The Clinical Profile of Adenosine Deaminase (ADA) in Tuberculous and Non-Tuberculous Ascites.

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ABSTRACT

The objective of this study is to determine the clinical utility of ascitic fluid ADA (adenosine deaminase) in diagnosing tuberculous ascites and also it's sensitivity, the specificity and significance for diagnosing tuberculous ascites at cut-off value of 32U/L.This study is carried out in Dr.PDMMC,Amravati with 25 patients diagnosed as tuberculous ascites as per the diagnostic criteria were taken as cases and 25 patients of ascites due to non-tuberculous etiology were taken as control. There ADA values are determined by colorimetric method of Galanti and Giusti, before starting any antituberculous treatment. There were 14(56%) males and 11 (44%) females of tuberculous ascites in the case study group. The results were statistically analyzed by applying chi square test. The result is labeled statistically significant if p value calculated was <0.05. In this study, the mean ADA value in tuberculous ascites patients was 55.80 ± 25 U/L and the range was from 28 to 133 U/l. While in non-tuberculous ascites patients the mean ADA value was 15±8.9 and range was 2.8 to 35. At the cutoff value of ADA at 32U/L , the sensitivity was 92% and the specificity was 88% for diagnosing tuberculous ascites. The test is highly significant with Chi-square value of 32.04, (P <0.001) ; d.f – 1 .The study shows that the ADA determination is a simple test with high sensitivity and specificity that can be routinely used to diagnose tuberculous ascites and to differentiate between tuberculous and non-tuberculous cause of ascites.

INTRODUCTION

The first documented case of ancient tuberculous peritonitis was described in human in 1843[1]. .Tuberculous peritonitis is a frequent cause of ascites and significant health problem in the developing countries , and if left untreated may reach mortality rates of 50 – 60%. Over the last few years there is an increase in both pulmonary and extrapulmonary tuberculosis also in developed countries, the part of world where TB generally was rare[1,2] . This is partly a result of increasing travel and migration and also to the rising number of HIV patients who are susceptible to opportunistic intections [1,2]. Abdominal tuberculosis is far common in India than is commonly believed[3].Though pulmonary presentation is the most frequent,extrapulmonary presentation is not unusual,affecting 10 – 15% of immunocompetent patients and 50 – 70% of AIDS patients [4]. Peritonial tuberculosis is currently the sixth most frequent extrapulmonary location and it increases proportionally to the rising incidence ot TB worldwide[4,5]. Tubercular peritonitis constitutes 4 – 5 % of all patients with extra pulmonary TB and occurs in 0.1 – 3.5 % of cases of PTB and one of the most common forms of extra pulmonary TB. [1] . Approximately 20-25% of cases of tuberculosis involving the GI tract have simultaneous pulmonary disease[6] . Mycobacterium TB is the most commonly isolated organism in present era [8] . Ascites , which is a pathological intraperitoneal fluid accumulation, complicates a variety of disorders besides tuberculosis like parenchymal liver disease, neoplasm, congestive cardiac disorders, nephrosis, pancreatitis and myxedea[7].

The insidious nature of tuberculous peritonitis often causes diagnostic challenge and other conditions presenting with ascites greatly hampers diagnosis [2] .Because of the non-specific symptoms and signs, it's diagnosis is often delayed [9] . Non invasive diagnostic methods have low sensitivity and specificity or are

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extremely time consuming and invasive methods like laparoscopy and laparotomy increases mortality amoung seriously ill patients [2,10]. The diagnosis of abdominal TB is made difficult because of the paucity of MTB in peritonial fluid[11]. Therefore, new techniques which are rapid and less invasive, but maintains high sensitivities and specificities ,and positive/negative predictive values need to be evaluated to aid in the diagnosis of tuberculous peritonitis [11].

Recenty ADA determination has been found to be effective method for the diagnosis of tuberculous peritonitis [2] .Since 1978 when adenonine deaminase (ADA) activity was found to be high in tuberculous pleural exudates, ADA has been used in the diagnosis of tuberculous effusion [12]. ADA analysis is a simple and inexpensive calorimetric test that can be performed on body fluids [12].

ADA is an enzyme of purine catabolism with catalyses the conversion of adenosine to inosine and is secreted by lymphocytes and to a lesser extent by macrophages during activation of the cell immune response to mycobacterium antigens [2] . ADA is a purine degrading enzyme, widely distributed in tissues and body fluids [4]. However , the most important biologic activity is related to lymphoid tissue, because ADA is necessary for proliferation and differentiation of T lymphocytes [4] . T lymphocytes has ADA levels 10 – 12 times higher than B lymphocytes and activity varies depending on proliferative status and maturity of cells [4] . During lymphocyte proliferation, the enzyme activity varies inversely to the maturity state of lymphocytes and relates to the intensity of stimulation and the maturation state of the lymphocytes, due to the immune response against MTB [4] . ADA enzyme is also found in erythocytes and cerebral cortex [10].

Material and Methods –

Material

1). This study was carried out in Dr.Panjabrao Deshmukh Memorial Medical college and Hospital, Amravati during the study period from August 2008 to February 2010.

2). All males and females above 18 years who fulfills our inclusion/exclusion criteria and were admitted for ascites with primary cause in Medicine and Chest & T.B wards of Dr. P.D.M.M.C, hospital Amravati were included in the study. The patients were mainly from proper Amravati city and peripheral villages.

3). During the study period only total 25 number of cases were diagnosed as of tuberculous ascites as per the diagnostic criteria. Then the remaining first 25 cases of ascites due to non-tuberculous etiology were taken as control and there ADA values are determined by colorimetric method of Galanti and Giusti, before starting any antituberculous treatment. There were 14(56%) males and 11 (44%) females of tuberculous ascites in the case study group with male to female ratio was 1.27:1.

4). All the patients of ascites admitted have to fulfills the following criteria and should have to undergo the following required investigations to reach the final diagnosis.

*** Eligibility Criteria**

Age- 18 years and above.Gender – Both.

*** Inclusion Criteria**

Male or female above 18 years of age.The subject is able to understand the information and is willing to take part in the study .

*** Exclusion Criteria**

Patients who were not willing to get done the procedures/investigations specified by the protocol or to follow the instruction of the study personnel.

***Diagnostic Criteria**

Absolute - Ascitic fluid smear positive for AFB.

Suggestive Criteria

a) Exudative ascitic fluid.

b) Clinical features suggestive of tuberculosis like fever,weight loss, diarrhoea, diarrhoea alterating with constipation.

c) Evidence of extra abdominal tuberculosis,sputum positivity for AFB, Tuberculin test positive ,History of contact with tuberculosis in family.

d) USG findings suggestive of tuberculosis like ascites with internal septations / loculations / intestinal thicking / strictures / abdominal lymphadenopathy.

***Laboratory investigations-** CBC,ESR,LFT,Serum Creatinine, Random Blood Sugar, Serum Albumin, HIV and HbSAg were done in every patient.

*Chest X – ray Posteroanterior (PA) view, USG abdomen & pelvis were done in every patient.

* Mantoux test and Sputum for AFB were caried out in every patient suspected of tuberculosis.

*Ascitic fluid, fresh and aseptically withdrawl, examined for:-

Total protien , TLC , DLC.

Ascitic fluid cytology for malignant cells.

Ascitic fluid smear for Acid Fast Bacilli and Gram staining for organisms.

Ascitic fluid ADA levels were done in every patient.

*Serum TBIgM ,TBIgG , Serum amylase , Serum lipase, Biopsy , 2- D ECHO and CT- Abdomen were caried out in selected patients to confirm/support the diagnosis.

* After examine the whole data, patients were classified into tuberculous and non-tuberculous ascites.ADA values are get done by colorimetric nethod of Galanti and Giusti in every patient.ADA values are done before starting any antituberculous treatment.

Methods

CBC – Done by Electronic cell counter,ESR – Done by Westengreen's method,LFT – Done by Semi-automatic analyser,Serum protein –Done by Colorimetric Method,Random Blood Sugar –Done by Semi-automatic analyser.

Ascitic fluid, fresh and aseptically withdrawl, investigated for:

Total protein – Done by Colorimetric method (Value > 2.5 was considered as exudative and value <2.5 considered as transdative.)

TLC/DLC – Done by manual count by Naeubaeur's chamber.

*** Ascitic fluid ADA levels are done by the colorimetric method of GIUSTI,before starting any antituberculous treatment. The results are reported as U/L.**

Principle

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 coloured indophenol complex formed is directly proportional to the amount of ADA present in the sample.

$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Ammonia} + \text{inosine}$$

$$\text{Ammonia} + \text{Phenol} + \text{Hypochlorite} \xrightarrow[\text{Medium}]{\text{Alkaline}} \text{Blue Indophenol complex}$$

SATISTICAL METHODS

* Summary measures like mean, standard deviation were calculated for continuous variables and percentage proportions were obtained for categorical variable.

* The standard deviation (S.D) is calculated from the basic formula;

S.D $\sqrt{\sum(x-\overline{x})2}$ (For size < 30), Arithmetic mean, n= Total number.

***Results were statistically analyzed by applying chi square test. The result is labeled statistically significant if p value calculated was <0.05.**

OBSERVATION

1) Age of the patients was in the range of 20 to 70 years of age. The mean age for the patients was 38.64±13.46 years. Majority of the patients 9 (36%) belonged to the age group of 20 -30 years of age. There are 14 (56%) males and 11 (44%) females in the study . Male to female ratio was 1.27:1.

2) Out of 25 cases of abdominal tuberculosis fever was present in 25(100%) ,abdominal distention was present in25(100%), weight loss in 21(84%), cough in 17 (68%), abdominal pain in 11(44%) , diarrhoea in 8(32%),constipation in 2(8%), contipation alterating with diarrhoea in 3(12%) and no patient presents with gastrointestinal bleeding. Thus fever and abdominal distention are the most common complaint in this study and is present in all cases.

3) The past history of pulmonary tuberculosis was found in 11(44%) cases, out of which 10(40%) was sputum positive and 1(4%) was sputum negative. Out of 25 patients, family history of tuberculosis is found in 8(32%) of cases and all were sputum positive cases.

4) Anaemia was present in 22(88%) of cases, out of which 12(48%) were male and 11(44%) were females.The mean haemoglobin level was 8.72±2.39gm/dl .

5) ESR was raised in 24 (96%) of cases. Mean ESR

was 56.56+/- 32.36 mm in the first hour. The range was from 18 to 126 mm at the end of one hour.

6) Tuberculin test was positive (induration >10mm after 72 hours) in 20 (80%) of cases.

7) Serum albumin levels were decreased (<3.5 g/dl) in 20 (80%) of cases. The mean serum albumin level was 2.83+/- 0.82 g/dl.The range was 1.4 to 4.8 g/dl. In 17(68%) of patients the albumin levels were less then 3 g/dl.

8) Abnormal chest X-ray was found in 23(92%) of cases.Pulmonary infilterates were seen in total 12(48%) of cases, out of which 5(20%) have unilateral and 7(28%) have bilateral chest infiltrations. Fibrocavitory lesions were seen in total 9(36%) of patients, out of which 5(20%) had unilateral fibrocavitory lesion and 4(16%) had bilateral lesions. Pleural effusion was present in 7(28%) of cases out of which 2(8%) had isolated pleural effusion and 5(20%) had pleural effusion with pulmonary infilterates.

9) In 25 (100%) of cases, ascitic fluid protein level were more than 2.5 g/l. The mean ascitic fluid protien concentration was 4.21 ± 0.97gm/dl. The range was from 2.62 to 5.7 g/dl.

10) The total cell count in tubercular ascites fluid was ranged from 56 to 1750 cells. The average cell count was 520.16 cells . Maximum number of patients 9(36%) had cell counts between 301 – 600 cells. 22(88%) of patients had lymphocytic predominance cell count.

11) AFB staining was performed for each and every sample of tubercular ascitic flluid, and in only 1(4%) patient AFB staining was positive for Acid fast bacilli.

12) ADA level was done in all patients.The mean ADA value in tuberculous ascites patients was 55.80 ± 25 U/L and the range was from 28 to 133 U/l. While in non-tuberculous ascites patients the mean ADA value was 15±8.9 and range was 2.8 to 35. . When the cut-off value for ADA was set at 32 U/L , 23 (92%) of tuberculous ascites patients had ADA value more than 32 U/L,2(8%) of cases had ADA values less than 32 U/L, 3(12%) of non-tuberculous ascites had ADA values more than 32 U/L and 22(88%) of non-tuberculous ascites had ADA values less than 32 U/L. So, at the cutoff value of ADA at 32U/L , the sensitivity and specificity of diagnosing tuberculous ascites was 92% and 88% respectively. The test is very highly significant with Chi-square value of 32.04, (P <0.001) ; d.f – 1.

CONCLUSIONS

ADA determination in ascitic fluid is a very useful and realiable test for diagnosing tuberculous ascites. The method of ADA estimation is simple, cheap and doesn't require elaborate laboratory arrangement except a simple colorimeter.The values of ADA higher than 32 U/l have high sensitivity(92%) and specificity(82%).

It can be routinely used to differentiate between tuberculous and non-tuberculous cause of ascites.

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