Serum - Ascites Albumin and Cholesterol Gradients in Differential Diagnosis of Ascites

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Abstracts: Background: Differential diagnosis of ascites is a common clinical problem. Less expensive biochemical techniques are required to differentiate ascites with unknown etiology. Aim: To evaluate the diagnostic efficiency of ascitic fluid cholesterol, serum ascites albumin gradient (SAAG) and serum ascites cholesterol gradients (SACG) in differentiating cirrhotic, tuberculous and malignant ascites. Methods: 50 patients (25 with hepatic cirrhosis, 15 with tuberculosis and10 with malignancy) were evaluated for ascitic fluid total protein, albumin, cholesterol, SAAG and SACG. Results: The mean ascitic fluid cholesterol was significantly higher in malignant ascites when compared with cirrhosis and tuberculous ascites (p= 0.0001 each). The difference between tuberculous and cirrhotic ascites was also significant (p= 0.001). The mean value of SAAG was significantly higher in cirrhosis when compared with tuberculous and malignant ascites (p= 0.0001; p= 0.001 respectively) but the difference between tuberculous and malignant ascites was not significant The mean SACG was significantly lower in malignant compared to tuberculous and cirrhotic ascites (p= 0.0001; p= 0.001 respectively). The difference between tuberculous and cirrhotic ascites was not significant. Conclusion: SAAG is a better marker to differentiate cirrhotic ascites from tuberculous and malignant ascites. Ascitic fluid cholesterol and SACG are better markers to differentiate malignant ascites from cirrhotic and tuberculous ascites. [Vyakaranam S et al. NJIRM 2011; 2(3) : 22-28]

Key Words: Cirrhosis, Ascitic fluid cholesterol, Tuberculous peritonitis, Malignant ascites.

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Introduction: Ascites is a common clinical complication of various diseases. The most important cause of ascites is cirrhosis (80%) followed by malignant peritonei (10%), tuberculous peritonitis (2%), congestive cardiac failure, nephrotic syndrome, others (3%).[1, 2]

Differential diagnosis of ascites is the common clinical problem confronting the physicians.[3.] The effective way of diagnosis is ascitic fluid analysis. Various parameters like Total Protein,[2,4-8] Albumin,[5] Cholesterol,[7,9-12] Amylase [12,14], Lactate dehydrogenase(LDH)[12], Adenosine deaminase (ADA)[13] were used to differentiate ascites. A new physiologically based approach to classify ascites by albumin gradient between serum and ascitic fluid (SAAG) has completely replaced the traditional way of classification as transudate (ascitic fluid total protein ≤2.5gm %) and exudate (ascitic fluid total protein >2.5gm %) [5,8,15]. A high albumin gradient (≥1.1gm %) is usually associated with increased portal pressure as in cirrhosis and a low gradient (<1.1gm%), in conditions where ascites is not related to portal hypertension, but due to peritoneal chafe- as in malignant peritonei, tuberculous peritonitis, metastatic peritoneal implants etc [5,15,16]. In patients with low albumin gradient the ability to differentiate malignant ascites from other etiologies is a major clinical problem. Although cytology is considered as a gold standard for malignancy, its diagnostic sensitivity is only 64%. [17]

Several studies have proved an elevated ascitic fluid cholesterol levels in patients with malignant ascites. Along with it, serum ascites cholesterol gradient (SACG) too aids in differential diagnosis of ascites. [17, 19, 20] Only a few studies have related the serum & ascitic fluid -total protein, albumin, cholesterol & their gradients (SAAG, SACG) in differential diagnosis of ascites.

The present study was done to differentiate ascites due to hepatic cirrhosis, tuberculosis peritonitis &

malignancy by estimating Serum & Ascitic fluid - Total protein Albumin, Cholesterol & their gradients (SAAG, SACG).

Material and Methods: This is a cross-sectional study done in Gandhi Hospital, Secunderabad, from August 2008- February 2009. A total of 50 patients with clinically significant ascites were included in the study conveniently from 2 hospitals. Purposefully patients admitted to the wards of General medicine and Gastroenterology in Gandhi Hospital and MNJ Cancer hospital were selected on a continuous basis. The study was carried with the permission of the institutional ethics committee. The study population was divided in to 3 groups A, B & C.

- Group A had twenty five (25) patients with hepatic cirrhosis due to chronic alcoholism, confirmed by history, abdominal ultrasound scan and altered liver function tests (elevated serum bilirubin, alanine transaminase, asparatate transaminase, alkaline phosphatase).
- Group B had fifteen (15) patients with tuberculous peritonitis, confirmed by history, cytology showing lymphocytes & elevated ADA in ascitic fluid, chest X-ray, ultrasound scan of abdomen and Mantoux test.
- Group C had ten (10) patients with malignant ascites confirmed by positive ascitic fluid cytology or histopathological examination of peritoneal biopsy material obtained at laparoscopy and ultrasound scan of abdomen, an elevated Ca-125 in levels in ovarian carcinoma.

Patients with spontaneous bacterial peritonitis, ascites due to other etiologies such as nephrotic syndrome, bud-chiari syndrome, malnutrition, mixed causes of ascites (cirrhosis with tuberculosis, cirrhosis with malignancy) were excluded from the study. An informed consent was taken from all the cases. All the patients were admitted and clinical history was obtained. A detailed clinical examination was carried & a base line investigation - CBP, CUE, LFT, ECG, and ultrasound scan of abdomen were performed.

Under strict aseptic conditions blood samples were collected, by venous puncture, into properly labelled plain polystyrene tubes. The samples were collected, handled and transported to the lab according to the guidelines given by clinical and laboratory standards institute/ NCCLS (National Clinical Chemistry Lab Standards) [21, 22]. The blood sample was centrifuged at 10,000 rpm for 10 min and serum was collected

The ascitic fluid was collected simultaneously by abdominal paracentesis [23, 24, 25] following the guidelines given by AASLD (American Association of the Study of Liver Diseases) [1]. Prior to procedure the patient was asked to empty the bladder. Ascites was confirmed by physical examination. The patient was laid in semi recumbent position. The preferred site for the tap was on the left side of the flank two finger breaths cephaloid and two finger breaths medial to anterior superior iliac spine (distended caecum may be present on the right side, hence avoided) The site was painted with Iodine solution and draped. Skin and deeper tissues were infiltrated with 2% xylocaine. The skin was retracted caudally and a 1.5 inch 22-gauge needle on syringe was inserted into the anesthetized area and advanced while aspirating. A 5ml of fluid was aspirated and the needle was withdrawn quickly and the caudal skin retraction is released, allowing the skin to return to its normal position so that the entrance and exit needle sites form a "Z-tract" to minimize ascites leakage. The fluid was transferred to the sterile polystyrene tubes. The fluid was centrifuged and the supernatant was collected.

The serum and AF samples thus collected were analyzed on the same day .The serum total protein was estimated by Biuret method [26] and serum albumin by Bromocresol green method[27] with Qualigen Diagnostics kit. The serum cholesterol was estimated by enzymatic, CHOD-POP end point assay [28] by Autospan liquid gold diagnostics kit.

Quality control: The results were evaluated by comparison with standards of known concentration. Measures were taken by checking the kit-to-kit variability and the repeatability was checked by duplicate testing. The intra and inter assay coefficients of variation for all the parameters was maintained <5%.

Statistical analysis: The data was processed in MS EXCEL and analysis was carried out using SPSS (17th version). The results were statistically analyzed by unpaired Student't' test and by Pearson's correlation coefficient. A two tailed probability value of < 0.05 was taken as indicating significance. The critical values for ascitic fluid cholesterol and SACG were obtained by hypothetical Receiver Operation Characteristics (ROC) curves.[29, 30]

Result: Of the twenty five patients (25) in group A (hepatic cirrhosis) twenty three (23) were males and 2 were females. Their age groups ranged from 37-48 yrs and the mean age was 42.5±5.5 yrs. In group B, which included 15 patients with tuberculous peritonitis nine were males and 6 were females. Their age groups ranged from 28-48 years and the mean age was 38±10yrs. Group C included malignant ascites in which seven were females and 3 were males their age group ranged from 36-60yrs and the mean age was 48±12yrs .The etiology of ascites in this group was ovarian carcinoma (n=5), carcinoma cervix (n=2), carcinoma colon (n=2) and of unknown etiology (n=1).None of the patients in group C had hepatic metastasis.

The results of serum and ascitic fluid analysis in all the three groups of patients included in this study are shown in Table 1.

Serum total protein: Serum total protein was higher in patients with malignant ascites (6.0±0.44gm %) when compared to cirrhosis group (5.5±0.73gm %) and tuberculous peritonitis group (5.92±0.48gm %) but this difference was not statistically significant (p >0.05) (Table: 1).

Table: 1 Serum and ascitic fluid -Total Protein, Albumin, Cholesterol and Serum- ascitic fluid gradients in the three study groups.

Parameter		Cirrhosis (n=25)	Tuberculosis (n=15)	Malignancy (n=10)	P-value
Total Protein (gm %)	Serum	5.5±0.73	5.92±0.48	6.01± 0.44	C vs. T : NS, C vs. M : NS,T vs. M : NS
	Ascitic fluid	1.85± 0.48	3.43±0.58	3.83±0.66	C vs. T: 0.001,C vs. M: 0.001, Tvs M : NS
Albumin (gm %)	Serum	2.61±0.65	3.46±0.37	3.81±0.28	C vs.T: 0.0001,C vs.M:0.001,T vs. M : 0.01
	Ascitic fluid	1.24±0.54	2.74±0.47	3.05±0.35	C vs. T: 0.001, C vs.M:0.0001,T vs. M : NS
SAAG (gm %)		1.38±0.23	0.76±0.26	0.78±0.04	C vs.T: 0.0001, C vs. M: 0.001,T vs. M : NS
Cholesterol (mg %)	Serum	128.72±30.1	133.06±21.73	168.6±33.79	C vs.T : NS,C vs. M :0.001 T vs. M : 0.003
	Ascitic fluid	31.4±10.0	46.66±11.59	120.3±36.63	C vs.T:0.001,Cvs.M:0.0001,T vs M :0.0001
SACG (mg %)		96.48±23.53	86.13±20.85	44.3±16.75	C vs.T : NS.Cvs. M:0.0001, T vs. M : 0.001

P-value <0.05 indicates Significance (S)

Ascitic fluid total protein: The ascitic fluid total protein concentrations were low in cirrhosis (1.85±0.48gm%) when compared to tuberculous (3.43±0.58gm%) and malignant ascites (3.83±0.66gm%). The difference between cirrhosis and other two groups was statistically significant (p=0.001 for each). The difference between tuberculosis and malignancy was not significant (p >0.05) (Table 1). With a critical value of 2.5gm%, five patients (20%) with cirrhotic ascites had higher values and two patients (8%) with tuberculous ascites had values less than 2.5gm%. None of the patients with malignant ascites had lower values. The cut off value of 2.5gm% had sensitivity (80%), specificity (92%), positive predictive value (90%), negative predictive value (82%) and diagnostic accuracy (86%) (Table: 2).

Table 2 Diagnostic values of various parameters in separating cirrhotic from non-cirrhotic (tuberculous and malignant) ascites.

Parameter	Cut-off value	Sensitivity (%)	Specificity (%)	Positive Predictive value	Negative Predictive value	Diagnostic accuracy
AF Total protein	<2.5gm%	80%	92%	90%	82%	86%
AF Albumin	<2.0gm%	76%	92%	90.4%	79.3%	84%
SAAG	>1.1gm%	96%	92%	92.3%	95.8%	94%

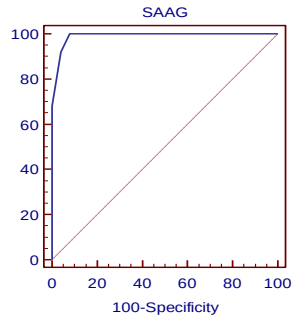
Serum albumin: Serum albumin levels were significantly low in cirrhotic ascites (2.61 ±0.65gm %) when compared with tuberculous (3.46±0.37gm%) and malignant ascites (3.81±0.28gm%) (p =0.0001, p =0.001 respectively). The difference between tuberculosis and malignant ascites was also significant (p =0.01; Table 1).

Ascitic fluid albumin: Ascitic fluid albumin levels were significantly low in cirrhosis group (1.24±0.54gm %) when compared with tuberculous and malignant groups (p =0.001; p =0.0001 each Table 1).The difference between tuberculosis (2.74±0.47gm %) and malignant (3.05±0.35gm %) groups was not significant (p >0.05) (table 1). With a critical value of 2gm%, six patients (24%) in cirrhotic group had higher values. One patient (6.66%) with tuberculous ascites and one patient (10%) with malignant ascites had values < 2gm%. At the above cutoff value sensitivity was 76%, specificity was 92%, positive predictive value was 90.4%, negative predictive value was 79.3% and diagnostic accuracy was 84% (Table 2).

Serum /ascites albumin gradient (SAAG): The difference in the SAAG was significantly higher in cirrhotic group (1.38±0.23gm %) when compared with tuberculosis and malignant ascites (p =0.0001, p =0.001 respectively) where as the difference between tuberculosis (0.76±0.26gm%) and malignancy (0.78±0.04gm %) was not statistically significant (p >0.05; Table 1). With a critical value of 1.1gm %, only one patient (4%) with cirrhotic ascites had value <1.1gm% where as only two (13.33%) patients with tuberculous ascites had higher values. None of the patients with malignancy had values >1.1gm%.The cutoff value obtained from ROC curves was 1.1gm % (fig: 1). With this sensitivity was 96%, specificity was 92%, positive predictive value was 92.3%, negative predictive value was 95.8% and diagnostic accuracy was 94% (Table 2).

Serum cholesterol: Serum cholesterol was significantly higher in patients with malignant ascites (168.6±33.79 mg%) compared to cirrhotic (128.72±30.1mg%) and tuberculous ascites (133.06±21.73mg%) (p=0.001 and 0.003 respectively); whereas the difference between cirrhosis and tuberculosis groups was not statistically significant (p >0.05) (Table 1).

Graph 1: Receiver operation characteristics (ROC plots) for Serum- ascites albumin gradient



Ascitic fluid cholesterol: The ascitic fluid cholesterol was significantly elevated in malignant ascites (120.3±36.63mg %) when compared with cirrhosis (31.4±10.0mg %) and tuberculosis (46.66±11.59mg %) (p=0.0001 each). The optimal diagnostic efficiency was obtained with a cutoff point of 62mg % (fig: 2). With this cutoff one patient (10%) with malignant ascites had lower value and one patient (2.5%) with non-malignant group had higher value. At a cut off level of 62mg%, sensitivity was 90%, specificity was 97.5%, positive predictive value was 96%, negative predictive value was 97.5% and diagnostic accuracy was 96% (Table 3). The difference between cirrhotic and tuberculous group was also significant (p= 0.001) (table 1).There was considerable overlap between the values and no critical value could be ascertained between tuberculous and cirrhotic ascites.

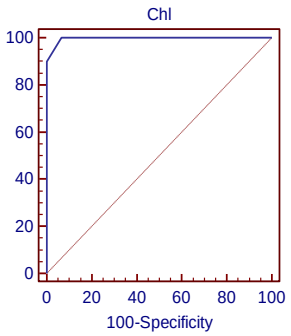
Serum ascites cholesterol gradient (SACG): The malignant group had lowest SACG (44.3±16.75mg %) when compared with cirrhosis (96.48±23.53mg %) and tuberculosis (86.13±20.85mg%) (p=0.0001,p=0.001 respectively). The difference between cirrhosis and tuberculosis group was not significant (p >0.05) (table 1). Taking a critical value of 53mg% only one patient (10%) with malignant ascites had higher value and two patients (5%) in non-malignant group had lower values. The cut off level obtained from ROC curves was 53mg% (fig: 3). At this cutoff sensitivity was 90%, specificity was 95%, positive predictive value was 81.8%, negative predictive value was 97.4% and diagnostic accuracy was 94% (Table 3).

Table 3 Diagnostic values of various parameters in separating malignant from Non-malignant (tuberculous and cirrhotic) ascites

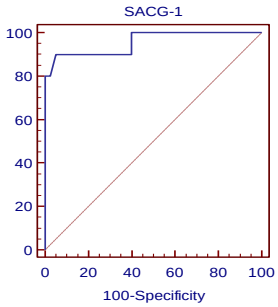
Parameter	Cutoff value	Sensitivity (%)	Specificity (%)	Positive predictive value	Negative predictive value	Diagnostic accuracy
AF Cholesterol	>62mg%	90%	97.5%	96%	97.5%	96%
SACG	<53mg%	90%	95%	81.8%	97.4%	94%

Sensitivity, Specificity, Predictive values and Diagnostic accuracy: The critical values, obtained by ROC curves, for ascitic fluid total protein (2.5gm %), albumin (2.0gm %), cholesterol (62mg %), SAAG (1.1gm %) and SACG (53mg %). The sensitivity, specificity, predictive values and diagnostic accuracy were calculated (Table 2&3). Ascitic fluid cholesterol had highest diagnostic accuracy (96%) of all parameters, with a sensitivity of (90%) and specificity of (97.5%, Table 3). SAAG had maximum sensitivity (96%) and a diagnostic accuracy of 94%. Of all the parameters ascitic fluid albumin had lowest sensitivity (76%) and diagnostic accuracy (84%) followed by ascitic fluid total protein sensitivity (80%) and diagnostic accuracy (86%).

Graph 2: Receiver operation characteristics (ROC plots) for Ascitc fluid cholesterol.



Graph 3: Receiver operation characteristics (ROC plots) for Serum- ascites cholesterol gradient



Discussion: Ascites is an important clinical finding; its appropriate treatment depends on proper diagnosis. This study is focused on evaluation of the efficiency of various conventional diagnostic parameters to differentiate cirrhotic, tuberculous and malignant ascites from each other and also to propose ascitic fluid cholesterol and SACG as new diagnostic parameters.

Our study has reinforced the observations of earlier studies stating a limited value of transudate and exudate concept based on ascitic fluid total protein in differentiating cirrhotic from non-cirrhotic ascites and of no value in differentiating malignant and tuberculous ascites[18,31,32]

SAAG was adopted as a newer and more physiological approach to classify ascites on the basis of presence or absence of portal hypertension [15,16,18]. Hoefs etal [33] established a cutoff value of 1.1gm%, it was supported by our and various other studies[15, 16, 18.] Ascites is one of the important sequels of portal hypertension; secondary to cirrhosis. SAAG ≥1.1gm% can differentiate cirrhotic from non-cirrhotic ascites. Similar results were observed in our study, with a critical value of ≥ 1.1gm% SAAG differentiated cirrhotic from non-cirrhotic ascites with a diagnostic accuracy of 94%. Presently SAAG is included in the guidelines of investigations recommended on the management of ascites in cirrhosis by American Association of the Study of Liver Disease (AASLD) [1] and British Society of Gastroenterology[2].

Various studies have proposed the significance of ascitic fluid cholesterol in differentiating malignant ascites. Prieto etal[18] showed that ascitic fluid cholesterol concentrations were significantly higher in patients with peritoneal metastases and was superior to ascitic fluid total protein, lactate dehydrogenase and SAAG for discriminating ascites from that due to liver disease; however there was no difference in ascitic fluid cholesterol between those with liver disease and those with superimposed hepatocellular carcinoma. The etiology for the elevated cholesterol levels in malignancy is due to the increased vascular permeability increased cholesterol synthesis and

release from malignant cells implanted on peritoneum[17, 31,34]. In our study ascitic fluid cholesterol concentrations were significantly elevated in malignant ascites when compared to cirrhotic and tuberculous ascites. With a critical value of >62mg%, the diagnostic accuracy was 96%. This is supported by the study done by Sood A etal[7], stated that tuberculous and malignant ascites are difficult to differentiate because several markers express similar patterns with notable exception of ascitic fluid cholesterol. In their study the cutoff value was 54.5mg% and diagnostic accuracy of 93.18%. A variation in critical values for ascitic fluid cholesterol was observed in different studies. Satya Rana etal[17] (>70mg %) had diagnostic accuracy of 94%, Sharatchandra etal[19] (>67mg %) had a diagnostic accuracy of 96%, R. Gupta etal [31] (> 55mg %), a diagnostic accuracy of 94%. These variations in the cutoff levels could be attributed to the selection of patients, serum cholesterol levels and to the extent of peritoneal implants.

Our study showed significantly lower levels of SACG in malignant ascites when compared to cirrhotic and tuberculous ascites. With a critical value of 53mg% SACG differentiated malignant ascites from cirrhotic and tuberculous ascites by a diagnostic accuracy of 94%. Unlike ascitic fluid cholesterol SACG could not differentiate cirrhotic from tuberculous ascites. Only few studies have mentioned the significance of SACG. Our study was consistent with the study done by Ranjith etal [20]; SACG with a cutoff value of 63.5mg% had sensitivity (93.3%) and specificity (90.3%). R.Gupta etal[26] also found that SACG can differentiate tuberculous ascites from malignant ascites; due to considerable overlap of figures a cutoff limit could not be ascertained.

Conclusion: In the present study SAAG differentiated cirrhotic from tuberculous and malignant ascites. In view of good diagnostic efficiency we propose that ascitic fluid cholesterol and SACG can be used as an effective parameter to differentiate malignant ascites. These are simple and cost effective method for diagnosing the etiology of ascites in developing countries.

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