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Biochemical markers of tubercular ascites

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ABSTRACT

The diagnosis of abdominal tuberculosis requires a high index of suspicion due to its vague symptomatology. Early diagnosis of tubercular ascitis is crucial to prevent progression of disease to its advanced stages, thereby preventing the fatal complication like intestinal obstruction, fistulas and peritonitis. The objective of our research work is to evaluate the role of biochemical parameters such as Adenosine Deaminase (ADA), IgG, Lactate, Total protein and albumin, Glucose, Cholesterol and pH in diagnosis of abdominal tuberculosis. Ascitic fluid samples were taken from patients admitted in medicine wards of SSK Hospital after informed consent. A total of 100 patients meeting the selection criteria were enrolled in the study. The biochemical investigations performed for the ascitic fluid samples were Adenosine Deaminase (ADA), IgG against 38 kDa mycobacterial antigen, Lactate, Total protein, albumin, Glucose, Cholesterol and pH. In the 79 patients that had been followed up, with ATT response as a reference, a highly significant association was observed with ascitic fluid assays ADA, IgG, Serum Ascitic Albumin Gradient(SAAG), Cholesterol, Lactate and pH. Adenosine Deaminase (ADA) and IgG against 38 kDa mycobacterial antigen can be used as corroborative markers for diagnosis of extra pulmonary paucibacillary tubercular ascitis where conventional methods like smear microscopy and culture frequently fail to establish the diagnosis.

Keywords: Abdominal tuberculosis, Adenosine Deaminase, IgG against 38 kDa mycobacterial antigen.

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INTRODUCTION

Abdominal tuberculosis can have a varied presentation, frequently mimicking other diseases. The diagnosis of abdominal tuberculosis requires a high index of suspicion due to its vague symptomatology [1].The clinician must look for tuberculosis and confirm or exclude this treatable disease in any patient who present with symptoms related to gastrointestinal tract. Initial symptoms of abdominal tuberculosis like fever, pain, diarrhea, constipation, weight loss, anorexia and malaise are non-specific and non-alarming [2]. Thus the primary disease progresses to the advanced stages leading to complications like ascites, obstruction, fistulas and peritonitis. This increases the morbidity and worsens the prognosis. Ascites as a presenting complaint is seen in 21 -30 % of patients suffering from abdominal tuberculosis [3].However, its multiple etiologies like cirrhosis, malignancies, cardiac and renal pathologies, present a diagnostic challenge to the clinicians. An early and accurate diagnosis is imperative to initiate early intervention and treatment. In the absence of any rapid and reliable method of diagnosis, most of the time, treatment is started on presumptive diagnosis.

Conventional diagnosis of tuberculosis employs the microscopic identification of Acid Fast Bacilli (AFB) in smears stained by Ziehl-Neelsen technique and culture in Lowenstein-Jensen medium. Culturing of bacilli has a specificity approaching 100 % and is considered as the gold standard. It also permits susceptibility testing of isolates to various drugs [4, 5]. However diagnosis by these methods is difficult in paucibacillary samples like ascitic fluid besides the long period needed for growth in culture.

Hence there is a need for an early and reliable marker of diagnosis of abdominal tuberculosis.

The objective of our research work is to evaluate the role of biochemical parameters such as Adenosine Deaminase (ADA), Lactate, Total protein, albumin, Serum Ascitic Albumin Gradient (SAAG),Glucose, Cholesterol and pH in diagnosis of abdominal tuberculosis.

MATERIALS AND METHODS

The study was conducted jointly in the Departments of Biochemistry, Microbiology and Medicine, Lady Hardinge Medical College and Associated Hospitals, New Delhi, India after Institutional ethical clearance.

A total of 100 patients meeting the selection criteria were enrolled in the study. Ascitic fluid samples were taken from patients admitted in medicine wards of SSK Hospital after informed written consent.

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Selection criteria-

Any patients presenting with unexplained ascites were selected for study initially. Ascitic fluid samples were collected under all aseptic conditions by abdominal paracentesis. The ascitic fluid samples were subjected to routine cytology (Inflammatory cell count like total leukocyte count, polymorphonuclear neutrophils and differential counts) and routine biochemical analysis for ascitic fluid (Total protein, albumin).

Of the above patients, all patients with exudative ascites (Total protein >2.5 gm/dl) were included in the study. Patients with cardiac and chronic liver disease were included in the study only if the ascitic fluid indicated an increased inflammatory cell count.

Diagnosed cases of cancer, which could present with ascites were excluded from the study.

The patients were subjected to detailed history and clinical examination routine and special investigations.

Venous blood samples were also collected under aseptic condition and are processed to get the desired serum .These serum samples were further subjected to routine biochemical investigations (Glucose, Liver function tests like total and direct bilirubin, Alanine aminotransferase, Aspartate aminotransferases, Alkaline phosphatase, Kidney function tests like urea, creatinine, uric acid, Electrolytes like sodium, potassium, calcium and phosphate, Total protein and albumin and lipid profile like cholesterol ,triglyceride) .

The biochemical investigations performed for the ascitic fluid samples were Adenosine Deaminase (ADA), Lactate, Total protein, albumin, Serum Ascitic Albumin Gradient (SAAG), Glucose, Cholesterol and pH. The pH of ascitic fluid was measured immediately by pH paper and then the ascitic fluid samples were stored at -20^{0}C in aliquots for ELISA until batch analysis. Microbiological tests like smear microscopy by ZN staining for AFB and culture in LJ medium was also done.

Serological tests like IgG antibody against 38 kDa antigen of *Mycobacterium tuberculosis* complex was done.

Ascitic fluid analysis

1. Adenosine Deaminase (ADA)-

Principle - Adenosine Deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with phenol and hypochlorite in an alkaline medium to form a blue

indophenols complex with sodium nitroprusside acting as a catalyst. Intensity of the blue coloured indophenols complex formed is directly proportional to the amount of ADA present in the sample.
The test was performed by using Microexpress ADA-MTB kit with the instructions provided by the manufacturer.
Interpretation- Normal < 30 U/L, Positive > 30 U/L
Ascitic fluid lactate, total protein, albumin, glucose and cholesterol were measured using Beckman CX 9 Autoanalyzer.

2. Lactate- Measured by lactate oxidase method.

Lactate reacts with oxygen in presence of the enzyme lactate oxidase to form pyruvate and hydrogen peroxide. This hydrogen peroxide then reacts with p-aminophenazone and p-chlorphenol in the presence of peroxidase to form a red colored chromogen. The increase in absorption at 546 nm is proportional to the lactate concentration.
Lactate > 25 mg/dl indicates infected fluid.

3. Total protein –Measured by Biuret method.

Cupric ions, in alkaline medium, interact with protein peptide bonds resulting in formation of a colored complex. The increase in absorbance is measured at 546nm.
Total protein >2.5 gm/dl indicates exudates and <2.5 gm/dl indicates transduate.

4. **Albumin**- Measured by Bromocresol green (BCG) dye binding method.

Albumin is positively charged at pH lower than its isoelectric point, and has affinity for anionic dyes.BCG with pH range 3.8-5.4 binds to albumin. Reaction between albumin and dye BCG produces a colour change from yellow to blue green changing absorption at 578nm which is proportional to the albumin concentration. Yellow is the monovalent anion and blue is divalent anion that changes the absorption reading with albumin.
Serum Ascitic Albumin Gradient (SAAG) >1.1 gm/dl indicates portal hypertension and SAAG < 1.1 gm/dl indicates non-portal hypertension.

5. Cholesterol- Determined after enzymatic hydrolysis and oxidation.

The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. Change in absorbance is measured at 500nm.
Ascitic fluid cholesterol is < 55 mg/dl in cirrhosis.

6. Glucose – Measured by glucose oxidase method.

Glucose is determined after enzymatic reaction in the presence of glucose oxidase. The hydrogen peroxide formed reacts under catalysis of peroxidase, with phenol and 4-amino phenazone to red violet quinoneimine dye as indicator and absorbance is measured at 540 nm.

7. Quantitative assay of antibodies (IgG) against 38-kda antigen of *Mycobacterium tuberculosis* complex

Estimation of IgG antibodies was carried out using Pathozyme-Myco kit. Pathozyme-Myco kit is Enzyme Linked immunoassays for the detection of antibodies against *Mycobacterium* species.

Principle: A recombinant 38-kDa protein and a highly purified antigen derived from *Mycobacterium tuberculosis* are bound to the surface of microtitration wells. Test sera diluted 1/100 are applied. Specific antibodies to *Mycobacterium* species bind to the antigens in the wells. Unbound material is washed away and anti-human IgG antibody conjugated to Horseradish Peroxidase is applied. The conjugate binds to the human antibodies which are bound to the antigen. Unbound material is again washed away. On addition of the substrate, stabilized 3, 3', 5, 5', Tetamethyl Benzidine (TMB), a colour will develop, the intensity of which is determined by the amount of the antimycobacterial antibody in the sample. The enzyme reaction is stopped by the addition of dilute sulphuric acid and the absorbance is then measured at 450nm.

Interpretation: ≥ 400 U/ml considered as positive and < 400 U/ml as negative.
The data were analyzed by using SPSS version 19 and p value < 0.05 was considered as significant.

Observations and Results

In our study, we observed the following ascitic fluid investigations results.

Ascitic fluid Investigations (n=100)

	Number of patients	Percentage (%)
Adenosine Deaminase(ADA) (>30 U/L)	83	83
IgG (> 400 U/ml)	82	82
SAAG (< 1.1 gm/dl)	29	29
Cholesterol (>55 mg/dl)	61	61
Lactate (<25 mg/dl)	62	62
pH (>7)	73	73

A significant number of patients in our study had raised levels of ADA and IgG antibodies to 38 kDa tubercular antigen.

Follow-up of patients

100 patients were enrolled in our study on the basis of clinical suspicion of tubercular ascites and biochemical investigation like total protein. All were put on therapeutic trial (category I / $2(HRZE)_3$+ 4 HR as per Revised National tuberculosis Control Programme (RNTCP),India) of anti-tubercular therapy(ATT).Patients were followed up after 3 months to see the response to therapy. 21 patients were lost to follow up. 79 patients were left in the study to see the response to therapy. Out of these, only 35 patients responded to therapy.

Comparative analysis of ascitic fluid parameters in ATT responders (n=35)

	Positive	Percentage (%)	p value
ADA(>30 U/L)	34	97.1	0.0001
IgG (>400 U/ml)	32	91.4	0.001
SAAG (<1.1 gm/dl)	14	40	0.007
Ascitic fluid / serum Glucose ratio(<0.96)	22	62.9	0.0475
Cholesterol (>55 mg/dl)	24	68.6	0.004
Lactate (<25 mg/dl)	31	88.6	0.0001
pH(> 7)	22	62.9	0.0001
Smear microscopy (ZN stain)	1	2.9	0.870
Culture(LJ medium)	0	-	-

In the 79 patients that had been followed up, with ATT response as a reference, a highly significant association was observed with ascitic fluid assays ADA, IgG, SAAG, Cholesterol, Lactate and pH.

DISCUSSION

In our study we found only one patient showed positivity for AFB smear while culture was negative. This correlates with work done by *Singh et al* [5] in tubercular ascites in which smear positivity was less than 3%.

A cut off level of ascitic fluid pH < 7.35 was used to exclude other bacterial causes of ascitis [6]. As reported a significant association of SAAG (<1.1 gm/dl) with tubercular ascitis [7]. Cholesterol levels of ascitic fluid were analyzed to exclude out cirrhosis again showed a significant association with tubercular ascitis [7]. A value of ascitic fluid lactate >25 mg/dl indicates other bacterial etiology [6] and in our study 89.6 % of the patients had values < 25 mg/dl.

The ascitic fluid analysis of our study shows a highly significant association with ADA, IgG, lactate and pH .The levels of ADA observed in our studies are in accordance with earlier studies [8, 9]. A cut off level of 60 U/L increases the specificity and sensitivity to 90 % for tubercular infection[10]. A good concordance was also seen for the levels of IgG against 38kDa antigen with the studies, sensitivity and specificity of 81 % and 88% respectively with a

diagnostic accuracy of 84% [9]. The other parameters such as ascitic fluid pH, cholesterol and lactate levels showed a significant association in tubercular ascitis and they can be used as corroborative diagnostic aids [6, 7].

Thus, Adenosine Deaminase (ADA) and IgG against 38 kDa mycobacterial antigen can be used as corroborative markers for diagnosis of extra pulmonary paucibacillary tubercular ascitis where conventional methods like smear microscopy and culture often fail to establish the diagnosis. The significance of our study is that Adenosine Deaminase (ADA) and IgG against 38 kDa mycobacterial antigen can be used for early diagnosis as it is time saving and for early treatment as abdominal paracentesis is a simple safe procedure with immense diagnostic potential.

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