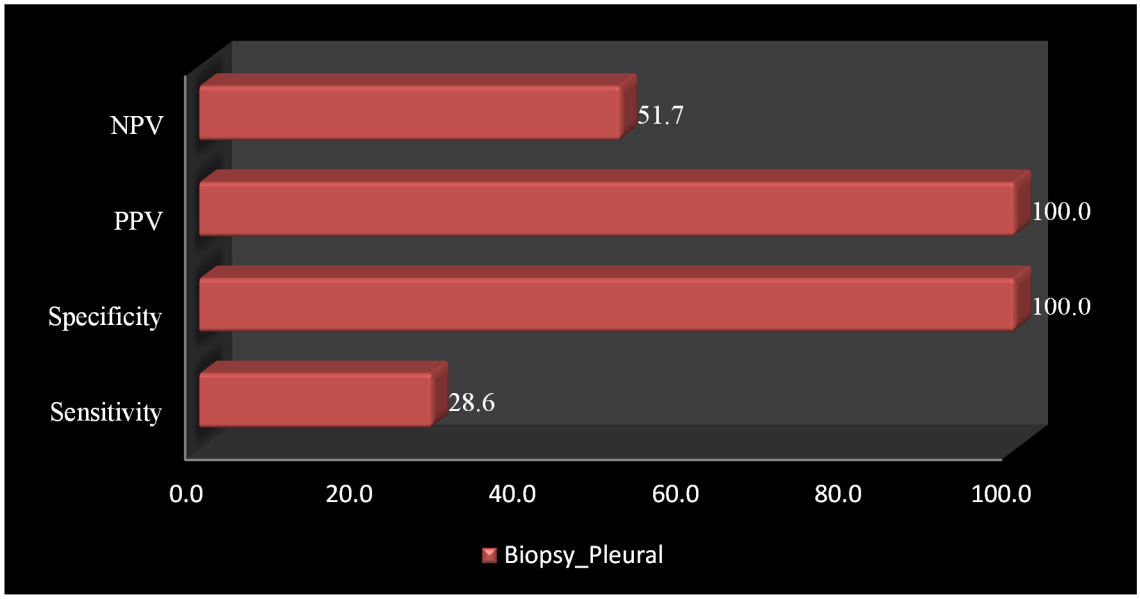

Figure 4. Sensitivity, specificity, PPV and NPV of pleural biopsy in tuberculous effusions.

available standard, which is taken as the “gold standard.” In case of pleural TB and other pauci-bacillary forms of M. tuberculosis infection, the “gold standards,” culture and biopsy, present significant limitations in sensitivity and in the time and the clinical expertise required to determine the etiology.

Ziehl-Neelsen staining although is rapid and inexpensive but lacks sensitivity (Valdés et al., 1998; Pai et al., 2003) and can be used to detect acid fast bacilli in concentration of > 10^4 organisms per ml (David, 1975). The culture, although sensitive and more specific, takes a long period for positive results and can detect 10 - 100 viable organisms per sample (Valdés et al., 1998). This led to the development of rapid yet sensitive and specific tests. PCR and ADA are the most commonly studied methods for rapid diagnosis of pleural TB, but the sensitivity and specificity of these tests have varied over a wide range in various studies. Our study comprised of 57 cases of lymphocytic exudative pleural effusions, 39 diagnosed as tubercular and 18 as non- tubercular

effusions. The average age of patients with tuberculous pleurisy is increasing and the disease is now commonly seen in middle age group. 79% (31 of 39) pTB patients in our study were younger than 50 years of age, and 41% (16 of 39) were in the age group of 20 - 40. The mean age of the patients of tubercular pleurisy in our study was 37 years. The results were contrary to the study done by Aho et al. (1968) and Sibley (1973) who found that mean age of the patients of tubercular pleurisy was 28 years and 25 years respectively and consistent with the results Berger and Meija (1973) where mean age was 35 years showing the increasing trend in age of tuberculous pleurisy patients.

In our patients of pTB out of 39 patients 27 (69.2 %) were males and 12 (30.8%) were females. Our results were similar to the results of Jose (1998) who found in their study that 66.7% of patients of pTB were males and 33.3 % were females. Productive cough (82.1%) and fever (79.5%) were the most common presenting symptoms of tuberculous effusions patients and both the symptoms in combination were present in 69% of patients of pleural TB (27 of 39). Similar results were observed by Berger (1973). Moudgil et al. (1980) also observed that cough was present in 70% of their cases.

In this study the pleural fluid white cell count was between 100-1000/mm^3 in 82.1% of the patients. 34 of 39 patients had pleural fluid total leukocyte count below 2500. This was contrary to the findings of Berger (1973) who reported the pleural fluid white blood cell count between 1,000 and 6,000/cu mm in 23 out of 41 patients (58.9%). Pleural fluid protein concentration of more than 5gm/dl was seen in 64.1% and elevated lactic acid dehydrogenase (LDH) of more than 400 U/L was seen in 77.4% patients of pTB in our study . Similar results were reported by Berger (1973). for both pleural fluid protein and LDH. Calnan et al. (1951) found a glucose concentration less than 60 mg/100 ml in 52% of the patients (13 out of 25) including seven with less than 30 mg/100 ml. They stated that a pleural glucose concentration of 30 mg/100 ml or less was diagnostic of tuberculosis andthat the diagnosis of tuberculosis was unlikely if the value was above 60 mg/100 ml. Barber et al (1957) found glucose concentrations between 10 and 48 mg/100 ml in 70 cases of tuberculous pleurisy. The results in our study showed a pleural fluid glucose of less than 60mg/dl in 74.3% of the patients of pTB and none had values less than 30 mg/dl.

ZN staining of sputum has a sensitivity of 10.2% in our study; results were consistent with the study conducted by Berger (1973) where sputum smear positivity was 8%. Our results were also in accordance with those of Chan (1991). All the 39 patients of pTB received and responded to antitubercular treatment. Seven of these patients were considered as tubercular only on the basis of clinical response to ATT (as per our broad case definition). Similar response to ATT has been broad case definition). Similar response to ATT has been reported by Dutt et al. (1986) where all patients had responded to ATT.

Demonstration of an elevated pleural fluid adenosine deaminase (ADA) level is useful in establishing the diagnosis of tuberculous effusions. ADA is an enzyme involved in purine catabolism. It is found in the majority of the cells, but particularly in lymphocytes, where its concentration is inversely related to the degree of differentiation. High levels of ADA have been found in patients with tuberculous pleurisy (Piras et al., 1978). In a study done by Reechaipichitkul et al. (2001) the pTB diagnosis by ADA activity levels in the pleural fluid had a sensitivity of 80% and specificity of 80.5%. Canbolat et al. (1999) showed that sensitivity of ADA (adenosine deaminase) assay was 91.7% and specificity was 94.5%, whereas Ocana et al. (1983) reported specificity of ADA to be 100% when pleural fluid ADA levels were above 70 U/L.

In our study, ADA activity measurements also yielded good results in the diagnosis of pTB (sensitivity of 89.7%). however, ADA activity values may be increased due to other clinical entities. Valdes et al[12] have also reported high levels of ADA in patients with other causes of pleural effusions (mainly lymphomas, adenocarcinomas, systemic lupus erythematosus and pneumonia).

The ADA cutoff value indicative of tuberculosis is subject to debate, since the literature presents a great variation of these values, ranging from 30 to 50 U/L; higher cutoff values (70 U/L) have been described Bañales et al. (1991). It is difficult to define a universal cutoff value for ADA activity. This test has to be validated for each region, and eventually for every service where the test is to be used. We defined the best cutoff value as 40U/L as was done by Danielle et al. (2003) in their study. With the decline in the prevalence of TPE, the positive predictive value of pleural fluid ADA also declines, but the negative predictive value actually increases. Therefore, the measurement of the pleural fluid ADA level could be used to rule out a tuberculous etiology of lymphocytic pleural effusions, regardless of the rate of prevalence of the disease.

Polymerase chain reaction is being increasingly used in the rapid diagnosis of tuberculous effusions considering the paucibacillary nature of the pleural fluid and the fact that PCR can diagnose different species of mycobacteria. In this study, of the 39 patients who met the criteria for the diagnosis of pTB using the broad case definition, PCR findings were positive in 20 patients. The PCR protocol used in this study was based on the amplification of a portion of the *M tuberculosis* genome located in the *MPB-64 gene* sequence. In our study, the sensitivity of PCR was 51.3% and specificity was 94.4%. These data are in agreement with those obtained by De Wit et al. (2000), Folgueira et al. (1993), Pao et al. (1990) in that the PCR sensitivity ranged from 13 to 100% and

Table 2. Comparison of sensitivity,specificity,and predictive values of the diagnostic methods used in the study.

Sensitivity, specificity, and predictive values of the diagnostic methods used in the study.				
	Sensitivity	**Specificity**	**PPV**	**NPV**
ADA	89.7%	50%	79.5%	69.2%
PCR	51.3%	94.4%	95.2%	47.2%
ADA and PCR(either/or)	97.4%	50%	80.9%	90%
Pleural Biopsy	28.6%	100%	100%	51.7%

specificity ranged from 88 to 100%. In more recent studies using pleural fluid, PCR has a sensitivity of 70% and specificity of 100% by Nagesh et al. (2001) and 50% sensitivity and 61% specificity by Reechaipichitkul et al. (2000).

Our results suggest a low PCR sensitivity compared to ADA, but PCR specificity was much better than any other technique used in this study except that of pleural biopsy which was 100% specific. (Table 2) PCR results vary significantly according to the material studied, as well as with the extraction method used (Grange, 1989). The fact that the pleural fluid is paucibacillar can partially explain the small number of positive results observed in this study. Other possible explanations for low PCR sensitivity are the presence of polymerase inhibitors in the pleural fluid and the sampling errors. In this study, PCR finding was positive in a sample in which the tuberculosis diagnosis could not be confirmed. This patient was diagnosed as a case of small cell lung cancer on pleural biopsy. Even though evaluation of pleural biopsies is considered a good approach for the diagnosis of pTB, in our study 37 patients underwent pleural biopsy during their diagnostic evaluation; 21 were diagnosed as pTB by our Broad Case Defination. The characteristic histopathologic findings (granulomas) which helped in making the correct diagnosis were present in six biopsy samples only (sensitivity 28.6%; specificity 100%). Results of needle pleural biopsy have been reported positive in 50 - 80**%** of cases of tuberculous pleurisy (Light, 1995; Danielle et al., 2003). The high specificity (100%) in our study can be explained by the fact that we considered the presence of caseating granulomas in pleural biopsy samples as diagnostic of pleural tuberculosis.

The combination of ADA and PCR (either/or) improved the sensitivity to 97.4% which was much higher than pleural biopsy (28.6%), PCR (51.3%) and even ADA (89.7%) but at the cost of specificity which dropped to 50%. Comparison of sensitivity and specificity of this combination to ADA, PCR and to the “gold standard” pleural biopsy supports the feasibility of using the PCR of pleural fluid together with determination of ADA activity as the first diagnostic approach in circumstances in which pleural tuberculosis is suspected and access to procedures such as needle biopsy of pleura is limited.

REFERENCES

Aggarwal AN, Gupta D, Jindal SK (1999). Diagnosis of tuberculous pleural effusion. Indian J Chest Dis Allied Sci., 41(2): 89 - 100.

Aho K, Brander E, Patiala J (1968). Studies for primary drug resistance in tuberculous pleurisy. Scand J Respr Dis.,63:111-14.

Bañales, JL, Pineda PR, Fitzgerald M (1991). Adenosine deaminase in the diagnosis of tuberculous pleural effusions: a report of 218 patients and review of the literature. Chest; 99: 355 - 357

Barber LM, Xlazzadi L, Deakins DD (1957). Glucose level in pleural fluid as a diagnostic aid. Dis Chest. 31:680 – 687.

Berger HW, Meija E (1973). Tuberculous pleurisy. Chest. 63:88 - 92.

Calnan WL, Winfield BJO, Crowley SlF (1951). Diagnostic value of the glucose content of serous pleural effusions. Br hied J. 1: 1239-1240,

Canbolat O, Ulusdoyuran S, Ozgen G, Ceyhan I, Gümüşlü F, Akbay A (1999). The comparison of adenosine deaminase activity values with polymerase chain reaction results in patients with tuberculosis. . J. Clin. Lab. Anal., 13(5):209 - 12.

Chan CH, Arnold M, Chan CY (1991). Clinical and pathological features of tuberculous pleural effusions and its long term consequences. Respiration; 58:171 - 175.

Danielle ML, Keny JBC, Benedito AL, da Fonseca (2003). Combined use of the polymerase chain reaction and detection of adenosine deaminase activity on pleural fluid improves the rate of diagnosis of pleural tuberculosis. Chest. 124: 909 - 914.

David HL (1976). Bacteriology of the mycobacterioses. US Govt. Printing office Washington D.C.

De Wit D, Maartens G, Steyn L (2000). A Comparative Study Of The Polymerase Chain Reaction And Conventional Procedures For The Diagnosis Of Tuberculous Pleural Effusion. Tuber Lung Dis., 73(5): 262 - 67.

Dutt AK, Moers D, Stead W (1986). Short course chemotherapy for pleural tuberculosis. Chest; 90:112.

Folgueira L, Dlgado R, Palenque E (1993). Detection of mycobacterium tuberculosis DNA in clinical samples by using polymerase chain reaction. J. Clin. Microbiol., 31:1019 - 1021.

Giusti G (1974). Adenosine deaminase. Bergmeryer , HU eds. Methods of enzymatic analysis 1974, 1092-1099 Academic press. New York , NY:

Grange JM (1989). The rapid diagnosis of paucibacillary tuberculosis. Tubercle. 70:1 - 4

Harries AD (1990). Tuberculosis and human immunodeficiency virus infection in developing countriesLancet; 335(8696):1044.

Jose MM (1998) Tuberculous pleurisy: a study of 39 patients. P.8

Kataria YP, Khurshid I (2001). Adenosine deaminase in the diagnosis of tuberculous pleural effusion. Chest. 120: 334 - 336

Light RW (1995). Pleural diseases 3rd ed. ,154-166 William & Wilkins. Baltimore, MD:

Mario C, Raviglione, Richard J.O’Brien (2001). Tuberculosis. In. Fauci, Braunwald, Kasper, Hauser, Longo, Jameson, Loscalzo. Eds.Harrisons Principles of Internal Medicine,Tata McGraw Hill ,17th ed.vol 1 p1010 .

Moudgil H, Sridhar G, Leitch AG (1980). Reactivation disease The commonest form of tuberculous effusion in Edinburg, 1991.Respr Med., 88:301-304

Nagesh BS, Sehgal S, Jindal SK, Arora SK (2001). Evaluation of

polymerase chain reaction for detection of Mycobacterium tuberculosis in pleural fluid. Chest. 119(6):1737-41.
Ocana I, Martinez-Vazquez JM, Segura RM (1983). Adenosine deaminase in pleural fluids: test for diagnosis of tuberculous pleural effusions. Chest ; 84:51 - 53
Pai, M., Flores, L.L., Riley, L.W. and Colford, J.M. (2003): Nucleic Acid Amplification tests in the diagnosis of tubercular pleuritis. BMC Infec. Dis. ,4: 6-11.
Pao CC, Yen TSB, You JB (1990). Detection and identification of mycobacterium tuberculosis by DNA amplification. J. Clin. Microbiol., 28:1877-1880.
Piras MA, Gakis C, Budroni M (1978). Adenosine deaminase activity in pleural effusions: an aid to differential diagnosis. BMJ., 2: 1751-1752
Reechaipichitkul W, Kawamatawong T, Teerajetgul Y (2001). Diagnostic role of pleural fluid adenosine deaminase in tuberculous pleural effusion. Southeast Asian J. Trop. Med. Public Health. 32: 383 - 389
Reechaipichitkul W, Lulitanond V, Sungkeeree S, Patjanasoontorn B (2000). Rapid diagnosis of tuberculous pleural effusion using
polymerase chain reaction. Southeast Asian J. Trop. Med. Public Health. 31(3): 509 - 14.
Rossman MD, Mayock RL (1993). Pulmonary TB, in Schlossberg D, Ed. Tuberculosis. Springer verlag, New York, p. 95.
Sahn SA (1995). The diagnostic value of pleural fluid analysis.Seminars in Respir and Crit Care Med., 16(4): 269 – 78.
Sibley JC (1973). A study of 200 cases of tuberculous pleurisy with effusion. Am Rev Tuberc., 62:314-323.
Singh R, Singh RS, Tripathi AKG (2004). Circadian Periodicity of Plasma Lipid Peroxides and Anti-Oxidant Enzymes in Pulmonary Tuberculosis. Ind. J. Clin. Biochem.m 19: 14 - 20.
Udwadia, Zarir f; sen, tiyas (2010). Pleural tuberculosis: an update ,current opinion in pulmonary medicine: 16(4): 399 - 406.
Valdés L, Alvarez D, San José E, Juanatey JR, Pose A, Valle JM, Salgueiro M, Suárez JR (1995). Jun: Value of adenosine deaminase in the diagnosis of tuberculous pleural effusions in young patients in a region of high prevalence of tuberculosis. Thorax. 50(6): 600 - 3.
Valdés L, Alvarez D, San José E, Penela P, Valle JM, García-Pazos JM, Suárez J, Pose (1998). A.Tuberculous pleurisy: a study of 254 patients. Arch Intern Med., 12; 158(18): 2017- 21.
Valdes L, Alvarez D, Valle JM (1996). The etiology of pleural effusions in an area with high incidence of tuberculosis. Chest. 109: 158 - 162
Villegas MV, Labrada LA, Saraiva NG (2000). Evaluation of polymerase chain reaction adenosine deaminase and interferon-γ in pleural fluid for the differential diagnosis of PTB. Chest. 118: 1355 – 1364.