Original Research Article

Diagnostic role of adenosine deaminase (ADA) in ascites

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Abstract

Background: 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 diagnostic role of ascitic fluid ADA activity in differentiating between tubercular and nontubercular ascites. **Material and method:** The descriptive study was conducted in ACPM Medical College Dhule Maharashtra India. Total 120 patients of clinically diagnosed ascites admitted in the hospital in Medicine ward and Intensive Care Unit were studied. Ascitic fluid ADA values are determined in all ascites patients. **Result:** In present study, out of 120 Ascites patients 15(12.5%) patients were having TB ascites. There was significant difference in the variable i.e. age; sex and biochemical parameters like Serum Albumin; ESR; TLC/cm; lymphocyte (%); Ascitic fluid biochemistry and microbiology and ADA. Sensitivity and specificity of ADA with cut off 30 U/L; 33 U/L and 40 U/L were high in diagnosing tuberculoses ascites i.e. about 90 %. Percent of false positive in all group were also minimum i.e. 1.9%. **Conclusion:** ADA estimation is specific, sensitive, predictive ancillary tool which serves as definitive supplementary and supportive test for the diagnosis of tubercular ascites.

Key Word: Adenosine deaminase; Ascites; Tuberculosis

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INTRODUCTION

Ascites is the pathological accumulation of free fluid within the peritoneal cavity. Ascites is manifestation of number of diseases. Traditional classification of ascites into "Exudative" and "Transudative" which involves estimation of ascitic fluid total protein, which is ≥ 2.5 g/dl in exudates and <2.5 g/dl in transudate.[1] Transudative ascites results due to increased hydrostatic pressure, reduced serum osmotic pressure or both and usually occurs in Cirrhosis, Congestive heart failure, Constrictive pericarditis, Inferiorvena cava obstruction, Hepatic vein obstruction (Budd-Chiari syndrome) or Nephrotic syndrome, Protein-losing enteropathy, Malnutrition.[1,2] Exudative ascites usually occurs as a result 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.[3] Adenosine deaminase (ADA) is a purine degrading genzyme, 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 also showed that the normal blood of humans and mammals contained ADA activity.[4]Most important biological activity is related to lymphoid tissue, because ADA is necessary for proliferation and differentiation of T lymphocytes. Tlymphocytes have ADA levels 10 to 12 times higher than B lymphocytes and activity varies depending on proliferative status and maturity of cells.[5] 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 ascites. Adenosine deaminase (ADA) activity was found to be high in pulmonary and

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extra pulmonary form of tuberculosis. Hence this prospective study is undertaken to determine diagnostic role of ascitic fluid ADA activity in differentiating between tubercular and nontubercularascites. [6,7]

METHODOLOGY

The descriptive study was conducted in ACPM Medical College Dhule Maharashtra India hospital during period from September 2010 to September 2016.Patients more than15 years of age; clinically diagnosed as ascites and willing to give inform consent were included in the study. Total 120 patients of clinically diagnosed ascites admitted in the hospital in Medicine ward and Intensive Care Unit were studied. Pretested questionnaire was used to obtain the detailed clinical history; general examination and systematic examination. The patients were subjected to the routine laboratory tests like CBC, ESR, LFT, Serum Creatinine, Random Blood Sugar, Serum Albumin, HIV and HbS Ag. Ascitic fluid: fresh and aseptically withdrawn, examined for (Before starting antituberculous treatment) TLC/DLC, Total protein, Albumin; Sugar-Cholesterol, LDH, CEA and CA 125 and Amylase Ascitic fluid ADA values are determined in all ascites patients before starting any antituberculous treatment. ADA levels done by micro express ADA-MTB KIT. The results were reported as U/L. (ADA values are determined before starting AKT/antituberculous treatment.) Principle of the test is based upon colorimetric method of Galanti and Giusti. Adenosine deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitropusside acting as catalyst. The intensity of the blue colourindophenols complex formed is directly proportional to the amount of ADA present in thesample.[4,6] Patients were classified into tuberculous and non-tuberculous ascites as per criteria given below. One or more of the following criteria were used for diagnosis. Ascitic fluid smear and/or culture positive for AFB; Exudative ascitic fluid and clinical features suggestive of tuberculosis like fever, weight loss, diarrhea, diarrhea alternating with constipation; evidence of extra abdominal tuberculosis, sputum positivity for AFB, tuberculin test positive, History of contact with tuberculosis in family ; USG findings suggestive of tuberculosis like ascites with internal septations; loculations; intestinal thickening; strictures; abdominal lymphadenopathy etc this criteria was used for diagnosis of tubercular ascites. Differences between tuberculoses ascites patients and non-tuberculoses ascites 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 test and Chi-square test for numerical and categorical variables, respectively. We calculated sensitivity, specificity, and positive and negative likelihood ratios at the suggested cut-off levels. Ninety-five percent confidence intervals (95% CI) were constructed for all estimates**.**

RESULTS

In present study, out of 120 Ascites patients 15(12.5%) patients were having TB ascites. Total 81 (67.5%) patients were due to Liver cirrhosis. Among them majority were alcoholic cirrhosis i.e. 78(96.3%) and 3 (3.7%) chronic hepatitis. Cardiac ascites patients and malignant ascites were 10(8.3) and 3(2.5%) respectively. Other 4(3.3%) patients were due to nephrogenic ascites and 2(1.7%) patients had nephrotic syndrome. Ascites due to PEM were in 2 (1.7%) cases. Pancreatitis, Spontaneous bacterial peritonitis (SBP) and SLE causes ascites in 1 (0.8%) case each.

Table 1: Comparisons of demographical and laboratory parameters between the ascites without and with tuberculosis group

Variable	Tuberculosis ascites (n:15)	Non Tuberculosis ascites (n:105)	P value (Sig)
Age (years) Mean ± SD	34.8 ± 5.4	45.9 ± 9.5	0.0001 (sig)
Sex (Male : Female)	9:6	88:17	0.028 (sig)
Serum albumin	3.2 ± 0.49	3.2 ± 0.44	0.867 (NS)
ESR	71.6 ± 19.9	20.9 ± 7.5	<0.0001 (Sig)
TLC/cmm	837.3 ± 283.7	258.7 ± 191.3	<0..001 (sig)
Lymphocyte (%)	69.1 ± 17.5	40.8 ± 15.7	< 0.03 (Sig)
	Ascitic Fluid analysis		
Total protein (gm%)	4.96 ± 1.19	2.03 ± 0.9	< 0.001 (Sig)
Raised LDH	15 (100%)	09 (8.6%)	< 0.000 (Sig)
Positive Ziehl Neelson stain	01 (6.7%)	00	<0.001 (sig)
	Culture and sensitivity of Ascitic fluid		
AFB positive	2(13.3%)	0	0.001 Sig
E coli sensitive to ceftriaxone	1 (1.0%)	0	
Culture Negative	104 (99.0%)	13 (86.7%)	
Adenosine Deaminase enzyme	66.3 ± 30.2	14.2 ± 6.31	<0.0001 (sig)

There was significant difference in the variable i.e. age; sex and biochemical parameters like Serum Albumin; ESR; TLC/cm; lymphocyte (%); Ascitic fluid biochemistry and microbiology and ADA. In non tuberculoses ascites patients, abdominal Distension in 95(90.5%),oedema on feet 77(73.3%); vomiting 37 (35.2%) were major symptoms. Anorexia 13(86.7%); Abdominal distension 11 (73.3%), pain 9 (60%), fever11 (73.4%) were major symptomsin tuberculoses ascites.

Table 2: Adenosine deaminase enzyme in tuberculoses and non tuberculoses ascites group

Adenosine Deaminase Enzyme (U/L)	Tuberculosis ascites (n:15)	Non Tuberculosis ascites (n:105)	P value (Sig)
ADA (U/L) : 30 U/L cut off			
< 30 U/L	1 (6.7%)	103 (98.1%)	P value < 0.0001
>30 U/L	14 (93.3%)	02 (1.9%)	Sig
ADA (U/L) : 33 U/L cut off			
< 33 U/L	1 (6.7%)	103 (98.1%)	P value < 0.0001
> 33 U/L	14 (93.3%)	02 (1.9%)	Sig
ADA (U/L) : 40 U/L cut off			
< 40 U/L	2 (13.3%)	103 (98.1%)	P value < 0.0001
> 40 U/L	13 (86.7%)	02 (1.9%)	Sig

In tuberculoses ascites 14 (93.3%) patients had ADA value more than 30 U/L and only 2 patients from non tuberculoses ascites group had value of ADA more than 30 U/L. Similar findings with value of ADA more than 33U/L and 40 U/L in non tuberculosis ascites group. ADA value more than 40 U/L cut off was in 13 (86.7%) patients.

Table 3: Validity of ADA at cut off 30, 33 and 40 U/L in diagnosing tuberculoses ascites

Validity	Cut off 30 U/L	Cut off 33 U/L	Cut off 40 U/L
Sensitivity	93.3	93.3	86.7
Specificity	98.1	98.1	98.1
Positive Predictive value (PPV)	87.5	87.5	86.7
Negative Predictive value (NPV)	99.0	99.0	98.1
Accuracy	97.5	97.5	96.7
Percent False negative	6.7	6.7	13.3
Percent false positive	1.9	1.9	1.9

Sensitivity and specificity of ADA with cut off 30 U/L; 33 U/L and 40 U/L were high in diagnosing tuberculoses ascites i.e. about 90 %. Percent of false positive in all group were also minimum i.e. 1.9%.

Table 4: Correlation ADA with TLC, Lymphocytes, Proteins Sugarand SAAG in Tubercular ascites

	Correlation coefficient (r)	P Value
ADA (U/L)	1	
TLC/cmm	0.730	0.000 (sig)
Lymphocyte	0.414	0.0000 (sig)
Proteins (gm%)	0.643	0.0000 (sig)
Sugar (mg/dl)	-0.618	0.000 (sig)
Serum Ascites Albumin Gradient (SAAG)	-0.526	0.0000 (sig)

In Tubercular Ascites patients ADA was positively correlated to the TLC, Lymphocytes and proteins level while ADA was found to be negatively correlated to the SAAG level and Sugar levels. The ADA had statistically significant ($p<0.01$) positive correlation with TLC, Lymphocytes and Proteins while statistically significant negative ($p<0.01$) correlation with SAAG values in ascites patient.

DISCUSSION

In present study, mean (SD) age of non tuberculosis ascites patients was 45.9 ± 9.5 and significantly differ TB ascites patients the mean age was 34.8 ± 5.4 years. Studies done by other was found similar finding i.e. tuberculosis ascites was more common in early age as compared to non tuberculosis ascites. Study done by Jalees Fatima *et al* (2010) (74) studied 55 patients of tuberculous ascites in which the age of the study subjects varied from 18–62 years and maximum patients (41.8%) were in 20-30 years age group followed by 31–40 years (30.9%) and 41-50 years (12.7%).[8] Malhotra V *et al* (52)(1992) in their clinic pathological study of abdominal tuberculosis, prospectively studied 99 patients with possible diagnosis of abdominal tuberculosis and found that the mean (± S.D) age of the patients was 31.6 ± 13.6

years with a range of 13 to 65 years.In this study, out of 15 patients with TB Ascites 9 (60%) were male and6 (40%) were female While in 105 Non tuberculous Ascites 88 (83.8%) were male and 17(16.2%) were female patients.[9] Similar findings were also observed by Kashyap R S *et al* and N.L.Patney *et al* i.e. male outnumbered the female.[10,11] Basedon systematic review of literature done by F M Sanai *et al*, found that abdominal pain was a common presenting symptom (64.5%) frequently accompanied by abdominal dissention.[12] Similar findings were also observed in our study. In our study mean serum albumin level was 3.2±0.4 gm/dl in non tuberculous ascites and tuberculous ascites both. N.L.Patney *et al* (54) studied 20cases of intestinal tuberculosis and found that the mean of total serumproteins was normal at 6.82 gm/dl but mean serum albumin was reduced to2.86 gm/dl (gm%).Only 5 cases (25%) had normal serum proteins pattern.[11]Basu S *et al* (2007) retrospectively studied medical records of 115patients 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.[13]In present study, ESR was significantly raised in patients of tuberculous ascites with mean 71.6 mm and that of non tuberculous ascites group with mean 20.9 mm. J. Ramesh *et al*(2008) studied 86 cases of abdominal TB and found the ESR to be elevated in 98% of cases.[14]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 ascites.[15] In this study, Culture and sensitivity of non tuberculous ascites revealed 1(1%) patient with E coli organism sensitive to ceftriaxone attributing to SBP. In 2 (13.3%) patients with tuberculous ascites culture and sensitivity was positive for AFB while in rest 13(86.7%) it was negative. There was statistically highly significant ($p<0.01$) difference in the results of culture sensitivity for AFB in Tuberculous and non tuberculous group. Studies of some workers showing sensitivity of ascitic AFB culture Malhotra V *et al*(1992) 22.22% ; Singh *et al*(1996) 83% ; Jalees Fatima *et al*(2012) 1.8% and .[8,16,17]Some of the above studies correlate with the present study and it can be said that culture and sensitivity of AFB in ascitic fluid varies, may be due to technical aspects of storage, transportation of sample and condition of media used. In present study, mean (SD) adenosine deaminase (ADA) level in tuberculosis ascitic fluid was 66.3 (± 30) and that of non tuberculosis ascites was 14.2 (± 6.31). 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* [6,18,19,20]. ADA determination in tubercular peritonitis has high sensitivity and specificity at determined values. In this study, comparison was done on available data taking different ADA cut off and it was found that ADA more than 30 U/L had sensitivity of93.3%, specificity of 98.1% and 87.5% positive predictive value and 97.5%accuracy in detecting tuberculous ascites. ADA more than 33 U/L had same results as to that of ADA 30 U/L. ADA more than 40U/L had lower sensitivity of 86.7%, specificity of 98.1% and lower positive predictive value of 85.7% in detecting tuberculous ascites. Study done by Burgess L J *et al*(2001) studied 178 paired ascites and serum specimens and found that the mean(range) ADA activity in the tuberculous group was 61.6(17.5 – 115.0) U/L and was significantly higher than in any other diagnostic group(<0.05). At cut-off value of 30 U/L the sensitivity and specificity was 94 % and 92 %, respectively. Dwivedi *et al* found the adenosine deaminase concentration in tuberculous ascitic fluid was 98.8 +/- 20.1 U/L, which was significantly more than that noted in cirrhotic (14 +/- 10.6 U/L) or malignant (14.6 +/- 6.7 U/L) ascitic fluids (p less than 0.001 for each). At a cut off value of greater than 33 U/L, the sensitivity, specificity, positive and negative predictive value, and the overall diagnostic accuracy for diagnosing tuberculous ascites were 100%, 96.6%, 95%, 100%, and 98%, respectively. [6] M.A. Sathar *et al* (1995) in their study of Ascites Fluid gamma interferon concentration and adenosine deaminase activity in tuberculous peritonitis found that ADA activity was significantly higher in the TB group (range: 0-8-318.50 U/L; mean: 101.84U/L) than in the control groups Cirrhosis (range: 4.1-29.3 U/L; mean: 13.49 U/L) and malignancies (range: 5.3-70.02 U/L; mean: 19.35 U/L).[18] In conclusion tuberculous ascites is one of the most common causes of exudative ascites in developing nations. Present study have shown higher frequency of tubercular ascites as 12.5%.. Major clinical manifestations of tuberculous ascites were abdominal distension followed by abdominal pain; fever; anorexia and recent weight loss. Adenosine Deaminase enzyme (ADA) estimation is a simple colorimetric test with high sensitivity and high specificity that can be routinely used to differentiate between tuberculous and non tuberculous causes of ascites. Although new techniques have been developed that help the diagnosis of tuberculous ascites like lysozyme, interferon gamma and PCR; considering all aspect such as sensitivity, specificity, efficacy, and availability and economy adenosine deaminase (ADA) enzyme assay is the most appropriate diagnostic test for

analysis of peritoneal fluid in resource- limited settings. Hence, ADA estimation is specific, sensitive, predictive ancillary tool which serves as definitive supplementary and supportive test for the diagnosis of tubercular ascites.

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