A STUDY OF SERUM ASCITES ALBUMIN GRADIENT IN THE AETIOLOGICAL DIAGNOSIS OF ASCITES

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ABSTRACT

BACKGROUND
Ascites is the pathological accumulation of fluid within the peritoneal cavity. It is one of the most common amongst the various clinical conditions, confronting not only a physician, but a surgeon and a gynaecologist too. It complicates a variety of disorders (1) which include cirrhosis, decompensated heart failure, nephrotic syndrome, peritoneal tuberculosis, disseminated carcinomatosis, pancreatitis, myxoedema, etc. In these conditions, ascites develops only as a consequence of the underlying illness. So the evaluation of the patients with ascites requires that the cause of ascites be established. A proper diagnosis is a prerequisite for the successful management of these patients.

The aim of this study is to differentiate ascites on the basis of serum ascites albumin gradient into high SAAG ascites > 1.1 g/dL and low SAAG ascites < 1.1 g/dL. To determine the sensitivity and specificity of serum ascites albumin gradient and that of ascitic fluid total protein in identifying the aetiology of ascites. To compare the diagnostic accuracy of serum ascites albumin gradient with the traditional marker - ascitic fluid total protein.

MATERIALS AND METHODS
The study of ‘Serum Ascites Albumin Gradient in the aetiological diagnosis of Ascites’ was carried out in the Department of General Medicine, ACSR Govt. Medical College Hospital, Nellore, Andhra Pradesh.

RESULTS
The results of the study “SAAG in the aetiological diagnosis of ascites” conducted in the medical wards of ACSR Govt. Medical Hospital has yielded an aetiological profile of the entire study group as follows: Liver cirrhosis - 56%, Decompensated heart failure - 16%, Tuberculous peritonitis - 10%, Malignant ascites - 8% including 3 cases of Peritoneal carcinomatosis and 1 case of Malignant deposit in liver, Nephrotic syndrome - 4%, Splenic abscess - 2%, Pancreatitis - 2% and Hypothyroidism - 2%.

CONCLUSION
The study “Serum ascites albumin gradient in the aetiological diagnosis of ascites” conducted among the fifty inpatients with ascites in the wards of the Department of General Medicine, at ACSR Govt. Medical College Hospital has concluded that the sensitivity and specificity of SAAG in the differentiation of different types of ascites are 94% and 91% respectively. The accuracy of SAAG in the aetiological diagnosis is 94%. The Serum Ascites Albumin Gradient (SAAG) is superior to ascitic fluid total protein (AFTP) in the differential diagnosis of ascites and it is statistically significant.

KEYWORDS
Ascites, Serum Ascites Albumin Gradient, Ascitic Fluid Total Protein.


BACKGROUND
Ascites is the pathological accumulation of fluid within the peritoneal cavity. It is one of the most common amongst the various clinical conditions, confronting not only a physician but a surgeon and a gynaecologist too. It complicates a variety of disorders (1) which include cirrhosis, decompensated heart failure, nephrotic syndrome, peritoneal tuberculosis, disseminated carcinomatosis, pancreatitis, myxoedema, etc. In these conditions, ascites develops only as a consequence of the underlying illness. So the evaluation of the patients with ascites requires that the cause of ascites be established. A proper diagnosis is a prerequisite for the successful management of these patients.

Diagnostic ascitic fluid aspiration is the most rapid and cost effective test for identifying the basic disease process. Before the 1980s, the Ascitic Fluid Total Protein [AFTP] concentration was used to classify ascites as either exudative [AFTP >/= 2.5 g/dL] or transudative [AFTP < 2.5 g/dL]. This classification is unable to correctly identify the aetiological factors responsible for its causation. Hence, this antiquated system of ascitic fluid classification is problematic and it offers only a little insight to the pathophysiology of the ascitic fluid formation.

Further, these drawbacks led to the development of a new approach to classify ascites, based on the difference between the serum and ascitic fluid albumin concentration [Serum
Ascites Albumin Gradient - SAAG. This newer concept classified ascites into two categories - High SAAG ascites with SAAG >= 1.1 g/dL in cases with portal hypertension and low SAAG ascites with SAAG < 1.1 g/dL in cases with ascites, unrelated to portal hypertension.

The serum ascites albumin gradient has been proved in multiple studies to categorise ascites better than either the ascitic fluid total protein or other parameters in ascitic fluid analysis. In view of the above, the present study is undertaken among the inpatients admitted with ascites in the medical wards of ACSR Govt. Medical College Hospital, to evaluate the value of SAAG in the aetiological diagnosis of ascites and also to compare its sensitivity and diagnostic accuracy with that of Ascitic Fluid Total Protein [AFTP].

MATERIALS AND METHODS
A total of fifty adult patients with ascites, admitted to the Department of General Medicine, ACSR Govt. Medical College Hospital, Nellore, within six months' period, whose aetiological diagnosis had not been known previously were studied prospectively. The protocol was approved by the Hospital’s Ethical Committee and an informed consent was obtained from all patients.

On entry, a detailed history and clinical examination were conducted. The fifty patients who satisfied the set criteria were included in the study. Paired ascitic fluid and serum samples were collected from them simultaneously and were examined for ascitic fluid albumin, ascitic fluid total protein and serum albumin with established methods of estimation - Bromocresol green and Biuret methods as described by Varley et al.

Inclusion Criteria
All patients with ascites due to any cause with normal coagulation profile.

Exclusion Criteria
Ascitic patients with severe coagulopathy or Disseminated Intravascular Coagulation (DIC).

Abdominal Paracentesis

After obtaining informed consent from the patient and relatives, diagnostic abdominal paracentesis was done. The patients were asked to empty the bladder prior to the procedure.

The skin of the abdominal wall was disinfected with an iodine solution. The skin and subcutaneous tissue were infiltrated with a local anaesthetic. A special technique was followed to prevent the leakage of fluid after the needle was withdrawn. This technique of needle insertion (Z tract) was accomplished by displacing the skin approximately 2 cms downward and then slowly inserting the paracentesis needle mounted on the syringe held in the other hand. The paracentesis needle is a steel 22-gauge needle about 1.5 inch in length. The hand holding of the syringes was used to stabilise the syringes and to retract the plunger simultaneously. The skin was released only after the needle had penetrated the peritoneum. When the needle was ultimately removed, the skin resumed the original position and sealed the needle pathway.

The needle was advanced slowly through the abdominal wall. Slow insertion helped to allow the bowel to move away from the needle, thereby avoiding bowel puncture.

Site of Needle Insertion

The needle was inserted into the left lower quadrant rather than the right lower quadrant, because the caecum may be distended with gas from lactulose therapy. In the presence of a surgical scar, the needle was placed several centimetres from the scar.

The ascitic fluid collected was sent for cell count in an EDTA bottle, biochemical analysis including total protein and albumin in a plain bottle and for culture in a blood culture bottle. Simultaneously, blood samples were collected from the patients and were sent for the estimation of serum albumin to the laboratory.

Calculation of SAAG

The serum ascites albumin gradient was calculated after measuring the serum and ascitic fluid albumin concentrations and simply subtracting the ascitic fluid value from the serum value.

To increase the accuracy of SAAG, specimens of serum and ascitic fluid were obtained simultaneously.

Correction of SAAG

To correct the SAAG in the setting of a high serum globulin level, the following formula was used.

Corrected SAAG = Uncorrected SAAG x 0.16 x (Serum globulin + 2.5)

Serum hyperglobulinaemia (Serum globulin > 5 g/dL) leads to a high ascitic fluid globulin concentration and can narrow the albumin gradient by contributing to the oncotic forces. A narrow gradient caused by high globulin levels occurs in one percent of ascitic fluid specimens.

Albumin Estimation - BCG Method

The serum and ascitic samples for albumin are fed into the autoanalyzer.

Total Protein (Biuret Method)

Principle

Peptide bonds of protein form a blue violet coloured complex with cupric ions in an alkaline medium. The intensity of the colour is proportional to the number of peptide bonds and the colour is read at 540 nm.

All the 50 patients further underwent ultrasonogram abdomen, which revealed the ultimate diagnosis for confirmation and comparing the diagnostic accuracies of SAAG and ascitic fluid protein.

Ultrasonography of the liver and portal venous system, which is a non-invasive imaging modality, helped to establish the diagnosis of portal hypertension. USG diagnosis of portal hypertension was based on demonstration of -

a. Dilated collaterals around the gastro-oesophageal junction and splenic hilum.

b. Splenomegaly and dilated portal vein > 14 mm in diameter and splenic vein > 12 mm.

It also helped in establishing the aetiology of portal hypertension by giving information about -
a. Hepatic architecture (Altered echo pattern with nodularity indicates cirrhosis, normal echo pattern in extrahepatic portal vein obstruction and non-cirrhotic portal fibrosis).
b. Patency of the portal and splenic veins (Portal vein thrombosis and portal cavernoma diagnostic of EHPVO).
c. Patency of hepatic veins and IVC. (Thrombosis or Budd-Chiari syndrome).

RESULTS
The results of the diagnostic ascitic fluid aspiration and the ascitic fluid analysis in all the fifty selected patients are being interpreted here.

The ascitic fluid specimens of forty eight patients were straw coloured, whereas two specimens were haemorrhagic. After complete workup, one turned out to be a case of chronic calcific pancreatitis and the other was a case of peritoneal carcinomatosis with primary in the large intestine.

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>SAAG &gt;/= 1.1</th>
<th>SAAG &lt; 1.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhosis</td>
<td>26</td>
<td>2</td>
</tr>
<tr>
<td>CCF</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Nephrotic Syndrome</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Liver Metastasis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Peritoneal Carcinomatosis</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>TB ascites</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Splenic abscess</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Above table classifies the ascites of the patients into two groups as exudative and transudative with the cut-off of ascitic fluid total protein as >/= 2.5 and < 2.5 respectively; 48% of cases presented as exudative and 52% of cases had transudative ascites.

After evaluating the patient with imaging studies and coming to the conclusion of the aetiology, the patients were further classified into high SAAG or low SAAG depending on the pathophysiology of ascites, whether related or unrelated to portal hypertension and exudative ascites or transudative ascites, which are depicted in tables.

Above table groups the individuals with ascites in the study population into high SAAG group and low SAAG group with a cut-off value of 1.1. About 74% of the people were in high SAAG group and the left out 26% were in low SAAG group.

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>AFTP &gt;/= 2.5</th>
<th>AFTP &lt; 2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhosis</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>CCF</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Nephrotic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Metastasis</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Peritoneal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinomatosis</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>TB Peritonitis</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Splenic Abscess</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Above table groups the ascites into high SAAG and low SAAG depending on the pathophysiology. Cirrhosis, decompensated heart failure, liver metastasis and hypothyroidism were grouped under high SAAG ascites. Peritoneal carcinomatosis, tuberculous ascites, nephrotic syndrome, splenic abscess and pancreatitis were grouped under low SAAG ascites.
The five variables calculated for both AFTP and SAAG are depicted in the above table. At a glance from this table, one could understand easily and with no doubt that SAAG is better than AFTP in determining the aetiology of ascites, which is the aim of the present study.

**DISCUSSION**

In a study conducted by U. H. Malabu et al, I. O. Olubuyide et al, M. E. Shaiu et al and F. Olawuyi et al (2006) in the Gastroenterology Unit, Department of Medicine, University College Hospital, Ibadan, Nigeria, the clinical profile was as follows: 44% liver cirrhosis, 23% TB peritonitis, 22% malignant ascites, 6% heart diseases and 5% nephrotic syndromes.

In another group of 132 people studied by Al-Knawy BA et al at the division of Gastroenterology, King Saud University, Abha, Saudi Arabia, the clinical ranking and profile were similar to the previous one with 69.7% liver cirrhosis, 10.6% peritoneal tuberculosis, 9.1% malignant ascites, 7.6% decompensated cardiac failure and 3% nephrotic syndrome.

In contrast to the two studies discussed above, decompensated heart failure ranked the second instead of tuberculous peritonitis in the present study group. On the other hand the major causes of ascites are liver cirrhosis and malignant ascites in the Western population, whereas tuberculous peritonitis leads the list in the Asians and Blacks.

However, it is important to note that there is a limitation to such studies in general. Statistics derived from hospital figures are biased and are only an approximate guide to the incidence of the disease in a community. What is seen in hospitals may represent only the tip of an iceberg. The present data must be interpreted in the knowledge of the defects inherent to such studies.

The clinical profile of the low SAAG ascites in the present study group was 39% of tuberculous peritonitis, 25% of peritoneal carcinomatosis, 15% of nephrotic syndrome and other causes like pancreatitis and splenic abscess which accounted for 7% each. The present study is comparable to a study conducted by Fariborz Mansour-Ghanaei et al, Afshin Shafaghi et al, Amir- Hossein Bagherzadeh et al, Mohammad-Sadegh Fallah et al among 148 patients over a 7 years period at the Gastrointestinal and Liver Diseases Research Centre, Gilan University of Medical Sciences, Gilan Province, Iran, which concluded that Tuberculous peritonitis should be considered in all patients with low gradient ascites in the developing countries. This is in contrast to the study conducted by Runyon et al, which stated malignant ascites as the commonest cause of low SAAG ascites in the developed countries.

Myxoedema ascites is a rare entity and hypothyroidism as a cause of ascites accounts for less than one percent. A case report and review literature by Jeong-Seonji et al and colleagues at the Department of Internal Medicine and Pathology, College of Medicine, The Catholic University of Korea, Seoul, Korea had stated that in a review of 51 well documented cases of myxoedema ascites the mean SAAG and ascites were 1.5 and 3.9 g/dL respectively. Similarly, the sixty eight years old hypothyroid female in our study presented with high protein and high SAAG ascites.

In the present study, the patients with malignant ascites presented as two groups - one with high SAAG ascites comprising 1 case of secondaries liver comprising about 25%.

### Table 1: Classification of Ascites as Exudative or Transudative

<table>
<thead>
<tr>
<th>Pathophysiology</th>
<th>High SAAG</th>
<th>Low SAAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal HT</td>
<td>36 (True Positive)</td>
<td>2 (False Negative)</td>
</tr>
<tr>
<td>Non-Portal HT</td>
<td>1 (False Positive)</td>
<td>11 (True Negative)</td>
</tr>
</tbody>
</table>

### Table 2: Comparison of SAAG and Portal Hypertension

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AFTP</th>
<th>SAAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>83%</td>
<td>94%</td>
</tr>
<tr>
<td>Specificity</td>
<td>63%</td>
<td>91%</td>
</tr>
<tr>
<td>Positive Predictive Value</td>
<td>42%</td>
<td>97%</td>
</tr>
<tr>
<td>Negative Predictive Value</td>
<td>92%</td>
<td>85%</td>
</tr>
<tr>
<td>Diagnostic Accuracy</td>
<td>68%</td>
<td>94%</td>
</tr>
</tbody>
</table>
The other group presented with low SAAG ascites consisting of 3 cases of peritoneal carcinomatosis comprising about 75%. A similar one,(9) "the study of the clinical pattern of ascites due to malignancy" was conducted in Qatar by Khan F Y et al, Ahmed M S et al, Loot A Q et al and Ascamawi M et al during the year 2005 - 2006, at Hamad General Hospital among 22 patients. The study revealed that SAAG was able to discriminate peritoneal carcinomatosis from other types of malignant ascites, since it is related to the genesis of ascites and it is very crucial in clinical practice. The diagnostic accuracy of SAAG in malignant ascites is 100% in our present study.

The results of the application of the two tests of interest, i.e.
1. Serum Ascites Albumin in Gradient (SAAG), and
2. Ascitic Fluid Total Protein (AFTP).

In the categorisation of aetiology of ascites in the study population are expressed in terms of sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy calculated by the appropriate formulae as already discussed.

The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of SAAG are 94%, 91%, 97%, 85% and 94% respectively as compared to 83%, 63%, 42%, 92% and 68% respectively with ascitic fluid total protein. These results clearly demonstrate that SAAG offers an excellent discrimination of the causes of ascites. Similar observations have been reported by other studies too.

In the study conducted by Al-Kanvy BA et al in Saudi Arabia, two other parameters, i.e. ascitic fluid lactic dehydrogenase and ascitic to serum ratio of total protein in addition to SAAG and AFTP were compared. Among all the four, SAAG had the highest 60 positive and negative predictive values (80% and 98%) against that of ascitic fluid total protein. These results clearly demonstrate that SAAG offers an excellent discrimination of the causes of ascites. Similar observations have been reported by other studies too.

There are two Indian studies,(9),(10) which were conducted in Aligarh and Allahabad regarding the comparison of the accuracies of SAAG and AFTP in diagnosing the aetiology of ascites. In the study of 76 patients at the Department of Medicine, JN Medical College, Aligarh Muslim University by M. Beg et al, S. Hussain et al, N. Ahmad et al and N. Akhtar et al, the diagnostic accuracy and sensitivity of SAAG were 96% and 68% against the respective values 60% and 66% of AFTP. In the other study conducted at the Department of Gastroenterology and Pathology, M.L.N. Medical College, Allahabad, by Gupta R et al, Misra SP et al, Dwivedi M et al, Misra V et al, Kumar S et al and Gupta SC et al, the diagnostic accuracies of AFTP and SAAG were found to be 88% and 92% respectively.

All these studies were based on the initial study(11) conducted by Bruce A. Runyon et al, Agnes A. Montano et al, Evanengolos A. Akriviadis et al, Mainor R. Antillon et al and Michelle A. Irving et al and John G. McHutchison et al among 901 patients in the University of Iowa, Iowa city in the year 1992. The diagnostic accuracy of SAAG and ascitic fluid total protein was 96.7% and 55.6% respectively.

In another study(12) conducted among 51 patients by Akriviadis E. A. et al, Kapnas D. et al, Hadjigravel M. et al, Missiou A. et al and Gouli S J et al, in the University of Thessaloniki, Hippocratical Hospital, Greece the diagnostic accuracy of SAAG was found to be 98% when compared to 52% - 80% in the four other diagnostic markers compared.

Thus, serum ascites albumin gradient (SAAG) is the single best test against Ascitic Fluid Total Protein (AFTP) in the differential diagnosis of ascites. The terms exudative and transudative can be replaced by high SAAG and low SAAG ascites. This result is similar to the results of all the studies conducted.(13),(14),(15),(16),(17)

CONCLUSION
The study "Serum ascites albumin gradient in the aetiological diagnosis of ascites" conducted among the fifty inpatients with ascites in the wards of the Department of General Medicine, at ASCR Govt. Medical College Hospital has concluded that -
1. The specificity and sensitivity of SAAG in the differentiation of different types of ascites are 94% and 91% respectively.
2. The accuracy of SAAG in the aetiological diagnosis is 94%.

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>SAAG</th>
<th>AFTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhosis</td>
<td>92%</td>
<td>68%</td>
</tr>
<tr>
<td>CCF</td>
<td>100%</td>
<td>50%</td>
</tr>
<tr>
<td>CRF</td>
<td>100%</td>
<td>50%</td>
</tr>
<tr>
<td>Malignant Ascites</td>
<td>100%</td>
<td>75%</td>
</tr>
<tr>
<td>TB Peritonitis</td>
<td>80%</td>
<td>80%</td>
</tr>
<tr>
<td>Miscellaneous (Splenic Abscess, Hypothyroidism &amp; Pancreatitis)</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Diagnostic Accuracies of SAAG & AFTP as per Aetiology

T = 2.72 (p < 0.05)

The diagnostic accuracy of SAAG and AFTP were calculated separately in the above table for each cause in the present study and they were compared statistically using student's 't' test to determine the 95% confidence interval. A p value < 0.05 is considered significant.

The calculated 't' value is 2.72 (p < 0.05). For 10 degrees of freedom the table value is 2.23 at 5% level of significance, which is lesser than 2.72. So the data is significant at 5% level of significance and implies that SAAG is a better diagnostic measure than ascitic fluid total protein.
3. The Serum Ascites Albumin Gradient (SAAG) is superior to Ascitic Fluid Total Protein (AFTP) in the differential diagnosis of ascites and it is statistically significant.

REFERENCES