

USEFULNESS OF SERUM ASCITES CHOLESTEROL GRADIENT (SACG), TOTAL PROTEIN (TP) RATIO AND SERUM ASCITES ALBUMIN GRADIENT (SAAG) IN DIFFERENTIATING TUBERCULOUS ASCITES AND CIRRHOTIC ASCITES

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ABSTRACT

Background: Differential diagnosis of ascites is a common clinical problem and is usually done by Serum Ascites Albumin Gradient (SAAG). However many other markers can also be utilized for the same.

Aims & Objective: This study was carried out to evaluate the diagnostic efficiency of ascitic fluid cholesterol, serum ascites albumin gradient (SAAG), Total protein Ratio and serum ascites cholesterol gradient (Chol gradient/ SACG) in differentiating cirrhotic and tuberculous ascites.

Material and Methods: The study included 48 patients admitted in St John's Medical Hospital, Bangalore, out of which 25 patients were diagnosed with tuberculous ascites and 23 patients were diagnosed with cirrhotic ascites. Serum and ascitic fluid (AF) albumin, Total protein (TP) and Cholesterol (Chol) were estimated. The SAAG, TP ratio, Serum ascites cholesterol gradient (SACG) were calculated. Significance was assessed at 5% level of significance. Cohen's d effect size has been computed and discrimination function analysis is done to determine the percentage of correct classification between cirrhotic and tubercular ascites.

Results: SAAG showed a sensitivity and specificity of 100% and 95.6% at cut off of >1.1g/dl TP ratio at a cut off > 0.5 showed sensitivity 100% and specificity 98%. Ascitic fluid Cholesterol is high in the tuberculous group and showed sensitivity and specificity of at a cut off value of 100% and 95.5%. Ascitic fluid TP showed a sensitivity and specificity of 100% and 96% at a cut off value of <2.5g/dl. Whereas SACG at a cut off value of <95mg% showed a sensitivity and specificity of 68% and 100% respectively. Their effect sizes were (3.18, 4.21, 3.21, 3.51, 1.00 respectively). Their % discriminations were (100%, 97.9%, 95.8%, 97.9%, 60.4%).

Conclusion: We conclude that SAAG is definitely the best marker along with TP ratio and AF cholesterol. However SACG is not a good marker to differentiate tuberculous ascites and cirrhotic ascites.

Key-Words: Serum Ascites Albumin Gradient (SAAG); Serum Ascites Cholesterol Gradient (SACG); Total Protein (TP) Ratio; Cirrhotic Ascites; Tuberculous Ascites

Introduction

Differential diagnosis of ascites is the common clinical problem confronting the physicians. The effective way of diagnosis is ascitic fluid analysis. Various parameters like Total Protein, Albumin, Cholesterol, Amylase, Lactate dehydrogenase (LDH), Adenosine deaminase (ADA) could be used to differentiate ascites. One of the approaches to classify ascites is by albumin gradient between serum and ascitic fluid (SAAG). A high albumin gradient (≥ 1.1 gm%) is usually associated with increased portal pressure as in cirrhosis and a low gradient (< 1.1 gm%), in conditions where ascites is not related to portal hypertension like tuberculous ascites.^[1]

Several studies have proved an elevated ascitic fluid cholesterol levels in patients with malignant ascites but not in tuberculous ascites.^[2] Along with it, serum ascites cholesterol gradient (SACG) too aids in differential diagnosis of ascites.^[3,4] However only a few studies have related the serum & ascitic fluid -total protein, albumin, cholesterol & their gradients (SAAG, SACG) in differential diagnosis of ascites

Hence we took up this study with primary objective to differentiate ascites due to hepatic cirrhosis and tuberculosis peritonitis by estimating Serum & Ascitic fluid -Total protein, Albumin, Cholesterol & their gradients (SAAG, SACG). Our secondary objective was to find the effect size indicating the best marker that aids in

categorizing the two groups and find their sensitivity and specificity.

Materials and Methods

Study Design: A Cross Sectional Comparative Study was done on 48 patients after strict inclusion and exclusion criteria, admitted in the Medical and Gastroenterology wards of St John's Medical College Hospital between October 2008 and January 2009 of which 23 were Cirrhotic ascites patients and 25 were Tuberculous ascites patients.

After obtaining written and informed consent venous blood samples and ascitic fluid samples were collected with aseptic precaution from these patients at the time of admission. Detailed clinical history was taken, physical examination and investigations e.g. AFB, cytological examination, biochemical examinations (glucose, protein, albumin) and, wherever possible, biopsy and histopathological examination, USG, X- ray chest, ECG, ECHO and other appropriate investigations were done. Only patients diagnosed with cirrhotic ascites and tuberculous ascites were included. The Ascitic Fluid and Serum were used to estimate Cholesterol, Total Protein and Albumin. SAAG, TP ratio and SACG were calculated. The utility of serum analytes and ascitic analytes, gradients, ratio were evaluated statistically.

Inclusion Criteria: All patients with ascites diagnosed as tuberculous ascites and cirrhotic ascites, which represents the exudative and transudative ascites.

Exclusion Criteria: All other causes of ascites like malignant, Spontaneous Bacterial Peritonitis (SBP) etc. were excluded as they have certain biochemical markers similar as tuberculous ascites.

Specimen Collection and Storage: Blood and ascitic fluids were collected with aseptic precaution and centrifuged. Serum and supernatant fluid were separated and Stored at - 20°C for 1 month.

- Albumin was estimated by the Spectrophotometer- Bromocresol Green (BCG) -dye binding method

- Total protein was estimated by Spectrophotometer- Biuret Method
- Total cholesterol was estimated by Spectrophotometer- Cholesterol oxidase method

Statistical Analysis: The Statistical software namely SPSS 16.0, Stata 8.0, Medcalc 9.0.1 and Systat 1.0 were used for the analysis of the data. Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean \pm SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Student 't' test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis). Cohen's d effect size has been computed and discrimination function analysis is done to determine the percentage of correct classification between cirrhotic and tubercular.

Results

Our study showed that when only serum analytes were assayed serum Total protein had the least effect size and % decimation indicating the least useful criteria in correctly classifying the two groups. Albumin with mean (1.81 ± 0.32 in cirrhotic) and (2.70 ± 0.62 in Tuberculous) was statistically significant with $p < 0.001$, it also showed the large effect size and % discrimination followed by serum cholesterol with mean. None of these serum analytes alone can be used to classify or differentiate the ascites. (Table 1)

Compared to serum analytes, ascitic fluid (AF) analytes showed large effect size and % discrimination. However in ascitic fluid both the total protein (1.20 ± 0.52 vs 4.79 ± 1.30) and albumin with mean (0.33 ± 0.14 vs 2.11 ± 0.64) had similar % discrimination and effect size. Both were statistically significant with $p < 0.001$ Indicating the ability to correctly differentiate the cirrhotic and tuberculous ascites. Ascitic fluid cholesterol was comparatively have less effect size and % discrimination (Table 2). Ascitic fluid TP showed a sensitivity and specificity of 100% and 96% at a cut off value of < 2.5 g/dl. Ascitic fluid albumin showed sensitivity and specificity of

100% and 44% at a cut off of < 2 g/dl.

Table-1: Comparison of Serum (Total Protein, Albumin, and Cholesterol (Chol) between Cirrhotic and Tuberculous Ascites Patients

Biochemical variables	Cirrhotic	Tuberculous	Significance	Effect Size	% Discrimination
Total Protein (g/dl)	6.92 ± 0.74	7.10 ± 0.94	t=0.728, p=0.470	0.21	54.2%
Albumin (g/dl)	1.81 ± 0.32	2.70 ± 0.62	t=6.167, p<0.001**	1.75	85.4%
Chol (mg/dl)	157.65 ± 32.96	176.16 ± 26.89	t=2.139, p=0.038*	0.61	68.8%

* Moderately significant (P value: 0.01<P 0.05); ** Strongly significant (P value: P≤0.01)

Table 2: Comparison of Ascitic Fluid (AF) (Total Protein, Albumin and CHOL) between Cirrhotic and Tuberculous Ascites Patients

Biochemical variables	Cirrhotic	Tuberculous	Significance	Effect Size	% Discrimination
Total Protein (g/dl)	1.20 ± 0.52	4.79 ± 1.30	t=12.369, p<0.001**	3.51	97.9%
Albumin (g/dl)	0.33 ± 0.14	2.11 ± 0.64	t=13.031, p<0.001**	3.71	97.9%
Chol (mg/dl)	32.91 ± 16.76	79.72 ± 11.66	t=11.306, p<0.001**	3.21	95.8%

* Moderately significant (P value: 0.01<P 0.05); ** Strongly significant (P value: P≤0.01)

Table-3: Comparison of Total Protein ratio, SAAG, CHOL Gradient between Cirrhotic and Tuberculous Ascites Patients

Biochemical variables	Cirrhotic	Tuberculous	Significance	Effect Size	% Discrimination
TP Ratio	0.17 ± 0.08	0.67 ± 0.13	t=15.974, p<0.001**	4.51	97.9%
SAAG (g/dl)	1.49 ± 0.32	0.58 ± 0.24	t=11.141, p<0.001**	3.18	100.0%
SACG (mg%)	124.74 ± 29.81	96.44 ± 26.14	t=3.503, p=0.001**	1.00	60.4%

* Moderately significant (P value: 0.01<P 0.05); ** Strongly significant (P value: P≤0.01)

Ascitic fluid Cholesterol is high in the tuberculous group. It showed mean of (32.91 ± 16.76 in cirrhotic vs 79.72 ± 11.66 in tuberculous) and showed sensitivity and specificity of 100 % and 95.5% respectively at a cut off value of 70 mg%.

TP ratio was calculated as Ascitic fluid TP: Serum TP. SAAG was calculated as Serum albumin- ascitic fluid albumin. SACG was calculated as Serm Chol- Ascitic fluid Chol.

When we compared all these we could find that SAAG was still the best with very large effect size and 100% discrimination followed by TP ratio. However SACG had small effect size in comparison and less % of discrimination (60.4%).

SAAG with a mean value of (1.49 ± 0.32 in cirrhotic ascites vs 0.58 ± 0.24 in tuberculous)

showed a sensitivity and specificity of 100% and 95.6% respectively at cut off of >1.1g/dl. TP ratio with a mean value (0.17 ± 0.08 in cirrhotic vs 0.67 ± 0.13) at a cut off > 0.5, showed sensitivity 100% and specificity 98%. Whereas SACG with mean value of (124.74 ± 29.81 in cirrhotic vs 96.44 ± 26.14 in tuberculous) showed a sensitivity and specificity of 68% and 100% respectively at a cut off value of < 95mg%.

Hence it is clear that SAAG and TP ratio and AF chol are better markers than SACG for differentiating cirrhotic ascites and tuberculous ascites.

Discussion

Ascites is an important clinical finding; its appropriate treatment depends on proper and prompt diagnosis. This study is focused on evaluation of the efficiency of various conventional diagnostic parameters to differentiate cirrhotic, tuberculous ascites from each other and also to propose the best marker as differentiating factor in ascites.

In our study SAAG showed a sensitivity and specificity of 100% and 95.6% at cut off of > 1.1 g/dl TP ratio at a cut off > 0.5 showed sensitivity 100% and specificity 98% specificity. Ascitic fluid Cholesterol is high in the tuberculous group and showed sensitivity and specificity of at a cut off value of 100% and 95.5%. Ascitic fluid TP showed a sensitivity and specificity of 100% and 96% at a cut off value of < 2.5 g/dl. Whereas SACG at a cut off value of < 95 mg% showed a sensitivity and specificity of 68% and 100 % respectively. Their effect sizes were (3.18, 4.21, 3.21, 3.51, 1.00 respectively). Their % discriminations were (100%, 97.9%, 95.8%, 97.9%, 60.4%).

Sapna Vyakaranam et al, showed in their study that SAAG had maximum sensitivity (96%) and specificity of 94%. Their other analytes like AF Cholesterol at a cut off of > 62 mg% showed sensitivity of 90% and specificity of 97.5%. SACG at cut off value of < 53 mg% showed sensitivity of 90% and specificity of 95%. They concluded that SACG could not differentiate cirrhotic and tuberculous ascites unlike AF chol and

commented that SAAG is the better marker to differentiate ascites.^[1]

B L Kanwy et al compared SAAG with the AF total protein and TP ratio, they found that the tuberculous group higher ascitic fluid total protein (aTP) concentration (4.77 ± 2.05 versus 1.98 ± 1.56 g/dL), ascitic to serum ratio of total protein (a/sTP) concentration (0.75 ± 0.43 versus 0.26 ± 0.19), a lower SAAG (0.6 ± 0.30 versus 1.71 ± 0.61). They concluded that SAAG is still the best marker to differentiate. This study has similar findings to ours.^[5]

M Beg et al observed that the serum albumin ascitic gradient had a diagnostic sensitivity of 94.73% and 96% specificity, compared to AFTP, which is 65.62% and 68% respectively. They concluded that Differential diagnosis should be based on SAAG than on TP ratio Our study showed at cut off of > 1.1 g/dl SAAG had better sensitivity and specificity (100% and 95.6% respectively) and TP ratio at a cut off > 0.5 showed sensitivity 100% and specificity 96%.^[6]

U H Malabu et al showed that AF total protein at >3.0 g/dL showed sensitivity and specificity of 63 % and 83% respectively. Total protein ratio >0.5 showed sensitivity and specificity of 61% and 65% respectively. And the SAAG at <1.1 g/dl showed sensitivity and specificity of 95% and 98%.^[7]

According to Fatoumata et al, AF cholesterol can be used to classify ascites into transudate and exudate using a threshold of 183g/L. We also had similar findings, with a cut off of 70mg% our study also showed sensitivity and specificity of 100% and 95 % respectively.^[8]

A variation in critical values for ascitic fluid cholesterol was observed in different studies. Satya Rana et al^[9] (> 70 mg%) had diagnostic accuracy of 94%, R. Gupta et al^[10] (> 55 mg %), a diagnostic accuracy of 94%. These variations in the cut off levels could be attributed to the selection of patients, and serum cholesterol levels Sharath Chandra L K et al showed that with a cut off of 64mg% the AF cholesterol had sensitivity and specificity of 96% each. And the SACG showed sensitivity and specificity of with 80% each at a

cut off of 64 mg%.^[4] They concluded stating that SALG (Serum ascites Lipid gradient) which also includes SACG could be used to differentiate ascites along with SAAG. We however differ in this aspect.

Limitations and Future Scope

Large multi-centric studies are better to evaluate. This study did not include malignant ascites which could have been the reason for better sensitivity and specificity. However future studies can be done on serosal fluids and effect size can be calculated to find out which analyte can correctly classify the serosal fluid accumulation.

Conclusion

Our study has reinforced the observations of earlier studies stating differentiation of ascites SAAG is the best based upon sensitivity, specificity, effect size and % discrimination. We differ with other studies in terms of TP ratio and SACG. We conclude that TP ratio is also equally good marker. However we differ with many recent studies which claim SACG also to be a good marker for differentiating the ascites. We also conclude that AF chol can be used along with SAAG and TP ratio for better diagnostic outcome.

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