e-ISSN: 2319-9865
p-ISSN: 2322-0104
Research and Reviews: Journal of Medical and Health Sciences

Analysis of Ascetic Fluid for Cytological and Biochemical Findings.

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Research Article

Received: 01/09/2013
Revised: 15/09/2013
Accepted: 30/09/2013

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Keywords: Ascites, Transudate, Exudate.

ABSTRACT

Ascites is a common clinical finding with a wide range of causes. Ascites refers to collection of excess fluid in the peritoneal cavity. It is clinically important to classify ascitic fluid into transudates and exudates because it is indicative of the underlying pathological process involved. The present study aims at evaluating the pathological findings in Transudative and Exudative ascites. This study is based on the evaluation of 250 ascitic fluid specimens which were received in department of pathology from various clinical departments in Rajindra Hospital Patiala. Detailed examination – physical, cytological, biochemical and microbiological (wherever indicated) was done. Results were compiled after careful examination. The most common clinical cause of ascitic fluid effusion as ascertained after examining 250 ascitic fluid specimens was liver cirrhosis (43.6%) followed by tuberculosis (24.4%). Most of the cases of tubercular (22.4%), malignant (4.8%) and acute infective ascites (10.4%) had pH < 7 while most of cases of cirrhotic ascites (27.6%) had pH >7. By smear examination, 42% were cirrhotic, 34.8% inflammatory, 8.4% cardiac and renal, 4.8% malignant and 10% were inconclusive. Most of the inflammatory cases were due to tuberculosis (70.1%). Most of malignant cases had primary in ovary (41.6%). The total protein content of ascitic fluid was significantly lower in cirrhotic cases as compared to tubercular, acute infective cases and malignant cases. Cirrhosis of liver is the most common cause of ascites while malignancy being the least common.

INTRODUCTION

The term “ascites” is derived from the Greek word Askitos meaning bladder or bag. Ascites is the pathologic accumulation of fluid within the peritoneal cavity [1]. It is not actually a disease but a symptom. Normally, there is just enough free fluid in the peritoneal cavity to lubricate the peritoneal surfaces. Ascites occurs when there is an imbalance of factors that favour the flow of fluid from vascular space and/or when there is exudation of fluid through infection or malignant implantation on the peritoneum. Ascitic fluid may accumulate rapidly or gradually depending upon the cause. Mild ascites may not produce any symptoms. Moderate ascites may just produce an increase in abdominal girth and weight gain. Large amounts of fluid can produce abdominal discomfort, appearance of hernias, particularly umbilical hernias and hinder the mobility of the patient. Elevation of diaphragm and restriction of its movements can produce breathlessness.

Paracentesis with ascitic fluid analysis is the most rapid, simple, safe & cost-effective method of determining the etiology of ascites. Paracentesis should be performed & ascitic fluid should be analyzed in all patients with new onset of ascites. The occurrence of symptoms, signs and lab evidence of infection in patients with ascites should also prompt paracentesis with ascitic fluid analysis [1].

It is clinically important to classify ascitic fluid into transudate and exudate because it is indicative of the underlying pathological process. Such a distinction allows appropriate investigations to be instigated, enabling

e-ISSN: 2319-9865
p-ISSN: 2322-0104
better patient management. Transudative ascites is the pathological fluid of non inflammatory origin. It occurs due to filtration of blood serum across physically intact vascular wall [2].

Common Causes of transudative ascites are cirrhosis, congestive heart failure, constrictive pericarditis, hepatic vein obstruction (Budd-Chiari syndrome), portal vein obstruction, nephrotic syndrome, malnutrition, protein losing enteropathy, myxedema

Exudative ascites is the pathologic fluid of inflammatory origin, often due to bacterial infection. It results from an active accumulation of fluid with in body cavities, associated with damage to the walls of capillaries [2]. Common causes of exudative ascites are ruptured viscus with or without an intra abdominal abscess, tuberculosis, bacterial peritonitis, pancreatitis, bile peritonitis, trauma, secondary peritoneal carcinomatosis, lymphomas, leukemias, primary hepatic tumours, primary mesotheliomas.

The risk for spontaneous bacterial peritonitis is 15% within the first 3 years after the onset of ascites [1].

Physical Examination

Physical examination includes various features.

Volume

Upto 50 ml of fluid is normally present in the mesothelial lined peritoneal cavity. It becomes clinically detectable when at least 500 ml has accumulated, but many litres may collect and cause massive abdominal distension.

Colour

The appearance to the naked eye of a fluid reveals clues about the cause of an effusion and nature of its cellular contents. Transudative ascitic fluid is generally light yellow. In portal hypertension and hypoalbuminemia – the fluid is clear and straw coloured. Exudative is clear to opaque and from colourless to milky or bloody in colour. Infection causes turbid ascites. Chylous ascites is of milky colour. Bloody ascites is usually due to malignancy, tuberculosis, pancreatitis, recent abdominal punctures, endometriosis and abdominal trauma. Dark brown fluid may indicate the presence of bile. A transudate will not clot. A cobweb coagulum is typical of tuberculosis.

Odour

It is characteristic in some cases. It can be foul smelling in case of purulent infection. It can have fecal odour, if ascites is due to intestinal perforation.

Specific gravity

This relates to the protein content of the fluid. It is less than 1.05 in transudative ascitic fluid and more than 1.05 in exudative ascitic fluid [2].

Reaction (pH)

It has been used mainly in the diagnosis of spontaneous bacterial peritonitis in patients with cirrhotic ascites. When pH is less than 7, it suggests infection. However there is no significant difference between the value of ascitic fluid pH in patients with spontaneous bacterial peritonitis and patients with malignant ascites. The acidic ascitic fluid can occur due to leakage of gastric fluid through perforation. Low pH is also found in patients with pancreatic ascites and tuberculous peritonitis.

Cytological Examination

The cytological examination of ascitic fluid has an important role in diagnosing the cause of ascites like [3,4]

- Malignancy- primary – mesotheliomas.
- Secondary- metastasis, lymphomas and leukemias.
- Specific chronic inflammatory conditions- tuberculosis.
- Non-specific chronic inflammatory conditions.
- Acute purulent ascites - acute appendicitis, acutepancreatitis.
- Parasites - microfilaria in endemic areas.

e-ISSN: 2319-9865
p-ISSN: 2322-0104

- Connective tissue disorders.

Microbiological examination

Ascitic fluid examination for acid fast bacilli identifies the organism in less than 3 % of cases. The frequency of positive ascitic fluid culture for mycobacterium tuberculosis is less than 20 % and may take upto 8 weeks for culture to yield definitive information [1]. The percentage of positive cultures can be markedly increased if ascitic fluid is inoculated directly into blood culture bottles at the bed side. In spontaneous bacterial peritonitis the gram's stain has a sensitivity of only 25% and routine cultures are positive in only about 50% cases.

Biochemical Examination

The biochemical examination of ascitic fluid includes estimation of glucose level, proteins level and adenosine deaminase [5,6].

Transudative ascitic fluid contains glucose in same concentration as that of blood. Exudative ascitic fluid has glucose level lower than transudatives, due to destruction or glycolysis of glucose by the action of bacteria and cells. Ascitic fluid glucose can drop significantly in severe infections like secondary peritonitis or late stage of spontaneous bacterial peritonitis. Low glucose can also be found in malignant ascites. In neoplastic effusion and secondary bacterial peritonitis, glucose level is <60 mg/dl.

Transudative ascitic fluid has low protein content (<3 gm %) and exudative fluid has relatively high protein content (>3 gm %). However, ascites with high protein content although a consistent finding in malignant ascitic fluid has also been reported in upto 25% of patients with chronic liver disease. Conversely, a relatively low ascites protein concentration may be found in patients with exudative ascites if there is hypoproteinemia as is common in Indian patients. Spontaneous bacterial peritonitis is often associated with low protein and a high albumin gradient. Ascites in cirrhotics converts from a transudate to an exudate during diuresis in 2/3 of patients. In plasmacytoma and multiple myeloma, globulin levels are increased. Globulin levels are also increased in chronic liver disease, connective tissue disorders like rheumatoid arthritis, systemic lupus erythematosis and many infections like kala-azar, lymphogranuloma venereum.

Adenosine deaminase is an enzyme of purine salvage pathway which catalyses the deamination of adenosine to inosine in the ribonucleoside catabolic pathway. The determination of ascitic fluid adenosine deaminase (ADA) activity has been shown to have a very high accuracy for diagnosing tuberculous peritonitis. Its biological activity is related to proliferation and differentiation of lymphocytes. Its levels are increased in tubercular peritonitis as a result of stimulation of T lymphocytes by mycobacterial antigens.

The distribution of ADA enzyme is ubiquitous but its physiological role is especially important in lymphoid tissues. The levels are ten times higher in lymphocytes than in erythrocytes.

RESULTS

Maximum number of cases (26.8%) were in the age group of 41- 50 years (fifth decade) followed by 20.8% in sixth, 18.8% in fourth, 17.6% in seventh, 8% in eighth, 5.6% in third and 2.5% in second decade respectively. Majority of the patients 69.2% were males and rest 30.8% were females. Maximum number of cases 43.6% had cirrhosis followed by 24.4% cases of tubercular ascites, 10.4% cases of acute infective ascites & 4.8% cases were of malignant ascites. Miscellaneous causes of ascites included cardiac ascites, ascites due to chronic renal failure, pancreatic ascites and cases of unknown cause. These accounted for 16.8% causes (Table-1). The physical examination of ascitic fluid samples had shown that maximum specimens 81.6% were straw coloured followed by blood tinged in 14.4% cases & others (cloudy & brown coloured) constituted 4% of the total. Most of the specimens 94.8% were odourless & only 5.2% fluids had foul smell. Tubidity was seen in 3.2% fluids and cobweb formation in 0.8% of the specimens. Majority of the cases of tubercular ascites 22.6%, all cases of acute infective ascites 10.4%, cases of miscellaneous ascites 12% and 4.8% cases of malignant ascites had pH < 7. While majority of cirrhotic ascites cases 27.6% had pH > 7 (Table-2).

On cytological examination of ascitic fluids, 42% were diagnosed as cirrhotic followed by 34.8% as inflammatory, 8.4% as cardiac & renal and 4.8% as malignant. 10% of examined specimens were inconclusive. Out of 12 cases (4.8%) of malignant ascites primary site of malignancy in 5 cases was ovary, while in 4 cases the primary site was large intestine and endometrium in 1 case. One case of lymphomatous ascites was also seen which on follow up was diagnosed as NHL (Table-3).

Biochemical investigations revealed that maximum number of cases 107 (42.8%) had glucose concentration between 121- 150 mg/dl, 95 (38%) cases with values between 60-90 mg/dl and 48 (19.2%) cases

e-ISSN: 2319-9865
p-ISSN: 2322-0104

had concentrations between 91-120mg/dl. Cases of tubercular, acute infective and malignant ascites had glucose concentration < 90mg/dl. Majority of the cases of cirrhotic ascites had glucose concentrations between 121-150mg/dl. The protein content in majority of cases 84 (33.6%) was between 1.0- 2.0 gm/dl followed by 77(30.8%) cases with values between 3.1- 4.0 gm/dl. 67 (26.8%) cases had values between 2.1- 3.0 gm/dl. It was only 22 (8.8%) cases which had values more than 4.1 gm/dl.

Table No 1: Clinical diagnosis of these cases

Category	Number of cases	Percentage(%age)
Cirrhotic ascites	109	43.6
Tubercular ascites	61	24.4
Malignant ascites	12	4.8
Acute infective ascites	26	10.4
Miscellaneous	42	16.8
Total	250	100

Table No 2: Physical Examination of ascitic fluid specimens

Physical Character	Category	Number of cases	Percentage (%)
Volume	< 5 ml	175	70
	5-10 ml	75	30
Colour	Straw	204	81.6
	Blood tinged	36	14.4
	Others	10	4
Odour	Foul smelling	13	5.2
	Odourless	237	94.8
Other characters	Turbidity	8	3.2
	Cobweb formation	2	0.8
	No special character	240	96

Table No 3: Cytological diagnosis of examined ascitic fluids

Category	Number of cases	Percentage (%age)
Cirrhotic	105	42
Inflammatory	87	34.8
{Tubercular	{61	
Acute infective}	26}	
Cardiac & Renal	21	8.4
Malignant	12	4.8
{Ovary	{5	
GIT	4	
Endometrium	1	
Lymphoma	1	
Unknown}	1}	
Inconclusive	25	10
Total	250	100

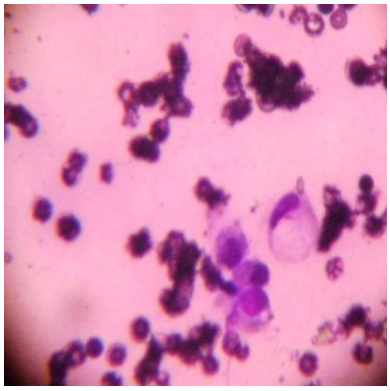
Table No 4: Transudative and Exudative fluids on the basis of protein content

Category	Transudative fluid protein<3gm/dl		Exudative fluid protein >3gm/dl	
	Number	Percentage	Number	Percentage
Cirrhotic ascites	109	43.6		
Tubercular ascites			61	24.4
Malignant ascites			12	4.8
Acute infective ascites			26	10.4
Miscellaneous ascites	42	16.8		

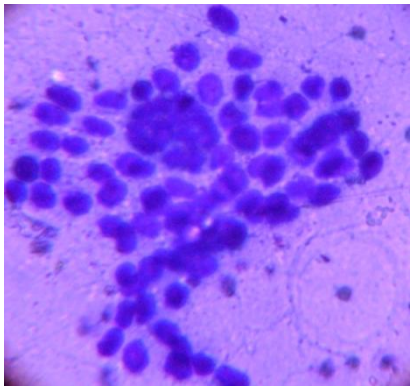
e-ISSN: 2319-9865
p-ISSN: 2322-0104

Chi-Square test value	p value	Significance
250.32	<0.001	Highly significant

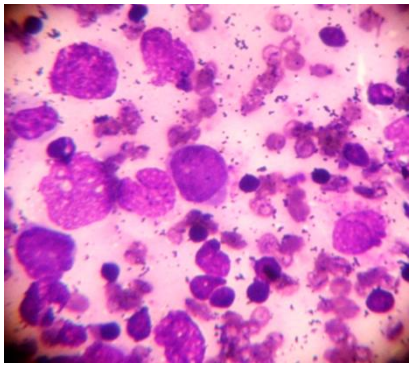
On basis of protein content of ascitic fluid using a discriminative value of 3.0 gm/dl, the categorisation into Transudative and Exudative ascitic fluid was done. Patients with cirrhotic ascites 109 cases had protein concentration < 3.0 gm/dl, while all 61 cases of tubercular ascites, 12 cases of malignant ascites and 26 cases of acute infective ascites had protein >3.0 gm/dl. The 42 miscellaneous cases had protein <3.0 gm/dl (Table 4).



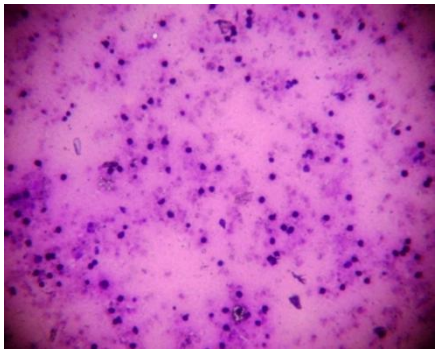
Photomicrograph of signet ring cells malignant ascitic fluid (MGG x 400)



Photomicrograph of papilla of formation in



Photomicrograph showing lymphomatous cells in malignant ascetic (MGG x 400)



Photomicrograph showing lymphocytes in tubercular ascitic fluid(MGG x 100)

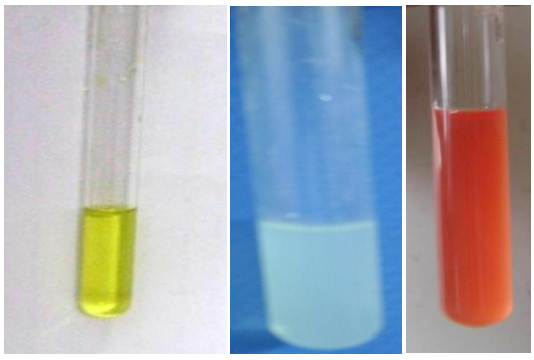


Figure 1: Gross appearance of ascitic fluids

e-ISSN: 2319-9865
p-ISSN: 2322-0104

Figure 2: Cobweb formation

DISCUSSION

Ascitic fluid analysis can be helpful and give clues in diagnosing certain disease entities. In our study, the incidence of ascitic fluid effusion was found more in males as compared to females. This sex wise distribution has also been recorded by Filik & Unal, Khan & Mahmood et al [7,8,9]. The relative frequency of normal straw coloured fluid was greater as compared to abnormal ones. This has also been documented by Barmeir et al [10]. Atalli et al found that cirrhotic ascitic fluid has higher pH than that of malignant and tubercular ascitic fluid and this corresponds with present study [11].

Hwangbo et al, khan & Mahmmod et al reported that liver cirrhosis is the most common cause of ascites , as in our study the maximum number of specimens of ascitic fluid revealed features of cirrhotic ascites [8,9,12]. Incidence of malignant ascites in our study is in concordance with Mahmmod et al. [9] Khan et al & Sherwani et al observed ovaries and gastrointestinal tract were amongst the most frequent causes of malignant ascites. This corresponds with the findings of present study [13].

Presents study reported with significantly lower ascitic fluid glucose levels as in concordance with study done by Attanasio [15]. Runyon et al reported lower ascitic fluid glucose in patients with malignant ascites as compared to cirrhotic ascites which is comparable to the results of the present study [16].

The estimation of ascitic fluid total proteins was the important criterion used to classify ascites. Jungst et al found that ascitic fluid protein levels had discriminative value of differentiating cirrhotic from non –cirrhotic ascites but not malignant from tubercular ascites, with lower protein concentrations in cirrhotic as compared to malignant and tubercular cases. This is in accordance with the present study [17].

CONCLUSIONS

While reviewing all the results, it is concluded that cirrhosis is the most common cause of ascites while malignancy is the least common cause. The exudative ascites is seen with tubercular acute infective and malignant ascites, with protein content >3.0 gm/dl and lower glucose levels while the transudative ascites as seen commonly with cirrhotic ascites has protein content <3.0 gm/dl and a higher glucose levels.

ACKNOWLEDGEMENT

We thank the patients, for their cooperation and technical staff of Pathology Department Government Medical College Patiala for processing the cytology smears and helping us to reach to our conclusion in this study.

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