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SERUM ASCITIC ALBUMIN GRADIENT & ASCITIC FLUID CHOLESTEROL - NEWER APPROACH TO EVALUATE ASCITES

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Abstract

Ascites is common clinical condition. The underlying pathogenesis involves various mechanisms. In present study we had investigated 100 cases of ascites with regard to clinical history, biochemical parameters & cytological examination. We observed that Liver cirrhosis was the most frequent cause of ascites (68%) with SAAG > 1.1mg/dl. Malignancy was noted in 6% cases which were confirmed by cytological examination & revealed cholesterol value > 50mg/dl.

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INTRODUCTION

The term ascites refers to collection of excess fluid in peritoneal cavity[1]**.** It usually becomes clinically detectable when at least 500 ml of fluid has accumulated in peritoneal cavity.

The etiological spectrum of ascites is vast & practically includes pathology of all systems. The earlier approach in differential diagnosis constituted separation of fluid on the basis of

protein concentration as Transudate (Protein< 2.5gm %) and Exudate (Protein > 2.5gm %) Transudative ascites supposed to be caused by liver diseases while neoplastic, tuberculosis & other inflammatory conditions contribute exudative ascites. But this concept has certain limitations & has been challenged in various occasions, especially in cirrhotic patients on prolonged diuretic treatment & patients with cardiac ascites &spontaneous bacterial peritonitis (SBP). SBP is often associated with low protein as endogenous antimicrobial activity/opsonic activity correlate closely with fluid's protein concentration. Fluids with protein <1gm% have essentially no opsonic & antibacterial activity. Hence better parameters were evolved like Serum ascitic albumin gradient (SAAG) which is difference between serum & ascitic fluid albumin concentration. Likewise Ascitic fluid cholesterol along with cytological examination offers an excellent discrimination between malignant & non-malignant ascites.

Therefore this study will be helpful to evaluate the diagnostic utility Serum ascitic albumin Gradient and Ascitic fluid cholesterol in association with cytological examination in differential diagnosis of ascites as well as correlate clinical, cytological and biochemical findings in cases of ascites.

MATERIALS AND METHODS

In this prospective study we evaluated 100 cases of ascites admitted to R.C.S.M.G.M.C. Kolhapur, during the period of September 2009 to August 2010 & include indoored patients in medicine, surgery, paediatrics & gynaecology department with distension of abdomen. Males &females form both urban &rural population were included. Relevant clinical data was collected from Hospital and Laboratory records.

DIAGNOSTIC APPROACH IN ASCITES [2]

a) History: Patient usually presents with distension of abdomen. In alcoholic cirrhosis patient usually have history of regular alcohol intake for a long duration.

If patient has cirrhosis of long duration and suddenly presents with ascites, diagnosis of hepatocellular carcinoma should be suspected. Ascites of Cirrhosis is usually painless while that of malignancy is associated with pain. Pain also occurs in ascites associated with pancreatitis or peritonitis.If there is history of intravenous drug abuse, blood transfusion, acupuncture, tattooing then Post necrotic cirrhosis is suspected. A history of congestive cardiac failure points towards cardiac ascites. Malignancies usually of breast, lung, colon, pancreas, ovary, liver or lung may manifest with ascites. So relevant history should be taken

As per the clinical presentation, physical examination & investigations patients were categorised into cirrhosis, Nephrotic syndrome, and Congestive cardiac failure, anaemia with hypoproteinemia, tuberculosis &malignancy.

***b) Examination*:** Abdomen appears distended; umbilicus may be flushed, everted or transversely stretched. There may be distended veins running vertically on anterior abdominal wall. Presence of splenomegaly suggests portal hypertension. Patient with liver diseases usually have pedal oedema whereas renal and cardiac patients have anasarca.

On Percussion: Puddle Sign – is the first sign to appear. In this flicking percussion of abdomen is done with simultaneous auscultation of abdomen with patient in knee elbow position. Later on, flank dullness with shifting dullness appears on accumulation of further fluid. There is horse-shoe shaped dullness followed by fluid thrill.

c) Collection of Ascitic Fluid [3]

i) Paracentesis: Diagnostic paracentesis is performed in most patients with new ascites.

A minimum of 30 ml is needed for complete evaluation and if possible at least 100 ml should be provided for cytological examination.

***ii) Diagnostic Peritoneal lavage*:** Useful in evaluation of abdominal trauma. A catheter is placed through a small incision into abdominal cavity. If less than 15 ml of gross blood can be aspirated, diagnostic peritoneal lavage can be performed by infusing 1 litre of saline or Ringer's lactate (20 ml/kg in children) Collect the fluid by gravity drainage. At least 600 ml fluid is collected.

iii) Peritoneal Dialysis: Dialysate fluid from patients with chronic renal failure is collected for checking the infection.

iv) Peritoneal Washings: This is performed intraoperatively to document early intra-abdominal spread of gynaecologic or gastric carcinomas. To prevent coagulation, the aspirated fluids should be collected in a container containing Acid Citrate Dextrose (ACD) or in a container to which added 5 units of heparin / mm^3 of aspirated fluid [4].

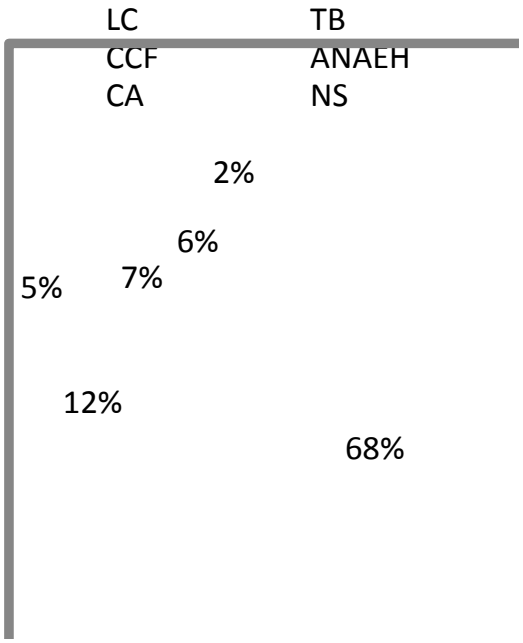
Ascitic fluid was collected by diagnostic paracentesis in different bulbs, plain for biochemistry & in EDTA for cytology. Simultaneous venous samples were collected. Ascitic fluid total protein was estimated by Biuret method by using Autopak kits by Biolab Diagnostics.

Ascitic fluid albumin & serum albumin were estimated by Bromocresol Green method while the fluid cholesterol determined by using the principle of hydrolysis of cholesterol esters & oxidation to yield a red coloured complex measured colorimetrically. From cytology bulb total & differential count was done using Nebeaur's chamber. Hematoxylin & Eosin

stained smears prepared from centrifuged deposit were examined for morphological details.

RESULTS

We observed that Cirrhosis (68%) was the most common cause of ascites (Fig.01)



LC- Liver Cirrhosis, TB- Tuberculosis, CCF- congestive cardiac failure, ANAEH- Anaemia,CA- Malignant ,NS- Nephrotic syndrome

Fig 1:Pie diagram showing Distribution of cases according to etiology

Grossly the tuberculous ascites was turbid while in malignant hemorrhagic & in other cases has clear, straw coloured or pale yellow appearance.(Fig.02)

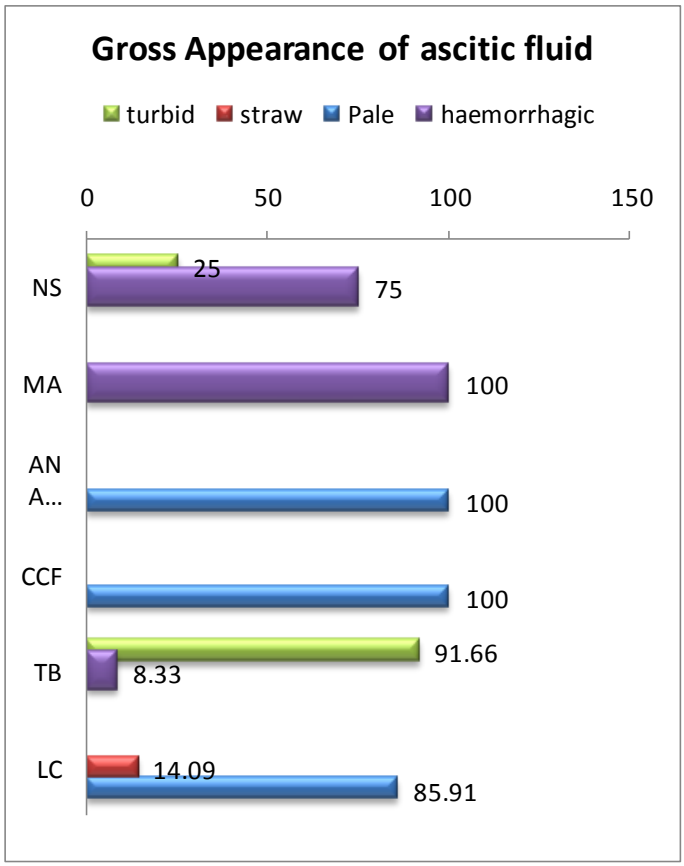


Fig 02: Showing gross appearance of ascitic fluid

The value of serum ascitic albumin gradient was found to be > 1.1gm/dL in while the Ascitic fluid cholesterol was higher in malignant one.(Table1) The total nuclear cell count was more than 100/mm^3 in maximum cases of tuberculosis & malignant ascites (Table 2) All 6 cases with higher cholesterol were confirmed to be malignant on cytology. (Fig.03)

SL. No.	Etiology	Ascitic fluid mean total protein (gm/dL)	Mean SAAG (gm/dL)	Mean Ascitic Fluid cholesterol (mg/dL)
1	Liver cirrhosis	1.76 ± 0.45	1.86±0.28	13.35±5.49
2	Tuberculosis	3.41 ± 0.56	0.95±0.24	30.08±6.57
3	Congestive Cardiac Failure	1.86 ± 0.54	1.96±0.41	17.20±6.72
4	Anaemia with hypoproteinnemia	1.50 ± 0.42	0.60±0.21	16.00±8.48
5	Malignancy	1.90±0.54	1.58±0.41	15.85±6.72
6	Nephrotic Syndrome	3.70±0.41	0.92±0.07	76.00±12.71

Table 1: Table showing Mean Values of biochemical parameters in ascites of different etiologies

SL. No.	Etiology	<100cells/mm^3	100 – 500 cells/mm^3	>500cells/mm^3
1	Liver cirrhosis	58(85.29)	8(11.76)	2(2.94)
2	Tuberculosis	2(16.66)	9(75)	1(8.33)
3	Congestive Cardiac Failure	5(100)	-	-
4	Anaemia with hypoproteinemia	6(85.71)	1(14.28)	-
5	Malignancy	1(16.66)	4(66.66)	1(16.66)
6	Nephrotic Syndrome	1(50)	1(50)	-

Table 2: Table showing Leucocytes count in ascitic fluid of different etiologies

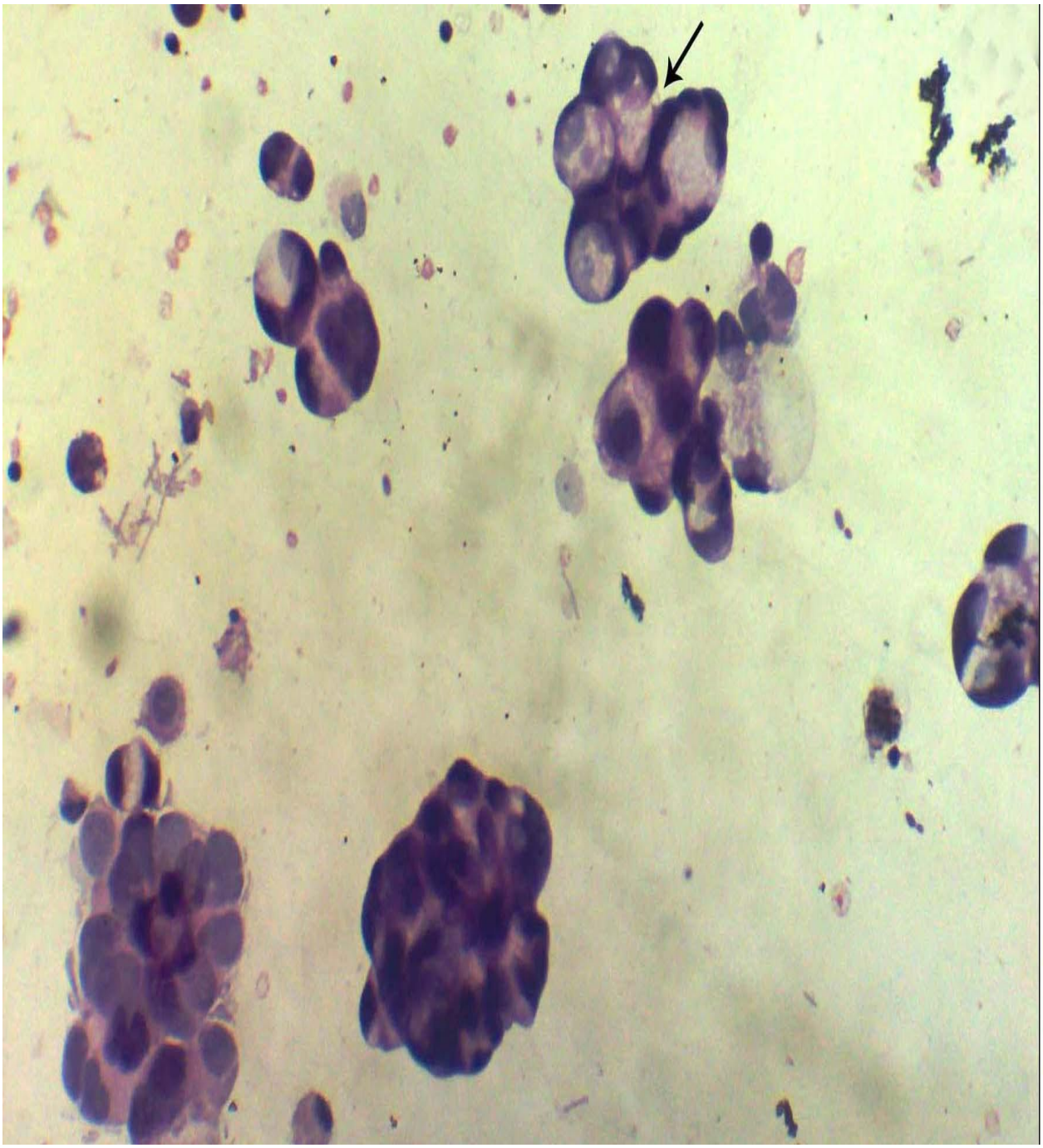


Fig 3: Balls of metastatic cells from Mucinious adenocarcinoma of stomach in Ascitic fluid the arrow shows signet ring cells. (H &E 40X)

DISCUSSION

Ascites is one of the most common clinical problems confronting a physician; especially if the clinical picture is not clear.

Careful history taking and clinical examination can provide clue to the etiology of ascites. But paracentesis remains the main stay of the investigation of a new onset ascites. The pathophysiology of ascites can be explained on the basis of Starling's law of fluid Exchange[5]. Starling suggested that interchange of fluid between blood & tissue space is controlled by balance between capillary hydrostatic pressure forcing the fluid into tissue space & osmotic pressure returning the fluid in vascular compartment.from this it is clear that there are 2 factors important for ascites – i) Plasma colloidal pressure ii) portal venous pressure. The accumulation of ascitic fluid in portal hypertension represents a state of total-body sodium and water excess, but the event that initiates this imbalance is unclear. Three theories have been proposed.

The *"underfilling" theory* suggests that the primary abnormality is inappropriate sequestration of fluid, within the splanchnic vascular bed due to portal hypertension and a consequent decrease in effective circulating blood volume. According to this theory, an apparent decrease in intravascular volume (under filling) is sensed by the kidney, which responds by retaining salt and water[6].The *"overflow" theory* suggests that the primary abnormality is inappropriate renal retention of salt and water in the absence of volume depletion. A third and more recent theory, the *peripheral arterial vasodilatation* hypothesis may unify the earlier theories and accounts for the constellation of arterial hypotension and increased cardiac output in association with high levels of vasoconstrictor substances that are routinely found in patients with cirrhosis and ascites. According to this theory, portal hypertension results in splanchnic arteriolar vasodilatation, mediated by nitric oxide, and leading to underfilling of the arterial vascular space and baroreceptor-mediated stimulation of renin-angiotensin, sympathetic output, and antidiuretic hormone release[7, 8]. Regardless of the initiating event, a number of factors contribute to ascites formation like increased levels of serum epinephrine & norepinephrine, renal vasoconstriction resulting in sodium retention[9,10]. Transudative ascites is caused by Cirrhosis, nephrotic syndrome while Exudative is contributed by Tuberculosis Spontaneous bacterial peritonitis & malignant ascites. In our prospective study we used newer parameter, SAAG and ascites due to portal hypertension was divided into high gradient (i.e. >1.1gm/dl) & low gradient

(i.e.<1.1gm/dl) which do not have portal hypertension[11,12]. The biochemical evaluation revealed Serum ascitic albumin gradient of <1.1 gm/dl in exudative ascites (Inflammatory conditions & malignancy) and ascitic fluid cholesterol in malignant ascites was found to be > 50mg/dl.

Advantages of Serum ascitic albumin gradient: - Serum ascitic albumin gradient correlates with only one physiological factor, the portal pressure[13]. It retains its accuracy even after diuresis and therapeutic paracentesis[14] and was found to be far superior to exudate-transudate concept in classification of ascites, accuracy for two methods were 96.7% and 55.6% respectively[15].

Hence according to Runyoun B.A. et al [14] 1992, serum ascitic albumin gradient should be taken as initial factor to classify ascites.

However, neither ascitic fluid total protein nor serum ascitic albumin gradient replace cytology and culture in confirming suspected peritoneal carcinomatosis or tuberculosis respectively[14]. Another parameter is Ascitic fluid Cholesterol. Low yield of cytology and low positive rates of direct smears for acid-fast bacilli have forced the workers to look for alternative methods in differential diagnosis of ascites[16]. Recently ascitic fluid cholesterol has been reported to offer excellent discrimination between ascites due to malignancy and other non malignant causes [17, 18].

Ascitic fluid cholesterol level is raised in malignancy due to increased leakage of plasma lipoprotein HDL and LDL cholesterol into peritoneal cavity. Ascitic fluid cholesterol and malignant ascites are correlated by cholesterol levels above 45 mg /dL and confirmed by cytological examination[19].

Mean ascitic fluid cholesterol level in cirrhotics was found 33± 21 mg/dL which in malignant ascites was found 95± 42 mg/ dL. [20].Taking cut off value of 48 mg/dL, sensitivity, specificity, positive, negative predictive values and diagnostic accuracy were found to be 96.5%, 96.6%, 93.3%, 98.3%, 96.6% respectively. Patients with tuberculous ascites, cardiac ascites and pancreatitis usually have values in range of values of malignant ascites giving false positive results. So, cytology is the single best test to order when peritoneal carcinomatosis is suspected with sensitivity 100%[21]. It was found that there is 100% correlation between ascitic fluid cholesterol with cut off value of 50 mg/dL in peritoneal carcinomatosis. We found mean asctic fluid cholesterol (76 ± 12.71) which is in accordance with other studies[22, 23]. (Cut of values used in all studies = 50mg/dl). We

observed Cirrhosis (68%) was the most comman cause of ascites & Nephrotic syndrome is least common (2%) which is in concordance with Nath et al & Khan F.Y.studies[24, 25].Incidence of tuberculosis as etiological agent in present study was found to be lower as compared to previous studies, because of increased awareness, early diagnosis and treatment of tuberculosis. According to present study ascites was found to be more common in males (73 %) which are in agreement with Nath et al, Khan FY et al and S Ali et al. [26].The student 'z' &'t' test of significance were applied between transudative and exudative group as well & malignant ascites respectively. The p value was found to be <0.0 1 &0.05 in malignant ascites which is highly significant[27].

CONCLUSION

The initial laboratory investigation of ascitic fluid should include an ascitic fluid cell count with differential, ascitic fluid total protein, and SAAG and Ascitic fluid cholesterol to come to exact etiology of ascites.The ascitic fluid should be classified into 'High Gradient' and 'Low gradient 'depending on value of Serum Ascites Albumin Gradient, replacing the previous 'Exudate-transudate' concept. These parameters are cost effective & can carried out at basic level also & confirmation should be done by cytohistological examination.

CONFLICTS OF INTEREST- None

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