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TO STUDY THE ROLE OF ASCITIC FLUID CHOLESTEROL IN DIFFERNTING MALIGNANT AND TUBERCULAR ASCITES

Medicine

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ABSTRACT

Ascites is defined as the presence of freet fluid within peritoneal cavity. In most cases ascites appears as a part of well recognized illness i.e. cirrhosis, congestive heart failure, tubercular peritonitis, nephrosis, disseminated carcinomas, etc

AIMS AND OBJECTIVES- To study ascitic fluid cholesterol levels in two types of ascites-Malignant and Tubercular , to study SAAG (serum ascites albumin gradient) in both types of ascites ,to compare diagnostic values of ascitic fluid cholesterol levels v/s SAAG and to find out specificity & sensitivity of ascitic fluid cholesterol levels in differenting malignant and tubercular ascites.

MATERIAL AND METHODS- The study was conducted on 100 patients of ascites of different etiologies admitted in Govt. Medical College and associated group of Hospitals, Kota of 15-65 years of either sex. The following study groups were made viz.:-

Group I 50 cases of ascites caused by Tuberculosis

Group II 50cases of ascites caused by Malignancies

The paracentesis was performed after proper positioning of patient . 150 ml. of ascitic fluid was drawn and examined for gross appearance, total protein, albumin, adenosine deaminase (ADA) (when indicated), sugar, cholesterol, total cell count, cell type, malignant cells, acid fast bacilli (AFB), Gram staining, aerobic and anaerobic culture (when indicated). Liver function test, Serum cholesterol, Sputum for AFB and culture (When indicated), ECG, Chest X-ray, X-ray abdomen, Ultrasonography of abdomen: Histopathological examination, Upper GI endoscopy, Ascitic fluid examination-Gross appearance, Colour, Total protein (Both Ascitic fluid and serum) estimated through Biuret method (Wottern, 1964) Albumin (Both for ascitic fluid and serum) By Dumans et al (Bromocresol Dye Method) Globulin Sugar Cholesterol Ascitic fluid cytology (Kolmer and Boerner) were also done when indicated. EXCLUSION CRITERIA - Following patients were excluded from the study :- Haemodynamically unstable patients: Blood pressure less than 90 mmHg. Arterial hypotension may result in a decrease in the portal pressure and a narrowing of the SAAG, Bleeding abnormality: Coagulopathy, When there is clinically evident fibrinolysis or clinically evident disseminated intravascular coagulation.

CONCLUSION- Ascitic fluid cholesterol is an easy, cheap and reliable biochemical parameter to differentiate tubercular ascites from malignant ascites.

KEYWORDS

Ascitic fluid cholesterol, biochemical parameter, tubercular ascites, malignant ascites

INTRODUCTION 1-

Ascites is defined as the presence of free fluid with in peritoneal cavity . In most cases ascites appears as a part of well recognized illness i.e. cirrhosis, congestive heart failure, tubercular peritonitis, nephrosis, disseminated carcinomas, etc. Ascites can only be treated by correction of underlying cause.

Thus evaluation of a patient with ascites is incomplete. unless the cause of ascites is established[.1]. Unless a positive diagnosis of malignancy or infection is confirmed by cytology or culture, a definite cause cannot be firmly established by conventional analysis of ascitic fluid. False negatives are a significant problem if this test is to be relied upon[.2]. The absence of malignant cells in ascitic fluid does not exclude malignancy. Malignant tumors may produce ascites without shedding malignant cells into ascitic fluids, e.g. blocking lymphatic or blood vessels or by setting up inflammation of the peritoneum . Malignant cells are rarely found in patients with di use hepatic metastasis in the absence of peritoneal implants and in patients with hepatocellular carcinoma superimposed on cirrhosis with portal hypertension. Low ascitic fluid volume has small yield & poor preservation of cell. At the same time, benign mesothelial cells may be growth stimulated & resulting "mesotheliosis" is some times impossible to distinguish from malignant cells by routine morphology alone.

Both malignant and tubercular ascites are exudative in nature with lymphocytic predominance and low SAAG values and cannot be di erentiated easily from each other[.1] Ascitic fluid ADA is significant high in tubercular peritonitis than due to other causes. Level above 32 mcg/L in ascitic fluid and above 54 mcg/L in serum suggest tuberculosis with a sensitivity of 100% & specificity of 92- 100%. Ascitic fluid ADA is high in malignancies of Breast, Esophagus, Liver, Colorectum[.3-8]. Studies have shown that parameters like ascitic fluid fibronectin and cholesterol are found superior to the conventional methods of ascitic fluid analysis in di erentiating ascites caused by malignancies from tuberculosis[.9,10,11]. Estimation of ascitic fluid cholesterol has been found useful in di erentiating ascites especially malignant ascites from tubercular ascit es.[12]. The pathogenesis of high ascitic fluid cholesterol is not clear. It is not a reflection of serum cholesterol concentration as the serum ascites cholesterol difference did not yield a better discrimination than the ascitic fluid cholesterol alone.[13]. The increased concentration of cholesterol in effusion is more specifically related to tumor involvement of the serosal cavity. This can be the result of various mechanisms that act together. The cholesterol may originate in cell membrane, perhaps as a result of disintegration of tumor cells and / or surrounding benign cell. It can also enter the cavity from the interstitial space because of obstructed lymph vessels or be related to increased permeability of the carcinomatous serous membrane or due to enhanced movement of plasma liporproteins into the peritoneal cavity. Raised cholesterol concentrations have also been reported in inflammatory conditions involving the peritoneum, acute pancreatitis and chronic cardiac congestion.

Ascitic fluid cholesterol has higher sensitivity in di erentiating etiology of ascites when compared to fibronectin levels in ascitic fluid (100 vs 93%) in diagnosis of malignant ascites[14].

The present study was performed to evaluate the role of ascitic fluid cholesterol estimation as a diagnostic tool in establishing the etiology of ascites.

AIMS AND OBJECTIVES 2

1. To study ascitic fluid cholesterol levels in malignant and tubercular ascites.
2. To study SAAG (serum ascitis albumin gradient) in ascites.
3. To compare diagnostic values of ascitic fluid cholesterol levels v/s SAAG.
4. To find out specificity & sensitivity of ascitic fluid cholesterol levels in diferential diagnosis of ascites in tuberculosis and malignancy.

MATERIAL AND METHODS 3

The study was conducted on 100 patients of ascites of different

etiologies admitted in Govt. Medical College and associated group of Hospitals, Kota.

100 cases of ascites of varied etiology were selected in the age group of 15-65 years of either sex.

The following study groups were made viz.:-Group I 50 Cases of ascites caused by Tuberculosis

Group II 50 cases of ascites caused by Malignancies

The paracentesis was performed after proper positioning of patient from left lower abdominal quadrant. By using aseptic technique 150 ml. of ascitic fluid was drawn and examined for gross appearance, total protein, albumin, adenosine deaminase (ADA) (when indicated), sugar, cholesterol, total cell count, cell type, malignant cells, acid fast bacilli (AFB), Gram staining, aerobic and anaerobic culture (when indicated).

EXCLUSIONCRITERIA-

Haemodynamically unstable patients: Blood pressure less than 90 mmHg, Bleeding abnormality: Coagulopathy, When there is clinically evident fibrinolysis or clinically evident disseminated intravascular coagulation.Peripheral venous blood was taken from patients just prior to paracentesis and sent for investigations for routine blood investigation,Liver function test , Serum cholesterol, Sputum for AFB and culture (When indicated), ECG , Chest X-ray , X- ray abdomen, Ultrasonography of abdomen, Histopathological examination, Upper GI endoscopy, Ascitic fluid examinatioth (gross apearrence,colour, total protein (both ascetic fluid and serum) estimated through Biuret method (Wottern.1964) albumin both in serum and ascetic fluid) By Dumanss et al (Bromocresol Dye Method), Globulin,Cholesterol, Sugar, Ascitic fluid cytology (Kolmer and Boerner)

DATA ANALYSIS AND RESULT 4

A cut off value for each parameter was calculated by following formula (Martin Prieto(1985), R. Garg et al (1993)

Cut off value =(2xSD) ± (mean value)
Cut off value = (2xSD) ± (mean value)

P value less than 0.05 was considered statistically significant

TABLE 1 AGE AND SEX DISTRIBUTION

Group	Age range (Years)										Age (Years) Mean ± S.D.				
	20-30		31-40		41-50		51-60		>60		Total				
	M	F	M	F	M	F	M	F	M	F	M	F	Male	Female	Total
I	4	1	5	6	15	6	10	1	2	-	36 (72%)	14 (28%)	41.22± 11.48	36.43± 10.12	39.88± 11.3
II	-	-	1	-	2	8	10	12	11	6	24 (48%)	26 (52%)	52.67± 9.96	52.69± 8.35	52.68± 8.87

Amongst 50 patients of group I (tubercular ascites), 36(72%) were male and 14 (28%) were female. In group II (malignant ascites), out of 50 patients, 24 (48%) were male and 26 (52%)were females. The highest number of cases in group I were in age group of 41-50 years (15& 6 respectively). In group II, the highest number of cases were in age group of more than 51 years (39 cases).

The mean age among patients of group I was 39.9 8±.14 years, in group II, 52.7±10.76 years.

TABLE 2 VARIOUS CAUSES DETECTED IN CAUSATION OF MALIGNANT ASCITES (GROUP) SUBJECTS

Gp N-50	Diagnosis	Male	Female	Total
A	Ovarian carcinoma with or without peritoneal implant	-	14 (28%)	14 (28%)
B	Secondaries in liver with peritoneal implant (Ca.Lung,Buccal Ca Adenocarcinoma of colon)	8 (16%)	-	8 (16%)
C	Carcinoma of gallbladder with peritoneal implant	2 (4%)	2 (4%)	4 (8%)
D	Carcinoma of gastrointestinal tract with peritoneal implant	4 (8%)	6 (12%)	10 (20%)
E	Hepatoma	2 (4%)	-	2 (4%)
F	Uterine carcinoma with peritoneal implant	-	2 (4%)	2 (4%)
G	Renal cell carcinoma with peritoneal implant	4 (8%)	-	4 (8%)
H	Unknown tumor with peritoneal implant	4 (8%)	2 (4%)	6 (12%)

Out of 50 cases of malignant ascites ,14(28%) had ovarian carcinoma,8 (16%) had secondaries of liver with primaries from carcinoma lung, buccal carcinoma, adenocarcinoma of colon. 4 (8%) had gall bladder carcinoma, 10 (20%) had gastrointestinal tract (gastric ca., ca. ampula of vator), 2 patient (4%) had hepatoma,6 (12%) had metastatic carcinoma of peritoneum of unknown origin,4 patients (8%) had renal cell carcinoma &2 patient (4%) had uterine carcinoma.

TABLE 3 ASCITIC FLUID, PHYSICAL APPEARANCE AND CYTOLOGICAL EXAMINATION

Gp	Physical Appear ance		Cell Count/ cumm (mean S.D.)	Predominant Cells		Mlignant cell positive	AFB Positivity
	Types	No.Of cases		Types	No.Of cases		
Gp I N-50	Clear Turbid/ Opalescent Haemor hagic	14 30 6	709.68 ±352.642	Polymorphs Lymphocyte RBC	16 (32%) 50(100%) 10 (20%)	Nil	8 (16%)
Gp II N-50	Clear Turbid/ Opalescent Haemor hagic	20 6 24	897.44 ± 581.629	Polymorphs Lymphocyte RBC	3 (12%) 42 (84%) 44 (88%)	22(44%)	Nil

Most of the patients in group I, 30 patients ascitic fluid was opalescent / turbid with mean cell count 709.68 ± 352.642 and predominance of lymphocytes(100%) with AFB positivity in 8(16%)cases. In group II physical appearance was mostly haemorrhagic with mean cell count 897.44±581.629 with predominantly RBC (88%) including malignant cells in 22(44%) cases.

Haemorrhagic fluid was in group I showed 6 cases and group II showed 22 cases of haemorrhagic fluid. AFB negativity was found in group IInd.

TABLE -4 COMPARISION OF TOTAL PROTEIN VALUES OF ASCITIC FLUID V/S SERUM

Group	Asctic fluid total protein(gm/dl)	Serumtotal protein(gm/dl)	A/S total protein ratio
Group I	3.43 ± 0.883	6.084 ±0.708	0.551 ±0.117
Group II	3.76± 0.880	5.99± 0.886	0.605± 0.105

The mean value of ascitic fluid total proteins, serum total protein and ascitic fluid total proteins and serum total protein ratio were in tubercular ascites the mean values were 3.43 ± 0.883, 6.084 ±0.708 and.605 ± 0.117 respectively.

These mean values were 3.76 ± 0.880, 5.99 ± 0.896 and. 605 ± 0.105 respectively in malignant ascites . The statistical evaluation has also been depicted.

TOTAL-5 Incidence of Positivity of Malignant Cells In Ascitic Fluid In Relation to Nature/ Site of Malignancy

S. NO.	Etiology (N-50)	Malignant cells present	Malignant cells absent
1	Ovarian carcinoma with or without peritoneal implant N1= 7(28%)	8(57%)	6(43%)

2	Secondaries in liver with peritoneal implant (Lung Ca.Buccal Ca., Adenocarcinoma of colon) N2 =8 (16%	4(50%)	4(50%)
3	Carcinoma of gallbladder with peritoneal implant n3 =4 (8%)	2(50%)	2(50%)
4	Carcinoma of gastrointestinal tract with peritoneal implant N 4 =10 (20%)	4(40%)	6(60%)
5	Hepatoma N5=2(4%)	-	2(100%)
6	Uterine carcinoma with peritoneal implant. N 6 =2 (4 %)	-	2(100%)
7	Renal cell carcinoma with peritoneal implant N7 =4 (8%)	2(50%)	2(50%)
8	8 Unknown tumor with peritoneal implant N 8 =6 (12%)	2(33%)	4(67%)

Out of 50 cases of malignant ascites, which were proved by histo pathological examination, only 22 patients (44%) had evidence of malignant cells in ascitic fluid and rest of 28 cases did not demonstrate microscopic evidence of malignant cells. Thus microscopic examination of ascitic fluid does not exclude the diagnosis of malignant ascites especially in freshly detected cases.

TABLE -6 Comparative values of presence /absence of malig- nant cells in Ascitic fluid with levels of serum cholesterol, ascetic fluid cholesterol and SAAG

Group II	Cytological examination	Ascitic fluid cholesterol (mg/dl)	Serum cholesterol (mg/dl)	SAAG (gm/dl)
II-A	Malignanat cell present (n1=22,	84.31 +_ 23.84	179.82+_ 23.28	0.94 +_0.22
II-B	Malignant cells not present (n2=14, 56%)	78.62+_ 24.22	188.23+_ 20.98	0.84+_0.29
Statistical significance II-A v/s II-B		P>0.05(NS)	P>0.05(NS)	P>0.05(NS)

TABLE-7 DISTRIBUTION OF SAAG

SAAG (gm / dl)	Group-1	Group-2
	No. of patients	No. of patients
0-1.1	46(92%)	46(92%)
1.1	4(8%)	4(8%)

Group	Serum cholesterol (mg/dl) (Mean S.D)		
	Male	Female	Total
Group I	168.22+_16.50	185.71+_31.12	173.12+_21.15
Group II	185.76+_17.43	183.38 +_ 27.50	184.53+_ 22.89

TABLE-8

There was no significant difference among both groups studied (P>0.05). Similarly there was no significant difference between males and females among both groups studied (P>0.05).

TABLE -9 Distribution of ascitic fluid cholesterol

ascitic fluid cholesterol (mg/dl) (Range)	Group I N-50	Group II N-50
	No. of patients	No. of patients
<25	14 (28%)	-
26-47	28 (56%)	2 (4%)
48-60	6 (12%)	8 (16%)
>60	2 (4%)	40 (80%)

TABLE 12 Diagnostic Value Of Individual Screening Test For Diffrentiating Of Tubercular Ascites From Malignant Ascites

Parameters	Cut off value	Sensitivity (%)	Specificity (%)	Positive predective value (%)	Negative predictive value (%)	D. A. (%)
Total protein	2.5	5	95	50	50	50
A/S total protein ratio	0.5	20	90	66.66	52.94	55
Cytology	Malignant cells	45	100	100	64.51	72.5
SAAG	1.1	10	90	50	50	50
Cholesterol	48	95	95	95	95	95
A/S Cholesterol	0.29	75	95	93.75	79.16	85

D.A.: Diagnostic Accuracy

DISCUSSION 6

Ascites appears as a part of well recognized illness ie. Cirrhosis, congestive heart failure, tubercular peritonitis, Nephrosis, disseminated carcinomas etc.

Evaluation of a patient with ascites is incomplete unless the cause of ascites is established.

Certainly estimation of SAAG has more discriminatory power in differentiating transudative (cirrhotic and others) from exudative (tubercular and malignant) ascitis than the ascitis total protein concentration and ascitic serum cholesterol ratio, while estimation of ascitic fluid cholesterol is a simple biochemical parameter of great diagnostic value at a cut o value of > 48mg/dl to di erentiate malignant from tubercular ascites. However, histopathological confirmation is required in all the case of final diagnosis.

In our study male and female had almost equal incidence of malignant and other ascites which was similar to Garg R et al and Sood et al in cirrhoticand tubercular ascites, while higher incidence of malignant ascites in female than male. Predominent cell type in ascetic fluid was lymphocytes in both groups, exceptin group II where it was RBC which was similar to the study by Simon B et al.[16].

Similarly cytological ecamination for malignant cells in group –II was positive in 22 out of 50(44%). patients whereas sensitivity, specificity , positive predictive value, negative predictive value and diagnostic accuracy was 44%100%, 100%, 64.51%, and 72.5% respectively. Although the specificity was very high (100%) but the sensitivity was very low (44%). Though cytological examination of malignant cells is considered as the gold standard in terms of diagnostic specificity but with low negative predictive value (64.51%), it cannot be used as a good screening diagnostic tool. These results are comparable with studies of Gerber AL et al . Rommette et al , R Garg et al , Giuseppe Castardo et al.[17,18,15,19]

The cytological examination for AFB in group -I was positive in –I was positive in 8 out of 50 (16%) patients, whereas sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy was 20%, 100%, 100%, 55.55% and 54% respectively. The specificity was was very high (100%) but the sensitivity was very low (44%). Though it cannot be used as a good screening diagnostic tool. Our study is comparable with study of Gonella JS et al . [20]. Direct smear of ascetic fluid for AFB gives poor results.

Ascitic fluid : serum total protein has low diagnostic accuracy and sensitivity in comparison of tubercular with malignant ascites. The.serum ascites albumin gradient was < 1.1 gm/ dl in tubercular and malignant ascites. Similar observation was noted by Pierre Pare et al[.21].

The ascetic fluid cholesterol estimation at the cut off value of 48 mg/dl had showed highest sensitivity (95%), specificity (95%), Positive predictive value (95%), Negative pedictive value (95%) and diagnostic accuracy (95%) in di erentiating from tubercular from malignant ascites This is result is similar to the observation made by Garg R et al and Gupta R et al. [15]

There was no significant di erence in the serum cholesterol level in group I, group II, and there was no correlation between serum and ascitic fluid cholesterol. Similar findings are sustained by Mortenson PB et al, where as Martin Prieto et al and Guiseppe Castaldo et al have found high concentration of serum cholesterol in malignant ascites group than non malignant ascites group . [14,11,19]

CONCLUSION 7 –

Asitic fluid cholesterol is an easy, cheap and a reliable biochemical parameter to di erentiate tubercular ascites from malignant ascites.

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