WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 6, 831-839. Research Article ISSN 2277 – 7105

ADA ESTIMATION IN BODY FLUIDS OF TUBERCULOSIS PATIENTS

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Article Received on
10 June 2014,

Revised on 05 July 2014,
Accepted on 30 July 2014

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ABSTRACT

Aim: The aim of the study is to determine the ADA level in tuberculous patient and figuring cut off value for ADA to use it as a diagnostic marker. **Methods:** ADA levels were estimated in serum and body fluids of 100 tuberculous and 100 non-tuberculous diseased patients. **Results:** Serum ADA for tubeculous patients (19.32±2.15 U/L) is significantly higher than non- tuberculous patients (10.55±0.90 U/L). Also the ADA in Pleural fluid (29.61±3.93), Pericardial Fluid (28.53±1.02), Peritoneal Fluid (27.91±2.23), CSF(28.40±1.86) in tuberculous patient is significantly higher than non tuberculous diseased pleural (10.33±1.28), peritoneal (10.11±0.53), pericardial (10.46±1.00) and CSF (10.07±0.88). The cut off value suggested is 15 U/L which yelds the sensitivity and specificity of about 98% and 100 % respectively. The ratio of body fluid ADA to serum ADA in subjects with tubercular cases is significantly higher than and non-tubercular disease patients. **Conclusion:** Conventional markers of TB i.e. serum ADA levels are significantly higher in tuberculous patients as compared with the healthy subjects and subjects with other disease. It is also concluded from the study that the Body fluid (pleural fluid, pericardial fluid, peritoneal fluid and CSF) ADA to serum ADA ratio is significantly higher in TB cases as compared to that with non-tubercular cases. The cut-off value suggested for the parameters studied in the study yields a high sensitivity and specificity for the diagnosis of tubercular infection.

KEY WORDS: Mycobacteria, pleural, ascites, parapneummonic, monocytes, peritonitis, empyema, meninigits.

Ada Estimation In Body Fluids Of Tuberculosis Patients

TB continues to be a major cause of mortality and morbidity in developing countries. Although lung is the most frequent organ to be involved**,** inflammation of serous membranes is equally common. The definitive diagnosis is established when typical histological features can be demonstrated or *mycobacteria* can be isolated from the body fluids or from sputum or from gastric lavage. Various other methods**,** such as gel electrophoresis**,** radiometric assay and polymerase chain reaction are also available. It is well documented that isolation of *mycobacteria* and culture is difficult and time consuming (1) and other tests are complex and technically more demanding**,** most of the times diagnosis is based on radiological features**,** clinical features and positive tuberculin testing. Recently**,** attempts are on to find most simple techniques which are reliable more feasible**;** less costly and giving quick results and are largely comparable with standard techniques. In the present study, ADA (Adenosine deaminase) estimation in blood and body fluids of tubercular patients are studied.

MATERIALS AND METHODS

The cases under study included pulmonary and other sites tuberculosis (Group A), non tubercular disease cases (Group B). The entire two group in the study had sample size of 100 (n=100) and having Gaussian distribution in the selected population. Blood samples of the controls /study group was collected for ADA estimation by venipuncture serum was used for analysis of ADA. Body fluids like pleural, peritoneal, and pericardial and CSF fluids all were collected in sterile tubes from patients wherever possible. ADA estimation was done using Giusti and Galanti modified method(2) Level of significance was calculated by applying student‘t’ test (2 tailed).

RESULTS

The data obtained is used for statistical analysis and is tabulated as follows -

Table No. 1: Showing serum ADA levels in Group A and B

	Group A (n=100)	Group B (n=100)	P Value
ADA (U/L)	19.32±2.15	10.55±0.90	<0.05

Table No. 2: Showing types of fluid and number of subjects in tubercular and non-tubercular disease group

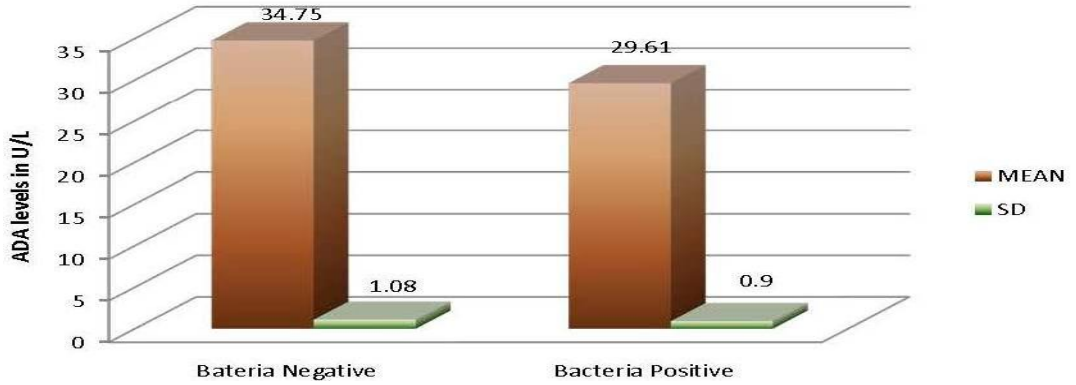
Type of Fluid	Group A	Group B
Pleural fluid	53	53
Pericardial Fluid	21	21
Peritoneal Fluid	21	21
CSF Fluid	29	29

Table No. 3: Comparison of ADA levels in body fluids of group B and C subjects (mean±S.D.).

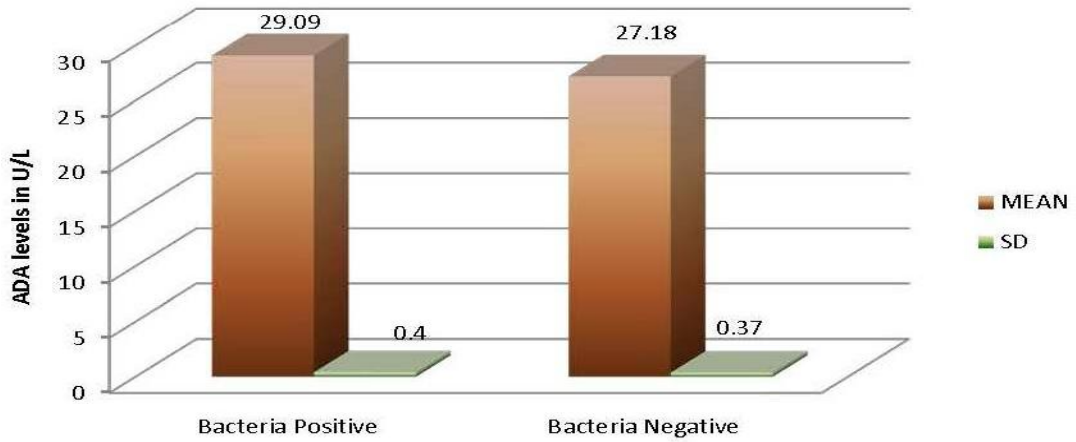
ADA (mg/dl)	Group A (n=100)	Group B (n=100)	P Value
Pleural fluid	29.61±3.93	10.33±1.28	<0.05
Pericardial Fluid	28.53±1.02	10.11±0.53	<0.05
Peritoneal Fluid	27.91±2.23	10.46±1.00	<0.05
CSF	28.40±1.86	10.07±0.88	<0.05

Table No. 4: Showing Comparison of Bacteria positive body Fluid ADA and with bacteria negative body fluid ADA.

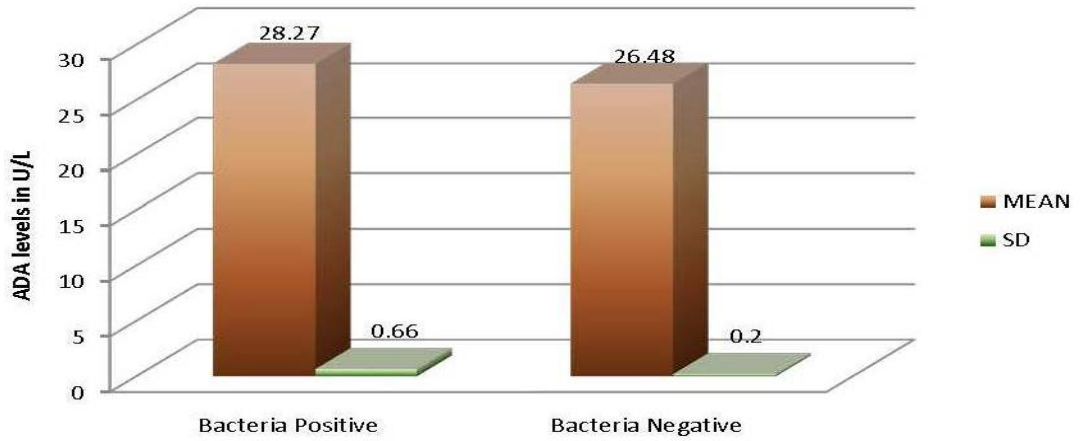
Body Fluid	Bacteria positive	Bacteria negative	P value
Pleural Fluid	34.75±1.08	29.61±0.90	<0.05
Pericardial Fluid	29.09±0.40	27.18±0.37	<0.05
Peritoneal Fluid	28.27±0.66	26.48±0.2	<0.05
CSF	29.31±0.39	27.50±0.36	<0.05



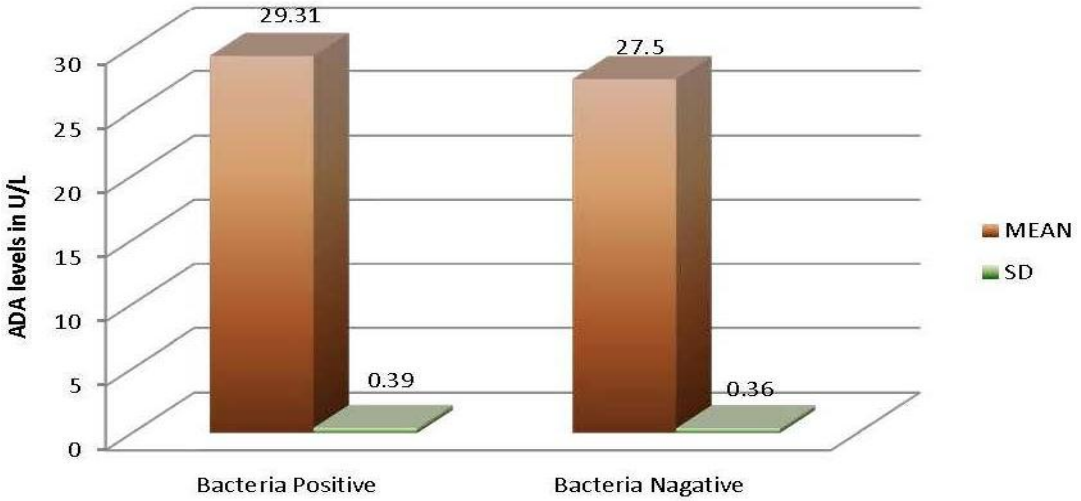
Graph No. 1 Comparison of bacteria positive to bacteria negative pleural fluid ADA



Graph No. 2 Comparison of bacteria positive to bacteria negative pericardial fluid ADA



Graph No. 3 Comparison of bacteria positive to bacteria negative peritoneal fluid ADA



Graph No. 4 Comparison of bacteria positive to bacteria negative CSF ADA

Table No. 5: Showing comparison of ratio of body fluid ADA to serum ADA in subjects with tubercular and non-tubercular diseases.

	Group A	Group B	P value
ADA PF/ADA SERUM	1.53±0.22	0.98±0.13	<0.05
ADA PCF/ADA SERUM	1.52±0.19	0.98±0.08	<0.05
ADA PET/ADA SERUM	1.49±0.19	0.96±0.08	<0.01
ADA CSF/ADA SERUM	1.44±0.14	0.96±0.03	<0.01

Table No. 6: Cut-off points for various biochemical parameters for diagnosis of TB.

Parameter	Cut-off level
Serum ADA	>15.00 IU/L
PF ADA/Sr.ADA ratio	>1.0
PCF ADA/Sr.ADA ratio	>1.0
PET ADA/Sr.ADA ratio	>1.0
CSF ADA/Sr.ADA ratio	>1.0

Table No. 7: Showing sensitivity and specificity of the parameters studied for the diagnosis of tubercular infection.

Parameter	Sensitivity	Specificity
Serum ADA	98%	100%
PF ADA/sr.ADA	100%	96%
PCF ADA/sr.ADA	100%	100%
PET ADA/sr.ADA	100%	100%
CSF ADA/sr.ADA	100%	100%

DISCUSSION

A total 53 patients were analyzed for pleural fluid, 21 each for pericardial and peritoneal fluids and 29 for CSF. The fluid findings were compared that with the corresponding non-tuberculous cases. Pleural, pericardial, peritoneal fluid and CSF ADA (U/L) levels are 26.04±0.72, 20.25±1.02, 18.82±0.94, 11.90±0.11 which is significantly higher in group A than and group B. In group A the pleural fluid ADA to serum ADA ratio in our study is 2.34±0.07 U/L, pericardial fluid ADA to serum ADA 1.81±0.09 U/L, peritoneal fluid ADA to serum ADA 1.68±0.09 U/L, CSF ADA to serum ADA which significantly differs from that compared to group B.

In the present study, it was found that, the sensitivity and specificity for serum ADA concentration is 98% and 100% and for pleural fluid/serum ADA concentration 100% and 96% respectively. In addition, a cut-off point of 15.3 IU/L for pleural ADA test yielded as sensitivity and specificity of 85.8% and 82.3% and at a cut-off point of 0.66 for pleural fluid/serum ADA ratio yielded 83.3% and 83.2% respectively in their study which is comparable to present study's cut-off point of 15 IU/L which yeilded the sensitivity and specificity of pleural ADA concentration for the diagnosis of exudates to be 98% and 100% respectively and at cut-off point of 1.0 for pleural fluid/serum ADA concentration 100% and 96% respectively. Hence, the cut-off point established in this study yielded a better sensitivity and specificity as compared to the other study. (3) The measurement of ADA activity in body fluids has been reported to be useful in the diagnosis of tuberculous effusion. This test will be of particular value in situations where it is not possible to do a pleural biopsy because of the small extent of the effusion or when standard diagnostic procedures including pleural biopsy have given negative results.The present study showed that, ADA activity is increased in the body fluid of 89% of patients with tuberculous effusion with levels ranging from 20 to 30U/L. Similar results have been reported by other workers. (4,5) but with some higher magnitude. High ADA levels have been reported in patients with empyema and parapneumonic effusion.

The ADA levels in malignant effusion are generally low. ADA is widely distributed in lymphoid tissue, especially in the thymus, lymph nodes, spleen, and gastrointestinal tract. The enzyme is found in a higher concentration in T-cells than in the B-lymphocytes. In TB and other infections the activity of ADA is increased after activation of T cells by antigenic stimulation. T-cells are found more abundantly in pleural fluid than in the peripheral blood of patients with tuberculous effusion. (6,7) However, there no correlation was found between ADA activity and the total number of T-cells and it is thought that ADA activity is related to the stage of maturation of the T-cells. (8) It has been shown that ADA is required in the maturation of the early T-cells (9) in the thymus as well as in the differentiation and maturation of T cells and B-lymphocytes and monocytes (9, 10, 11) after antigenic stimulation. The increased ADA activity is thought to be due to a local activation of T-lymphocytes and monocytes. (12) The assay of ADA can be recommended as a useful diagnostic test of tuberculous pleurisy. Unlike pleural biopsy, ADA 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 (5 ml) is required to perform the assay, which can be done in most clinical laboratories. The present results confirm the high sensitivity and specificity of ADA test for early diagnosis of TB in cases of serous effusions. The selective enzyme increase in tuberculous peritonitis strongly supports the concept that the study of ADA activity is of great value for identifying the tuberculous etiology of ascites (13,14) ADA has been proposed to be a useful surrogate marker for TB in pleural, pericardial and peritoneal fluids. Almost all research workers have shown sensitivity and specificity of 90% to 100% for the value of ADA in pleural fluid using different cut off levels. Gupta VK et al (1992) studied 53 cases of pleural effusion out of which 36 were of tuberculous etiology. (15) The mean ADA level in tuberculous was 50.75 U/L while in malignant and parapneumonic effusion it was 14.47 U/L and 28.65U/L respectively. The sensitivity and specificity for diagnosing TB were 100% and 94.1% respectively. Burgess LJ (1995) showed ADA activity in tuberculous effusion was higher than in any other diagnostic group. (16) At a level of 50 U/L the sensitivity and specificity for the identification of TB was 90% and 89% respectively Strankinga WFM (1987) et al investigated 10 patients with TB pleurisy and 76 patients with pleural effusions of other etiology. The ADA activity in the tuberculous patients was significantly higher than in the other groups while the exception of those with empyema. They also found that, specificity 87% and sensitivity 100% of this test for TB are high when a reference limit of more than 53 U/L is taken. Voight (2002) studied 41 cases with bacteriologically confirmed TB and 41 cases with other causes. The mean ADA level for tubercular etiology was 99.8 U/L

with sensitivity and specificity for diagnosis tubercular ascites was 95% and 98% respectively. (18) Dwivedi et al (1990) studied 49 patients with ascites of which 19 were of tubercular etiology with mean ADA level of 98.8 U/L. At an ADA level >33 U/L the sensitivity, specificity, positive and negative predictive values were 100%, 96.6%, 95%and 100% respectively.(19) A total 24 ascites cases were studied by Gupta et al (1992) of whom 7 were due to tubercular etiology with an ADA level of >30 U/L and sensitivity and specificity of 100% and 94.1% respectively.(15) The sensitivity and specificity for tubercular ascites on the basis of ADA level were 100% and 97% respectively as per the study of Bhargawa et al (1990) studied pericardial fluid ADA level along with histopathology of pericardial biopsy and found a cut off ADA level of 40 U/L in pericardial fluid which has sensitivity of 93% and specificity of 97% in diagnosis of tubercular pericardial effusion.(20,) Mathur et al (2005) found that sensitivity and specificity of ADA level for diagnosis of tubercular pericardial effusion was 100% and 83.3%. (21)

The specificity of ADA for diagnosis of tubercular pericarditis was low and which was attributed to number of cases of pericardial effusion. Rana et al (2004) found that mean ADA levels in CSF were highest in TB meningitis patients as compared to pyogenic meningitis and aseptic meningitis.(22) Ribera et al (1987) has also demonstrated similar findings in their study of adult TB meningitis patients.(23) Baheti et al (2001) found that CSF - ADA level 6.5 IU/L as a cutoff value exhibited a sensitivity of 95.83%, specificity of 92.85% for the diagnosis of tuberculous meningitis. (24) In addition to this, they also found the positive predictive value of test is 95.83% with overall accuracy being 94.73%. Cut-off CSF - ADA level of 6.5 IU/L may differentiate tuberculous from non-tuberculous meningitis. They observed a statistically significant difference in the CSF - ADA levels of TBM and other groups of meningitis and controls ($p< 0.001$). Though demonstration of AFB in CSF, CSF culture, CSF cytochemistry, and CT scan are the various means to diagnose TBM, the CSF - ADA estimation is a cost-effective and reliable means to establish a diagnosis of TBM.

CONCLUSION

Conventional markers of TB i.e. serum ADA levels are significantly higher in tuberculous patients as compared with the healthy subjects and subjects with other disease. The body fluids (pleural, pericardial, peritoneal, CSF) levels of serum ADA is higher in those with tubercular fluid accumulation as compared to that with non-tubercular fluid accumulation. It is also concluded from the study that the Body fluid (pleural fluid, pericardial fluid,

peritoneal fluid and CSF ADA to serum ADA ratio is significantly higher in TB cases as compared to that with non-tubercular cases. The cut-off value suggested for the parameters studied in the study yields a high sensitivity and specificity for the diagnosis of tubercular infection.

REFERENCES

1. Shah N, Aslan N (1992). Adenosine deaminase activity levels and its diagnostic value. Pakistan medical journal 251: 217-221.
2. Giusti G, Galanti B (1974). Adenosine deaminase. In: Hergmyer HU (RD). Method of enzymatic analysis. New York: Verlag Chemic Weinhein and Academic Press 1092-1099.
3. Jadhav, J. Bardapurkar (2007). Diagnostic value of adenosine deaminase to differentiate exudates and transudates Indian Journal Physiol Pharmacol 2007: 51 (2):170-174.
4. Paliwal R, Shah KV (1998). Serum adenosine deaminase es-timation in patients with Pulmonary Tuberculosis and other non tuberculosis respiratory conditions. Indian J of Tuberculosis. 1998; 45(3): 174.
5. Alatas F, Uslu S, Moral H, Alatas O, Metints M., Erginel S, Uegun I (2003). Serum adenosine deamise activity in Pulmonary tuberculosis. Tuberk Toraks 2003; 51(3) ; 277-81.
6. Pettersson T, Klockars M, Weber TH (1991): Diagnostic value of cerebrospinal fluid adenosine deaminase determination. *Scand J Infect Dis* 1991, 23:97-100.
7. Pettersson T, Ojala K, Weber TH (1984). Adenosine Deaminase in diagnosis of pleural effusions. Medline. 215: 299-304.
8. Ocana I, Desenlla MJM, Capdulla C (1966). Adenosine deaminase activity in pleural fluids Clinical infectious diseases 96: 84-91.
9. Shore A, Dosch HM, Gelfand EW (1981). Role of adenosine deaminase in the early stages of precursor T cell maturation. Clin Exp Immunol 1981; 44: 152-5.
10. Chechik BE, Schrader WP, Fernandes B, Sengupta S, Gelfand EW (1985) Association of Adenosine Deaminase with Differentiation of Normal and Leukemic T and B Lymphocytes Annals of the New York Academy of Sciences Volume 451, Deaminase in Disorders of Purine Metabolism and in Immune Deficiency pages 169–179, October 1985.
11. H. R. Miller and E. P. Adams (1977) Reassortment of lymphocytes in lymph from normal and allografted sheep. Am J Pathol. Apr 1977; 87(1): 59–80.

12. Kim J, Park Y, Kim Y, Kang S, Shin J, Park I, Choi B (2003). "Miliary tuberculosis and acute respiratory distress syndrome". *Int J Tuberc Lung Dis* 7 (4): 359–64.
13. Martinez-Vasquez JM, Ocana I, and Ribera E, (1986) Adenosine deaminase activity in diagnosis of tuberculous peritonitis. Gut. 27, 1049-1053.
14. Martinez-Vazquez JM, Ocana I, Ribera E, Segura RM, Pascual C (1986) Adenosine deaminase activity in the diagnosis of tuberculous peritonitis Gut, 1986, 27, 1049-1053
15. Gupta VK, Mukerji S, Datta SK (1992). Diagnostic evaluation of ADA in tuberculous peritonitis. *JAPI* 1992; 40 (6): 387-389.
16. Burgess LJ (1995). Use of adenosine deaminase as a diagnostic tool for tuberculous pleurisy. *Thorax* 1995 June; 50 (6):672-674.
17. Strankinga WFM, Nauta JJP, Straub JP Stam J (1987) Adenosine deaminase activity in tuberculous pleural effusions: A diagnostic test Tubercle Volume 68, Issue 2, June 1987, Pages 137-140.
18. Voight MD, Kalvaria L, Trey C, (1989) et al. Diagnostic value of ascites adenosine deaminase activity in tuberculous peritonitis. *Lancet*; 1: 751 – 754.
19. Dwivedi M., Misra SP, Misra V, et al (1990). Value of adenosine deaminase estimation in the diagnosis of tuberculous ascites. *Am J Gastroenterology* 1990; 85: 1123-5.
20. Bhargava DK, Gupta M Nijhawan S (1990). Adenosine deaminase in peritoneal tuberculosis, diagnostic value in ascitic fluid and serum. *Tubercle* 1990; 71: 121-126.
21. Mathur PC, Tiwari KK, Trikha S Tiwari D (2006) Diagnostic Value Of Adenosine Deaminase (Ada) Activity In Tubercular Serositis *Indian J Tuberc 2006; 53:92-95*
22. Rana SV, Singhal RK, Singh K Kumar L (2004) Adenosine deaminase levels in cerebrospinal fluid as a diagnostic test for tuberculous meningitis in children Indian Journal Of Clinical Biochemistry, 2004, 19 (2) 5-9
23. Ribera E, Varzuez M. (1990) Activity of Adenosine deaminase in cerebrospinal fluid for diagnosis of tuberculosis. Chest 54:53-82.
24. Rajesh Baheti, Purnima Laddha, RS Gehlot, CSF - Adenosine Deaminase (ADA) Activity in Various Types of Meningitis, Journal, Indian Academy of Clinical Medicine, October-December 2001;2(4):285-87.