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## **Assessment of adenosine deaminase levels and lymphocyte counts in tubercular ascitis**

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**Abstract**---Ascitis is the pathological accumulation of free fluid within the peritoneal cavity. Ascitis is manifestation of number of diseases. Traditional classification of ascitis into “Exudative” and “Transudative” which involves estimation of ascitic fluid total protein, which is  $\geq 2.5$  g/dl in exudates and  $<2.5$  g/dl in transudate. To estimate and compare the ascitic fluid ADA levels between the tubercular ascitis and non-tubercular ascitis and to correlate the levels of ascitic fluid ADA with total protein, albumin, sugar, SAAG and lymphocytes in subjects with tubercular ascites. In our study, we included a total of 140 subjects, out of which 21 (15%) subjects had tubercular ascitis. Out of 140 subjects 105 of them had cirrhosis of liver and 35 of them had ascitis due to other causes. Biochemical analysis for Protein, albumin, LDH and Glucose were performed on Automated Biochemistry Analyser and ADA was estimated by ADA-MTB kit method. The mean levels of total protein and albumin in Group T were  $5.4 \pm 0.98$  and  $3.4 \pm 0.96$  and in Group NT were  $2.2 \pm 1.1$  and  $3.2 \pm 1.1$  respectively. There were statistically significant elevated levels of total protein and decreased albumin in Group T as compared to Group NT. We found that the 85.7% had elevated ADA levels in Group T and 14.2% had ADA levels  $<40$  U/L whereas in Group NT 98.44% had ADA levels  $<40$  U/L and 1.55% had ADA levels  $>40$  U/L and there was positive correlation of ADA levels with TLC, lymphocytes, proteins whereas ADA levels had negative correlation with sugar and SAAG.

ADA estimation is specific, sensitive, predictive ancillary tool which serves as definitive supplementary and supportive test for the diagnosis of tubercular ascitis.

**Keywords**---tubercular ascitis, protein, albumin, glucose, adenosine deaminase, lymphocytes.

## **Introduction**

Ascitis is the pathological accumulation of free fluid within the peritoneal cavity. Ascitis is manifestation of number of diseases. Traditional classification of ascitis into “Exudative” and “Transudative” which involves estimation of ascitic fluid total protein, which is  $\geq 2.5$  g/dl in exudates and  $<2.5$  g/dl in transudate.<sup>1-4</sup> Adenosine deaminase (ADA) is a purine degrading enzyme, widely distributed in tissues and body fluids. Conway and Cooke (1939) were first to study the distribution of ADA in various organs of the rabbit and showed that the normal blood of humans and mammals contained ADA activity ADA is secreted by lymphocytes and to a lesser extent by macrophages during activation of the cell immune response to mycobacterium antigens this raised activity of ADA is assessed by different methods by various workers to diagnose tubercular ascitis. Adenosine deaminase (ADA) activity was found to be high in pulmonary and extra pulmonary form of tuberculosis. Hence this prospective study is undertaken to determine the levels of ascitic fluid ADA activity in differentiating between tubercular and non-tubercular ascitis and to correlate the levels of ADA with biochemical parameters and lymphocytes in ascitic fluid in subjects with tuberculosis ascitis.<sup>5-7</sup>

## **Objectives of the study**

The objectives of this study include:

- To estimate and compare the ascitic fluid ADA levels between the tubercular ascitis and non-tubercular ascitis.
- To correlate the levels of ascitic fluid ADA with total protein, albumin, sugar, SAAG and lymphocytes in subjects with tubercular ascitis.

## **Materials and Methods**

### **Source of Data**

A Hospital Based observational study conducted on “*Assessment of Adenosine Deaminase Levels and Lymphocyte Counts in Tubercular Ascitis*” was conducted from January 2019 to January 2021 at our tertiary care hospital. We included a total of 140 subjects suffering from ascitis due to various causes. Out of which 21 were tubercular ascitis and 129 were non-tubercular ascetic subjects.

### **Laboratory Analysis**

Biochemical analysis for Protein, albumin, LDH and Glucose were performed on Automated Biochemistry Analyser and ADA was estimated by ADA-MTB kit

method. Cytological examination (cell count, cell type, malignant cells) and Microbiological demonstration of AFB by ZN stain and AFB culture was done by conventional LJ method. After all relevant investigations, lymphocytic exudates were segregated with >50% lymphocytic proportion of all nucleated cells. ADA level cut-off >40IU/ L were considered as tuberculous exudates which were confirmed by AFB stain and AFB culture subsequently. ADA level cut off value <40IU/L were studied for cytological examination for malignant cells and relevant investigation to confirm non-tuberculous lesion.

### Statistical Analysis

Data were expressed as mean  $\pm$  SD. The Student *t* test was used for the comparison. Differences between tuberculosis ascitis patients and non-tuberculosis ascitis patients with regard to the biochemical parameters were compared using their actual values and dichotomized according to the cut-off points suggested. To test for significance, we used the Mann-Whitney U test and Chi-square test for numerical and categorical variables, respectively.

### Results

We included a total of 140 subjects in our study, out of which 21 (15%) subjects had tubercular ascitis. Out of 140 subjects 105 of them had cirrhosis of liver and 35 of them had ascitis due to other causes which include CCF with ascitis, malignant ascitis, nephrogenic, pancreatitis and spontaneous bacterial peritonitis. Out of 105 liver cirrhosis subjects majority of the subjects (98/105) had alcoholic liver disease and 7/105 had cirrhosis secondary to HBV and HCV infections. We divided subjects into two groups group T tuberculosis ascitis subjects and group NT non-tuberculosis ascitis group.

Table 1  
Shows comparisons of demographic profile and laboratory parameters of study subjects

Parameters	Group T (n = 21)	Group NT (n = 129)	P value
Age (in years)	36.4 $\pm$ 6.3	46.8 $\pm$ 7.3	HS
Male: Female ratio	14:7	91:38	S
Biochemical parameters			
Total protein (g/dL)	5.4 $\pm$ 0.98	2.2 $\pm$ 1.1	HS
Serum albumin (g/dL)	3.4 $\pm$ 0.96	3.2 $\pm$ 1.1	NS
Elevated LDH (U/L)	20 (95.2%)	12 (9.3%)	HS
ADA (U/L)	68.3 $\pm$ 28.4	16.3 $\pm$ 4.4	HS
Pathological and microbiological parameters			
ESR	78.6 $\pm$ 18.4	18.4 $\pm$ 8.4	HS
TLC/cmm	778.6 $\pm$ 318.4	358.4 $\pm$ 205.4	HS
Lymphocyte (%)	68.6 $\pm$ 18.4	38.6 $\pm$ 20.4	HS
AFB positive	3 (14.2%)	0	S

It is evident from the above table that there were statistically significant differences in demographic and laboratory parameters between T and NT groups.

Table 2  
Adenosine Deaminase levels cut off as <40 U/L and >40 U/L in study subjects

Parameters	Group T (n = 21)	Group NT (n = 129)	P value
<40 U/L	3 (14.2%)	127 (98.44%)	HS
>40 U/L	18 (85.7%)	2 (1.55%)	

It is evident from the table that 85.7% had elevated ADA levels in Group T and 14.2% had ADA levels <40 U/L whereas in Group NT 98.44% had ADA levels <40 U/L and 1.55% had ADA levels >40 U/L.

Table 3  
Shows the correlation of ADA with TLC, Lymphocytes, Protein, SAAG and Sugar levels in Group T

Parameters	Correlation coefficient	P value
ADA (U/L)	1	HS
TLC/ cmm	0.789	HS
Lymphocyte	0.523	HS
Proteins (gm%)	0.689	HS
Sugar (mg/dL)	-0.632	HS
Serum Ascites Albumin Gradient (SAAG)	-0.598	HS

It is evident from the above table that there was positive correlation of ADA levels with TLC, lymphocytes, proteins whereas ADA levels had negative correlation with sugar and SAAG.

## Discussion

Ascitis is the pathological accumulation of free fluid within the peritoneal cavity. Ascitis is manifestation of number of diseases. Traditional classification of ascitis into "Exudative" and "Transudative" which involves estimation of ascitic fluid total protein, which is  $\geq 2.5$  g/dl in exudates and  $<2.5$  g/dl in transudate. Transudative ascitis results due to increased hydrostatic pressure, reduced serum osmotic pressure or both and usually occurs in Cirrhosis, Congestive heart failure, Constrictive pericarditis, Inferior vena cava obstruction, Hepatic vein obstruction or Nephrotic syndrome. Exudative ascitis usually occurs because of increased capillary permeability and is usually associated with tuberculosis, malignancy, pancreatitis, and myxoedema. Peritoneal tuberculosis is currently the sixth most frequent extra pulmonary location and it increase proportionally to the rising incidence of TB worldwide.<sup>8-12</sup>

The World Health Organization estimates that 10.0 million new cases of tuberculosis, equivalent to 133 cases per 100,000 population, occurred worldwide in 2017. Previous studies have demonstrated the usefulness of adenosine deaminase (ADA) measurements in the diagnosis of tuberculosis in body fluids, including cerebrospinal, pleural, peritoneal, and pericardial fluids. In our study, we included a total of 140 subjects, out of which 21 (15%) subjects had tubercular ascitis. Out of 140 subjects 105 of them had cirrhosis of liver and 35 of them had ascitis due to other causes which include CCF with ascitis, malignant ascitis, nephrogenic, pancreatitis and spontaneous bacterial peritonitis.<sup>12-14</sup>

The mean age of the subjects in group T was  $36.4 \pm 6.3$  as compared to group NT which was  $46.8 \pm 7.3$ . This finding was similar to the study conducted by Jalees Fatima et al (2010). The tubercular ascitis was more common in younger age group. Similarly, Malhotra V et al (1992) in their clinio pathological study of abdominal tuberculosis, prospectively studied 99 patients with possible diagnosis of abdominal tuberculosis and found that the mean age of the patients was  $31.6 \pm 13.6$  years with a range of 13 to 65 years. In this study, out of 21 subjects with TB Ascitis 14 (66.6%) were male and 7 (33.3%) were female, while among 129 Non-tuberculous Ascitis 98 (75.9%) were male and 38 (29.4%) were female patients. Similar findings were also observed by Kashyap R S et al and N.L. Patney et al i.e. male outnumbered the female.

The mean levels of total protein and albumin in Group T were  $5.4 \pm 0.98$  and  $3.4 \pm 0.96$  and in Group NT were  $2.2 \pm 1.1$  and  $3.2 \pm 1.1$  respectively. There were statistically significant elevated levels of total protein and decreased albumin in Group T as compared to Group NT. This finding was similar to the studies conducted by N.L.Patney et al and Basu et al. N.L.Patney et al. studied 20 cases of intestinal tuberculosis and found that the mean of total serum proteins was normal at 6.82 gm/dl but mean serum albumin was reduced to 2.86 gm/dl (gm%). Only 5 cases (25%) had normal serum proteins pattern. Basu S et al (2007) retrospectively studied medical records of 115 patients diagnosed with abdominal TB and observed hypoalbuminaemia (serum albumin  $<3$  gm/dl) in 67.83% of cases with range 1.92 -5.86 and median 2.62 gm/dl. Result of the present study correlate well with the above studies and suggests that serum albumin was decreased in majority of patients with abdominal tuberculosis.<sup>14-16</sup>

We found significantly elevated ESR in patients of tuberculous ascitis with and that of non-tuberculous ascitis group. This finding was similar to the study conducted by J. Ramesh et al (2008). He studied 86 cases of abdominal TB and found the ESR to be elevated in 98% of cases. Uzunkoy A et al studied records of 11 patients diagnosed as abdominal tuberculosis and found that the average ESR was 50 mm/h. The results of the present study are consistent with the above studies, so it can be concluded that ESR is raised in most of the patients diagnosed with tuberculous ascitis raised in most of the patients diagnosed with tuberculous ascitis. In this study, there were significantly elevated levels of TLC, lymphocytes in Group T as compared to Group NT. ADA, an enzyme required for purine degradation, is distributed in systemic tissues and body fluids. ADA levels in the ascitic fluid have been reported as a potential marker for the diagnosis of tuberculous peritonitis, with a sensitivity of 100% and specificity of 97% in

previous studies, and sensitivity of 100%, specificity of 96.0%, PPV of 53.3%, and NPV of 100% in this study. It is important for clinicians to understand the reason for this low PPV. The most important activity of ADA involves the proliferation and differentiation of T lymphocytes, and T lymphocytes have approximately 10 times higher ADA levels than B lymphocytes. Therefore, ADA levels can theoretically increase in cases of effusion (including pleural, peritoneal, and cerebrospinal fluid) due to infection (especially tuberculosis), lymphoproliferative disorders, and rheumatic diseases. In our study, we found significantly elevated levels of ADA levels in ascitic fluid in Group T as compared to Group NT.<sup>17-19</sup> Ascitic fluid ADA levels  $\geq 40$  IU/L showed an excellent sensitivity, despite a relatively low specificity, for the diagnosis of tuberculous peritonitis. Similar high value of adenosine deaminase was also observed in Dwivedi et al; M.A.Sathar et al Voigt MD et al ; Fernandez- Rodriguez et al; Gupta B K et al P C Mathur et al; Sharma SK et al and Agrawal S et al. ADA determination in tubercular peritonitis has high sensitivity and specificity at determined values.

### **Conclusion**

ADA estimation is specific, sensitive, predictive ancillary tool which serves as definitive supplementary and supportive test for the diagnosis of tubercular ascitis.

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