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Research Article

UTILITY OF ASCITIC FLUID CHOLESTEROL LEVELS IN ALIGNANT ASCITES - RESEARCH ARTICLE

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ABSTRACT
The differential diagnosis of malignant ascites by traditional methods has been recently challenged. We had studied 100 cases of ascites (27 were of malignant ascites) to evaluate the role of ascitic fluid cholesterol levels as a marker in malignancy.100 cases of ascites were studied with regard to SAAG, ascitic fluid cholesterol levels and fluid cytology. FNAC & biopsy was performed wherever mandatory to confirm the role of biochemical marker. We found The sensitivity & specificity of raised ascitic fluid cholesterol levels in diagnosing malignant ascites was found to be 88.88% & 100% respectively (cut-off value 70mg/dl), which was proved by cytology & biopsy ($p = 0.005$ was significant).So to conclude Raised ascitic fluid cholesterol levels can be used as an excellent marker in malignancy, the efficacy of which was proved on cytology & biopsy.

INTRODUCTION

Ascites is one of the most common clinical problems confronting a clinician and ascitic fluid analysis is the most effective way to diagnose it (Beg *et al.*, 2001). The earlier approach in differentiating causes of ascites was based on the concept of transudate (ascitic fluid proteins <2.5 gm/dl) and exudate (ascitic fluid proteins >2.5 gm/dl). This concept has recently been challenged (Akriviadis *et al.*, 1996) as it has certain limitations: i) The ascitic fluid total protein of most cardiac ascites samples (traditionally expected to be transudate) was high. ii) Ascitic fluid total proteins of most spontaneously infected samples (traditionally expected to be exudate) was low (Beg *et al.*, 2001; Runyon *et al.*, 1992) iii) The efficacy has also been challenged in conditions like prolonged diuretic therapy and, sometimes, in normal ascitic fluid too. Moreover, it offers little insight into the pathophysiology of as cites (Beg *et al.*, 2001).The difference between serum & ascites albumin concentration (SAAG) is thought to directly reflect the colloid osmotic pressure gradient & indirectly the degree of portal hypertension (Bandar *et al.*, 1997) Thus it has been widely suggested that SAAG is an excellent marker of portal hypertension than ascitic fluid protein concentration (Pare *et al.*, 1983). Patients with a gradient $\geq$ 1.1 gm/dl have portal hypertension while those with a gradient of < 1.1 gm/dl do not. The accuracy of such determinations is 97% (Runyon *et al.*, 1992). However, SAAG is not able to differentiate between malignant ascites & tuberculous ascites (Alba *et al.*, 1995), Fluid cytology has low sensitivity for malignancy as the differentiation between reactive atypical mesothelial cells and malignant cells is sometimes difficult (Jain *et al.*, 1966) So there is a need for more specific & a highly sensitive new marker in presumptive diagnosis of ascites. Ascitic fluid cholesterol level, with a diagnostic sensitivity of 89.65% & specificity of 100% (Sood *et al.*, 1995 & Jungst *et al.*, 1992) promises to be one of them. An enhanced movement of plasma lipoproteins into peritoneal cavity could cause the raised cholesterol levels. It has also been suggested that a minor fraction of cholesterol in malignant ascites might be derived from cell membranes and thus contribute to elevated ascitic fluid concentrations in malignant ascites (Jungst *et al.*, 1992) According to recent studies, using a cut-off value of 70 mg/dl, the specificity is 100% and sensitivity is 96% in diagnosis of malignancy (Rana *et al.*, 2005). Thus, the present study was undertaken to evaluate the diagnostic utility of ascitic fluid cholesterol levels in diagnosis of malignant ascites to prove the efficacy of these parameters with the help of cytology & biopsy.

MATERIALS AND METHODS

Ascitic fluid was collected in all patients by paracentesis done under sterile condition using a 21-gauge needle. All samples were subjected to physical & chemical examination. The chemical examination constituted total protein, albumin & ascitic fluid cholesterol estimation. The sera of all patients were also tested simultaneously to determine the SAAG value. The protein concentration was determined by using Autopack albumin kits from Ames which used bromocresol green method (BCG method) by the principle that Albumin in buffered solution reacts with anionic bromocresol green (BCG) with a dye binding reaction to give a proportionate green color which is measured at 628 nm, the final color is stable for 10 min. The ascitic fluid cholesterol levels were determined by enzymatic method by using autopack cholesterol kits from Ames ,the principle of which is cholesterol esters are hydrolyzed by cholesterol ester hydrolase to free cholesterol and fatty acids. The cholesterol so produced and pre existing one are oxidized by cholesterol oxidase into cholestenone and hydrogen peroxide. Hydrogen peroxide in presence of peroxidase oxidase the chromogens (4 amino phenazone /phenol) to a red coloured compound which is read at 510 nm. The color of the reaction is stable for 2 hours, if not exposed to direct sunlight. Cytology – Smears from the centrifuged sediments of all fluids were examined by routine H&E staining. In cases of raised ascitic fluid cholesterol levels (> 70 mg/dl), with the high suspicion of malignancy, non invasive modes of diagnosis like USG/ CT scan were done. Then the primary tumour was confirmed with guided FNAC, endoscopy, colonoscopy, laparoscopic biopsy to prove the efficacy of raised cholesterol levels in malignant ascites. The biopsies were performed in patients with raised ascetic fluid cholesterol levels. The statistical analysis was done by student's't' test. The diagnostic sensitivity & specificity of each parameter was calculated.

RESULTS

The mean SAAG VALUE was 0.93 Serum/ascites albumin gradient (SAAG). Comparison of Ascitic fluid cholesterol levels in study groups

Groups	**Ascitic fluid cholesterol**		
Group I	< 70 mg/dl	> 70 mg/dl	Mean cholesterol (mg/dl)
Cirrhosis	43	00	25.6
Cardiac failure	10	00	30
SBP	06	00	33
Group II			
Tuberculosis	11	00	42.5
Malignancy	03(Mean value was 66 mg/dl)	24	83.5
Nephrotic syndrome	03	00	23.5

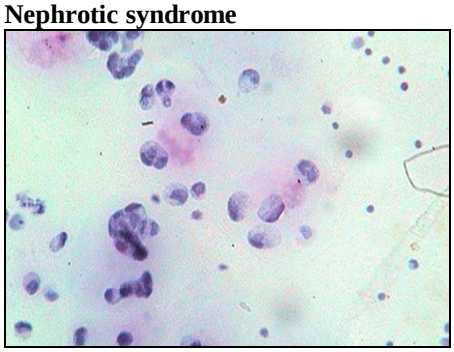

Figure 1: Ascitic Fluid Cytology Showing Scattered Signet Ring Cells [40 X]

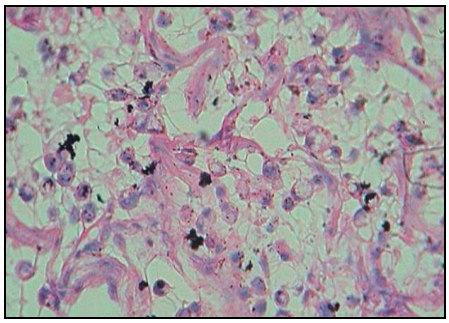

Figure 2: Biopsy Showing Peritoneal Deposits of Signet Ring Cells [40 X]

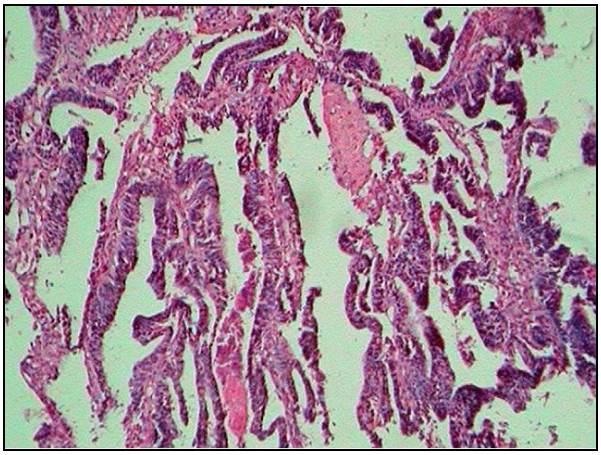

Figure 3: Biopsy Showing Grade II Adenocarcinoma of Colon [10 X]

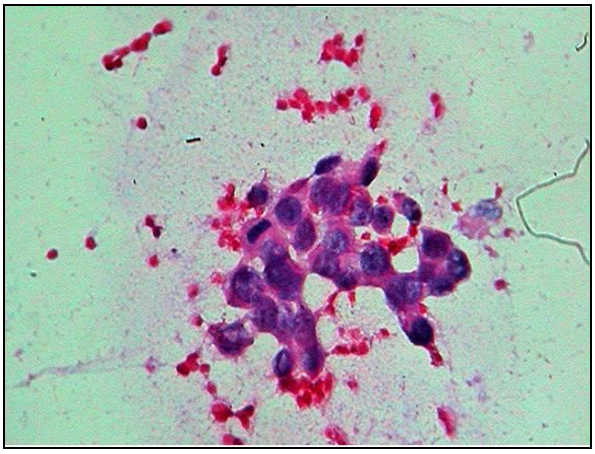

Figure 4: FNAC Showing Hepatocellular Carcinoma [40 X]

Ascitic fluid cholesterol levels in almost all the cases of non-malignant ascites were < 70 mg/dl. 24 out of 27 malignant cases had values > 70 mg/dl. Thus the sensitivity & specificity of this parameter was found to be 88.88% & 100% respectively in diagnosing malignant ascites. P = 0.005 which was significant.

Cytology & Biopsy Correlation with Biochemical Parameters

15 out of total 27 cases of malignant ascites showed malignant cells on smears with a sensitivity of 55.56% only. Thus in view of raised cholesterol levels > 70 mg/dl in all cases with high suspicion of malignancy, non invasive modes of diagnosis like USG/ CT scan were done and the primary was confirmed with guided FNAC & biopsy. We found 06 cases of signet ring cell carcinoma of stomach (Figure 01, 02), 06 of adenocarcinoma of stomach, 09 of colon carcinoma (Figure 3), 03 were diagnosed as Hepatocellular carcinoma on guided FNAC (Figure 4) & in three cases there was an occult primary, which revealed malignant cells on fluid cytology. Thus there was 100% correlation between raised cholesterol levels >70mg/dl with cyto-histopathology.

DISCUSSION

The results of this study show that SAAG is an excellent discriminator in differential diagnosis of ascites as it has a sensitivity of 96% & specificity of 100%. Other workers have observed similar utility (Runyon, Goyal *et al.*, 1989; and Gupta *et al.*, 1995). If the gradient is $\geq$ 1.1 g/dl the underlying cause is almost always related to portal hypertension. The application of albumin gradient disregards the traditional concept of transudate versus exudate as it provides a more rational approach separating ascitic fluids into two categories on the basis of presence or absence of portal hypertension (Beg *et al.*, 2001 and Runyon *et al.*, 1992)

The albumin gradient retains its utility in spontaneous bacterial peritonitis & cardiac ascites (Beg *et al.*, 2001). Thus the present study reinforces the conclusion of reports, which showed that albumin gradient is superior to traditional concept of transudate & exudate based on ascitic fluid total protein concentration. The utility of albumin gradient in non-alcoholic liver disease has been debated (Bandar *et al.*, 1997) however the present study test has proved to have significant utility in ascites caused by both alcoholic & non-alcoholic liver disease. Though SAAG is an excellent discriminator, it has certain limitations. It does not differentiate between tuberculous & malignant ascites. Consequently there is still need for tests like cytology, culture for mycobacteria or biopsy, wherever indicated, to differentiate between these two (Alba *et al.*, 1995). The level of AFTP, apart from the transudate - exudate concept, has some value in certain cases. A low level of AFTP implicates a high risk of spontaneous bacterial peritonitis (Alba *et al.*, 1995)

although having very few limitations; SAAG is superior to previously proposed concept, not only because of high diagnostic accuracy but also in providing a better approach to the pathophysiology of ascites. Thus the concept of transudative and exudative ascites should be replaced by high gradient ascites (related to portal hypertension) & low gradient ascites (not related to portal hypertension). As SAAG has limitations in differentiating tuberculous from malignant ascites & because fluid cytology has less sensitivity in detecting malignancy (Jain *et al.*, 1966) we propose a new highly sensitive biochemical marker in the form of raised ascitic fluid cholesterol levels in malignant ascites, having 88.88% sensitivity & 100% specificity using a cut-off value of 70 mg/dl and 100% correlation with cyto-histopathology (Rana *et al.*, 2005). Thus, to conclude raised ascitic fluid cholesterol level can be used as one of the best presumptive marker in the diagnosis of malignant ascites.

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