

TO STUDY THE USEFULNESS OF SERUM AND ASCITIC FLUID C-REACTIVE PROTEIN (CRP) AND ASCITIC FLUID CHOLESTEROL LEVEL IN DIFFERENTIAL DIAGNOSIS OF MALIGNANT, NON-MALIGNANT AND TUBERCULAR ASCITES

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ABSTRACT

BACKGROUND

Ascites is a common clinical problem. However, the capability to distinguish malignant from non-malignant and tubercular causes of ascites using available biochemical techniques would obviate many expensive and time-consuming diagnostic studies on patients presenting with ascites of unknown aetiology. Therefore, this study was planned to evaluate usefulness of serum & ascitic fluid C-Reactive Protein (CRP), SAAG and ascitic fluid cholesterol level in diagnosis of malignant, non-malignant and tubercular ascites.

MATERIALS AND METHODS

We conducted a prospective observational study in 80 patients, those admitted and willing to give consent in the department of Medicine, LLR & Associated Hospitals, GSVM Medical College, Kanpur from December 2015 to October 2017. All patients underwent full investigations to make the diagnosis of ascites malignant (20) and nonmalignant (n=54), tubercular (7) and non-tubercular (47) groups. 6 patients were excluded because the ascitic fluid analysis was suggestive of bacterial peritonitis. The data was processed in MS Excel and analysis was carried out using SPSS Ver. 23.

RESULTS

We found that in the non-malignant group mean value of ascitic fluid CRP was 0.29 ± 0.12 vs 3.16 ± 1.42 (mg/l) in malignant group, SAAG was 1.81 ± 0.70 vs 0.87 ± 0.34 (mg/l) in non-malignant vs malignant group. The mean value of ascitic fluid cholesterol in malignant group was 100.85 ± 34.28 vs 6.7 ± 2.5 in non-malignant group (p value <0.01) and the mean value of ascitic fluid cholesterol in malignant group was 100.80 ± 34.28 vs 32.43 ± 15.7 in tubercular group, so ascitic fluid cholesterol is highly specific (100%) and sensitive (65%) at cut off value of 100 mg/dl in differentiating benign and malignant causes of ascites.

CONCLUSION

SAAG, Ascitic fluid CRP, and cholesterol having high specificity, can be used for differentiating between non-malignant and malignant ascites. It can also be used to differentiate tubercular ascites from malignant ascites.

KEYWORDS

Ascites, malignant, non-malignant, tubercular, non-tubercular, ascitic fluid cholesterol and serum, SAAG and ascitic fluid C-Reactive Protein (CRP).

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BACKGROUND

Ascites is the pathological accumulation of fluid within abdominal cavity, which can present a challenging diagnostic problem¹. Malignant ascites accounts for about 10% of all cases of ascites and is usually caused by ovarian, endometrial, lung, breast, colorectal, pancreatic, hepatobiliary, and primary peritoneal carcinomas.^{2,3} The differentiation between malignancy-related ascites (MRA) and non-malignant ascites (NMA) is important for further diagnostic and therapeutic procedures.^{4,5}

Cytodiagnostic investigation of ascitic fluid is characterized by a high specificity but a low sensitivity in detecting malignant disease because only a few neoplastic cells are present in the fluid^{6,7} or processing of specimens is suboptimal with lysis of tumour cells. To increase diagnostic sensitivity, cytologic evaluation has been coupled with the analysis in serum and ascitic fluid for total protein, various enzymes, fibronectin, tumour antigens, and lipids.^{2,8,9} Despite the availability of all above factors, differential diagnosis of ascites is a common clinical problem and is usually done by serum ascites albumin- gradient (SAAG).¹⁰

CRP stands for c-reactive protein it is classical member of pentraxin family. It is acute phase protein that is synthesized by liver & increases in inflammatory process. Because of synthesis in liver, production of CRP in patient with chronic liver disease is expected to be lower than in patients with liver disease. This may result in difference in interpreting CRP levels in patient with portal and non-portal hypertension ascites. In malignant ascites a common cause of non-portal

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hypertension ascites with intensive inflammation process, values of CRP could be guide in discriminating the underlying the cause of ascites.¹¹

Papers about the detection of ascitic cholesterol have been published a lot and they have shown a relatively high diagnostic efficiency in differential diagnosis of MRA.^{2,3} However, conflicting results have been reported and the exact role of ascitic fluid cholesterol concentration remains unclear.

Therefore, we performed this study to establish the usefulness of serum & ascitic fluid C-reactive protein (CRP) and ascitic fluid cholesterol level in differential diagnosis of malignant, non-malignant and tubercular ascites.

Aims & Objectives

To study the usefulness of serum & ascitic fluid C-reactive protein (CRP) and ascitic fluid cholesterol level in differential diagnosis of malignant, non-malignant and tubercular ascites.

Study Design

Prospective observational study.

MATERIALS AND METHODS

This prospective observational study was carried out in the Post Graduate Institute of Medicine, GSVM. Medical College, Kanpur during the period of December 2015 to October 2017. Those patients willing to give consent were admitted in the department of medicine, LLR & Associated Hospitals, GSVM Medical College, Kanpur. Informed consent taken from all patients.

Total 80 patients of ascites were included in this study. Among these 80 patients, 74 were included in the study and 6 excluded because the ascitic fluid analysis was suggestive of bacterial peritonitis. In all patient all related investigations like complete blood count, liver profile, serum amylase and lipase, serum creatinine alfa-fetoprotein, CA-125, Prothrombin Time/INR, lipid profile, serum CRP, urine albumin, 24 hours urinary protein and ascitic fluid examination for the biochemical analysis for albumin, total protein, cholesterol, glucose, amylase, LDH, CRP and ADA, cytological analysis for cell counts and differential count, and microbiological analysis for gram stain, ZN stain, malignant cells and bacterial culture were performed.

Other investigations like USG abdomen, upper GI Endoscopy, CT scan abdomen and pelvis, echocardiogram, thyroid profile, ECG, 2D echo, X ray chest. Upper GI endoscopy, FNAC of the peritoneal nodules and liver biopsy, other tumour markers, were done in selected cases where it was needed.

The patients aged >18 yrs. and clinical features suggestive of ascites were included in this study. Bacterial peritonitis, presence of another co-existing disease/drug therapy that would alter serum cholesterol and CRP levels and those patients who are already on treatment (chemotherapy &/or radiotherapy) were excluded in this study.

Statistical analysis: The data was processed in MS Excel and analysis was carried out using SPSS Ver. 23. Quantitative variables were analysed using mean and SD. The results were statistically analysed by Student 't' test. A two-tailed probability value of <0.05 was taken as indicating significance.

RESULTS

In our study, we included 80 patients of ascites. Among these 80 patients, 74 were included in the study and 6 excluded because the ascitic fluid analysis was suggestive of bacterial peritonitis. The patients were divided into two groups malignant (n=20) and non- malignant (n=54). The non-malignant group was further subdivided into two groups tubercular (n=7) and non-tubercular cases (n=47).

The mean age of the patients who took part in study was 45.81±12.52 years. The lowest age was 20 years and highest was 85 years. Among 74 cases, 43 (58%) were males and 31 (42%) were females.

Aetiology	Total number (n=74)	%
Cirrhosis	40	54.05
Tubercular ascites	7	9.45
Decompensated Heart failure	4	5.4
Chronic kidney disease	2	2.7
Nephrotic syndrome	1	1.35
Hepatocellular Carcinoma	4	5.4
Carcinoma Pancreas	2	2.7
Carcinoma Prostrate	1	1.35
Carcinoma Ovary	8	10.8
Carcinoma Bladder	1	1.35
Carcinoma Gall Bladder	3	4.05
Colorectal Carcinoma	1	1.35

Table 1. Distribution of patients according to aetiology

Out of 74 patients, 40 (54.05%) were diagnosed as cirrhosis and 20 were diagnosed with malignancy. Out of 20 malignant cases, 8 cases were of carcinoma ovary (n=8; 40%).

	Non-Malignant* (N=47)	Malignant (N=20)	P Value
Ascitic Cholesterol (MG/DL)	6.7 ±2.5	100.85± 34.28	<0.01

Table 2. Ascitic fluid cholesterol mean value with sd in non-malignant and malignant group

*Tubercular cases are not included in the non-malignant group.

For the non-malignant group mean value of ascitic fluid cholesterol (mg/dl) is 6.7±2.5 vs 100.85 ±34.28 in malignant group. The student t-test is applied, p value is <0.01 indicating that ascitic cholesterol is significantly higher in malignant group.

	Malignant (n=20)	Tubercular (n=7)	P value
Ascitic fluid cholesterol(mg/dl)	100.85 ±34.28	32.43 ±15.7	<0.01

Table 3. Ascitic fluid cholesterol mean value with SD in malignant and tubercular group

For the malignant group mean value of ascitic fluid cholesterol (mg/dl) is 100.85±34.28 vs 32.43±15.7 in tubercular group. The student t-test is applied, p value is <0.01 indicating that ascitic cholesterol is significantly higher in malignant group.

	Non-tubercular (n=47)	Tubercular (n=7)	p value
Ascitic fluid cholesterol(mg/dl)	6.7 ±2.5	32.43±15.7	<0.01

Table 4. Ascitic fluid cholesterol mean value with SD in non-tubercular and tubercular group

For the non-tubercular group mean value of ascitic fluid cholesterol (mg/dl) is 6.7±2.5 vs 32.43±15.7 in tubercular group. The student t-test is applied, p value is <0.01 indicating that ascitic cholesterol is significantly higher in malignant group.

	Non-malignant* (n=47)	Malignant (n=20)	P value
Ascitic CRP(mg/l)	0.29 ±0.12	3.16± 1.42	<0.01
Serum CRP(mg/l)	4.40± 2.18	8.5± 3.4	<0.01

Table 5. Showing ascitic and serum CRP mean value with SD in non-malignant and malignant group

Tubercular cases are not included in the non-malignant group.

For the non-malignant group mean value of ascitic fluid CRP (mg/l) is 0.29±0.12 vs 3.16±1.42 in malignant group. The student t-test is applied, p value is <0.01 indicating that ascitic CRP is significantly higher in malignant group.

For the non-malignant group mean value of Serum CRP (mg/l) is 4.40 ± 2.18 vs 8.5 ± 3.4 in malignant group. The student t-test is applied, p value is <0.01 indicating that Serum CRP is significantly higher in malignant group.

	Non-malignant (n=54)	Malignant (n=20)	P value
Prothrombin time(sec)	18.11 ±4.39	13.45± 1.93	0.04
SAAG(g/dl)	1.81± 0.70	0.87 ±0.34	0.03

Table 6. Showing prothrombin time, SAAG mean value with SD in non-malignant and malignant group

For the non-malignant group mean value of Prothrombin time (sec) is 18.11 + 4.39 vs 13.45 +1.93 in malignant group. The student t-test is applied, p value is 0.04 indicating that Prothrombin time is significantly higher in non-malignant group.

For the non-malignant group mean value of SAAG (g/dl) is 1.81+0.70 vs 0.87+0.34 in malignant group. The student t-test is applied, p value is 0.03 indicating that SAAG is significantly higher in non-malignant group.

The sensitivity and specificity of ascitic fluid cholesterol at cut off value of 100 mg/dl was 65% and 100% respectively. The positive predictive value (PPV) and negative predictive value (NPV) was 100% and 74.07% respectively.

DISCUSSION

One of the most common challenges in the treatment of ascites patients is to reveal the cause of the disease as rapidly as possible for appropriate management. Ascitic fluid can accumulate as an exudate or a transudate, that plays a pivotal role in the diagnosis and management of ascites.

The search for novel biochemical markers in the serum and/or ascitic fluid is still under investigation. In this

approach, cholesterol and CRP have shown to be promising markers.

In our study, we included 80 patients of ascites. Among these 80 patients, 74 were included in the study and 6 excluded because the ascitic fluid analysis was suggestive of bacterial peritonitis. The patients were divided into two groups malignant (n=20) and non-malignant (n=54). The non-malignant group was further subdivided into two groups tubercular (n=7) and non-tubercular cases (n=47).

The ascitic fluid cholesterol was compared in malignant and non-malignant (excluding tubercular cases) group. The mean cholesterol level was found to be 100.85 mg/dl in malignant group and 6.7 mg/dl in non-malignant group with a P-value of <0.01. With a cut off value of ascitic fluid cholesterol 100 mg/dl, the specificity was 100% and sensitivity 65%. Rana SV et al. also reported that the mean ascitic cholesterol level was significantly higher in malignant ascites than in non-malignant ascites, with a cut off level of 70 mg/dl for ascitic fluid cholesterol.

The specificity (100%) and diagnostic efficiency (94%) of ascitic fluid cholesterol is better than the 84% specificity and 86% diagnostic efficiency of serum ascitic albumin gradient. The specificity is found to be similar as Rana SV et al. and sensitivity is lower than their study because the cut off value of ascitic fluid cholesterol used in our study was higher (100 mg/dl vs 70 mg/dl).

Almost similar results were reported by Sastry AS et al.¹²the ascitic fluid cholesterol was found higher in malignant ascites (128± 8.10) vs 51.40±8.3 in non-malignant cases.

The ascitic fluid cholesterol has sensitivity 88% vs 65% in our study, specificity is 96% vs 100% in our study. Almost similar result was also reported by Anita R. Bijoor et al.¹³ studied the ascitic fluid and serum concentration of total cholesterol, total proteins and albumin in a group of 45 patients. Non-malignant ascites patients had ascitic fluid cholesterol values of 19.41±8.33 mg/dl, as against the malignancy related ascites patients, who showed levels of 95.87±1.24 mg/dl.

Study performed by Vyakaranam S et al¹⁴ also supports our study in which the ascitic fluid cholesterol level above 62 mg/dl give the diagnostic accuracy of 96%.

Zhu H et al,¹⁵ meta-analysed the literature on using ascitic cholesterol as diagnostic tests to help identify MRA (malignant related ascites). Meta-analysis included 8 studies involving 743 subjects. Summary estimates for ascitic cholesterol in the diagnosis of MRA were as follows: sensitivity, 0.82 (95% CI 0.78 to 0.86); specificity, 0.90 (95% CI 0.87 to 0.93).

In our study we also compared the ascitic fluid cholesterol (mg/dl) between malignant (n=20) and tubercular group of ascites (n=7). The mean level of ascitic fluid cholesterol found in malignant group was 100.85 vs 32.43 in tubercular group which is statistically significant (p<0.01). Similar results were found in a study performed by Sood A et al,¹⁶ cholesterol was estimated in ascitic fluid of 44 patients (29 malignant and 15 tubercular). Mean ascitic cholesterol level was significantly higher in malignant ascites (89.52 mg/dl) as compared to tubercular ascites (35.07 mg/dl). Ascitic fluid cholesterol estimation is a reliable and simple test for differentiating malignant ascites from tubercular ascites.

We also observed that the non-malignant group mean value of SAAG (g/dl) is 1.81 ± 0.70 vs 0.87 ± 0.34 in malignant group, which is significant. Dharwadkar, Kavitarati et al.¹⁰ also reported that SAAG is definitely the best marker along with total protein ratio and ascitic fluid cholesterol.

In our study, mean ascitic CRP (mg/L) level in malignant group (n=20) is 3.16 vs 0.29 in non-malignant group (n=47; excluding tubercular case) which is statistically significant (p value <0.01). Ahmed Abdel-Razik et al.^{17,18} also reported that ascitic and serum CRP were significantly elevated in malignant ascites than benign ascites group respectively. At a cut-off value of 7.3 ng/ml, serum CRP levels had specificity of 77.3% and sensitivity of 84.7% for detecting malignant ascites respectively. Similarly, Yuskel et al.¹⁹ also showed that mean baseline serum and ascites levels of CRP were significantly higher in Group 2 (low gradient ascites) compared to those in Group 1 (high gradient ascites) (p = 0.021, p = <0.0001, respectively).

In our study, we also compared the ascitic fluid cholesterol (mg/dl) between rest of non-malignant (n=47) and tubercular group of ascites (n=7). The mean level of ascitic fluid cholesterol found in rest of non-malignant group was 6.7 vs 32.43 tubercular group which is statistically significant (p<0.01). This finding needs further studies.

CONCLUSION

Ascitic fluid cholesterol having high specificity, can be used for differentiating non-malignant and malignant ascites. It can also be used to differentiate tubercular ascites from malignant ascites. Serum and ascitic CRP can be used as biomarker for malignant ascites. Along with ascitic fluid cholesterol, ascitic and serum CRP as well as SAAG, all these parameters can be used to differentiate between malignant and non-malignant ascites.

Hence, these parameters being simple and cost effective, can be widely used to differentiate non-malignant and malignant ascites, even in small centers with limited diagnostic facilities.

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