

ORIGINAL ARTICLE

Diagnostic Value of Adenosine Deaminase in Tuberculous Pleurisy

Abbasali Niazi¹, Mosayeb Shahryar², Behzad Narouie^{*3}, Khalilallah Danesh³, Roya Alavi-Naeini⁴, Mostafa Dahmardehei⁵, Mohammad Ghasemi-rad⁶, Nikol Mladkova⁷, Hamideh Hanafi-bojd²

¹Pathology Department, Zahedan University of Medical Sciences, Zahedan, Iran.

²Internal Medicine Department, Zahedan University of Medical Sciences, Zahedan, Iran.

^{3*}Researcher of Clinical Research Development Center, Ali-Ebne-Abitaleb hospital, Zahedan University of Medical Sciences, Zahedan, Iran.

⁴Infectious and Tropical Disease Department, Zahedan University of Medical Sciences, Zahedan, Iran.

⁵Surgery Department, Zahedan University of Medical Sciences, Zahedan, Iran.

⁶Genius and talented organization, Student Research Committee (SRC), Urmia University of Medical Sciences, Urmia, Iran.

⁷Charles University in Prague, Czech Republic.

ABSTRACT

The diagnosis of tuberculous pleurisy represents a clinical challenge due to its unspecific clinical presentation and the insufficiency of traditional diagnostic methods for accurate diagnosis of this disease. We investigated the potential use of adenosine deaminase (ADA) activity in the determination of tuberculous pleural effusion.

We analyzed pleural effusion samples of 85 patients with biochemically confirmed forms of exudative pleural effusions. Using the ROC curve, we determined an optimal cut-off point for the diagnosis of TB pleural effusion.

58 exudative samples were of non-tuberculous (non-TB) etiology and 27 effusion samples were caused by tuberculous (TB). There was a statistically significant difference ($p < 0.0001$) between the mean of pleural fluid ADA level among the TB and the non-TB patients. The prevalence of TB pleurisy in the studied population was 31%. The cut-off point value for the diagnosis of TB effusion was >35 IU/L, with a sensitivity of 70.3% and a specificity of 91.3%. The positive predictive value (PPV) was 79.1% and the negative predictive value (NPV) was 86.8%.

Pleural fluid ADA assay is a sensitive and specific test suitable for rapid clinical diagnosis of TB pleurisy.

Key words: adenosine deaminase, tuberculosis, diagnostic value, pleural effusion

INTRODUCTION

Historical background

Tuberculous is one of the oldest diseases known to affect mankind and remains one of the most important chronic infectious diseases with respect to morbidity, mortality and financial burden. Although it has been identified as far back as Neolithic humans, only the crowded living conditions of the early industrial age favored its subsequent global spread. The primary etiologic agent in human infection is *Mycobacterium tuberculosis*, an aerobic Gram-positive bacterium with slow growth rate that was first described by Robert Koch in 1882.

Current epidemiologic situation

Despite great advances that have been achieved in tuberculous treatment and prevention, it remains a leading cause of death in the world today. The World Health Organization (WHO) estimates that globally about 9 million new cases of active tuberculous arise and more than 2 million deaths occur from the disease every year, making it is the second most common infectious cause of death, preceded only by HIV infection [1].

The disease affects all age groups, with most childhood cases occurring among children younger than 5 years. Commonly, its primary site of infection is the lungs, although in one third of cases it also can affect other organs.

Pleural effusion

Pleural effusion (PLE) is clinically is characterized by shortness of breath, chest pain, dyspepsia and cough. Various different diseases may cause a pleural effusion, such as congestive heart failure or pneumonia, but tuberculous (TB) remains an important cause of pleural effusion and 31% of TB patients develop PLE. PLE is more common in HIV positive TB patients and it occurs more frequently with the increase of age between 5 and 45 years. TB-related pleural effusion is one of

the most manageable types of exudative pleural effusion. Pleura can be affected in TB patients either primarily or secondarily by the infection of the lungs [2,3].

Exudative pleural effusion

Pleural effusions can be classified as transudate or exsudate. To determine the characteristics of effusion fluid, pleural fluid protein is divided by serum protein and pleural fluid LDH is divided by serum LDH. If the range of fluid protein to serum protein is greater than 0.5, or if the rate of effusion LDH to serum LDH is greater than 0.6, the effusion would be considered an exudative pleural effusion. Concordantly, if the rates were lower, the effusion would be considered a transudative effusion. The most common causes of exudative pleural effusion are: parapneumonic etiology, TB and other infections, malignancy, pulmonary embolism, abdominal disease (4).

The diagnosis of tuberculous effusion

The diagnostic techniques available for pleural effusion include a pleural fluid smear, culture, pleural biopsy, interferon-gamma (INF-gamma) and adenosine deaminase (ADA).

Pleural effusion smears, culture and pleural biopsies are positive in 25-30%, 75% and 25% of cases respectively in primary TB. While in secondary TB, pleural fluid smears, culture and pleural biopsies are positive in 50%, 60% and 25% of cases, respectively. In a sputum testing assay, sputum TB smear and culture are rarely positive - only in 25% and 33% of cases respectively in primary TB, and in 50% and 60%, respectively in secondary TB (5).

On the other hand, the Polymerase chain reaction (PCR) represents a highly sensitive and specific diagnostic technique in patients with a negative smear and positive culture. PCR also gives positive results in 70% of patients that have been previously affected by TB, but did not develop the active form of the disease. This significantly high rate of false positive results inevitably leads to diagnostic constraints (6).

Adenosine deaminase

Adenosine deaminase (ADA) is a polymorphic enzyme involved in purine catabolism, catalyzing the irreversible deamination of adenosine and deoxyadenosine converting it to inosine and deoxyinosine, respectively by the removal of an amino group. (7) Inosine and deoxyinosine is subsequently deribosylated and converted into hypoxanthine.

High concentrations (more than 40 IU/l) of ADA can be detected in tubercular pleural effusions. There are two important isoforms of ADA: ADA1 and ADA 2. ADA 1 is found in most body cells, while the more specific ADA2 namely reflects the activity of monocytes and macrophages and is therefore used as marker in the diagnosis of tuberculous pleural effusions. The main activity of ADA in tubercular pleural effusions is due to ADA2. Gakis et al., showed that the low activity of 2-ADAo/ADA can be important in the differentiation between tubercular pleural effusion and pleural effusions of different etiology with high activity of ADA (8, 9). The measurement of ADA levels is a simple and inexpensive method that provides results in a short amount of time.

Considering the prevalence of TB in the Iranian province of Sistan and Baluchestan, the disease considerably affects both the patients' lives and the economy of those regions in a negative way. In order to combat the disease by providing proper TB treatment, accurate diagnosis of TB must be established first.

Since the routine testing using smears, pleural culture, PCR and pleural biopsy does not have the required sensitivity and specificity necessary for the accurate diagnosis of TB based on tubercular pleural effusion, it is necessary to develop a simple, accessible, inexpensive and fast diagnostic method with higher sensitivity and specificity for TB than those of currently available routine tests. Hence the aim of this study was to assess the level of adenosine deaminase (ADA) in patients with pleural effusion and to evaluate its value in the determination of tubercular pleural effusion

METHODS

A. Patients

This descriptive analytic study was conducted during the year 2007-2008. Overall, 85 patients that were referred to Ali Ebn Abitaleb hospital and Bu Ali hospital in the district of Zahedan, Iran with exudative pleural effusions were enrolled in the study. Patients were divided into two groups: Patients with pleural effusions due to TB and patients with pleural effusion due to different etiology.

After the research study design and its goals were fully described to the patients, written informed consent was obtained from each participant.

An anterior-posterior (AP) chest X-ray image was then acquired from each patient. Patients with pleural effusions of considerable amount shown in the AP image of the thorax, or patients with pleural effusion greater than one centimeter in the lateral decubitus would be considered for thoracentesis (pleural tap), unless they had congestive heart failure.

Patients with congestive heart failure showing pleural effusion along with fever and chest pain were also considered for thoracentesis.

Thoracentesis was explained the patient and performed after consent from was obtained. Patients were sitting upright, and a 50ml needle was inserted in the second intercostal space below the scapula and in the posterior axillary line. On all pleural fluid samples, the following analyses were performed: The cell count and differential (number of white cells), protein level and LDH level were all determined. The fluid was also stained according to Gram, smear, and microbacterial culture for mycobacterium was performed.

5ml of the pleural fluid were immediately put on ice and sent for measurement of the ADA level.

Simultaneously, serum protein and LDH levels were measured. The classification of the samples as exudative pleural effusion or transudative pleural effusion was based on Light's criteria (10). According to Light, exudative pleural effusions meet at least one, whereas transudative effusions meet none of the following criteria:

1. Pleural fluid protein level divided by serum protein level ratio greater than 0.5
2. Pleural fluid LDH level divided by serum LDH level ratio greater than 0.6
3. Pleural fluid LDH greater than two-thirds the upper limit of normal for serum LDH.

After patients with exudative pleural effusion were selected, additional diagnostic tests were were ran in order to confirm the final diagnosis of TB. The additional testing included: PCR of the pleural fluid for TB, pleural biopsy, detection of immunologic factors such as Anti dsDNA, RF and ANA in the serum in uncertain cases to exclude rheumatoid disease etiology, such as collagen vascular disease. Response to treatment was evaluated in patients with high probability of TB infection based on clinical presentation, spiral CT or perfusion scan of the lungs was performed in cases with suspected pulmonary embolism in addition to bronchoscopy and bronchoalveolar lavage.

Exclusion criteria were the following: Other than Persian race, patients with transudative pleural effusion, patients with still unknown etiology of pleural effusion after all mentioned diagnostic tests were performed and patients with hemothorax.

Eventually, 85 patients with exudative pleural effusion of known etiology were selected for the study. Among those, 27 patients were diagnosed with TB and subsequently placed in the TB group, and 58 remaining patients were placed in the non-TB group.

B. Diagnostic criteria

1. Tuberculous pleural effusion:

- Detection of *Mycobacterium tuberculosis* in pleural effusion or in pleural biopsy
- Smear-positive pleural liquid or pleural biopsy
- Detection of tuberculous granuloma in pleural biopsy specimens
- PCR of the pleural effusion positive for *Mycobacterium tuberculosis*
- Response to treatment with antituberculous medication in patients with clinical and radiological findings consistent with active TB infection
- Sputum specimen positive for TB when non-tuberculous etiology of the exudative pleural effusion was excluded

2. Pleural effusion in the non-TB group:

2.1 Pleural effusion of malignant etiology:

Patients with current malignant tumor after the exclusion of other potential causes of pleural effusion, histological or cytological findings of malignant tumors

2.2 Parapneumonic effusion

Pleural effusion in patients with acute fever, diagnosed with pneumonia or pulmonary abscess and responding appropriately to antibiotics, after TB or malignancy was excluded

2.3 Empyema

Purulent pleural effusion on thoracentesis and positive bacterial culture of parapneumonic effusion.

2.4 Pulmonary embolism

Perfusion scans consistent with pulmonary embolism in the absence of the other potential causes of exudative pleural effusion

2.5 Other causes

Subdiaphragmatic abscess, collagen vascular disease, pancreatitis and other causes when above mentioned etiologies were excluded

Measurement of ADA

5ml pleural effusion specimen were obtained during thoracentesis in a plastic syringe, preserved on ice and send to a laboratory for analysis. The sample would first be centrifuged and stored at -20 degrees Celsius overnight then analyzed the following day. The analysis consisted of two separate steps : First adenosine was deaminated by ADA and ammonia was released, second the ammonia formed by the catalytic activity of adenosine deaminase was coupled to the reaction catalyzed by glutamate dehydrogenase. The reduced reabsorption measured at 340 nm wavelength (conversion of NADPH to NADP+) has a direct relationship to the concentration of ADA enzyme.

Statistical Analysis

ADA levels were analyzed and compared in the two groups. Descriptive values were used to describe the following: mean, standard deviation, frequency, minimum and maximum. Since the two groups were normal when a non-parametric test, such as One-sample Kolmogorov-Smirnov test was used, we used Student's T-test to determine if there is a difference between the mean value of factors in the TB and non-TB group. Receiver operating characteristics (ROC) was also drawn to determine cut-off points.

RESULTS

During a time period of 12 months, altogether 200 patients were diagnosed with pleural effusion. 115 of the patients were excluded from the study due to above mentioned exclusion criteria 85 patients with the confirmed diagnosis of exudative pleural effusion, consisting of 21 females (25%) and 64 males (75%) were enrolled in the study. 27 patients (31.7%) were diagnosed with TB and in 58 patients (63.3%) TB was not detected. The age of patients in TB group was between 16 and 92 years (mean of 43 years) and in non-TB group between 21 and 99 years (mean of 54 years). In non-TB group, 26 patients (44.8%) were diagnosed with paraneoplastic pleural effusion and 24 patients (41.3%) with parapneumonic effusion (Table 1)

The following parameters were analyzed: Protein level, lactate dehydrogenase (LDH) level, complete and differential cell count and lymphocyte-to-neutrophil ratio and those were compared in both groups (Table 2).

The data were analyzed using Student's T-test and there was no significant correlation between them.

Mean AD Alevel in pleural effusion samples in the TB group was 39.63 ± 14.96 IU/l and 22.11 ± 9.33 IU/l in non-TB group, which is statistically significant ($p < 0.001$). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ADA with the cut off value of 35 IU in the diagnosis of tuberculous pleural effusion were 70.3%, 91.3%, 79.1% and 86.8%, respectively. The post-test probability of TB was 0.78. The same parameters were also calculated for ADA cut off point of 30 IU based on the ROC (Figure 1), which seems to be more accurate and obtained values are presented in Table 3.

Table 1. The absolute and relative frequency of patients with exudative pleural effusion per etiology.

Etiology	Number of patients	Percent (%)
Pulmonary TB	27	31.7
Malignancy	26	44.8
Parapneumonic	24	41.3
Empyema	6	10.3
Pulmonary emboli	6	10.3
Collagen vascular disease	4	6.8
Subdiaphragmatic abscess	2	3.4
Total	85	100

Table 2. Comparison between mean protein level, LDH level, and cell count in pleural effusion in TB and non-TB groups

	TB	Non-TB	P-value
Protein	2798 ± 1488	1843 ± 1541	0.304
LDH	713 ± 383	580 ± 340	0.714
WBC	2908 ± 2175	1730 ± 838	0.113
Lymphocytes	70 ± 31.9	59 ± 30.1	0.761
Neutrophils	32 ± 29	38 ± 30	0.869
Neutrophils/lymphocytes	4.56 ± 2.41	3.71 ± 1.55	0.813

Table 3. Sensitivity, specificity, PPV and NPV in TB pleural effusion in two cut-off values

	Cut-off of 30 IU/L	Cut-off of 35 IU/L
Sensitivity	88.8%	70.3%
Specifity	86.2%	91.3%
PPV	75%	79.1%
NPV	94.3%	86.8%
Likelihood ratio	6.43	8.08
Prevalence	31.7%	31.7%
Pre-test odds	0.46	0.46
Post-test odds	2.95	3.75
Post-test probability	0.74	0.78

Table 4. ADA level in pleural effusion of TB patient, sensitivity ,specifity, PPV, NPV in previous reports

Investigator	Country	Cut-off (U/L)	Sensitivity (%)	Specificity (%)	PPV	NPV	Mean ± S.D.(range)
Aoki et al [12]	Japan	45	81.8	89.3	75	93	56.6 ± 19.8
Teo and Chio [13]	Singapore	50	96	81	65	98	88.3 ± 25.8
Reech et al [14]	Thailand	48	80	80.5	71.4	86.8	93.2±56.5
Sharma et al [15]	India	35	83.3	66.6	-	-	94.8 ± 57.5
Burgess et al [16]	South Africa	50	91	81	84	89	103.25 ± 36.1
Banales et al [17]	Spain	70	98	96	94	99	123.25 ± 39.4
Valdes et al [18]	Spain	47	100	95	85	100	107.5 ± 37.9
Orphanido et al [19]	Greece	40.6	79	93.5	86	90	85.6 ± 48.9

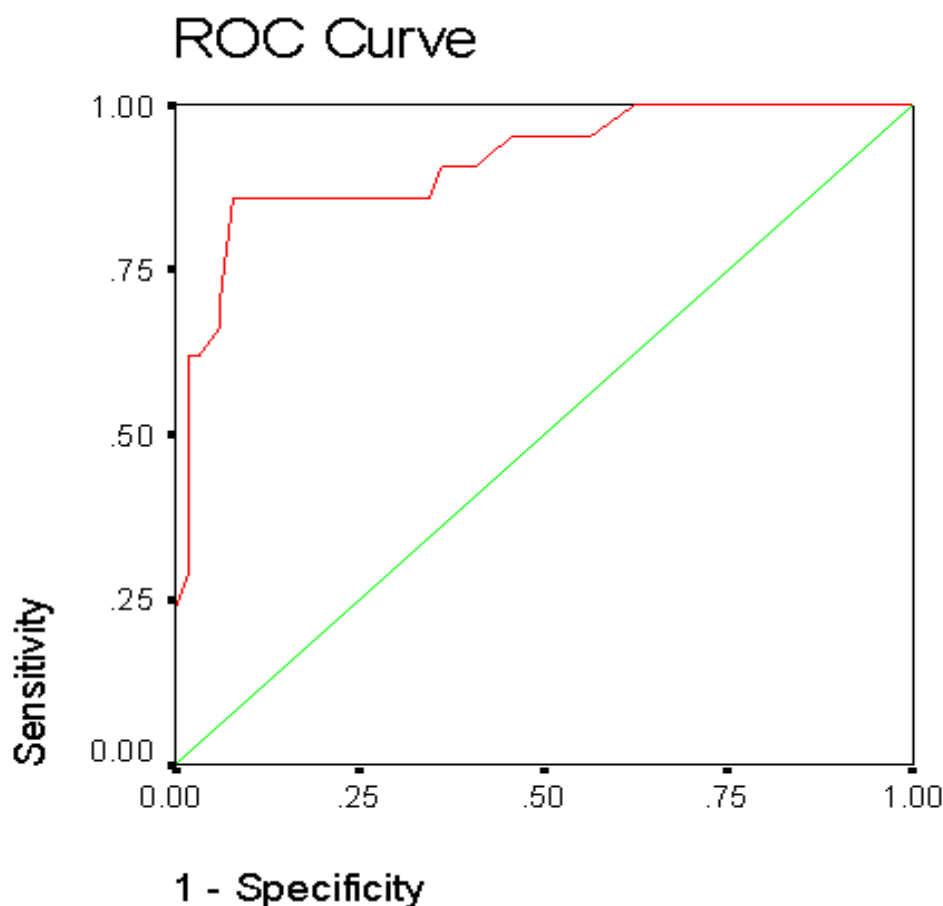


Figure 1: ROC for the determination of ADA level cut-off point in the diagnosis of TB pleural effusion

DISCUSSION

Tuberculous pleural effusion is most commonly caused by delayed cell-mediated hypersensitivity reaction. The small number of mycobacteria present in pleural effusions is the reason for obstacles related to direct finding and identification of *Mycobacterium tuberculosis* in tuberculous pleural effusion.

The probability of accurate diagnosis of TB pleural effusion by Ziehl-Neelsen stain is only 0.5%-1%, 23%-86% with microbiological culture of the fluid specimen and 51%-84% if microbiological culture is combined with pleural biopsy (9). According to our results, mean ADA level in pleural effusion in the TB group was 39.63 ± 14.96 IU/l and 22.11 ± 9.33 IU/l in the non-TB group, which was statistically significant ($p < 0.001$).

Detection ADA levels in pleural effusion has been introduced as a highly sensitive and specific test for the diagnosis of TB pleural effusion. However, the diagnostic value depends on the prevalence of TB in related area, concrete laboratory methods and the ethnic origin of studied population (9). A study conducted in India in 2001 has shown that the level of ADA in pleural effusion in TB group was 95.8 ± 57.5 IU/l and 72.2 ± 30 IU/l in non-TB group. The sensitivity and specificity were 40% and 100% respectively (16), demonstrating higher level of mean ADA level in both groups in comparison with our result. This may be due to higher prevalence of TB in India compared to Sistan and Baluchistan province. In our study, ADA was of higher sensitivity but of lower specificity in comparison with the Indian study.

A review article by... analyzed all studies on the value of ADA level in the diagnosis of TB pleural effusion between 1966 to 1999 and, reported the sensitivity of 74.1-100% and specificity of 50-100% of ADA in the diagnosis of TB pleural effusion (11). This corresponds with the results of our study.

Mr. Liyth has proposed that the low level of ADA in pleural effusions among people from Asian countries will reduce the value of this test in the diagnosis of TB pleural effusion. So far studies

from Singapore and Japan have shown higher sensitivity and specificity compared to studies from Thailand and India, which is in concordance with this hypothesis [12].

Another study conducted in China on ADA in TB pleural effusion using the cut-off point of 55.8 IU/l has shown the sensitivity, specificity, PPV and NPV of 87.3%, 91.8%, 82.1%, 94.4%, respectively (9). Results of other studies on this subject are summarized in Table 4.

Our study conducted among the population of Sistan and Baluchistan province in Iran has demonstrated the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ADA level with the cut-off point of 35 IU/l in the diagnosis of TB pleural effusion of 70.3%, 91.3%, 79.1% and 86.8%, respectively. The specificity is high at this cut-off point, but the sensitivity of considerably low with respect to the diagnosis of TB pleural effusion. In three patients with the ADA level of 30-35 UI/l and with the 35IU/l ADA cut-off point, the diagnosis of TB pleural effusion was not established. Hence based on this study's the ROC curve, the ADA cut-off point value of 30 IU/l was optimal for the diagnosis of TB pleural effusion. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ADA level with the cut-off point of 30 IU in the diagnosis of TB pleural effusion was 88.8 %, 86.2%, 75% and 94.3%, respectively.

CONCLUSION

The mean ADA level in TB pleural effusion and determined optimal cut-off point demonstrated by this study is lower than those reported in previous studies from other countries. However, this did not affect the sensitivity and specificity of the test.

Our study also shows that specific ADA levels employed as diagnostic clue in determination of TB pleural effusion should be carefully determined for each country's population or even a region. As demonstrated in this study, no universal cut-off point of the ADA level can be established as a marker for the diagnosis of tuberculous pleural effusion.

Since the measurement of ADA levels represent a quick, inexpensive, accessible and valuable test, our recommendation is to establish an individual cut-off point for the ADA levels in pleural effusion for specific regions and populations by conducting analogical studies.

ACKNOWLEDGEMENTS

The authors would like to thank the Clinical Research Development Center, Ali-Ebne-Abitaleb hospital, Zahedan University of Medical Sciences and the Genius and Talented Student Organization, Urmia University of Medical Sciences for their support during work on this paper and to Jason Burhman, University of Illinois, Chicago for corrections.

REFERENCES

1. Raviglione M, Luelmo F. (1996). Update on the global epidemiology of TB. *Curr Issues Public Health*; 2:192-197
2. Mario C. Tuberculosis. In: Brouwold B and et al. (2005). *Harisons principles of internal medicine*. 16th edition. USA: McGraw-Hill press; p.957, 1567
3. Khatami K. (2001). Pleural tuberculosis. *Shiraz E-Medical journal* .vol.2, no5.
4. Richard W. (2005). Disorder of the pleural. In: Brouwold B and et al. *Harisons principles of internal medicine* 16th edition. USA: McGraw-Hill press; v2, p.1513-7.
5. David W. Mycobacterial diseases. In: Mandell L, Bennett E. (2000). *Principles and practice of infectious diseases*. 5th edition. USA: Churchill Livingstone; .p.2600.
6. Reiohard E. Pleural effusion In: Mandell L. Bennett E. (2000). *Principles and practice of infectious diseases*. 5th ed. USA: Churchill Livingstone; p.726-7.
7. Yagawa K, Okamura J. (1981). Role of adenosine deaminase in activation of macrophages. *Infect Immun*. Apr; 32(1):394-7.
8. Shibagaki T, Hasegawa Y, Saito H, et al . (1996). Adenosine deaminase isoenzyme analysis in pleural effusion. *J Lab Clin Med*. Apr; 127(4):348-52
9. Chen ML, Yu WC, Lam CW, et al. (2004). Diagnostic value of pleural fluid adenosine deaminase activity in tuberculous pleurisy. *Clinica Chimica Acta* ; 341:101-107.
10. Richard W, Light MD. (2002). Pleural Effusion. *N Engl J Med* June; 346 (25):1971-77
11. Goto M, Noguchi Y, Koyama H, et al. (2003). Adenosine deaminase and pleural effusion. *Ann Clin Biochem*; 40:344-8.
12. Andreson N, Hirpsime L and et al. (2005). ADA2 isoform of adenosine deaminase from pleural fluid. *FEBS letters*; 579:643-647.
13. Aoki Y, Katoh O, Nakanishi Y, et al. (1999). A comparison study of IFN-gamma, ADA and CA125 as the diagnostic parameters in tuberculous pleuritis. *Respir Med* 199; 88:139-14.

14. Teo S, and Chio L. (1987). Adenosine deaminase in pleural fluid an enzymatic test for tuberculous pleural effusion. *Sing Med J*;28:220-224.
15. Reechaipichitkul W, Kawamatawong T, Teerajetgul Y , et al. (2001). Diagnostic role of pleural fluid adenosine deaminase in tuberculous pleural effusion. *Southeast Asian J Trop Med Public Health*; 32:383-389.
16. Sharma S, Suresh V, Mohan A, et al. (2000). A prospective study of sensitivity and specificity of adenosine deaminase estimation in the diagnosis of tuberculous pleural effusion. *Indian J Chest Dis*; 43:149-155.
17. BurgessL, MaritzF, Roux I ,et al. (1996). Combined use of pleural adenosine deaminase with lymphocyte/neutrophil ratio Increased specificity for the diagnosis of tuberculous pleuritis. *Chest*; 109:414-419.
18. Bañales JL, Pineda PR, Fitzgerald JM, et al. (1991). Adenosine deaminase in the diagnosis of tuberculous pleural effusions. A report of 218 patients and review of the literature. *Chest*; 99:355-357.
19. Valdés L, San José E, Alvarez D ,et al. (1993). Diagnoses of tuberculous pleurisy using the biologic parameters adenosine deaminase, lysozyme, and interferon gamma. *Chest*; 103:458-465.
20. Orphanidou D, Gaga M, Rasidakis A ,et al. (1996). Tumour necrosis factor, interleukin-1 and adenosine deaminase in tuberculous pleural effusion. *Respir Med*; 90:95-98.
21. Gorguner M, Corci M, Gorguner I. (2000). Determination of adenosine deaminase activity and its isoenzymes for diagnosis of pleural effusion. *Respirology*; 5(4):321-4.
22. Nagesh BS, Sehgal S, Jindal SK , et al. (2001). Evaluation of polymerase chain reaction for detection of mycobacterium tuberculosis in pleural fluid. *Chest Jun*; 119 (6):1737-41.
23. Lee YC, Rogers JT, Rodriguez RM, et al. (2001). Adenosine deaminase level in none tuberculous Lymphocytic pleural effusions. *Chest Aug*; 120(2):356-61.
24. Adreasyan NA , Hairapetian HL, Sargisova YG ,et al. (2002). Activity of adenosine deaminase and its isoforms in pleural fluid in tuberculous pleuritis . *Med sci monit*; 8(10):708-12.
25. Lima DM, Colares JK, da Fonseca BA. (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 Sep*;124(3):909-14
26. Diacon AH, Van de Wal BW, Wyser C , et al. (2003). Diagnostic tools in tuberculous pleurisy. *Eur Respir J* ;22: 589-91.
27. Nun A, Hripsime L and et al. (2002). Activity of adenosine deaminase and its isoforms in pleural fluid tuberculous pleuritis. *Med Scin Monit*; 8(10):CR706-712.
28. Burgess LJ, Maritz FJ, Le Roux I, et al. (1996). Combined use of pleural adenosine deaminase with lymphocyte/neutrophil ratio. *Chest*; 109: 414-19.