DIAGNOSTIC VALUE OF ADENOSINE DEAMINASE ACTIVITY IN TUBERCULOSIS & NON TUBERCULOUS LYMPHOCYTIC BODY FLUIDS

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ABSTRACT

INTRODUCTION
The diagnosis of tuberculosis continues to be a challenge. More than 90% of TB effusions show a lymphocytic predominance, hence TB requires exclusion in any lymphocytic exudative effusion where no alternative cause is found.

AIMS & OBJECTIVE The objective of the present study was to validate the usefulness of adenosine deaminase (ADA) levels in lymphocytic body fluid (lymphocyte count >50%) in the diagnosis of TB.

METHOD AND MATERIAL
It was a prospective study and 300 lymphocytic body fluids were studied during the period of 1 year from June 2007 to August 2008 at department of Pathology. ADA level was estimated in all these samples.

RESULTS
Among 300 cases studied, including all age group and male predominance and using a cut off level of 55 unit/L of pleural fluid ADA, specificity 86.67%, the sensitivity 93.51%, false positive rate was 6.73%. For ascitic fluid, using a cut off level of 60 units/L of, specificity 94.44%, the sensitivity 92%, false positive rate was 14.30%. For CSF ADA, using a cut off level of 10 units/L of, specificity 97.56%, the sensitivity 88.57%.

CONCLUSION
We found that ADA when combined with lymphocyte >50%, remains a useful test in the diagnosis. ADA level were significantly reduced with antitubercular therapy, so we can use ADA as a prognostic marker of tuberculosis.

KEYWORDS
Body fluid, Adenosine deaminase (ADA), Tuberculosis (TB).

HOW TO CITE THIS ARTICLE: Nanwani P, Kapoor A, Khatri S. Diagnostic value of adenosine deaminase activity in tuberculosis & non tuberculous lymphocytic body fluids. J. Evid. Based Med. Healthc. 2016; 3(15), 554-561.
DOI: 10.18410/jebmh/2016/126

INTRODUCTION: Tuberculosis (TB) is a leading cause of preventable morbidity and mortality from an infectious agent.(1) It affects equally irrespective of age. The interaction of HIV with mycobacterium tuberculosis has led to its resurgence in developed nation & has increased the burden of TB cases in developing countries.(2,3)

The traditional answer to diagnose TB is to perform a needle biopsy for the histologic study & culture, which can lead to the diagnosis 86% of the time.(4) These procedures combined with culture of body fluids, have been reported to provide microbiologic confirmation of mycobacterium tuberculosis as often as 90% of the time.(5) Tuberculosis culture results can take time, even with BACTEC (Becton Dickenson Microbiology System; Cockeysville, MD) and Gen Probe (Gen Probe; San Diego, CA) technology. A significant minority of patients (10-20%) will not have positive culture results or granuloma on biopsy specimen. An ideal test for tuberculosis should be minimally invasive, of high accuracy & quick to perform.

More than 90% of TB effusions show a lymphocytic predominance, and hence TB requires exclusion in any lymphocytic exudative effusion where no alternative cause found. ADA level relates to the intensity of stimulation & the maturation of the lymphocytes, due to the immune cellular response against Mycobacterium tuberculosis. ADA level measurement in body fluids has emerged as an attractive alternative for the diagnosis of TB because of the description of fast determination method, such as the technique described by Giusti in 1981.

Since 1978 ADA has been used in the diagnosis of tuberculous effusion, which is simple & easy to perform. The level of ADA an enzyme, found in most cells, is increased in tubercular pleural effusion, pericardial effusion, ascitic fluid & CSF. This determination has acquired popularity as a diagnostic test in high incidence area for tuberculosis because it is non - invasive.(6)

Submission 06-01-2016, Peer Review 22-01-2016,
Acceptance 08-02-2016, Published 22-02-2016.
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DOI: 10.18410/jebmh/2016/126

High levels of ADA also have been reported in non-infectious conditions associated with body fluid lymphocytosis, including;

- Malignant conditions (e.g. adenocarcinoma, leukemia & lymphoma)
- Collagen vascular disease (e.g. Rheumatoid pleuritis & Systemic lupus erythematosus).
- Lymphocyte-rich body fluids associated with live intracellular microorganisms also could have elevated ADA pleural fluid levels, including those caused by coccidioidomycosis and histoplasmosis, which are endemic mycoses in vast areas of the United States, which make the test less useful in countries with low prevalence of tuberculosis.[7]

Therefore, an increased ADA level should not be considered as an equivalent to the presence of mycobacteria in the body fluids. A higher rate of false positive test results can lead to the unnecessary administration of antitubercular therapy or a delay in making an alternative diagnosis.

In lymphocyte-dominant pleurisy, peritonitis, ADA is released in the presence of live intracellular Mycobacterium tuberculosis. False positive diagnoses by ADA level determination could significantly reduce if ADA measurement is limited to lymphocytic body fluids.[6]

Aims & OBJECTIVE: This study was undertaken with the following aims & objectives:

- To determine the diagnostic value of ADA level in lymphocytic ascites, pleural effusion, CSF & pericardial fluid due to various causes.
- Study the ADA level in a variety of non-tubercular & tubercular lymphocytic effusions to find out false positive cases.
- To study whether any correlation exists between ADA activity and routine chemical & cytological examination of body fluids.
- To study the prognostic value of ADA in known tuberculous patients.

METHODS AND MATERIAL: The present study was carried out on patients admitted in different departments of pathology in hospital. Cases of all ages & both the sex were taken. All the lymphocytic body fluids (pleural, pericardial, peritoneal fluids & CSF) received in department of pathology from different departments, of varying aetiology from June 2007 to August 2008 were analysed. Body fluids with lymphocyte count <50% were excluded.

The body fluids were analysed in terms of glucose, protein, and pH levels, Gram stain, and cytopathology examination. Stain for AFB were also performed. An aliquot of the body fluid was set aside for ADA determination.

Inclusion Criteria for TB patients were:

1. Patient present with systemic symptoms like fever, malaise, chronic cough & haemoptysis.
2. No other accountable cause for effusion or CNS infection.
3. Presence of an exudates, (protein 3 gm % or more in pleural & ascitic fluid, and >45 mg/dl in CSF), increased cell count with lymphocytosis.
4. Radiological evidence of presence of a shadow suggestive of pulmonary tuberculosis.
5. Cytological evidence.
6. Histological evidence.
7. Response to anti tubercular drugs.
    1. Bacteriological evidence – AFB seen in fluid or sputum.
    2. Past history of TB.

Inclusion Criteria for non-tubercular patient were: Not having any symptoms of illness, no other positive finding according to above mentioned inclusion criteria for tuberculosis.

METHODS:

1. **Examination of the CSF, pleural fluid, ascitic fluid & pericardial fluid under following headings:**
    - Physical examination was done under following headings - quantity, colour, appearance, odour, blood- present/absent, coagulum- present/absent.
    - Biochemical examination.
        - Protein test by turbidimetric method.
        - Sugar test by GOD/POD method.
    - Microscopic examination of fluids for total cell count. Field stained, air dried simple centrifuge preparations was used for differential cell count
    - Cytological examination – all fluids were subjected for cytological examination. We used Papanicolaou staining method.
    - Histological examination - H&E stained slides were examined for presence or absence of chronic granulomatous reaction.

2. **Ziehl Neelsen staining of fluids, sputum for the detection of AFB.**

3. **ADA activity determination in all body fluids:** Determination of ADA levels was carried out in Clinical Pathology Laboratory using the Giusti method. After collection of the sample, 0.02 mL of the body fluid was placed into a test tube and 0.2 mL of an adenosine solution were added. The mixture was incubated at 37ºC for 60 minutes, and the reaction was then interrupted by the addition of a phenol-nitroprusside solution and a hypochlorite solution. The resulting solution was subsequently incubated at 37ºC for 15 minutes. The reading of the amount of ammonia liberated by ADA action was performed with the aid of a spectrophotometer at a wavelength of 620 nm. Each series of tests was carried out under a reaction control and a negative control for each sample. The readings were converted to U/L in order to make the statistical calculations.

The ADA sensitivity and specificity was determined by using the final diagnosis of TB (confirmed or not) as the gold standard. Mean ADA values in the different groups were compared using the Student's t-test. Correlation was analysed with Pearson's correlation test, $p < 0.05$ was considered significant.

RESULTS: The present study was conducted on 300 body fluids with lymphocyte count > 50% to study the diagnostic & prognostic value of ADA levels & to study whether any correlation exists between ADA activity and routine chemical & cytological examination of body fluids.

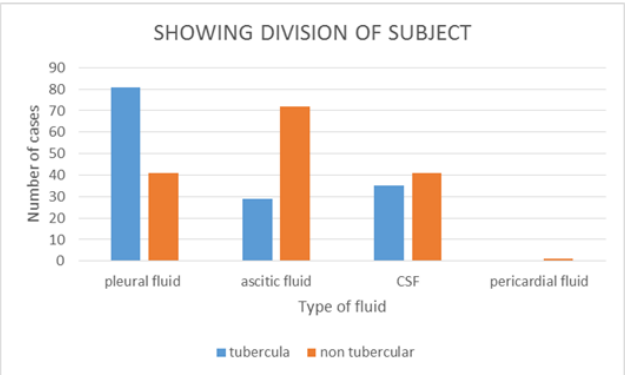


Figure 1

We have divided all the cases on the basis of type of fluids – pleural, ascitic, CSF, & pericardial fluid. 122 pleural fluids, 101 ascitic fluids, 76 CSF & 01 pericardial fluid were included in the study. They were further divided in tubercular & non tubercular sub groups, in which 81 cases were of tubercular pleural effusion, 29 tubercular ascitis, 35 tubercular meningitis. Remaining 155 lymphocytic body fluids were included in nontubercular subgroups.

Evidence of tuberculosis	Breakup data
Sputum positive and MT positive	30
Sputum positive and MT negative	18
Sputum negative and MT positive	15
Histological evidence present	02
Cytological evidence present	03
Treated cases responded to anti tubercular therapy	10
No evidence (diagnosis made on the basis of only clinical history and chest X-ray finding)	03
Total cases	**81**

Table 1: Revealed division of tubercular pleural effusion (81 case) based on evidence of tuberculosis

Evidence of tuberculosis	Breakup data
MT positive	16
Histological evidence present	03
Cytological evidence present	05
Treated cases responded to anti tubercular therapy	03
No evidence (diagnosis made on the basis of only clinical history and chest X-ray finding)	02
Total cases	29

Table 2: revealed division of tubercular ascites (29 case) based on evidence of tuberculosis

Evidence of tuberculosis	Breakup data
MT positive	28
Treated cases responded to anti tubercular therapy	02
Sputum positive (cases of disseminated tuberculosis)	05
Total cases	35

Table 3: Revealed division of tubercular meningitis cases (29 cases) based on evidence of tuberculosis

We found Mantoux test positivity in 45 cases of pleural effusion, 16 cases of ascitis & 28 cases of tuberculous meningitis. Pleural biopsy histological examination revealing granulomatous inflammation is frequently used as an inclusion criterion for pleural TB. In present study, 02 cases of pleural effusion & 03 cases of ascitis on laparoscopic biopsy specimens showed granulomatous inflammation, 03 cases of pleural effusion & 05 cases of ascitis with lymphadenopathy, on FNAC found to have granulomatous lesion, suggestive of tuberculosis.

The maximum number of cases was in the age group 31-40 years i.e. 23.67%, followed by. 23.33% in the age group 21-30 years. Out of total 300 cases, males were 188 & females were 112. Out of 145 tubercular cases 62.76% were males & 37.24% were females. In tubercular cases we found that male to female ratio was 1.68:1.

We divide the cases according to ethnic group, & observed that 90.33% patients were Hindu & 09.66% were Muslim. Our observation not corroborated with the work of Patidar R.C., 1984. The difference in ethnic group distribution could be due to wide variation in selection criteria in different studies.

Group	No. of subjects	% in pleural effusion group	Range of pleural -effusion ADA level (U/L)	Mean pleural fluid ADA level (U/L)
Tubercular cases	81	66.39	35–150	85.97
Nontubercular cases	41	33.61	1.25-150	39.33

Table 4: ADA activity in pleural fluid

Table 4 reveals that pleural fluid ADA levels are considerably higher in tubercular pleural effusions than other groups.

ADA level in tubercular cases is higher than in other lymphocytic effusions.

The mean ADA level in tubercular pleural effusion is 85.97U/L versus 39.33 U/L in non-tubercular cases.

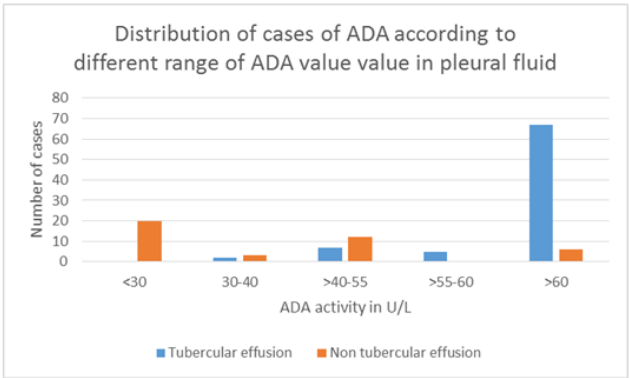


Figure 2

The above graph shows, out of 81 cases, 67 cases of tubercular effusion have ADA level >60 U/L & out of 41 nun tubercular pleural effusion cases, 20 cases have ADA level <30U/L.

Group	No. of subjects	% in ascitic fluid group	Range of ascitic fluid ADA level (U/L)	Mean ascitic fluid ADA level (U/L)
Tubercular cases	29	28.71	43.75-150	87.04
Nontubercular cases	72	71.29	1.16-150	22.44

Table 5: ADA activity in ascitic fluid

Table 5 shows that ascitic fluids ADA levels are higher in tubercular group.

The mean ADA value in tubercular ascetic fluids is 87.04 versus 22.44 U/L in non-tubercular ascitic fluids.

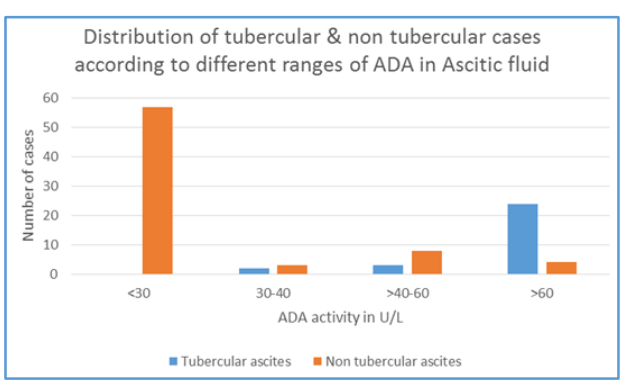


Figure 3

The above graph shows 24 cases out of 29 cases (87.76%) of tubercular ascites have ADA level > 60 U/L & 57 cases out of 72 cases of nontubercular ascites have ADA level < 30U/L.

Groups	No. of patients	% in CSF groups	Range of CSF ADA value (U/L)	Mean ADA level (U/L)
Clinically suspected tubercular cases	34	44.74	7.1-41.2	16.71
Other cases	42	55.26	0.99-14.72	5.16

Table 6: ADA activity in CSF

Table 6 shows that CSF ADA levels in cases of tubercular meningitis is considerably higher than non-tubercular meningitis.

The mean ADA in tubercular meningitis is 16.71 U/L versus 5.16 U/L in non-tubercular meningitis.

Range of ADA	Tubercular meningitis		Nontubercular meningitis	
	No. of cases	%	No. of cases	%
<10	04	11.43	40	97.56
>10	31	88.57	01	2.44
Total	**35**	**100**	**41**	**100**

Table 7: Distribution of clinically suspected tubercular & other cases according to different range of ADA in CSF

The above table shows that 88.57% cases of tubercular meningitis have ADA level >10U/L & 97.56% cases of non-tubercular meningitis cases have ADA level <10U/L.

Group	No. of cases	Transudative fluids %	Mean ADA level (U/L)	No. of cases	Exudative fluids %	Mean ADA level (U/L)
Tubercular PF	01	1.23	45.02	80	98.77	86.48
Nontubercular PF	15	36.58	20.10	26	60.98	63.42
Tubercular AF	01	3.45	71.50	28	96.55	84.57
Nontubercular AF	38	52.78	13.95	34	47.22	34.54

Table 8: correlation between pleural & ascitic fluids protein level versus their ADA levels

Out of the 223 cases of pleural effusion & ascites, applying the standard criteria laid by Bright et al, 27.80% i.e. 62 cases fell in the transudate group & 72.20% i.e. 161 cases fell in the exudate group.

Cytological study	Tubercular pleural effusion		Nontubercular pleural effusion		Tubercular ascitic fluids		Nontubercular ascitic fluids	
	No. of cases	Mean ADA level U/L	No. of cases	Mean ADA level U/L	No. of cases	Mean ADA level U/L	No. of cases	Mean ADA level U/L
Inflammatory smear with lymphocytic predominance	81	85.97	33	33.18	29	87.04	63	17.38
Smear with malignant cells	Nil	-	08	64.69	Nil	-	09	57.86
Haemorrhagic smears	Nil	-	01	150	Nil	-	1	150

Table 9: Correlation between pleural & ascitic fluids cytology & their ADA level

The above table shows that all tubercular PF & AF on cytology show inflammatory smears with lymphocytic predominance.

Groups	No. of cases	Mean ADA level in treated tubercular cases U/L
Pleural fluid	10	52.27
Ascitic fluid	03	43.38
CSF	02	13.75

Table 10: mean ADA level in patients already received Anti-tubercular therapy of various study groups

Groups	Mean ADA level in untreated tubercular cases U/L	Mean ADA level in treated tubercular cases U/L
PF	85.97	52.27
AF	87.04	43.38
CSF	16.88	13.75

Table 11: Comparison between mean ADA level in treated & untreated tubercular cases

The above table shows that the mean ADA level is high in untreated cases compare to treated cases.

DISCUSSION: Identifying tuberculosis is a common clinical problem with multiple pitfalls. Three relatively new techniques have been reported to make the diagnosis of tuberculosis-

1. Polymerase chain reaction (PCR) – has a relatively low sensitivity in body fluids (0.42 to 0.81) but it is fairly expensive.[8]
2. Interferon Gamma level – the sensitivity of an elevated interferon Y level appears better (0.89-0.99), but there have been relatively few studies of its use and the assay is expensive.
3. Lysozyme – lysozyme body fluids to serum ratio have been reported to improve the sensitivity and specificity of this test.

ADA analysis is simple and inexpensive calorimetric test that can be performed on body fluids. The isoenzyme ADA 2 is elevated significantly in body fluids with activated lymphocytes, such as from tuberculosis. False positive results can occur in lymphoma, rheumatoid arthritis, systemic lupus erythematosus, adenocarcinoma etc. A different isoenzyme ADA-1 is elevated in the presence of empyema.[9] However, use of the isoenzyme assay is more expensive and not readily available. By doing ADA assay only in lymphocytic effusion, we can avoid most false positive results. Valdes et al, in a study of 350 effusion samples, found no significant difference in the usefulness of ADA-2 over total ADA, and thus concluded that "the extra labor involved in the estimation of ADA2 would not be justified in clinical practice."

Sl. No	Study	Year	No. of patients	Cut-off value of ADA IU/L	Sensitivity	Specificity
1.	Piras et al[10]	1978	54	30	100	100
2.	Ocana et al[11]	1983	182	45	100	97
3.	Segura et al[12]	1989	600	71	100	92
4.	Valdes et al[13]	1993	405	47	100	95
5.	De Olivera et al[14]	1994	276	40	91	88
6.	Burger et al	1995	462	50	90	89
7.	Valdes et al[15]	1996	350	47	100	91
8.	Villena et al[16]	1996	228	33	90	85
9.	Perez-Rodriguez[17]	1999	103	40	89	92
10.	Villegas et al[18]	2000	140	45.5	88	86
11.	Sharma et al[19]	2001	75	35	83	67
12.	Lima et all[20]	2003	45	40	68	2
13a.	Present study	2008	300	60	85.90	86.36
13b.	Present study	2008	300	55	93.51	86.6

Table 12: comparison of mean pleural fluid ADA level with other author's studies

The discrepancies in the result among the reported studies can be attributed to the use of different methods of ADA analysis, with the most frequent being the calorimetric assay by Giusti and Galanti. The specificity is increased when the lymphocyte count (>50%) is considered in conjunction with an ADA concentration >55U/L, hence for suspicious cases of TB, increased level of ADA could facilitate diagnosis.

Sl. No.	Name of author's	Tubercular ascites	Malignant ascites	Other cause of ascites	Statistical significance
1.	Martinez[21] 1986	108.5	6.3	0.01	P<0.001 i.e. HS
2.	Gupta et al[22] 1988	158.10	15.6	.44	-

3.	Michael D. Voigt[23] 1989	99.8±49.1	14.8±8.4	14.8±8.4	Cut-off point of 32.3U/L sensitivity 95% & specificity 98%
4.	Bhargava et al[24] 1990	141.03±61.5	19.7±13.5	10.0±7.8	Cut-off point of 36U/L sensitivity 100% & specificity 97%
5.	Arnoldo Riquelme[25], 2006	95± 35.5	20.5±10.5	12±8.6	Cut-off point of 36 to 40U/L sensitivity 100% & specificity 97%
6.	M.A. Sathar, et al[26] 1995	101.84 U/L	19.35 U/L	13.49 U/L	Cut-off point of >30U/L sensitivity 93% & specificity 96%
7.	Present study	87.0±35.14	57.86 U/L	22.53±27.12	Cut-off point of 60U/L sensitivity 82.76% & specificity 94%

Table 13: comparison of mean ascitic fluid ADA level with other author's studies

Using a cut off level of 60 units/L of ascitic fluid ADA, value has been reported to show the specificity 94.44%, the sensitivity 92%, PPV 85.70% & NPV 93.15. The false positive rate of ADA of tubercular lymphocytic ascites was 14.30% which is slightly higher (t = 9.9199, P<0.0001).

The determination of ADA activity in CSF of TBM patients using cut off value of 10 U/L can be useful for the early differential diagnosis of TBM and it is cost effective with a false positive rate of 3.13%. The specificity of ADA in TBM is 97.56 %, sensitivity 88.57 %.

Sl. No.	Name of author	Tubercular meningitis	Non-tubercular meningitis
1.	Piras et al[10]	12.35±3.78	2.63±1.8
2.	Malan et al	15.7±21.67	12.5±12.33
3.	Ribera et al[27]	15.7±4.3	1.2±1.9
4.	Agrawal et al[28]	31.13±10.24	8.6±1.47
5.	Present study	16.71±9.42	5.17±2.75

Table 14: Comparison of CSF- ADA with other studies

From the above observation from the different authors, it is concluded that ADA activity is much higher in tubercular meningitis than that for nontubercular meningitis.

Correlation with Protein Content: In the present study as indicated in table 8. The mean ADA activity in tubercular & non- tubercular transudative effusion is less than that of exudative effusion. Present study shows that there is significant correlation between ADA level & protein content of fluids. Y.C. Gary Lee, MBChB, Jeffrey T. Rogers, 2001 also concluded that the ADA level were significantly lower in transudative effusions than in other exudative effusions. Goyal N.R. et al 1990, concluded that there is no statistically significant correlation of fluid ADA level with protein content. Out of 35 patients clinically suspected of tubercular meningitis, in 07 cases proteins were raised within twice the normal limit, in 16 cases these was 2 to 4 times increase of CSF protein content. The mean ADA activity is variable in different groups & does not show any correlation with protein content of fluids.

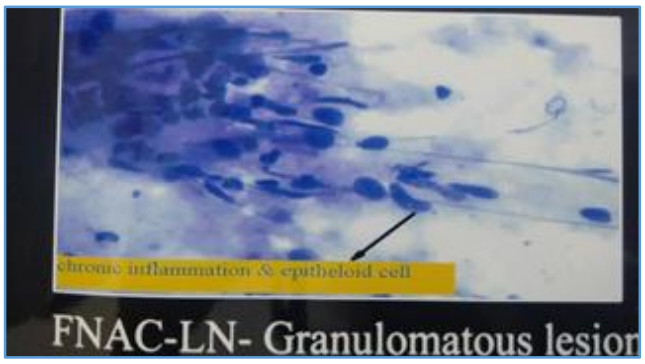
Whereas, Satya Vati Rana, Raj Kumar Singhal*, Kartar Singh & Lata Kumar, 2004 found a positive correlation of ADA levels in CSF with CSF proteins. Malan et al. found a positive correlation between CSF ADA levels, CSF proteins and CSF pleocytosis in patients of TBM. Christina Mann et al found a positive correlation of CSF-ADA with protein content in tubercular cases but there is no correlation in study done by Agrawal et al.

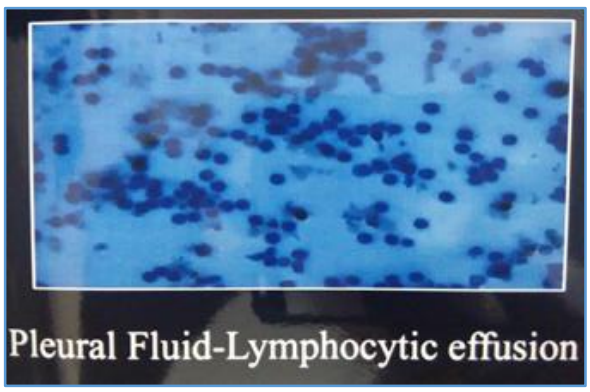
According to (Table 9), the mean ADA activity is high in malignant pleural & ascitic fluids but mean ADA activity in tubercular body fluid is significantly higher. The two-tailed P value equals 0.0407 by conventional criteria, this difference is considered to be statistically significant, (t=2.1227). One non-tubercular pleural fluid & one non-tubercular ascitic fluid were hemorrhagic. The mean ADA activity in hemorrhagic fluids is 150 U/L, which is falsely positive.

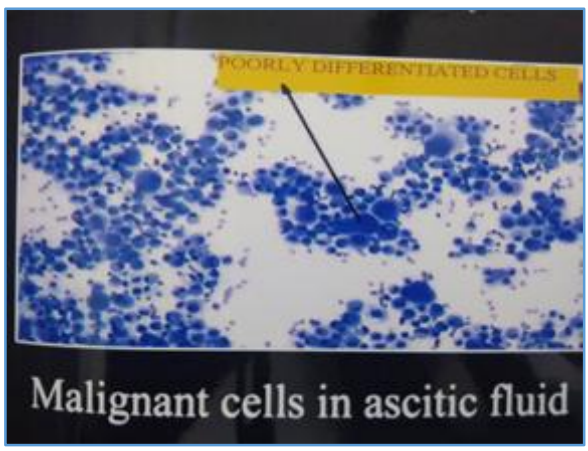
We also studied, the ADA activity in treated cases of tuberculosis, who responded well to antitubercular therapy. From 300 cases included in the study 10 cases of tubercular pleural effusion, 03 cases of tubercular ascites & 2 cases of tubercular meningitis already received antitubercular therapy & they responded well to treatment, their ADA level was found to be less compare to tubercular group.

The mean ADA in untreated cases of tubercular pleural effusion is 85.97±30.67 versus 52.22±5.75 in treated cases of tubercular pleural effusions. The two-tailed P value equals 0.0008 by conventional criteria; this difference is considered to be extremely statistically significant. It is concluded that ADA level significantly reduced after treatment with antitubercular therapy.

CONCLUSION: We found that when combined with differential cell counts and lymphocyte >50%, remains a useful test in the diagnosis of tuberculous pleural effusion, ascites & tubercular meningitis. ADA level were significantly reduced with antitubercular therapy, so we can use ADA as a prognostic marker of tuberculosis.







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