ORIGINAL ARTICLE

Pleural Fluid Adenosine Deaminase for Diagnosis of Tuberculous Pleural Effusion

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ABSTRACT
BACKGROUND AND OBJECTIVES: Diagnosis of tuberculous pleural effusion (TPE) is a common clinical problem with multiple pitfalls. Adenosine deaminase (ADA) analysis is a simple and inexpensive test that can be performed on body fluids.The isoenzyme ADA-2 is elevated significantly in pleural fluid with activated lymphocytes. To evaluate the diagnostic value of ADA in tuberculous pleural effusion present study was conducted. **METHODS:** Total 80 patients with age >12 yrs of either sex with pleural effusion were enrolled for study. CBC, sputum examination, chest X-ray and thoracocentesis were performed. Cell count, cyto-pathological examination, glucose-proteins levels, Gram's and Z-N staining were performed. Pleural fluid ADA levels were done. Patients were divided in to tuberculous or non-tuberculous(NTB) group according to their finding. **RESULTS:** Total 52 patients were diagnosed as having TPE and 28 patients as NTB effusion. The mean age in the study population was 39 ± 17.13 years. Dyspnea was the most common presenting complaint. In TPE group, the other presenting complaints were anorexia, cough, weight loss, expectoration, and chest pain. NTB group also showed the same trends in presenting complaints except fever. The mean pleural fluid ADA level was 54.97±23.51 U/L in TB group were higher in comparison with NTB group. It was statistically significant with 'p' value of <0.05. **CONCLUSION:** The level of ADA in TPE is much higher than the levels in effusion of other etiology. Pleural fluid ADA is adequately sensitive and specific test and at the same time, inexpensive and easy to perform.
Keywords: Tuberculosis, Tuberculous Pleural Effusion, Non-Tuberculous Pleural Effusion, ADA analysis.

INTRODUCTION

Tuberculosis (TB) has affected mankind for over 5000 years and the disease continues to be a major cause of morbidity and mortality in developing country. Although the mycobacterium tuberculosis bacilli has been discovered over a century back (1882, Robert Koch) and drugs have been available for more than 70 years, but TB still remain one of the leading cause of death in developing country. The 1990 World Health Organization (WHO) report on the Global Burden of Disease ranked TB as the seventh most morbidity-causing disease in the world and expected it to continue in the same position up to 2020.[1] TB has the dubious distinction of being the most persistent scourge, of humankind.[2]

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Worldwide statistics are staggering: WHO estimates that 9.27 million new cases of TB occurred in 2007 (139 per 100000 populations), compared with 9.24 million new cases (140 per 100000 populations) in 2006. Of these 9.27 million new cases, an estimated 44% or 4.1 million (61 per 100000 populations) were new smear positive cases.[3] The situation is more complicated when one considers countries such as India, China, Indonesia, Nigeria and South Africa, where TB disproportionately affects the young population.[3] Thus India is the highest TB burden country globally, accounting for one fifth of the global incidence and 2/3rd of the cases in south East Asia. Nearly 40% of the Indian population is infected with the TB bacillus. Each year, 1.9 million new cases of TB occur in the country, of which about 0.8 million are infectious new smear positive pulmonary TB cases.[4] More than 80% of the burden of tuberculosis is due to premature death, as measured in terms of disability-adjusted life years (DALYs) lost. Every day, more

than 5,000 people develop TB disease, and nearly 1,000 people die of TB, i.e. 2 deaths every 3 minutes. As per WHO estimates in 2006, nearly 322,000 persons in India died of tuberculosis (mortality rate 28 per 100,000 persons), which was estimated at over 500,000 annually.[5] India accounts for one-third of the global TB burden, with 1.8 million developing the disease each year and nearly 0.4 million dying due to TB annually.Tuberculosis usually affects lung, but extra pulmonary tuberculosis (EPTB) is also common. In India, EPTB forms 10 to 15 percent of all types of TB.[6] Extra Pulmonary tuberculosis, is defined as, TB of any organ other than the lungs, such as the pleura, lymph nodes, intestines, genitourinary tract, skin, joints and bones, meninges of the brain, etc. Diagnosis should be based on culture-positive specimen from the extra-pulmonary site, histological, radiological, or strong clinical evidence consistent with active extra pulmonary TB. Pleurisy is classified as extra pulmonary TB. A patient diagnosed with both sputum smear positive pulmonary and extra pulmonary TB should be classified as pulmonary TB.[7] Identifying tuberculous pleuritis is a common clinical problem with multiple pitfalls. One third of patients with this condition can have a negative tuberculin skin test.[8] Pleural fluid culture results can be positive in 25% of patients.[9] Without treatment, the natural history of tuberculous pleuritis is spontaneous resolution with a high rate of recurrence (65%) of active tuberculosis disease.[10] The AIDS epidemic has reminded us of the importance of identifying tuberculosis and treating it.[11] The traditional answer to this clinical problem is to perform a needle pleural biopsy for both histologic study and culture, which can lead to the diagnosis of tuberculous pleuritis 86% of the time.[12] These procedures, combined with cultures of pleural fluid and sputum, have been reported to provide microbiologic confirmation of Mycobacterium tuberculosis as often as 90% of the time.[13] Tuberculosis culture results can take time, even with BACTEC (Becton Dickenson Microbiology Systems; Cockeysville, MD) and Gen-Probe (Gen-Probe; San Diego, CA) technology.[14] A significant minority of patients (10 to 20%) will not have positive culture results or granulomas on biopsy specimen. Four relatively new techniques have been reported to help in the diagnosis of tuberculous pleuritis: Adenosine Deaminase (ADA), Lysozyme, Interferon gamma, and Polymerase Chain Reaction (PCR). Surprisingly, polymerase chain reaction has a relatively low sensitivity in pleural fluid (0.42 to 0.81)[15-17] and is fairly expensive. The sensitivity of an elevated interferon level appears better (0.89 to 0.99)[18-19], but there have been relatively few studies of its use and the assay is expensive. Lysozyme pleural fluid to serum ratios have been reported to improve the sensitivity of the pleural fluid ADA.[20] ADA analysis is a simple and inexpensive colorimetric test that can be performed on body fluids. [21] ADA, an enzyme that catalyzes the conversion of adenosine to inosine, is found in most cells. The isoenzyme ADA-2 is elevated significantly in pleural fluid with activated lymphocytes, such as from tuberculosis. False-positive results can occur with lymphoma, rheumatoid arthritis, systemic lupus erythematosus, and rarely adenocarcinoma.[22] Different isoenzyme (ADA-1) is elevated in the presence of empyema.[23] However, use of the isoenzyme assay is more expensive and not readily available. By not ordering an ADA assay of empyema fluid, one can avoid most false-positive results due to an elevated ADA-1 [22]. To evaluate the diagnostic value of ADA in tuberculous pleural effusion present study was conducted.

MATERIALS AND METHODS

The present study was conducted in the Department of Pathology, Medical College Baroda, Vadodara, India. Total 80 patients with age more than 12 yrs of either sex with pleural effusion, admitted in Department of Pulmonary Medicine were

enrolled for study, during a period of 19 Months from June 2013 to January 2015. After getting the informed consent, a detailed history and thorough physical examination was performed. In all of them complete blood count (CBC), sputum examination for Acid Fast Bacilli (AFB) and chest radiography were done. Thoracocentesis was performed for pleural fluid along with needle biopsy in all the patients. Cell count, cyto-pathological examination for malignant cells, estimation of glucose and proteins, Gram's staining and Ziehl-Neelsen (Z-N) staining for acid fast bacilli (AFB), were performed on pleural fluid. Pleural fluid and pleural biopsy specimens were cultured on Lowenstein - Jensen (LJ) medium. ADA levels were estimated in all the pleural fluid samples using Giusti's method.[21] Diagnosis of tuberculous pleural effusion was based on presence of any one of the following criteria; Mycobacterium tuberculosis identified in a culture of the pleural fluid and/or sputum, Caseating granulomas in the absence of any clinical evidence of sarcoidosis, tularemia or fungal infection in the pleural tissue or a response to antituberculous drugs revealed by an improvement of clinical symptoms and/or a clearing of chest radiograph. Remaining patients were considered as non tuberculous. From each patient about 10 ml of pleural fluid was collected in plain and EDTA bulbs. Samples were assessed for ADA; in case of delay the samples were stored at 4°C up to 4 days of collection.

Statistical analysis:

Results were expressed as mean, median, S.D., and percentages. ADA levels between tuberculous and non tuberculous patients were compared using appropriate Student t-tests. Sensitivity and specificity was calculated for various cut-off levels of ADA and a receiver – operator characteristic curve showing [1 - Specificity] on the x - axis and sensitivity on the y - axis using different cut-off levels of pleural fluid ADA levels was plotted to arrive at the most appropriate cut-off level.

OBSERVATIONS

In the present study, total 80 patients of age >12 yrs of either sex with pleural effusion, diagnosed clinically and radiologically were included. Based on the chief complaints, past medical and family history as well as laboratory evaluation including haemoglobin, total and differential leucocyte count, ESR, chest radiography and pleural fluid analysis, clinical diagnosis was achieved for every patient.

Table 1: Number of cases according to various causes of pleural effusion

Group	Cause of pleural effusion	Number of cases
TB	Tuberculous	52
NTB	Non-tuberculous 1. Cardiac 2. Renal 3. Hepatic 4. Empyema 5. Malignant	 06 05 05 06 06
Total		80

(TB- Tuberculous, NTB- Non tuberculous)

Table 2: Clinical scenario of cases according to their presenting complaints.

	TB		NTB		Total	
	No	%	No	%	No	%
Presenting Complaints						
Cough	46	88.46	20	71.43	66	82.5
Expectoration	34	65.38	09	32.14	43	53.75
Fever	47	90.38	16	57.14	63	78.75
Dyspnea	49	94.23	26	92.86	75	93.75
Chest Pain	32	61.54	19	67.86	51	63.75
Weight loss	37	71.51	12	42.86	49	61.25
Anorexia	46	88.46	21	75.0	67	83.75
Multiplicity		5.6		4.39		5.18
Past History						
TB	17	32.67	-	-	17	21.25
DM	02	3.85	04	14.28	06	7.50
Hypertension	01	1.92	02	7.14	03	3.75
IHD	01	1.92	02	7.14	03	3.75
CHF	-	-	01	3.57	01	1.25
Malignancy	-	-	05	17.85	05	6.25
Liver Cirrhosis	08	15.38	04	14.28	12	15.0
Family History						
TB	06	11.54	-	-	06	7.50
Personal History						
Smoking	32	61.53	14	50.0	46	57.50
Sputum for AFB						
Positive	10	19.23	-	-	10	12.5
HIV status						
Positive	06	11.54	-	-	06	7.50

(TB- Tuberculous ,NTB- non tuberculous, DM- Diabetes mellitus, IHD- Ischemic Heart Disease, CHF- congestive Heart failure , AFB- acid fast bacilli)

Out of these 80 patients, 52 were diagnosed as having tuberculous (TB) pleural effusion based on the preset criteria mentioned under materials methods. Rest of the patients i.e. 28 patients were grouped as non-tuberculous (NTB). Among the non-tuberculous patients, 6 patients were due to cardiac, empyema, and malignant causes each and 5 patients due to renal and hepatic etiology (Table 1). The mean age in the study population was 39 ± 17.13 years. Dyspnea was the most common presenting complaint. In the TB group other presenting complaints of fever, anorexia, cough, weight loss, expectoration, and chest pain were present in that decreasing order of frequency. NTB group also showed the same trends in presenting complaints except fever, which was present in 57.17% patients only (Table 2). None of the patients in either group had haemoptysis.

There was no statistical significant difference in the mean haemoglobin concentration between the TB and NTB groups including NTB subgroups viz. cardiac, hepatic, renal, empyema, and malignant (Table 3). On the other hand, the mean ESR of around 71 mm in first hour in TB group was statistically highly significantly higher than NTB group. Whereas, the mean total leukocyte count in the blood at around 8500/mm was statistically significantly higher in the TB group as compared to NTB group. The empyema subgroup with total cell counts in the range of 15,000/mm3 showed highly significantly higher counts than the TB group. Mean sugar level in pleural fluid of TB group was lower as compare to NTB group (79 mg% vs 81 mg %), while mean pleural fluid's protein level was higher for the TB group (4.4 gm% vs 2.75 gm%). Median total count was also higher in the TB group at 1280 cells/mm^3 as compare to NTB group which was 690 cells/mm^3. In the TB group RBCs were present on microscopic examination in only 13.46 % of cases as compared to 28.57 % of NTB group cases.

Table 3: Comparison of mean haemoglobin (Hb), Total count (TC), and ESR of TB group with various NTB subgroups

	Hb			TC			ESR		
	Mean ± S.D.	Mean Diff.	P-Value	Mean ± S.D.	Mean Diff.	P-Value	Mean ± S.D.	Mean Diff.	P-Value
TB	9.42 ± 1.73	-	-	8501.92 ± 2135.55	-	-	71.73 ± 34.13	-	-
NTB	9.65 ± 1.50	0.23 ± 0.15	NS	8892.86 ± 4819.40	390.94 ± 140.0	**	40.46 ± 28.68	31.27 ± 1.18	***
• Cardiac	9.58 ± 1.13	0.16 ± 0.74	NS	6400 ± 1888.92	2101.92 ± 164.27	***	20.67 ± 12.63	51.06 ± 2.09	***
• Hepatic	8.82 ± 1.71	0.60 ± 0.82	NS	6760 ± 1608.33	1751.92 ± 165.81	***	45.4 ± 24.82	26.33 ± 2.66	***
• Renal	8.74 ± 1.33	0.68 ± 0.81	NS	8280 ± 1676.90	221.92 ± 171.43	NS	43.2 ± 24.60	28.53 ± 2.68	***
• Empyema	10.00 ± 1.72	0.58 ± 0.76	NS	15186.67 ± 6460.16	7314.76 ± 392.01	***	45.83 ± 41.22	25.90 ± 3.08	***
• Malignant	10.83 ± 0.88	1.41 ± 0.73	NS	6750 ± 618.87	1751.92 ± 128.10	***	48.5 ± 31.51	30.39 ± 2.68	***
	NS	Non-significant					p > 0.05		
	*	Significant					p > 0.05		
	**	Significant					p < 0.01		
	***	Highly Significant					p < 0.001		

(TB- Tuberculous ,NTB- non tuberculous)

In TB group 90.38% patients had a exudate pleural effusion as compared to 46.43 % patients in NTB group (Table 4). Among the NTB group there was a great variation in the distribution as transudate and exudate. All cases of renal subgroup pleural effusions were transudate whereas those of malignant subgroup were exudate. Around 83.33% of empyema subgroup was exudate whereas same proportion of cardiac and hepatic subgroups had transudate pleural effusion. The mean pleural fluid's ADA levels of 54.97 ± 23.51 U/L in TB group were higher in comparison with NTB group as a whole as well as the cardiac, hepatic, renal, empyema and malignant subgroups (Table 5). It was statastically significant with 'p' value of less than 0.05.

Table 4: Difference between transudative and exudative pleural effusion.

	Transudate (≤2.9 g%)		Exudate (>2.9 g%)	
	N	%	N	%
TB	05	9.62	47	90.38
NTB	15	53.57	13	46.43
Cardiac	05	83.33	01	16.67
Hepatic	04	80.00	01	20.00
Renal	05	100.0	-	-
Empyema	01	16.67	05	83.33
Malignant	-	-	06	100.0

(TB- Tuberculous ,NTB- non tuberculous)

Table 5: Comparison of pleural fluid's ADA levels in TB group with various NTB subgroups

	Mean ± S.D. (U/L)	Mean Diff.	p – value
TB	54.97 ± 23.51	-	-
NTB	15.95 ± 10.74	39.02 ±4.75	p < 0.001
Cardiac	11.77 ± 8.40	43.20 ± 9.84	p < 0.001
Hepatic	-15.44 ± 8.58	39.53 ± 10.77	p < 0.001
Renal	8.34 ± 3.43	46.63 ± 10.71	p < 0.001
Empyema	23.28 ± 15.42	131.59 ± 10.00	p < 0.002
Malignant	23.23 ± 13.35	31.74 ± 9.94	p < 0.002

(TB- Tuberculous ,NTB- non tuberculous)

DISCUSSION

A definite diagnosis of tuberculous pleural effusion can be difficult to make because of the low sensitivity and/or specificity of noninvasive traditional diagnostic tools. In most series of patients with tuberculous pleural effusion, the results of pleural fluid staining for acid-fast bacilli are virtually always negative and pleural fluid cultures are positive for mycobacteria only in 25% of cases. On the other hand, a pleural biopsy specimen will demonstrate granulomatous pleuritis in 80% of patients with tuberculous pleural effusion, and when a culture of a biopsy specimen is combined with histologic examination, the diagnosis can be established in approximately 90% of cases.[22]

Adenosine deaminase is an enzyme in the purine salvage pathway required for converting adenosine to inosine. Its levels are ten times higher in lymphocytes than in erythrocytes and particularly so in T lymphocytes. Tuberculous pleural effusion is the result of cell mediated immune response to the presence of Mycobacterium tuberculosis and is characterized by the accumulation of the activated T lymphocytes and macrophages in the pleural space. In this tuberculous pleural effusion elevated levels of ADA have been noted by several authors.[23-31]

In the present study 80 patients of age more than 12 yrs of either sex with pleural effusion, diagnosed clinically and radiologically were subjected to pleural fluid analysis after thoracocentesis. The mean age in the study population was 39 ± 17.13 years. It ranged from 14 to 85 years in TB group and 16 to 85 years in NTB group. This is consistent with the observations by Nagaraja et al[24] and Ungerer et al [26]. The average age of patients with tuberculous pleurisy is increasing and the disease is now commonly seen in middle and old age. Out of these 80 patients, 52 (64%) were diagnosed as having tuberculous pleural effusion. Total 28 (36%) patients patients were grouped as non-tuberculous. Among the non- tuberculous patients, 6 (8%) patients were due to cardiac, empyema, and malignant causes each and 5 (6%) patients were due to renal and hepatic

causes each. High proportion of cases with tuberculous etiology is consistent with findings from other Indian studies.[32-37] This observation is possible due to high prevalence of tuberculosis in India.

In the present study males and females were present in a ratio of 7:2 in overall as well as both TB and NTB groups. This is in concordance with studies by Shaun K Teo [38] and Maldhure et al [37] on pleural effusion but was 19:11 in a study by Nagaraja et al[36] and 31:19 in a study by Reechaipichitkul et al [39].

The hemoglobin content and peripheral white blood cell count are usually near normal in tuberculous pleurisy. Only two patients in the series by Berger et al [8] had a haemoglobin level below 10 gm % and three patients had a white blood cell count above 12,000/mm^3. But in our study the mean haemoglobin concentration in TB and NTB group was 9.4 gm% and 9.6 gm%. The mean total leukocyte count in the blood was around 8500/mm^3 in the TB group of our study.

TB pleural effusion is typically clear and straw coloured; however, it can be turbid or serosanguinous but is virtually never grossly bloody. In our study, 90 % of the TB group patients had an exudate pleural effusion (pleural fluid protein >2.9 gm %) as compared to 46.43 % of NTB group. Among the NTB group there was a great variation in the distribution as transudate and exudate. In renal subgroup patients had transudate pleural effusions whereas those of malignant subgroup had exudates pleural effusion. Around 83 percent of cases in the empyema subgroup were exudate whereas same proportion of cardiac and hepatic subgroups had transudate pleural effusion. Pleural fluid glucose concentration was >60 mg/dL in 80% to 85% of cases in Valdes et al [18] study and <30 mg/dL in approximately 15% of cases in Epstein et al [23] study. In present study, in TB group mean pleural fluid sugar was 76.53 ± 33.36 mg % and NTB subgroups showed variable results. In present study pleural fluid protein levels that were higher for TB group (4.4 g/dL) as compare to NTB group (2.75 g/dL), which was correlating with study of Reechaipichitkul et al [39] and Antonangelo et al [40]. The mean ESR of around 71 mm in first hour in TB group was higher than NTB group. ESR, which is usually expected to be higher in a patient with tuberculosis, was < 30 mm in first hour in about 14% and >30 mm in first hour in about 86% cases of a study by Hussain et al [40].

The origin of the increased ADA activity found in tuberculous effusions is uncertain. Ocana et al [28] and Banales et al [29] have attributed the origin of these high levels to the fact that tuberculous pleurisy is a T-cell-mediated response. However, insignificant or inconclusive results have been obtained by Ocana et al [28] and Baganha et al for studies attempting to show a correlation between number of lymphocytes or lymphocyte populations and ADA levels. Ungerer et al [26] have suggested a monocyte-macrophage origin of ADA, reflecting an increased cellular activity or turnover. In our study, mean pleural fluid ADA level was 54.97 ± 23.51 U/L in TB group. This is consistent with Perez-Rodriguez et al [27] study, which was 54.7 ± 23.5 U/L. On the other hand in the NTB group the mean ADA level was 15.95 ± 10.74 U/L, which also correlates with Perez-Rodriguez et al [27] study (18.3 ± 43.2 U/L). The mean pleural fluid's ADA levels of 54.97 ± 23.51 U/L in TB group were statistically highly significantly higher as compare to NTB group. The observations are consistent with the findings of previous studies [29-31.]

The sensitivity and specificity of ADA depends on the prevalence of tuberculosis in the population as demonstrated by Valdes et al [30]. With the decline in the prevalence of tuberculous pleural effusion, the positive predictive value of pleural fluid ADA also declines, but die negative predictive value remains high. Thus, the measurement of the pleural fluid ADA level is an excellent test to rule out a tuberculous etiology of pleural effusion, irrespective of the rate of prevalence of the

disease. In areas in which the prevalence of disease is low, there is a higher likelihood of false-positive test results. The determination of a cut off value require a compromise between sensitivity and specificity and the considerations involved in this may vary from investigator to investigator depending upon the purpose for which the test is required. If ADA estimation is planned to be the definitive test for the diagnosis of tuberculosis, then a hundred percent specificity would be required. In our study the highest available sensitivity for a specificity of 100% was 63% and it occurred, at a cut off level of 45 U/L. On the other hand, if ADA estimation is planned as an initial phase screening test, then a high sensitivity with a reasonable specificity would be required. In our study, we obtained a sensitivity of 94% with a specificity of 82% at a cut off value of 30 U/L. Above or below this cut off value there were significant losses in sensitivity and specificity respectively, without a significant gain in specificity and sensitivity. So we opted for 30 U/L as the appropriate cut off.

In present study ADA-activity appear as a very good parameter for diagnosis of tuberculous effusion. Its sensitivity and specificity were very high. The levels of ADA achieved in tuberculous effusions were higher than in any other group ($p<0.001$). In nontuberculous effusions ADA-activity was always low. Thus, a value in the low range is of no diagnostic help, except in excluding tuberculosis. Some of the discrepancy in the results among the studies reported in the literature can be attributed to the use of different methods for ADA analysis. Consequently, when interpreting results, physicians should be conscious of the differing cut off levels that can occur with the different methods of ADA analysis [31]. One should be also aware that ADA levels in pleural fluid maintained at ambient temperature will decrease with time. A meta-analysis of 40 studies by Goto et al investigating ADA for the diagnosis of tuberculous pleuritis yielded the summary measure of test characteristics derived from the receiver operator characteristic curve where sensitivity equaled specificity at 92.2%. Similarly, a meta-analysis of 31 studies by Greco et al [25] on ADA in pleural tuberculosis yielded a joint sensitivity and specificity of 93%.

An ideal test for tuberculous pleurisy should be economic, minimally invasive, of high accuracy, and quick to perform. The ADA test has been hailed lately as the ideal test for the diagnosis of TB pleural effusion. However, this test has some limitations that should be taken into consideration when facing the challenge of diagnosing a pleural effusion of unknown origin. In practice, the ability to predict the presence or absence of disease from test results is dependent on the prevalence of the disease in the population tested, as well as on the sensitivity and specificity of the test. The advantages of ADA are that unlike pleural biopsy, it is not affected by sampling error e.g. when a non-involved area of the pleura is biopsied and only a small volume of pleural fluid (5mL) is required to perform assay.

CONCLUSION

The level of ADA in tuberculous pleural effusion is much higher than the levels in effusion of other etiology. Using 45 U/L as the cut off, it is possible to avoid invasive pleural investigation in as much as 63% of the patients to ascertain the diagnosis of tuberculosis. Thus, pleural fluid ADA estimation seems to have the potential for being a single test for the diagnosis of tuberculous pleural effusion. Pleural fluid ADA is adequately sensitive and specific test and at the same time, inexpensive and easy to perform in the field setting.

REFERENCES

1. Murray, Christopher JL, Lopez, Alan D. The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries and risk factors in 1990 and projected to 2020: summary - WHO Geneva, Switzerland, 1996; W 74 96GL-1/1996.

2. Narain JP (ed.): Tuberculosis - epidemiology and control. World Health Organization, Regional Office for South East Asia, New Delhi, India, 2002; SEA/TB/2002. 248:15-18.
3. Global tuberculosis control: Epidemiology, Strategy, Financing: WHO report 2009. Geneva: World Health Organization; 2009. Available http://www.who.int/tb/pubtications/global_report/2009/en/. Accessed 09/12/2009.
4. TB India 2008. RNTCP Status Report. Available http://www.tbcindia.org /docuinents.asp. Accessed 16/08/2009.
5. Global tuberculosis control: Surveillance, Planning, Financing: WHO Report 2006. Geneva: World Health Organization; 2006. Available at;URL:http://www.who.int/tb/publications/global_report/2006/en/index.Accessed09/12/2009.
6. Stelianides S, Belmatoug N, Fantin B. Manifestations and diagnosis of extrapulmonary tuberculosis. Rev Mal Respir. 1997; 14 Suppi 5:S72-87.
7. Wipa Reechaipichitkul, Theerasuk Kawamatawong. Diagnostic role of pleural fluid adenosine deaminase in tuberculous pleural effusion.Khon Kaen University, Khon Kaen 40002, Thailand. Vol 32 No.2 June 2001 383-389
8. Berger HW, Mejia E. Tuberculous pleurisy. Chest 1973; 63:88-92.
9. Scharer L, Mclement-JH. Isolation of tubercle bacilli from needle biopsy specimens of parietal pleura. Am Rev Respir Dis 1968; 97:466-468.
10. Roper WH, Waring JJ. Primary serofibrinous pleural effusion in military personnel. Am Rev Respir Dis 1955; 71:616-634.
11. Brudney K, Dobkin J. Resurgent tuberculosis in New York City. Am Rev Respir Dis 1991; 144:745-749.
12. Bueno CE, Clemente G, Castro C, et al. Cytologic and bacteriologic analysis of fluid and pleural biopsy specimens with Cope's needle. Arch Intern Med 1990; 150; 1190-1194.
13. Seibert AF, Haynes J, Middleton R, et al. Tuberculous pleural effusion: twenty-year experience. Chest 1991; 99:883-886.
14. Schluger NW, Rom WN. Current approaches to the diagnosis of active pulmonary tuberculosis. Am J Respir Crit Care Med 1994; 149:264-267.
15. Lassence A, Lecossier D, Pierre C, et al. Detection ofmycobacterial DNA in pleural fluid from patients with tuberculous pleurisy by means of the polymerase chain reaction; comparison of two protocols. Thorax 1992; 47:265-269.
16. Querol JM, Minguez J, Garcia-Sanchez E, et.al. Rapid diagnosis of pleural tuberculosis by polymerase chain reaction. Am J Respir Crit Care Med 1995; 152:1977-1981.
17. Villena V, Rebollo MJ, Aguado JM, et al. Polymerase chain reaction for the diagnosis of pleural tuberculosis in immunocompromised and immunocompetent patients. Clin Infect Dis 1998; 26:212-214.
18. Valdes L, Alvarez D, San Jose E, et al. Tuberculous pleuritis: a study of 254 patients. Arch Intern Med 1998; 158: 2017-21.
19. Villena V, Lopez-Encuentra A, Echave-Sustaeta J, et al. Interferon-gamma in 388 immunocompromised and immunocompetent patients for diagnosing pleural tuberculosis. Eur Respir J 1996; 9:2635-2639.
20. Villena V, Navarro-Gonzalvez JA, Garcia-Benayas C, et al. Rapid automated determination of adenosine deaminase and lysozyme for differentiating tuberculous and non-tuberculous pleural effusions. Clin Chem 1996; 42:218-221.
21. Giusti G. Adenosine deaminase. In: Bergmeyer HU, ed. Methods of enzymatic analysis. New York, NY: Academic Press, 1974; 1092-1099.
22. Light RW. Pleural diseases. 3rd ed. Baltimore, MD: Williams & Wilkins, 1995.

23. Epstein DM, Kline LR, Albelda SM, Miller WT. Tuberculous pleural effusions. Chest 1987; 91(1): 106-9.
24. Loddenkemper R. Thoracoscopy - state of the art. Eur Respir J 1998; 11: 213-21.
25. Greco S, Girardi E, Masdangelo R, Capoccetta GB, Saltini C. Adenosine deaminase and interferon gamma measurements for the diagnosis of tuberculous pleurisy: a meta-analysis. Int J Tuberc Lung Dis 2003; 7: 777-86.
26. Ungerer JPJ, Oosthuizen HM, Relief JH, Bissbort SH. Significance of Adenosine Deaminase Activity and Its Isoenzymes in Tuberculous Effusions. Chest 1994; 106; 33-37.
27. Perez-Rodriguez E, Perez Walton IJ, Sanchez Hemandez JJ, Pallares E, Rubi J, et al. ADAj/ADAp ratio in pleural tuberculosis: an excellent diagnostic parameter in pleural fluid. Respir Med 1999; 93(11): 816-21.
28. Ocana I, Martinez-Vazquez JM, Segura RM, Femandez-De-Sevilla T, Capdevila JA. Adenosine deaminase in pleural fluids. Test for diagnosis of tuberculous pleural effusion. Chest 1983; 84; 51-53.
29. Banales JL, Pineda PR, Fitzgerald JM, Rubio H, Selman M, et al. Adenosine deaminase in the diagnosis of tuberculous pleural effusions. A report of 218 patients and review of the literature. Chest 1991; 99; 355-7.
30. Valdes L, Alvarez D, Jose ES, Juanatey JR, Pose A, et al. Value of adenosine deaminase in the diagnosis of tuberculous pleural effusions in young patients in a region of high prevalence of tuberculosis. Thorax 1995; 50; 600 -3.
31. Riantawan P, Chaowalit P, Wongsangiem M, Rojanaraweewong P. Diagnostic Value of Pleural Fluid Adenosine Deaminase in Tuberculous Pleuritis With Reference to HIV Coinfection and a Bayesian Analysis. Chest 1999; 116; 97 - 103.
32. Raj B, Chopra RK, Lal H, Saini AS, Singh V, et al. Adenosine deaminase activity in pleural fluids--a diagnostic aid in tuberculous pleural effusion. Indian J Chest Dis Allied Sci 1985; 27(2): 76 - 80.
33. Chopra RK, Singh V, Lal H, Saini AS, Chawla RK, et al. Adenosine deaminase and T lymphocyte levels in patients with pleural effusions. Indian J Tuberc 1988; 35: 22-4
34. Gupta DK, Suri JC, Goel A. Efficacy of Adenosine deaminase in the diagnosis of pleural effusions. Indian J Chest Dis Allied Sci 1990; 32:205-8.
35. Subhakar K, Kotilingam K, Satyasri S. Adenosine deaminase activity in pleural effusions. Lung India 1991; 9: 57 - 60.
36. Nagaraja MV, Ashokan PK, Hande HM. Adenosine deaminase in pleural effusions. J Assoc Physicians India 1992; 40: 157-9.
37. Maldhure BR, Bedarkar SP, Kulkami HR, Papinwar SP. Pleural biopsy and Adenosine deaminase inpleural fluid for the diagnosis of tuberculous pleural effusion. Indian J Tuberc 1994; 41: 161-5.
38. Teo SK, Chio LF. Adenosine deaminase in pleural fluid – an enzymatic test for tuberculous pleural effusion. Singapore Medical Journal 1987; 28: 220-224.
39. Reechaipichitkul W, Kawamatawong T, Teerajetgul Y, Patjanasoontom B. Diagnostic role of pleural fluid adenosine deaminase in tuberculous pleural effusion. Southeast Asian J Trop Med Public Health 2001; 32: 383 - 9.
40. Antonangelo L, Vargas FS, Seiscento M, Bombarda S, Teixera L, et al. Clinical and laboratory parameters in the Differential diagnosis of pleural effusion Secondary to tuberculosis or cancer. Clinics 2007; 62(5); 585-90.