Journal of Cardiovascular Disease

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 04, 2023

ORIGINAL RESEARCH

Relevance of Cytology, Biochemical Parameters and CBNAAT in Differential Diagnosis of Ascitic and Pleural Fluid – A Prospective Study

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ABSTRACT

Background: Accumulation of fluid other than blood in pleural, peritoneal and pericardial cavities is known as Effusion. The classification of the fluid as exudate or transudate is the first step in evaluation of its etiology. **Aims and Objectives**: The aim of study is to evaluate the usefulness of biochemical parameter, cytology and cartridge based nucleic acid amplification test (CBNAAT) in differential diagnosis of ascitic and pleural fluid.

Material and Method: A study was carried out on 100 body fluid samples including 62 samples of ascitic fluid and 38 samples of pleural fluid. Clinical details were obtained and the samples were send to cytology where physical examination, staining (giemsa and pap staining), microbiology (CBNAAT) and biochemistry (total protein, fluid sugar, LDH) parameters were assessed.

Result- Out of 100 samples of body fluid, male to female ratio was 3.2:1. The most frequent etiology was found to be reactive in both type of effusion (49%) followed by inflammatory (46%) and malignancy (5%). Out of 38 pleural fluid samples, 84% belongs to exudate and (16%) belongs to transudate, classified on the basis of Light's criteria. Three were 3 malignant effusions and three cases were found CBNAAT positive. Out of 62 ascitic effusion, 37% were transudate and 63% were exudate and two cases were diagnosed as malignant effusions.

Conclusion: Ascitic fluid SAAG and LDH is found be helpful in diagnosis of different etiologies for effusion. For pleural fluid, light's criteria is found beneficial. Cytological diagnosis aids in early detection of malignancy found in body fluids. CBNAAT was found to be specific for detection of tuberculosis.

Keywords: Effusion, Transudate, Exudate

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INTRODUCTION

The history of serous effusion cytology dates back to almost early 19th century. Investigators Lucke and Klebs first recognized presence of malignant cells in Ascitic fluid. Quincke gave the description of Ovarian and Lung cancer cells in serous effusions. Since then effusion cytology have become the first line investigation of a suspected neoplastic effusion.Our body comprises of three serosal cavities which are pleural, peritoneal and pericardial cavity. These serous cavities are lined by outer parietal and inner visceral layer of epithelium(1). Normal amount of lubricating fluid in these cavities is approximately 50 ml. Accumulation of excess fluid in these cavities or spaces lead to effusion. It occurs when there is imbalance between fluid formation and removal(2).

Ascitic fluid effusion is classified into transudate and exudate. Transudative effusion occurs due to either increased hydrostatic pressure or due to decrease oncotic pressure. Most common causes of transudative effusion is cirrhosis. Other causes are beingn congestive heart failure, constrictive pericarditis, hepatic vein obstruction (Budd-Chiari syndrome), portal vein obstruction, Nephrotic syndrome, malnutrition etc. Exudative effusions are mostly inflammatory in etiology with common causes being tuberculosis, bacterial infection of gut, trauma, secondary peritoneal carcinomatosis, lymphomas, leukemia, primary hepatic tumor, mesotheliomas etc.(3). Studies have mentioned the reliability of ascitic fluid studies to identify the etiology of the diseases, i.e. benign or malignant(4). No laboratory test as of now is completely able to differentiate malignant ascites from ascites associated with cirrhosis or due to any other non malignant cause. It is important to differentiate between these conditions as it is of considerable clinical significance for further diagnostic and therapeutic management of patient. Common parameters used in the differential diagnosis of ascitic fluid are Lactate dehydrogenase (LDH), albumin, total protein(5) and serum ascites albumin gradient (SAAG)(6). Full analysis and comparison of these biochemical parameters, cytology and cartridge - based nucleic acid amplification test (CBNAAT) is needed to study the differential diagnosis of causes if ascitic fluid effusion.

Pleural effusion refers to excessive or abnormal accumulation of fluid in the pleural space. Pleural transudates have low protein concentration and can result from left heart failure, volume overload in critical care setting, chronic kidney disease patients, and atelectasis (collapsed lung) causing hydrostatic pressure differences across the pleural membranes. Exudative pleural effusions can occur due to pneumonia, malignancy (especially carcinoma, lymphoma or mesothelioma), tuberculous pleurisy, pulmonary embolism and other inflammatory disorders. Inspite of careful evaluation, in 19% cases no cause may be found, hence leading to diagnostic dilemma. As per Lights(7) criteria the exudative pleural effusion are identified by one or more of the following-

1. Pleural fluid LDH>2/3rd of upper limit of serum value
2. Pleural fluid to serum LDH >0.60 and LDH >200units in pleural fluid,
3. Pleural fluid protein to serum protein >0.50.

Tuberculosis (TB) is a highly contagious bacterial infection caused by *Mycobacterium tuberculosis* (MTB), affecting about 1/3rd of the world population. TB can affect both the pleural and peritoneal spaces. Nucleic acid amplification tests (NAATs) are molecular diagnostic methods based on amplification of mycobacterial nucleic acid. They provide results within a day, and are more specific and sensitive than Acid-Fast Bacillus Smear

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(AFB) smear. NAATs were originally designed for respiratory specimens, they can also be used on specimens from other TB sites like ascitic fluid samples[8].

Role of cytological examination in diagnosis of tuberculosis is widely recognized and well documented. Cytology mainly helps to identify the presence or absence of tumor cells, inflammatory cells, granuloma in absence of malignancy. Hence it has an important implication and often affects treatment[9].

This study is an attempt to compare cytology, biochemical parameter, CB-NAAT, clinical and radiological findings in cases of pleural and peritoneal effusion and to evaluate the causes for the same.

It is also an attempt to find the commonest non malignant cause of effusion in our setting using biochemical parameters (LDH, Sugar, SAAG), cell cytology and CBNAAT and to evaluate importance of serum to fluid albumin gradient (SAAG) in establishing differential diagnosis of ascites.

MATERIAL AND METHODS

All the fluid sample along with blood sample from different wards and ICU, submitted in the Central Pathology Lab of Department of Pathology from 1st January 2021 to 30th June 2022 were included in the study.

Physical examination included Quantity, Colour, Transparency, presence of coagulum or blood was noted. Samples were submitted to cytology and two centrifuged smears (centrifuged at the rate of 3000rpm for 2-3 minutes) prepared and stained with papanicolaou and giemsa stain. Sample was also submitted for microbiology for acid fast bacilli (AFB) culture (Lowenstein Jensen Medium) and cartridge based nucleic acid amplification test (CBNAAT) and biochemistry section for further testing(LDH, Sugar, SAAG).

Age, sex, site of collection, relevant history and investigation (ultrasound/radiological investigations) were compiled with various fluid parameters. All datas were recorded and analysed.

RESULT

Most cases of ascitic fluid fall under the age group ranges between 41-50 years (22 cases) followed by 31-40 years (16 cases) and minimum number of cases found below 20 years of age (2 cases). Among Pleural fluid most cases are seen under 41-50 & 51- 60 years of age (9 cases each), followed by 31-40 years (6 cases). Least number of pleural fluid cases were seen above 70 years of age (1 case) as shown in table 1. Most cases of ascitic and pleural fluid were seen amongst males.

Table 1: Age group distribution

Age group	AF		PF	
	Frequency	Percent	Frequency	Percent
<20	2	3.23	5	13.2
21-30	7	11.3	5	13.2
31-40	16	25.81	6	15.8
41-50	22	35.49	9	23.7
51-60	4	6.46	9	23.7

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61-70	4	6.46	3	7.9
>70	7	11.3	1	2.6
Total	62	100	38	100

Chisquare: 13.181,df=6 p=0.040

Approximately 63% cases of ascitic fluid falls under exudative category and 37% of cases under transudative. While in Pleural fluid 84.3% of cases were fall under exudate category while transudate shares only 15.7% as seen in table 2.

Table 2: Transudate and exudate in ascitic fluid and pleural fluid

	AF		PF	
	Frequency	**Percent**	**Frequency**	**Percent**
Transudate	23	37.1	6	15.79
Exudate	39	62.9	32	84.21
Total	100	100	100	100

Chisquare:5.195, df=1 p= 0.023

Most cases of ascitic fluid fall under the category of Reactive effusion (53%), followed by chronic inflammatory pathology (CIP) (34%), acute inflammatory pathology (AIP) (8%), Malignancy (3.25%) and Acute on CIP (1.62%). While maximum number of cases in pleural fluid fall under Reactive effusion (42%) followed by CIP (37%), AIP (10.5%), Malignancy (8%), Acute on CIP (2.6%) as shown in table 3.

Table 3: Cytological diagnosis

Cytology diagnosis	**AF**		**PF**	
	Frequency	**Percent**	**Frequency**	**Percent**
Acute on CIP	1	1.62%	1	2.64%
AIP	5	8.07%	4	10.53%
CIP	21	33.88%	14	36.85%
Malignancy	2	3.23%	3	7.9%
RE	33	53.235	16	42.115

Chisquare: 4.084,df=5; p =0.5370

Amongst the Light's criteria parameters studied for pleural fluid, we observed the sensitivity, specificity, area under curve(AUC), positive predictive value(PPV), negative predictive value(NPV) and accuracy of 93.75%, 96.88%, 0.885, 96.77%, 71.43% and 92.11% respectively of fluid to plasma LDH ratio to distinguish transudate from exudate. Similarly, for fluid to plasma protein ratio, sensitivity of 96.88%, specificity of 50%, AUC value of 0.734, PPV of 91.18%, NPV of 75% and accuracy of 89.47% was observed. Pleural fluid LDH showed sensitivity of 90.63%, specificity of 66.67%, AUC value of 0.786, PPV of 93.55, NPV of 57.14 and accuracy of 86.84% to distinguish transudate from exudate as shown in table 4.

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Table 4: Parameters of Light's Criteria

	Fluid and plasma LDH ratio	Fluid: Plasma protein ratio	Fluid LDH > 200
Sensitivity	93.75	96.88	90.63
Specificity	83.33	50	66.67
AUC	0.885	0.734	0.786
Positive Predictive Value	96.77	91.18	93.55
Negative Predictive Value	71.43	75	57.14
Accuracy	92.11	89.47	86.84

The mean value of light's criteria parameters in transudates and exudates of pleural fluid is shown in table 5 with significant P value. Transudate's parameters were of less value than exudate for fluid to plasma protein ratio, fluid to plasma protein LDH and fluid LDH, which was found to be statistically significant.

Table 5: Mean value of Light's Criteria in Pleural fluid

	Transudate	Exudate	p-value
Fluid: Plasma protein ratio	0.54±0.12	0.75±0.12	0.0004
Fluid and plasma LDH ratio	0.37±0.19	2.02±1.68	0.0225
Fluid LDH	101.5±71.63	485.07±276.31	0.0019

For ascitic fluid, mean value of SAAG (0.52±0.24) was lower in transudate compared to exudate (mean SAAG value of 1.58±0.45, p=<0.0001) which was found to be statistically significant, shown in table 6. SAAG value was found to be raised among cirrhotic causes of effusion.

Table 6: Mean value of SAAG in Ascitic Fluid

	Transudate	Exudate	P-Value
SAAG	1.58±0.45	0.52±0.24	<0.0001

Table 7: Mean serum LDH and p value in AF and PF

		Mean serum LDH Value	p value
Ascitic Fluid (62)	Transudate (23)	176.39 ± 69.55	<0.0001
	Exudate(39)	247.31 ± 68.49	
Pleural Fluid (38)	Transudate (6)	245.33 ± 36.36	0.5825
	Exudate(32)	271.25 ±112.24	

The mean serum LDH value in ascitic fluid transudate and exudate was 176±69.55 and 247.31±68.49 with significant p value of less than 0.0001, while in pleural fluid, it was found to be 245±36.36 for transudate and 271±112.24 for exudates with p value of 0.5825 respectively (not statistically significant) (Table 7).

In ascitic fluid, 15 cases (7 transudate and 8 exudate) were found to have sugar level less than 60mg/dl, 17 cases (5 transudate and 12 exudate) had sugar level in between 60-90 mg/dl, 15 cases (6 transudate and 9 exudate) had sugar level in between 91-120 mg/dl, 10

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cases (3 transudate and 7 exudate) had sugar level in between 120-150 mg/dl and 5 cases (2 transudate and 3 exudate) had glucose more than 150 mg/dl (Table 8).
Among pleural fluid, 14 cases (3 transudate and 11 exudate) had sugar level less than 60mg/dl, 17 cases (2 transudate and 15 exudate) had sugar level in between 60-90mg/dl, 3 cases (all exudate) had sugar level in between 91-120 mg/dl, 2 cases (all exudate) had sugar level in between 120-150 mg/dl, 2 cases (1 transudate and 1 exudate) sugar level had sugar level greater than 150mg/dl, shown in table 8. Glucose value among the ascitic and pleural fluid was found to be high in exudates as compared to transudates.

Table 8: Glucose level in both Ascitic and Pleural fluid

Glucose level	Ascitic fluid		Pleural fluid	
	Transudate	Exudate	Transudate	Exudate
<60 mg/dl	7	8	3	11
60-120 mg/dl	5	12	2	15
91-120 mg/dl	6	9	0	3
121-150 mg/dl	3	7	0	2
>150 mg/dl	2	3	1	1
Total	23	39	6	32

Out of 62 cases of ascitic fluid, two cases were found malignant, while in pleural fluid 03 out of 35 were found malignant. In both ascitic and pleural fluid malignancy was associated with exudative effusion (Table 9).

Table 9: Malignancy and non malignancy in ascitic in pleural fluid

		Malignant	Non-malignant
Ascitic fluid (Total 62)	Transudate	00	23
	Exudate	02	37
Pleural (Total 38)	Transudate	00	06
	Exudate	03	29

Out of the 38 cases of pleural fluid, clinical suspicion of tuberculosis was mentioned in 9 cases. All of these 9 cases were subjected to AFB culture and CBNAAT. However among these, 5 Cases were positive on Culture out of which 3 cases were positive on CBNAAT. All of these cases fall under exudative category (Table 10). Hence CBNAAT showed sensitivity of 33%, Specificity of 75.86%, PPV value of 12.50% and NPV value of 91.67%. for diagnosis of tubercular pleural effusion. In present study, we observed 9.37% CBNAAT positivity in pleural fluid in study population (38 cases). There is not a even single case of CBNAAT positive case amongst ascitic fluids in the present study (Table 10).

Table 10: CB NAAT positivity in pleural fluid

PF(38)			
CBNAAT			
Positive(3)		Negative(35)	
Transudate	Exudate	Transudate	Exudate
0	3	6	29

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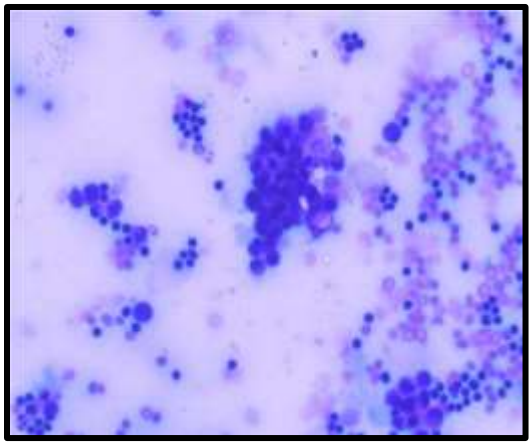

Fig. 1: Reactive effusion (100x) (MGG stain)

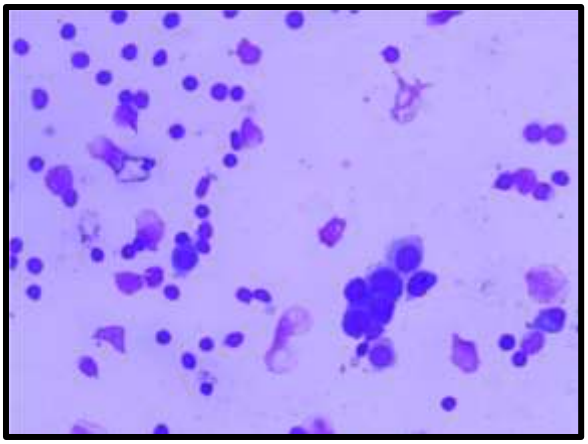

Fig. 2: Reactive effusion (400x) (MGG stain)

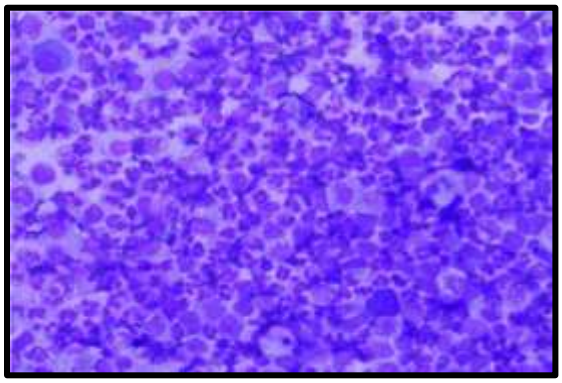

Fig. 3: Acute inflammatory cells (Empyema) at 400x (MGG stain)

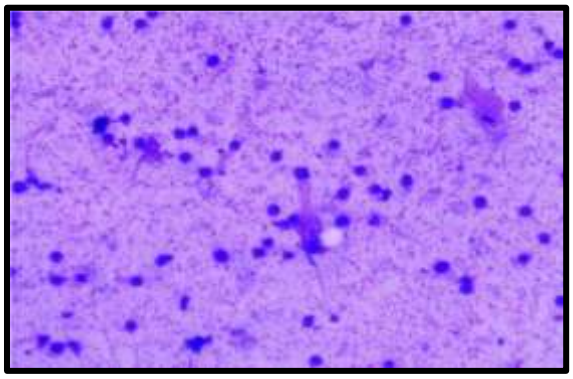

Fig.4 :Chronic inflammatory pathology (100x) (MGG stain)

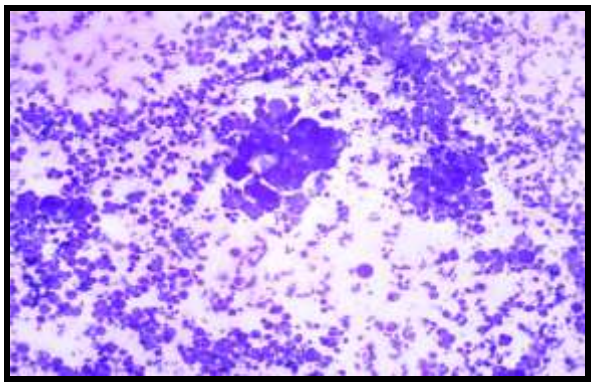

Fig:5 Adenocarcinoma 100x (MGG stain)

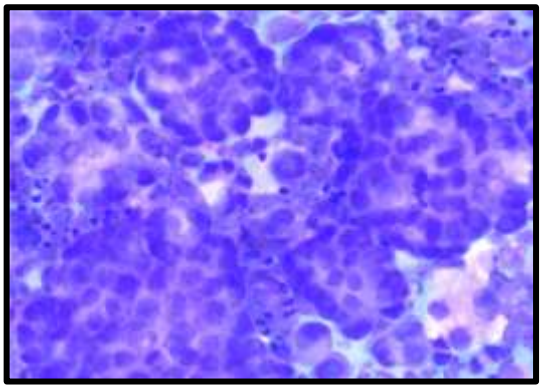

Fig:6 Adenocarcinoma 400x (MGG stain)

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 04, 2023

DISCUSSION

Ascitic fluid

Most of the cases of ascitic fluid fall under 3rd to 4th decade of life which was found to be 61.3% with male predominance while study done by Karthik et al (2020) showed 48% of cases with male predominance.(10) In our present study, we found 35.5% cases of transudate and 64.5% exudate. Similar findings were noted in study done by Anabela et al showed 45% transudate and 55% exudate.(11)

In our study, 43.5% of cases had infectious etiology while study done by Anabela et al had infectious etiology in 22% of cases only.(11) In our study, among ascitic fluid we found neoplastic etiology in 3.2% of cases only while study done by Anabela et al showed 19% and Bodal et al showed 4.8% neoplastic etiology(11,12), findings were quite similar with Bodal et al(12) study.

In our study, we 30.6% samples of ascitic fluid shows LDH value >400 IU which were predominantly exudative effusion while study done by Boyer et al showed 63% of cases with tubercular and malignant effusion had LDH value > 400 IU.(13) In our study, we found 80.6% of samples had serum fluid to serum LDH > 0.6 and 82% cases had SAAG value > 1.1 g/dl which was quite similar to study done by Anabela et al had 88%.(11)

Pleural fluid

In our present study, most of cases of pleural fluid were fall under 4th-6th decade which was found to be 71% with male predominance, similar findings were showed by Mohanty et al having 69.1% of cases with male predominance.(14) In our study, we found 15.7% cases of transudate and 84.3% cases of exudate while study done by Mohanty et al showed 15.2% transudate and 84.7% exudate and study done by Ambresh A et al showed 23.4% cases of transudate and 76.6% of exudate.(15) In our study, we found 50% of cases had infectious etiology while study done by Wang et al 78.9% cases had infectious etiology, results were quite different.(16) In our present study, we found 7.9% cases are malignant while study done by Mohanty et al showed malignancy in 13.3% of cases.(14)

In our present study, we found sensitivity of mean fluid LDH >200 IU was 90.63%, specificity of 66.6%, PPV of 93.5% and NPV of 57.14% to distinguish between transudate and exudate in pleural fluid while study done by Tarn Ac et al showed sensitivity of 71%, specificity of 100%, PPV of 100% and NPV of 61%.(17) In our present study, sensitivity of mean fluid plasma protein ratio was found to be 96.88%, specificity of 50%, PPV of 91.8% and NPV of 75% to distinguish between transudate and exudate in pleural fluid. Similar findings were noted by Tarn Ac et al showed sensitivity of 90%, specificity of 98%, PPV of 99% and NPV of 82%.(17) In our study present among pleural fluid we found sensitivity and specificity of CBNAAT to be 33% and 75.8% respectively while study done by Biswas et al showed sensitivity of 4.76% and specificity of 87.5%.(18)

CONCLUSION

In our Present study we analysed different parameters including biochemical parameters (Total protein, Albumin, Sugar, LDH, Fluid to Serum protein ratio, Fluid to serum LDH ratio, SAAG along with Culture and CBNAAT), Cytological and Microbiological test to establish correlation among differential diagnosis of ascitic and pleural fluid. In present study, SAAG turned out to be a good marker of cirrhotic causes of ascites. Ascitic fluid LDH is found helpful in diagnosis of exudate. For, Pleural fluid, Light's criteria is found

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 04, 2023

beneficial in differentiating Transudate and exudate. Glucose did not show much usefulness to that extent in differentiating transudate and exudate. Cytological diagnosis aids in early detection of malignancy found in body fluids. CBNAAT has high specificity for detection of tuberculosis in body fluids.

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