

LIVER CIRRHOSIS WITH ASCITES: STUDY ON IDENTIFYING RELIABILITY PARAMETERS FOR CLINICAL OUTCOME

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ABSTRACT

Background: Ascites is one of the major complications of liver cirrhosis which usually develop secondary to portal hypertension. **Aim:** The purpose of the current study was to evaluate the utility of ratios of few biochemical parameters between serum and ascetic fluid, whether these ratios are more reliable than the actual parameters in diagnosis and prognosis of these patients. **Study design:** Cross-sectional study. **Place and duration of study:** Owaisi Hospital and Research Centre, Hyderabad, India from July 2012 to December 2013. **Methodology:** Biochemical analysis of serum and ascetic fluid focussed on assessing liver function was done. A total of 350 cirrhotic patients of both genders were screened for ascites and 50 among them fulfilling inclusion and exclusion criteria were enrolled for this study. They were assessed for 34 demographic and laboratory parameters including liver function tests, renal function tests and serum electrolytes. We made an attempt to know whether ratio of some of the biochemical parameters in ascitic fluid-to-serum (AF/S) can be more reliable indicators and better prognostic markers in liver cirrhosis with ascites. **Results and Conclusion:** In this endeavour, the AF/S ratios of total proteins, albumin, ADA, LDH, GGT and α -amylase did not showed a clear advantage over the existing biochemical analysis in vogue.

Keywords: Cirrhosis, ascites, portal hypertension, liver function tests, reliability parameters.

INTRODUCTION

Ascites most often develops due to portal hypertension as a sequel to liver cirrhosis. Other common causes include malignancy and congestive cardiac failure. Appropriate management of ascites depends on diagnosis of its cause. Cirrhosis is most commonly caused by chronic alcohol consumption, Hepatitis B (HBV) or C (HCV) infection and is associated with poor quality of life, more prone for infections and may also develop end-stage renal disease (ESRD)¹.

The possibility of cirrhosis may be suspected by history of alcoholism, intra-venous drug abuse, sexual promiscuity and chronic viral hepatitis. A thorough physical examination to look for signs of hepatic damage and failure may be constructive. Abdominal examination may reveal presence of caput medusa and free fluid

in the peritoneal cavity. The single best test for diagnosing cirrhosis is liver biopsy, however, this invasive procedure carries a small risk of serious complications and therefore reserved for patients in whom type of liver disease or presence of cirrhosis on examination and non-invasive investigation is not clear. The routinely performed non-invasive investigations involve ultrasonography (USG) of abdomen and liver function tests. Though these tests give the structural and functional information about the liver, it does not focus on the fluid and electrolyte imbalance in ascites.

Approximately 50% of cirrhotic patients can develop ascites within 10 to 15 years. Once ascites develop, the prognosis worsens. It is estimated that approximately 50% of them may die within two years if they do not undergo liver transplant².

Patients with cirrhosis may have abnormal liver tests and evidence of renal failure. They may also have an elevated international normalized ratio (INR), hypoalbuminemia, thrombocytopenia, anemia and leukopenia. Liver transplantation is the treatment of choice in acute and chronic irreversible liver failure of varying etiologies. Surrogate markers of disease progression are needed for providing prognostic information to patients, optimizing referral time for liver transplant and serving as endpoints in clinical trials³. The most commonly used survival models to assess the degree of liver failure and to determine appropriate timing of liver transplantation are: the Child-Pugh score, Model for End Stage Liver Disease (MELD) score and Mayo Prognostic Model (Mayo R score)^{4,5}. The Child-Pugh score evaluates five parameters: ascites, encephalopathy, bilirubin, prothrombin time and albumin. The Child-Pugh classification provides superior results for periods exceeding one year. The MELD is a mathematical model which uses simple and objective variables such as serum bilirubin, serum creatinine and international normalized ratio (INR) of prothrombin time. One of the disadvantages of the MELD formula is the loss of prognostic accuracy beyond 3 months.

Non-invasive surrogate markers are having potential application in diagnostics as they do not pose the similar risks of pain and hemorrhage as liver biopsy. They can be performed frequently and provide a score, potentially capable of tracking progression from mild fibrosis through to end-stage cirrhosis. Serum fibrosis markers could potentially replace liver biopsy as the test of choice for determining the position of a patient along the spectrum of disease severity⁵.

The purpose of the present study was to evaluate the utility of ratios of few biochemical parameters between serum and ascetic fluid, whether these ratios are more reliable than the actual parameters in diagnosis and prognosis of these patients.

MATERIAL AND METHODS

A hospital based cross-sectional study from July 2012 to December 2013 was conducted. A total of 350 cirrhotic patients who came to our hospital were examined for the presence of ascites. The criteria for inclusion in the study were i) age group between 30-85 years ii) belonging to both genders and iii) clinical or ultrasonographic diagnosis of ascites. Patients having gross edema and fluid in interstitium secondary to chronic renal failure, hypoproteinemia, congestive cardiac failure and malignancies were excluded. Also those patients

receiving long-term diuretics were excluded. Only 50 patients who fulfilled the criteria were included for the study.

The study was approved by Institutional Review Board (IRB) and was carried out in accordance with Helsinki declaration. Informed consent was taken from all participants.

Categorization of ascites patients solely on clinical findings is difficult, however ultrasonography can complement the clinical findings. Accordingly, a grading system for ascites has been proposed by the International Ascites Club⁶ but the validity of the grading system has not yet been established.

- Grade 1: Mild ascites detectable only by ultrasound examination
- Grade 2: Moderate ascites manifested by moderate symmetrical distension of the abdomen
- Grade 3: Large or gross ascites with marked abdominal distension

In a modified Child-Pugh classification⁷, ascites has been scored as follows

- Grade 1: None at ultrasound
- Grade 2: Mild or controlled by diuretics
- Grade 3: Present despite administration of diuretics

Demographic data and clinical findings were noted. Blood and ascetic fluid was sent for evaluation. Biochemical analysis was done using Synchron CX7 (USA).

Variables studied

Liver function test (LFT)

Serum total proteins, serum albumin, serum total bilirubin, serum direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyltranspeptidase (GGT) were assessed for knowing the functional status of the liver.

Renal function test (RFT)

Blood urea and serum creatinine were assessed for renal function.

Serum electrolytes

Serum sodium, potassium and chloride were measured. Hyponatremia is an independent predictor of 3-month and 1-year mortality for patients with liver disease, 1mEq/L decrease in the serum sodium concentration between 125 and 140mEq/L is associated with upto 10% increase in mortality⁸.

Blood sugar

Random blood glucose levels were measured.

Ascitic fluid examination

After paracentesis, the ascetic fluid was sent to the lab in EDTA tube. Parameters that were assessed include

- **Serum-to-ascites albumin gradient (SAAG) determination**

The SAAG is calculated by subtracting the ascitic fluid albumin value from the serum albumin value which were obtained on the same day. The SAAG accurately identifies the presence of portal hypertension and is more useful than the protein-based exudate/transudate concept. The presence of a gradient ≥ 1.1 g/dL predicts that the patient has portal hypertension with 97 percent accuracy⁹.

- **Total cell count and differential count**

The total cell count with differential count was done as it is the single most useful test performed on ascitic fluid to evaluate for infection and initiate therapy. Antibiotic treatment should be considered in any patient with a corrected neutrophil count $\geq 250/\text{mm}^3$ ¹⁰.

- **Total protein concentration**

Ascitic fluid is classified as an exudate if the total protein concentration is ≥ 3 g/dL and a transudate if it is below this cut-off. Of late, the exudate/transudate system of ascitic fluid classification has been replaced by the SAAG, which is a more useful measure to know whether portal hypertension is present or not⁹. The total protein concentration may also help differentiate uncomplicated ascites due to cirrhosis from cardiac ascites, both of which have a SAAG ≥ 1.1 g/dL. In the case of ascites due to cirrhosis, the total protein is < 2.5 g/dL, whereas in cardiac ascites it is ≥ 2.5 g/dL. In patients with nephrotic ascites, the SAAG is < 1.1 g/dL, and the total protein is < 2.5 g/dL. Ascitic fluid to serum total protein ratio (ASTPR) and Ascitic fluid to serum albumin ratio (ASAR) may be better surrogate markers for prognosis in ascites than SAAG as the gradient may be a negative value where as ratio is always positive but either less than or more than zero.

- Measurement of glucose and lactate dehydrogenase (LDH) along with total protein is of value in differentiating spontaneous bacterial

peritonitis (SBP) from bowel perforation into ascites. The ascitic fluid glucose concentration is similar to that in serum unless glucose is being consumed in the peritoneal cavity by infective pathogens. The ascitic fluid/serum (AF/S) ratio of LDH is approximately 0.4 in uncomplicated ascites due to cirrhosis. In SBP, the ascitic fluid LDH level rises to 1.0¹¹. Ascitic fluid that has a neutrophil count ≥ 250 cells/ mm^3 and meets two out of the following three criteria are unlikely to have SBP and need immediate evaluation to determine bowel perforation¹²:

✓ Total protein > 1 g/dL

✓ Glucose < 50 mg/dL

✓ LDH greater than the upper limit of normal serum LDH

Some of the following additional tests to exclude cases suspected of complicated ascites were

- **Triglyceride concentration**

Chylous ascites is diagnosed if triglyceride content is greater than 200 mg/dL.

- **Bilirubin concentration**

Ascitic fluid bilirubin value greater than the serum suggests bowel or biliary perforation into peritoneal cavity.

- **α -amylase concentration**

Ascitic fluid amylase concentration will be about 40 IU/L in uncomplicated ascites due to cirrhosis and the AF/S ratio of amylase (ASAAR) about 0.4. Level increases in pancreatic ascites or bowel perforation¹³.

- **GGT concentration**

GGT concentration in ascetic fluid and ascetic fluid/serum ratio of GGT may be tested as prognostic marker.

- **Smear for acid-fast bacillus (AFB), culture and adenosine deaminase activity (ADA)**

Ascitic fluid smear microscopy for AFB has very low sensitivity. Culture and PCR of ascitic fluid for *Mycobacterium tuberculosis* has reasonable sensitivity. ADA of ascitic fluid is a useful non-culture method of detecting tuberculous peritonitis. ADA increases in TB, particularly in our country where the disease load is high¹⁴. Ascites to serum ADA gradient (ASADG) is calculated by subtracting serum ADA value from ascetic fluid value, a gradient of ≥ 3.8 IU/L predicts presence of portal hypertension with 99% accuracy⁹.

Ascites to serum ADA ratio (ASADAR) may be tested as prognostic marker.

Statistical analysis

The results are presented as means±standard deviations and confidence intervals of 95% for quantitative variables. As the mean values for reference normal range were not available, a rough estimate of statistical significance was obtained by looking at the overlapping confidence intervals.

RESULTS

The mean age of the test group was 56.58±6.78 years (95% CI: 54.65-58.51). Most of the patients belonged to middle and elderly age group. Male to female ratio was 39:11 (Male = 78%). Table 1 shows the various laboratory parameters considered for identifying reliability variables in ascites patients.

DISCUSSION

Many factors have been studied in relation to the survival of patients with liver cirrhosis and to improve forecasting models. Accordingly few prognostic indices were calculated that allowed estimating survival in patients with ascites and the models were able to accurately predict survival in considerable number of patients in those studies.

In the study by D'Amico¹⁵, it was reported that the Child-Pugh was the best predictor of mortality in cirrhosis. The univariate analysis showed that in addition to Child-Pugh; MELD, age, sex and liver cancer were associated with lower survival.

Age was the only variable that was predictive of survival in more than 10 studies⁴.

Fernández-Esparrach G et al¹⁶ showed that parameters evaluating renal function and systemic hemodynamics are of prognostic significance in cirrhosis with ascites. They also showed that four variables had independent prognostic value: renal water excretion, mean arterial pressure, Child-Pugh class and serum creatinine.

In the current study, thirty four demographic and laboratory variables including parameters assessing liver and renal function and the ratios of these parameters were analyzed as predictive factors of clinical outcome of these patients. Our subjects were mostly from the middle age group which reveals that the disease either develops or patients seek health attention due to symptoms during this period. Males were predominantly effected than females which is consistent with the findings of Said A et al¹⁷. Almost all the laboratory parameters were deranged from the normal

reference values. The biochemical assays of blood and ascetic fluid showed deranged sugar levels, liver function test and renal function tests. Among serum electrolytes, only serum sodium decreased in our patients' which is in agreement with the findings of Kim WR et al that hyponatremia is an independent predictor of survival in liver disease⁸. Various enzymatic activities in serum and ascetic fluid were found to be deviated from the normal levels and some of them falling in the range diagnostic of complicated ascites. Hence, these variables may not be accurate in assessing the prognosis.

In this study, we made an attempt to know whether ratio of some of the biochemical parameters in ascitic fluid to serum can be more reliable indicators and better prognostic markers in liver cirrhosis with ascites. Ratios were preferred over gradients, as the gradient may be a negative value where as ratio is always positive but either less than or more than zero. In every individual, the basal value of the parameters differs, from which the derangement is occurring. In uncomplicated ascites, transudate accumulates in the peritoneal cavity by the process of filtration of plasma due to change in the starling's forces. Hence, the concentration in the blood for a particular biochemical entity forms a good denominator for assessing the change in biochemical composition of ascetic fluid at each individual level.

We did not find any mention of the normal reference values in the literature for three ratios i.e. AF/S total protein, albumin and ADA. The mean AF/S total protein and albumin ratios were <1, showing the directionality that protein content in ascetic fluid is less than serum which complements the mean SAAG value less than 1.1 revealing the transudate nature of ascetic fluid. Ascitic fluid-to-serum ADA gradient was much higher than normal which was usually diagnostic of tuberculous peritonitis but our patients did not have any evidence of tuberculosis. The AF/S ADA ratio was found to be more promising as the mean was 1.55 (Range: 0.26 to 3.30). AF/S ratios of LDH, GGT and α -amylase were above the range of normalcy as mentioned in earlier works but did not confer a diagnostic and prognostic benefit.

LIMITATIONS OF THE STUDY

We did not take a control group. Also the patients were not followed over a period of time which might have been more informative and survival analysis could have been done.

CONCLUSION

Various biochemical parameters were deranged in cirrhosis with ascites. Our endeavour in finding whether the ratios between the ascetic fluid and serum of some biochemical substances are better markers than the absolute values did not yield favourable results. The ratios did not showed a clear advantage.

Conflict of interest

None

Consent

Informed consent was taken from all the enrolled patients.

Ethical approval

The study was approved by the Institutional Review Board.

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None

Table 1: Study parameters (n=50)

Parameter	Results		Our laboratory reference normal values	Whether statistically significant difference is present or not#
	Mean±SD	95% CI		
Random blood glucose (mg/dl)	103.33±22.05	97.22-109.44	Upto 150	May be absent
Serum total proteins (g/dl)	5.74±0.7	5.54-5.94	6-8	Present
Serum albumin (g/dl)	2.75±0.54	2.60-2.91	3-5	Present
Serum total bilirubin (mg/dl)	8.75±5.38	7.23-10.28	0.2-0.8	Present
Serum direct bilirubin (mg/dl)	4.68±3.43	3.70-5.66	0.0-0.2	Present
Serum AST (IU/L)	78.96±65.95	60.22-97.70	5-45	Present
Serum ALT (IU/L)	73.86±54.71	58.31-89.41	5-45	Present
Serum ALP (IU/L)	383.98±163.76	337.44-430.52	100-186	Present
Serum LDH (IU/L)	401.62±103.84	372.11-431.13	114-240	Present
Serum ADA (IU/L)	74.36±23.33	67.73-80.99	5-15	Present
Serum GGT (IU/L)	290.24±54.03	274.88-305.60	0-55	Present
Serum α-amylase (IU/L)	107.26±42.85	95.08-119.44	≤ 130	May be absent
Blood urea (mg/dl)	67.20±33.47	57.69-76.71	20-40	Present
Serum creatinine (mg/dl)	2.01±0.82	1.77-2.24	0.5-1.5	Present
Serum sodium (mEq/L)	121.20±6.22	119.43-122.97	135-150	Present
Serum potassium (mEq/L)	4.07±0.63	3.89-4.25	3.5-5.0	May be absent
Serum chloride (mEq/L)	89.92±6.12	88.18-91.66	90-110	May be present
Ascitic fluid glucose (mg/dl)	92.26±19.69	86.66-97.86	Upto 150	May be absent
Ascitic fluid total protein (g/dl)	3.91±0.71	3.71-4.11	Upto 3.0	Present
Ascitic fluid albumin (g/dl)	2.10±0.56	1.94-2.26	Upto 1.0	Present
Ascitic fluid ADA (IU/L)	104.54±32.66	95.26-113.82	Upto 30	Present
Ascitic fluid LDH (IU/L)	1261.58±4197.85	68.56-2454.60	Upto 50	Present
Ascitic fluid GGT (IU/L)	324.53±134.32	281.78-359.10	Upto 100	Present
Ascitic fluid α-amylase (IU/L)	178.20±42.99	165.98-190.42	Upto 40	Present
ASTPR	0.69±0.15	0.65-0.73	-	
SAAG (g/dl)	0.65±0.79	0.43-0.88	Upto 1.1	May be absent
ASAR	0.80±0.29	0.72-0.88	-	
ASADG (IU/L)	30.18±41.61	18.36-42.00	Upto 3.8	Present
ASADAR	1.55±0.72	1.36-1.74	-	
ASLDHR	3.19±10.13	0.31-6.06	Upto 0.4	May be present
ASGGTR	1.16±0.55	1.00-1.32	0.4	Present
ASAAR	1.83±0.60	1.66-2.00	0.4	Present

#Qualitatively the statistically significant difference is inferred from whether the Confidence Intervals between test group and normal reference values overlap or not.

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