

Study of Cholesterol Levels in Ascitic Fluid to Differentiate Malignant from Non-Malignant Effusion

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ABSTRACT

BACKGROUND

Collection of more than normal quantity fluid in a serous cavity is called effusion. It is classified as per location i.e. pericardial, pleural and peritoneal. Fluid collection in abdominal cavity is called ascites. Effusions are of two types - (1) Transudate (2) Exudate. Transudate develops as a result of physiological disturbances of circulation usually a rise in venous pressure or decrease in oncotic pressure, while exudate forms as a result of increased capillary permeability which is usually due to inflammation. Transudates usually have low specific gravity due to low protein content whereas exudates have high specific gravity due to high protein content. Transudates are usually associated with cardiac-, hepatic- or renal-disease. While exudates are caused by inflammatory conditions like tuberculosis and malignancies of pelvic and abdominal organs. Recently fibronectin and cholesterol levels of peritoneal fluid have been found to differentiate between ascites of different aetiologies. The estimation of fibronectin levels in fluid is sensitive but complicated. So, in this study we have taken the value of cholesterol for the aetiologic diagnosis of ascites as a simple procedure.

METHODS

The present descriptive study of 100 cases of ascites was conducted over a period of 12 months i.e. January. 2019 to January 2020. Various fluids were collected from OPD and IPD of Medical and Surgery units.

RESULTS

In the present study, 84% of the cases were transudates while exudates accounted for 16% of cases. Cirrhosis (65%) was the commonest cause among transudates. Other causes included congestive cardiac failure (09%), nephrotic syndrome (06%), & anaemias (04%). Exudative ascites was caused by tuberculosis in 10% cases & malignancy in 06% cases. Cholesterol level of >70 mg/dL was found in 82% of malignant ascites. So, it was concluded that ascitic fluid cholesterol levels were significantly greater in malignant cases in comparison to ascites caused by non - malignant aetiologies. Inference - Cholesterol concentration in ascitic fluid helps to differentiate ascites in malignancy, from non - malignant ascites.

CONCLUSIONS

Cholesterol estimation in ascitic fluid is a better parameter to differentiate malignant ascites from non - malignant ascites.

KEY WORDS

Cholesterol, Ascites, Transudate, Exudate

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BACKGROUND

There are serous cavities around delicate organs for their protection. Normally these cavities are either collapsed or contain very little lubricant fluid between the two layers i.e. the parietal and visceral layers. The visceral & parietal layer surface of each cavity are lined by membrane i.e. mesothelium, supported by connective tissue & an appropriate vascular & nervous apparatus.

In the absence of disease, the parietal and visceral layers of the mesothelium are separated by a thin layer of lubricating fluid that facilitates the movements of the two membranes against each other. Therefore the cavities are no true cavities but virtual cavities. The presence of gases and fluids will change them into true cavities. The viscera are external to the serous cavity & invaginate the visceral mesothelium. Thus the serous fluid normally lubricates & supports the visceral movement.

Whenever there is certain physical disturbance like increase in venous pressure or lowering of oncotic pressure or increase in the permeability of capillary membrane, the fluid starts accumulating in the potential spaces i.e. pleural, peritoneal or pericardial cavities. Slowly it starts exceeding the amount of fluid that is normally present in the cavity.

The two mechanisms that are involved in the development of effusions are

1. Increased venous pressure or drop in oncotic pressure in case of transudative effusion.
2. Increase in the permeability of capillary membrane in case of development of exudative effusion.

As there is Increase in the permeability of capillary membrane it allows more & more proteins & cells to seep into the fluids and hence there are higher protein levels and higher cell counts in the exudative fluids than the transudative ones. When exudates are due to infections microorganisms can be present in the fluids. In case of trauma foreign bodies may be identified in the fluids. When excessive fluid is collected in peritoneal cavity it is called ascites. There can be mild to massive increase of fluid in the peritoneal cavity due to liver, heart, inflammatory and malignant conditions.

The customary methods of investigation of causes of effusions are cytological examination of the fluid & biopsy of the peritoneum supplemented by biochemical and bacteriological examination. The previous approach in differential diagnosis was the protein concentration in the ascitic fluid & the cut off levels was 2.5 gm/dL above that was considered as exudates and it transudate. Certain other parameters like LDH & PH of ascitic fluid have been tried but of no use. A number of biochemical markers like hCG, CEA and CA - 125 are often measured in certain conditions the value of these procedures is still being debated. They sometime supplement but rarely replace the standard fluid examination.

Now a days, fibronectin and cholesterol levels of peritoneal fluid have been found to differentiate between ascites of different aetiologies. The estimation of fibronectin levels in fluid is sensitive but complicated. So, in this study we have taken the value of cholesterol for the aetiologic diagnosis of ascites as a simple procedure.

Objectives

1. To compare clinical diagnosis with biochemical and cytological findings.
2. To evaluate cholesterol concentration in ascitic fluid as a better diagnostic parameter.

METHODS

This is a descriptive study. One hundred patients with ascites attending the medical and surgical units, VIMS, Amroha district, for a span of twelve months from January 2019 - January 2020 were selected for the study. The study was approved by Ethics Committee and informed consent was obtained.

Collection of Fluids

1. Aseptic precautions taken.
2. Samples collected in two vials- one in EDTA for cytology & another one in plain vial for biochemistry.
3. Samples were processed as soon as possible.

Case Allocation

In present study 100 cases of ascites were taken for a span of 12 months i.e. January 2019 to January 2020. Various fluids were collected from OPD's and IPD's of Medical and Surgery units.

Considerations for Inclusion of Samples

- a. In cases of cirrhosis clinical history, Ultrasound and increased AST, ALT values were considered.
- b. For patients of nephrotic syndrome facial puffiness, pedal oedema. albuminuria raised urea/creatinine levels were taken as criteria.
- c. for Congestive Cardiac Failure Clinical findings along with ECG findings were included
- d. For anaemia clinical presentation, Hb% and low serum protein levels were taken into account.
- e. For tuberculosis raised ESR, H/O ATT drugs, X Ray (chest), ZN staining for acid fast bacilli & raised values of fluid proteins were considered as criteria.
- f. For malignancy clinical history, presence of neoplastic cells in fluid/ FNAC/ histopathology were considered.
- g. Fluids were examined for biochemistry & cytology.

For Cholesterol Estimation

Cholesterol concentration estimation was done by using Autopak cholesterol kits.

For Cytology

Total leucocyte count was done using readymade diluting fluid. Differential count was done using Leishman's stain. Cytology smears were made by centrifuging ascitic fluid for 05 minutes at 2000 rpm. Smears made from the sediment & stained by H&E stain and observed under microscope for morphology.

Technique

Cholesterol concentration was estimated with Cholesterol kits from Ames (Autopak).

Principle

Free cholesterol & fatty acids are formed by hydrolysis of cholesterol esters by cholesterol ester hydrolase. There is pre-existing cholesterol which along with the cholesterol produced by the above reaction is converted into cholestenone & hydrogen peroxide because of oxidation by cholesterol oxidase. H₂O₂ In the presence of peroxidase oxidises the chromogen to red colour complex to be read at 520 nm. This colour is constant for two hours unless kept in sun light.

Procedure for Cholesterol Estimation

- 1 tube was labelled as blank (B).
- 1 more tube was labelled as standard (S).
- Rest of the tubes were labelled as test (T) & numbered according to the number of samples of ascitic fluid.
- In each tube 1 ml of cholesterol reagent was poured after bringing the reagent to room temperature.
- In the tube B add 10 microlitre of distilled water, in the tube S add 10 microlitre of Cholesterol standard, In the tubes.

Labelled as T add 10 microlitre of supernatant from respective fluid samples. Keep all the tubes at 37* C for 10 minutes. Take their respective readings after calibration with standard. Note down all the readings.

Procedure for H & E Staining

1. Fix in Ether alcohol (1:1) for 25 - 30 minutes.
2. Stain with Haematoxylin for 3 - 4 minutes.
3. Differentiated with Acid alcohol (1%).
4. For Hydration - Keep for 4 - 6 mins. in running tap water.
5. Counter stain for 1 - 2 mins, with eosin.
6. To remove excess of eosin give two dips in tap water.
7. Blot the slide 3 - 4 times.
8. Keep for one & a half - two mins in 100% alcohol for dehydration.
9. Blot the slide.
10. Dip in clearing agent (Xylene) - 4 - 5 times.
11. Blot again
12. Mount in DPX.

Statistical Analysis

The data was formulated in MS - Excel spread sheet, data summarised, frequencies & means calculated, Fisher Exact test for significance was applied and the same is analysed by Epi Info 7 Software.

RESULTS

The common cause of ascites in 3rd and 4th decade were cirrhosis and tuberculosis. In 4th and 5th decade it was

malignancy. In males, cirrhosis of liver, nephrotic syndrome & malignancy were common reasons for ascites while tuberculosis, congestive cardiac failure and anaemia were common in females.

Sl. No.	Aetiology	Cases (Number)	Percentage
A	Cirrhosis of Liver	65	65
B	Peritoneal Tuberculosis	10	10
C	Congestive Cardiac Failure	09	09
D	Nephrotic syndrome	06	06
E	Anaemia hypoproteinaemia	04	04
G	Ascites in Malignancy	06	06

Table 1. Aetiological Distribution of 100 Cases of Ascites

Age in Years	Cirrhosis of Liver	Tuberculous Peritonitis	Congestive Cardiac Failure	Nephrotic Syndrome	Anaemia Hypoproteinaemia	Ascites in Malignancy
1 - 10	03	-	01	03	01	-
11 - 20	06	02	01	-	01	-
21 - 30	14	01	03	01	-	-
31 - 40	22	04	01	-	01	01
41 - 50	12	02	02	02	01	03
>50	08	01	-	-	-	02
Total Cases	65	10	09	06	04	06

2A. Age Distribution

Sl. No.	Aetiology	Cases (Total)	Male (Num.) %	Female (Num.) %
A	Cirrhosis of liver	65	48 73.8	17 26.2
B	Tuberculous peritonitis	10	04 40.0	06 60.0
C	Congestive Cardiac Failure	09	03 33.3	06 66.7
D	Nephrotic syndrome	06	04 66.7	02 33.3
E	Anaemia hypoproteinaemia	04	01 25.0	03 75.0
F	Ascites in Malignancy	06	04 66.7	02 33.3

2B. Sex Wise Distribution of Ascites

Table 2. Distribution of Cases According to Age and Sex

Colour of Fluid	Cirrhosis of liver	Tuberculous Peritonitis	Congestive Cardiac Failure	Nephrotic Syndrome	Anaemia Hypoproteinaemia	Ascites in Malignancy
Yellow	86.2%	-	100%	100%	100%	-
Amber coloured	12.3%	-	-	-	-	-
Turbid	1.5%	90 %	-	-	-	66.7
Haemorrhagic	-	10 %	-	-	-	33.3

Table 3. Gross Appearance of Ascitic Fluid in 100 Cases

In tuberculous peritonitis and in Malignancy fluid was mostly turbid while fluid was mostly clear yellow/amber coloured In the cases of cirrhosis of liver, congestive cardiac failure, nephrotic syndrome and anaemia. In tuberculosis (10% cases) and ascites in Malignancy (33.3 % cases) haemorrhagic fluid was found.

Aetiology	Cell Count Less than 100 Per Cubic mm	Cell Count 100 to 500 Per Cubic mm	Cell Count more than 500 per Cubic mm
Cirrhosis of liver	60 (92.3%)	04 (6.2%)	1 (1.5%)
Tuberculous peritonitis	03 (30.0%)	07 (70.0%)	NA
Congestive cardiac failure	09 (100%)	NA	NA
Nephrotic syndrome	06 (100%)	NA	NA
Anaemia hypoproteinaemia	04 (100%)	NA	NA
Ascites in Malignancy	04 (66.6%)	02 (33.4%)	NA

Table 4. Nucleated Cells in Ascitic Fluid of Various Aetiologies (%)

In tuberculosis (70% cases), in Malignancy (33.4% cases) and in cirrhosis (7.7% cases) nucleated cell count more than 100 per cubic mm was found in ascitic fluid while in remaining nucleated cells less than hundred cells per cubic mm were seen.

Aetiology	Ascitic fluid Cholesterol Concentration (mg/dL)	Mean Cholesterol Concentration (mg/dL)	
Cirrhosis of liver	6 - 38	21 ± 5.96	
Tuberculous peritonitis	16 - 40	26 ± 7.34	
Congestive cardiac failure	7 - 25	16 ± 6.72	p value
Nephrotic - syndrome	8 - 21	18 ± 11.18	<0.0001
Anaemia - hypo proteinemia	8 - 26	18 ± 9.07	highly
Ascites in Malignancy	48 - 92	61 ± 17.41	significant

Table 5. Mean Values of Cholesterol Concentration in Ascites of Different Aetiologies

Fisher Exact Test was applied In the above table, and p value was less than 0.0001 that is considered as very significant

Cholesterol Concentration	
Publishers	Mean Ascitic fluid cholesterol (mg/dL)
Albillos A. et al 1990	33 ± 21
Garg R. et al 1993	29.93 ± 9.21
Colli A. et al 1993	30.1 ± 3.3
Present study	21 ± 5.96

Comparison of Mean Cholesterol levels in Ascitic Fluid

Publishers	Mean Ascitic fluid cholesterol (mg/dL)
Albillos A. et al 1990	95 ± 42
Garg R. et al 1993	89.52 ± 49
Present study	61.00 ± 17.41

Table 6. Comparison of Cholesterol Concentration & Mean Cholesterol Levels in Ascitic Fluid between Other Studies and Our Study

In all the cases of non - malignant ascites total cholesterol values were less than 40 mg/dL; while in malignant ascites it was more than 48 mg/dL.

Statistically there is significant difference between ascitic fluid cholesterol levels in non - malignant (i.e. liver disease or tuberculosis) and ascites in malignancy. Thus, ascitic fluid total cholesterol concentration is helpful in drawing a line between non - malignant and ascites in malignancy.

In our study values of cholesterol in ascitic fluid was found to be greater in cases positive for malignant cells on cytology than those which were negative for malignant cells. So in doubtful smears ascitic fluid cholesterol level can guide us whether the smear is truly positive for malignant cells or not.

DISCUSSION

The clinical picture in most of the cases of ascites is usually not clear. Therefore, we need to investigate ascitic fluid for its aetiological classification which itself is quite often cumbersome. Transudative form is seen in various liver pathologies, CCF, anaemias and diseases of renal origin. In tuberculous peritonitis and malignancy exudate is common. In our study we found most of our cases (51%) in age group 30 to 40 yrs., which matches with study of Jain S.C. et al 1966.⁽¹⁾ i.e. (66%).

In the present study 64 % patients were male and 36 % were female that coincides with Nath et al 1966⁽²⁾ in which male and female percentage was 70% and 30% respectively. Findings of Nath et al 1966⁽²⁾ (58.6%) and those of Mehrotra M. P. and Mangal R. P. 1964⁽³⁾ (50%) cases of ascites were seen in cirrhosis that are comparable with our observation (i.e. 65% cases of ascites caused by cirrhosis). Now a days because of more social campaigning, early detection & treatment of tuberculosis we found only 10% cases of tuberculous ascites which is much less as compared to study by Jain S.C. et al 1966⁽¹⁾ (42%) and Mehrotra M.P. and R.P. Mangal 1964⁽³⁾ (30%).

Finding of Mehrotra M. P. and Mangal R.P. 1964⁽³⁾ (6%) correlates with our observation i.e. 09% cases of congestive cardiac failure as a cause of ascites. For unexplained reason CCF was found to be the cause of ascites in a study by Sikka et al 1967.⁽⁴⁾ (21.7%). Similar to the finding of Tito L et al 1988⁽⁵⁾ (56.7%) and Ljubi Ci. C.N. et al 1993⁽⁶⁾ (59.2%) cases cirrhosis as a cause of ascites in the age group of 30 to 40, we also found cirrhosis in (51%) cases as a cause of ascites.

Male to female ratio is more than one for cirrhosis as a cause of ascites. Cause of this is more consumption of alcohol in males. Mean cholesterol value in ascitic fluid in present study was found to be 21 + 5.96 mg/dL which resembles previous studies. In our study cholesterol levels found in ascitic fluid were between 8 and 21 mg/dL in cases of nephrotic syndrome while the value was 42 mg/dL in the only case reported by Albillos A. et al 1990 ⁽⁷⁾. Albillos A. et al 1990⁽⁷⁾ observed cholesterol levels in ascitic fluid as 81, 117, 100 and 94 mg/dL in the four cases of CCF they studied.

We found ascitic fluid cholesterol level as 7.25 mg/dL, the mean value being 16 + 6.72 mg/dL. Albillos A. et al 1990 ⁽⁷⁾ found values of cholesterol in ascitic fluid 110 and 115 mg/ dl in the two samples of tuberculous peritonitis. Sood A. et al 1995⁽⁸⁾ reported a mean cholesterol value of 35.07 + 9.72 mg/dL in the cases of tuberculous peritonitis. Ascitic fluid cholesterol in our study was 16 - 40 mg/dL with a mean value of 26 + 7.34 mg/dL, which is in accordance with observations of Sood A. et al 1995⁽⁸⁾. In study by Food N.C. 1956,⁽⁹⁾ Murphy 1972⁽¹⁰⁾ & Spieler et al 1985⁽¹¹⁾ 40% of the cases showed peritoneal metastasis. Nath et al 1966 ⁽²⁾ (8.57%), Sikka et al 1967⁽⁴⁾ (7.2%) and Mehrotra et al 1972⁽¹²⁾ (6.2%) findings i.e. malignancy as a cause of ascites are comparable to our study (06% cases of malignant ascites).

Although to determine malignancy in ascitic fluid cytology is very specific, but many a times the yield is very low. So in such cases the ascitic fluid cholesterol level can be tried to differentiate malignant from non - malignant ascites. Same was tried in one study by Garg R. et al 1993⁽¹³⁾ and differentiation of tuberculous lesion from ascites in malignancy in another series by Sood A. et al 1995.⁽⁸⁾ Garg R. et al 1993⁽¹³⁾ used a cut off level of cholesterol levels in ascitic fluid as 48 mg/dL to draw a line between malignant and non - malignant ascites. They found out it's sensitivity and specificity as 96.5% and 96.6% respectively.

Using a cut off level of 54.5 mg/dL Sood A et al 1995⁽⁹⁾ found the specificity and sensitivity of ascitic fluid cholesterol levels in differentiating tuberculous from malignant lesions as 100% and 89.65% respectively. In our study cholesterol levels were 48 - 92 mg/dL with a mean value of 61.00 + 17.41 mg/dL. Mean cholesterol in ascitic fluid was found to be 21 mg/dL in ascites due to cirrhosis and in tuberculosis it was 26 mg/dL. In ascites due to malignancy a mean ascitic fluid cholesterol level of 61 mg/dL was found. These values indicate that cholesterol levels in ascites due to malignancy are significantly higher than those found in non - malignant lesions. Moreover, it has been noted that cases positive for malignant cells had a higher ascitic fluid cholesterol (77.50 mg/dL) than those which were negative on cytology (51.25 mg/dL). Thus, in doubtful cases ascitic fluid cholesterol can help to guide us whether the smear is truly positive or not.

It is postulated that cholesterol is derived from malignant cell membrane Prieto M et al 1988.⁽¹⁴⁾ Relative blocking of peritoneal lymphatic drainage may also result in

sequestration of cholesterol macromolecule into the peritoneal cavity. Although the newer techniques like electron microscopy and immuno histochemistry bring down the false negative results on cytology, the techniques are quite complicated and more over they are not available at all centres. On the other hand ascitic fluid cholesterol level extraction is simple and cost effective method for aiding detection of malignant lesions causing ascites.

CONCLUSIONS

Statistically there is significant difference between ascitic fluid cholesterol levels in non - malignant (i.e. liver disease or tuberculosis) and malignant ascites. Thus, ascitic fluid total cholesterol concentration is helpful in differentiating malignant from non - malignant effusion. In the present study, cholesterol levels in ascitic fluid were found to be greater in smears positive/suspicious for malignant cells on cytology than those which were negative for malignant cells. So, in doubtful smears ascitic fluid cholesterol level can guide us in determining as to whether the smear is truly positive for malignant cells or not.

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