ADENOSINE DEAMINASE AND ITS ISOENZYMES IN ASCITES WITH DIFFERENT ETIOLOGIES

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Adenosine deaminase (ADA) activity increased in certain clinical conditions including tuberculosis and bacterial infections. In this study, the ADA isoenzymes patterns are assayed in ascites of different etiologies. Ninety two patients with ascites were selected and investigated to determine the cause of ascites. Total ADA and its isoenzymes are assayed spectophotometerically beside polyacrylamide gel electrophoretically. The total ADA in ascitic fluid secondary to parainfection in case of TB, abdominal cancer and liver cirrhosis is found to be 34.5±11.1, 87.6±23.6, 32.7±10.1 and 28.5±7.3 U L^{-1} respectivly. The ADA1m was 24.5±11.1, 4.3±1.9, 2.8±2.2 and 10.1±3.3 U L^{-1}. ADA1c was 7.8±3, 16.5±6.2, 4.7±1.3 and 10.1± .3 U L^{-1} respectivly. ADA2 was 2.2±3.9, 65.9±33.5, 26.2±6.2 and 3.3±9.7 U L^{-1} respectively. Hence it is concluded that total ADA above 41 U L^{-1} and ADA2 above 32 U L^{-1} in ascetic fluid have high sensitivity value in TB peritonitis. The ascitic fluid ADA1m/ADA more than 50 % has high specificty value in parainfective peritonitis. Total ascitic ADA<41 U L^{-1} and ADA2/ADA ratio >50 % have high specificity in abdominal cancer.

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Introduction

The ascite is an accumulation of fluid in the abdominal cavity. The two main ascites are transudate (total protein < 30 g L^{-1}) which is caused by liver cirrhosis (LC), and exudate (total protein > 30 g L^{-1}) which is caused by tuberculous peritonitis (TBP) and abdominal cancer (AC) respectively in Egypt.[1] TBP and AC require rapid recognition for the appropriate therapeutic management.[2] Clinically, etiological diagnosis of ascites is very difficult especially in exudative type because of the lack of specific differential clinical, radiological, or laboratory findings. Peritoneoscopy is the method of choice in the diagnosis of the cause of ascites.[3] However, the diagnostic failure rate of peritoneoscopy can reach as high as approximately 14 %; the main reason for failure is the interference from adhesions due to tumor, tuberculosis or previous surgery.[4]

Adenosine deaminase enzyme (adenosine amino hydrolase, EC 3.5.4.4. ADA) catalyzes the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine respectively. It is widely distributed in human tissues. ADA is critical in the development and function of immune system.[5,6] Clinical interest concering ADA enzyme has been reviewed due to the association between combined immunodeficiency and ADA deficiency.[5,6] ADA helps in proliferation and differentiation of lymphocytes especially T lymphocytes,[7] and is a significant indicator of active cellular immunity.[8] Thus, ADA has been proposed to be a useful surrogate marker for the diagnosis of tuberculosis (TB) because it can be detected in body fluids such as pleural,[5] pericardial,[9] cerebrospinal fluid,[10] and peritoneal fluid,[11] and elevated ADA levels have been reported in these cases.

Two isoenzymes of ADA, namely ADA1 and ADA2, have unique biochemical properties.[12] ADA1m isoenzyme is found as a monomer but ADA1c is a dimer.[13] The ADA1 isoenzyme is found in all cells, with the highest activity in lymphocytes and monocytes, whereas ADA2 isoenzyme is found only in monocytes.[14] The assay of ADA activity in ascites may be very useful in detecting the etiology of ascites, especially in the case of tuberculosis, which is characterized by an increase in activity.[15]

The purpose of this study is to evaluate the benefit of using peritoneal fluid ADA and its isoenzymes to clarify the final etiology of ascites.

Patients and methods

Study subject

Ninety-two patients, suffering from ascites with different etiology and had undergone abdominal ultrasonography and abdominal computed tomography (CT) scan with or without peritoneoscopy for diagnosis, were enrolled in this study. The study was carried out in Sohag Faculty of Medicine Hospital from January to July 2014.

All patients had laboratory tests such as complete blood count, serum liver function tests (albumin, bilirubin, AST, ALT and alkaline phosphatse), ascetic fluid albumin and Carcinoembryonic antigen (CEA) as tumor marker, cytology of ascetic fluid and ascetic fluid ADA. TBP was diagnosed on the basis of one of the following criteria: (1) ascetic fluid showed positive acid-fast bacilli stain and culture; (2) tuberculosis polymerase chain reaction test from ascites specimen was positive or (3) caseating granuloma was noted in the peritoneal biopsy specimen.

Abdominal cancer was diagnosed if cancer cells from ascetic fluid cytology were detected or cancer cells were documented from the peritoneoscopic biopsy specimen.

The Ethics Committee at Sohag University approved this study protocol and written consent was taken from all patients.

Kits supplied by Roche (Mannhein, Germany) was used to test serum and ascitic albumin, and seum bilirubin, ALT, AST and alkaline phosphatase spectrophotometrically, and ascetic fluid carcinoembryonic antigen (CEA) was assayed by Simple Step ELISA Kit from Abcam.

Total ADA assay

ADA activity was determined by the described spectrophotometric method.[16] Adenosine was used as a substrate and the amount of ammonia formed was measured by its reaction with phenol nitroprusside and alkaline hypochloride producing blue color that was read spectrophotometrically against water at 628 nm and ADA activity was calculated as U L^{-1} (Berthelot's reaction).[16]

ADA isoenzymes assay

All the three ADA isoenzymes (ADA1c, ADA1m and ADA2) were quantified by using the described polyacrylamide gel electrophoretic technique.[17,18]

For a 5 % gel, a phosphate buffer system consisting of a 0.1M bridge buffer and a 0.05 M gel buffer at pH 6.7 was used. 10 µl of the samples were applied to the gel. Electrophoresis was carried out horizontally at 200 mA for 2.5 hours at 4 °C. To make the isoenzymes visible an ADA staining reaction was used. The staining reaction mixture consisted of the following: 24 mg adenosine (Sigma), 1 U of nucleoside phosphorylase, and 0.5 U of xanthine oxidase (Boehringer), 12 mg 3-(4,5–dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide salt (Sigma), 1 mg phenazine methosulphate (Sigma) and 16 mg sodium phosphate in 8 ml staining buffer (0.3 M Tris, 0.2 M histidine HCl, pH 7.8). A cellulose acetate sheet soaked with this mixture was applied to the gel and incubated at 37 °C for 60 min.[19] The relative activity of each isoenzyme, determined by densitometric scanning, and the total ADA activity, determined with the spectrophotometric method, were then used to quantify each fraction.

Statistical analysis

Values for variables were presented as mean ± SD with ranges using *t*- test. *P* value less than 0.05 was considered as statistically significant. Data were analyzed using SPSS ver. 10. Sensitivity, specificity and efficiency were statistically calculated.

Results

Ninety two patients with ascites were diagnosed by abdominal ultrasonography and abdominal CT scan was selected. Of that population, 15 patients were diagnosed as parinfection (non TB or malignant ascites), 25 patients were diagnosed as TBP, 21 patients were diagnosed as malignant ascites due to primary or secondary abdominal tumors and the remaining 31 paitents with ascites secondary to liver cirrhosis. Parainfection ascites were secondary to congestive heart failure (5 cases), chronic renal failure (4 cases), continuous ambulatory peritoneal dialysis peritonitis (2 cases), systemic lupus erythematosus (SLE; 2 cases), or chronic pancreatitis (2 cases). Abdominal tumors group included 5 ovarian adenocarcinoma, 4 pancreatic cancers, 3 colorectal cancers, 3 advanced gastric cancers and 6 malignancies of unknown origin.

General clinical and laboratory characteristic of patients are shown in Table 1. Abdominal pain was the main complaint in parainfective ascites but, abdominal distension was apparent in liver cirrhosis, abdominal cancer and TB peritonitis. The complaints started from less than one month before in parainf and abdominal cancer groups than other groups. Laboratory characteristics showed significant low serum albumin and high serum bilirubin, ALT and AST in ascites secondary to liver cirrhosis. Also, SAAG was significantly higher in LC than other groups. Tumor marker (cercinoembryonic antigen; CEA) in ascites was significantly higher in abdominal cancer than other groups.

Table 2 lists the mean standard deviation (SD) and range of total ADA and its isoenzymes (ADA1m, ADA1c and ADA2) in ascitic fluids of patients with parainf, TB peritonitis, abdominal cancer and liver cirrhosis that were electrophoretically separated and photographed (Figure 1). All the tuberculous ascites had total ascitic fluid ADA activities of 41 U L^{-1} or more, whereas one of 21 peritoneal cancer cases and another one in parainf had ADA activities above this level (Figure 2). With diagnostic thresholds of 41 U L^{-1}, the sensitivity, specificity and efficiency of total ADA for tuberculosis were 100 %, 97 and 97.8 %, respectively. Also, ascitic fluid ADA2 activities of 32 U L^{-1} or more were found in 24 TB peritonitis and one abdominal cancer case. With diagnostic thresholds of 32 U L^{-1} of ADA2 in TB peritonitis, the sensitivity, specificity and efficiency of total ADA for tuberculosis were 96 %, 98.5 and 97.8 %, respectively. ADA2/ADA ratio more than 50 % had been found in 22 TB peritonitis and 3 peritoneal cancer case. With diagnostic thresholds of ADA2/ADA ratio more than 50 % in TB peritonitis, the sensitivity, specificity and efficiency of total ADA for tuberculosis were 88 %, 95.5 and 93.4 %, respectively. Less than 5.5 percent difference between the efficiency of ADA, ADA2 and ADA2/ADA ratio was significant.

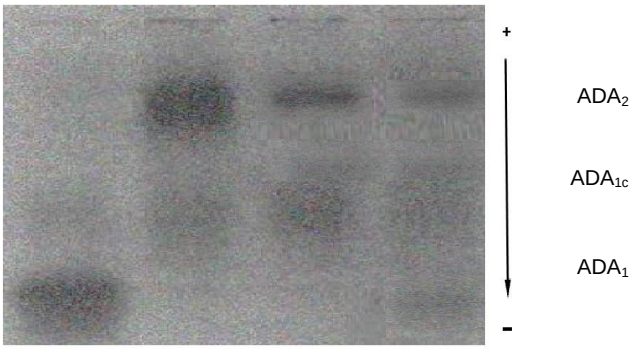

Figure 1. Photography of the electrophoretic pattern of ADA isoenzymes found in ascetic fluids parainfection (parainf), tuberculous peritonitis (TBP), abdominal cancer (AC) and liver cirrhosis (LC).

Table 1. General, clinical and laboratory characteristic patients with ascites with different etiology.

	Parainf *N* (15)	**TBP** *N* (25)	**AC** *N* (21)	**LC** *N* (31)
Age	45.4± 11.5	49.6± 13.6	54.9*± 14.6	38.7± 20.9
Gender (M/F)	(6/9)	(11/14)	(12/9)	(19/12)
Duration of symptoms (%)				
< 1 month	66.7	44	81	32.3
≥ 1 month	33.3	56	19	67.7
Abdominal pain	11 (73.3%)	2 (8 %)	7 (33.3 %)	2 (6.5 %)
Abdominal distension	3 (20 %)	22 (88 %)	19 (90.5 %)	30 (97 %)
Weight loss	1 (6.7 %)	20 (80 %)	18 (85.7 %)	12 (38.7 %)
Fever	9 (60 %)	5 (20 %)	3 (14.3 %)	8 (25.8 %)
Night sweating	1 (6.7 %)	16 (64 %)	1 (4.8 %)	1 (3.2 %)
Loss of appetite	1 (6.7 %)	22 (88 %)	19 (90.5 %)	13 (41.9 %)
Diarrhea	3 (20 %)	13 (52 %)	2 (9.5 %)	1 (3.2 %)
Serum albumin (g L^{-1})	39 ±14	41 ± 12	45± 12	21± 5*
Serum bilirubin				
Total (mg L^{-1})	10 ± 4	9± 3	13± 6	33± 27*
Direct (mg L^{-1})	2± 0.8	3± 0.6	3 ± 1	13± 5 *
Serum ALT (IU L^{-1})	22 ± 13.1	21± 12.3	28± 11.6	51.5± 23.1*
Serum AST (IU L^{-1})	20 ± 11.5	22 ± 14.2	26± 12.6	74 ± 13.1*
Serum alk. Ph. (IU L^{-1})	162± 12.6	156± 17.8	188± 22.3	144± 18.1
Ascitic leukocytes (mm^{-3})	3542.8± 342.2	5739.9± 253.7©	3645.8± 442.9	3101.5± 312.4
Ascitic lymphocytes (%)	23.5± 9.1	69.5± 16.9®	59.4± 15.3	21.4± 5.2
Ascitic albumin (g L^{-1})	28± 23	33± 19	38± 17	4± 2*
SAAG (g L^{-1})	11± 6.2	8.2± 2.4	7.3± 3.7	17.3± 5.3*
Ascitic CEA (ng mL^{-1})	3.4± 2	4.2± 2.1	579.3± 192.7∞	7.3± 4.7

alk. Ph: alkaline phosphatase; ALT: alanine transaminase; AST: aspartate transaminase; CEA: Carcinoembryonic antigen; M/F: Male/female; SAAG: Serum-ascites albumin gradient; *Significant change between LC group and other three groups. © Significant change between TB group and other three groups. ® Significant change between TB group and parainf and LC groups. ∞ Significant change between AC group and other three groups.

Table 2. Adenosine deaminase (ADA) activity in ascetic fluid.

	Total ADA (U L^{-1})		**ADA isoenzymes (U L^{-1})**					
			ADA_{1m}		**ADA_{1c}**		**ADA_2**	
	Mean ±SD	**Range**	**Mean ±SD**	**Range**	**Mean ±SD**	**Range**	**Mean ±SD**	**Range**
Parainf	34.5± 11.1	22-50	24.5± 11.1*	13-40.2	7.8± 3	2.4- 10.5	2.2± 3.9	1- 6.7
TBP	86.7± 23.6	41-130	4.3± 1.9	1.5- 6.3	16.5± 6.2	12.2- 19.4	65.9± 33.5©	31.9-104
AC	32.7± 10.1	22-44	2.8± 2.2	2.9- 7.8	4.7± 1.3	11.3- 26.1	26.2± 6.2®	18.3- 32.7
LC	28.5± 7.3	22-40.5	5.1± 1.3	3.8- 6.9	10.1± 3.3∞	6,2- 12.1	13.3± 9.7¥	10.2- 16.6

*Significant change between ADA_{1m} and other two isoenzymes in parainf. ©Significant change between ADA_2 and other two isoenzymes in TBP. ®Significant change between ADA_2 and other two isoenzymes in AC. ∞Significant change between ADA_{1c} and ADA_{1m} in LC. ¥Significant change between ADA_2 and ADA_{1m} in LC. ADA: Adenosine deaminase;

ADA1m/ADA ratio more than 50 % had been found in 14 parainf cases. With diagnostic thresholds of ADA1m/ADA ratio more than 50 % in parainf cases, the sensitivity, specificity and efficiency were 93.3 %, 100 and 97.8 %, respectively.

Total ascitic ADA < 41 U L^{-1} and ADA2/ADA ratio more than 50 % had been found in 20 cases of AC. With diagnostic thresholds of total ascitic ADA < 41 U L^{-1} and ADA2/ADA ratio more than 50 % in AC cases, the sensitivity, specificity and efficiency were 95.2 %, 100 and 98.9 %, respectively.

ADA deficient patients show defective cell mediated and humoral immunity.[6] The enzyme activity is more potent in T lymphocytes than B lymphocytes and is inversly proportional to the degree of T cell differentiation.[7] High serum ADA activity was reported in patients with some diseases in which cellular immunity is stimulated.[8] The increased serum ADA enzyme activity was detected in tuberculous patients secondary to activation of cell mediated immune system.[20]

Discussion

Lymphocytes and monocytes had comparable levels of ADA activity than other cell types or tissues. All the ADA activity in lymphocytes was attributed to ADA1 (ADA1m and ADA1c). The ADA2 was found only in monocytes.[13] ADA1m contributed approximately to 80 % to the total ADA activity, the residual activity was due to ADA2, with no ADAlc activity present in sputum of tuberculous patients.[14] Therefore, the finding in the current study is that the elevated ADA activity in TB peritonitis may be

secondary to stimulation in cellular immunity.[15] Also, ADA activity in tuberculous ascites was mainly due to ADA2 provides strong evidence that the ADA originates from the monocyte-macrophage cells either due to its turnover or activity.[6]

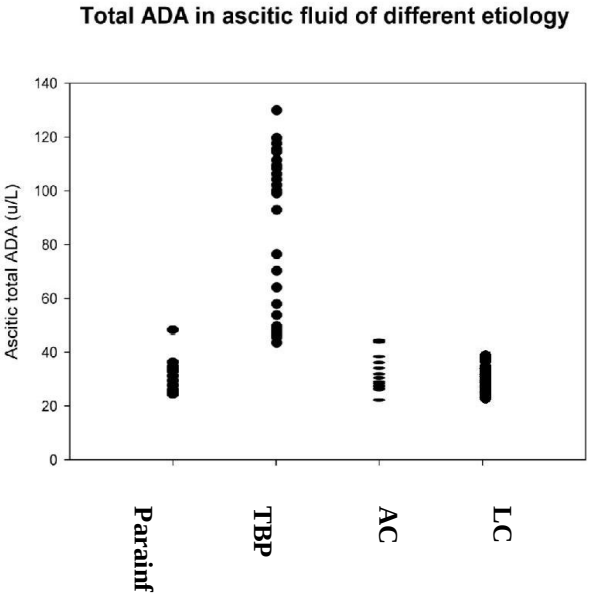

Figure 2. Total ADA activity of ascetic fluids in parainfection (parainf), tuberculous peritonitis (TBP), abdominal cancer (AC) and liver cirrhosis (LC).

The parainfective ascites cases exhibited elevated ADA levels, and the analysis of the isoenzymes profile proved that ADA1m was the predominant form contributing 71 percent of total ADA activity and 76 percent to ADA1 activity. These findings may be attributed to insufficient combining protein needed to convert all ADA1m to ADA1c was present. [5]

The differences in isoenzyme patterns between ascites of different etiologies could be indicative of different origins of ADA, or different mechanisms of release.[14] In the parainfective ascites, ADA probably originates from lymphocytes or neutrophils but, in TB peritonitis, macrophages derived monocytes were the most abundant cell type found in this type of ascites.

The elevated ADA activity in TB peritonitis may be secondary to stimulation in cellular immunity.[12]

The elevated ADA activity in ascites secondary to abdominal tumours may be due to increase in nucleic acid catabolism.[15]

Determination of ADA isoenzymes could help in distinguishing between different causes of ascites, especially between parainfective and non-parainfectve causes as found in this study.

Moreover TB peritonitis could be distinguished from other non-parainfective causes by high total ADA activities of 41 U/L or more (sensitivity, specificity and efficiency were 100 %, 97 and 97.8 %, respectively).

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