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Cytomorphologic Analysis of Body Fluids

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Abstract

Background: Many etiologies result in development of effusions in the serous cavities. Of these pleural, peritoneal, pericardial fluids and cerebrospinal fluid are the most common. Cytologic study is done to diagnose disease ranging from benign (infective and inflammation) to malignant effusion[1]. **Setting and Study Design:** Pleural, peritoneal, and cerebrospinal fluid were included and others were excluded. In this study the significance of fluid cytology in the diagnosis of various neoplastic and non neoplastic lesions in the cytology section was carried out. **Materials and Methods:** All samples received in the Department of Pathology - pleural fluid, ascitic fluid and CSF were evaluated. Gross Examination of fluids was done for total Volume, Colour and Clarity. Biochemical test correlation of fluid Adenosine De-Aminase (ADA) levels was done wherever necessary. Diagnosis was done by consultants who were posted in cytopathology section. **Results:** In this prospective study from August 2015-2017, total 146 cases were studied of which 65 cases were of ascitic fluid, 41 of pleural fluid and 40 cases of CSF. Of these 146 cases, benign cases were 134 (91.78%), malignant effusion 9(6.16%) and suspicious of malignancy were 3 (2.05%). Among 146 study cases, 136 (93%) cases were having similar cytomorphological pattern (singly scattered), other 10 cases showed various patterns such as 3-dimensional clusters, sheets, etc. **Conclusion:** Body fluid examination with correlation of various parameters like thorough clinical history and examination, different serum marker levels, primary malignancy if present and previous cytological diagnosis are very useful for the final diagnosis.

Keywords: Adenosine De-Aminase (ADA), Body Fluids, Cytomorphology, Neoplasia

1. Introduction

Many etiologies result in development of effusions in the serous cavities[1]. Pleural, pericardial, and peritoneal cavities are lined by the serous (or serosal) membrane (and hence the name "serous cavity"). The basic integral component of serosal membrane is a mesothelial cell which loosely rests on sub mesothelial stromal matrix tissue. Body fluids like pleural fluid, peritoneal fluid and Cerebro Spinal Fluid (CSF) are normally present with their constituents in particular proportions, in minimal quantities, within body cavities. These three cavities normally contain a small amount of thin fluid (serous fluid), which helps lubricate the membranes when they rub against each other, such as during breathing, etc. One of the challenging areas in clinical cytopathology is diagnosis by examination of exfoliated cells in serous effusions[1]. Carcinoma of the lung is the most common cause related to the presence of malignant effusions examined. Cytologic examination of a serous effusion helps in the diagnosis of cancer and for the staging and prognosis. Presence of cancer cells in the fluid indicates that cancer is advanced and chance of cure is less**.** In all the body fluids CSF examination with exfoliated malignant cells have worst prognosis.

Cytologic examination of pleural, peritoneal, and pericardial effusions helps in diagnosing inflammatory conditions of the serous membranes, parasitic infestations,

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and infection with bacteria, fungi, or viruses along with cancer[2]. Collection of effusions are comparatively simple[3]. Immunocytochemistry further helps in accurate diagnosis. Use of such ancillary techniques is on the rise now-a-days. Also, such methods will improve diagnostic accuracy[4].

2. Materials and Methods

The current cross-sectional study was carried in Department of Pathology, Tertiary Health care centre from August 2015 to November 2017. Total 146 cases were selected.

2.1 Eligibility Criteria

Inclusion criteria: Patient attending OPD and admitted under various departments and referred for cytomorphological diagnosis of pleural, peritoneal & cerebrospinal fluids.

Exclusion criteria: Body fluids except pleural, peritoneal and cerebrospinal fluid were excluded.

Methodology: All samples received - pleural fluid, ascitic fluid and CSF with detailed clinical information with regards to age, sex, history, provisional diagnosis etc. was taken.

Gross Examination of fluids is done for total volume, colour and clarity.

Biochemical Test – correlation of fluid ADA levels wherever necessary.

Collection of various fluids was done by:

- Peritoneal fluid is by abdominal paracentesis (paracentesis abdominis).
- Pleural fluid by thoracocentesis.
- Cerebrospinal fluid by lumbar puncture.

Staining was done using Papanicolaou stain (PAP stain) and Haematoxylin & Eosin (H&E) stain.

Conventional smear technique (Direct smears): The fluids were first centrifuged at 2000 rpm for 5 minutes. Supernatant was transferred to other tube. Sediment was used for direct smear preparation. After that prepared smears were appropriately labelled by a diamond glass marking pencil and stained according to need.

2.2 Fixation and Staining

2.2.1 Wet Fixed Smears

At least 2 smears were immediately kept in fixative (methanol) for 10 min and subsequently stained by H&E and PAP stains. In Present study after staining, smears were evaluated under headings of following parameters including other findings that were made on gross examination and other clinical data.

Assessment of the adequacy and representativeness of material in the smear was done.

Body fluids were examined under low power for cytomorphological features such as the overall cell population and predominant pattern. Cell morphology was studied under high power (Image 1-9).

2.3 Parameters

- Naked eye examination including colour and appearance.
- Cellularity.
- Architectural pattern.
- Abnormal cells.
- Previous Cytological diagnosis.
- Correlation with clinical diagnosis.
- Correlation of histopathological slides of neoplasms to diagnose metastasis for better evaluation.
- For Final report the clinical data and following investigations were correlated.

1. In suspected cases of tuberculosis correlation with serum or pleural fluid ADA levels.
2. Radiological findings
3. Hematological findings like ESR etc.

Thus, final diagnosis was given after due correlation of clinical, pathological, radiological and other data.

3. Results

Table 1. Distribution of cases according to type of fluid

Type of fluid	Frequency	Percentage
Ascitic	65	44.52%
CSF	40	27.40%
Pleural	41	28.08%
Total	**146**	**100.00%**

Out of total 146 samples studied 65 were of ascitic, 40 were of CSF and 41 were of pleural. Thus, maximum numbers of cases were that of peritoneal fluid (Table 1 and Figure 1).

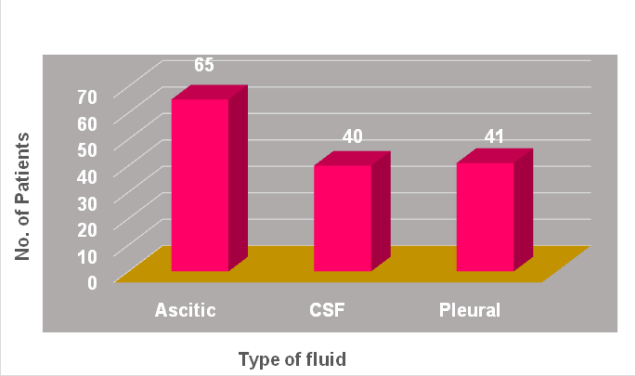

Figure 1. Distribution of cases according to type of fluid.

Table 2. Age distribution of all the cases in the study

Age groups in years	Frequency	Percentage
0-19	38	26.03%
20-39	43	29.45%
40-59	39	26.71%
60-79	24	16.44%
80-99	2	1.37%
Total	146	100.00%

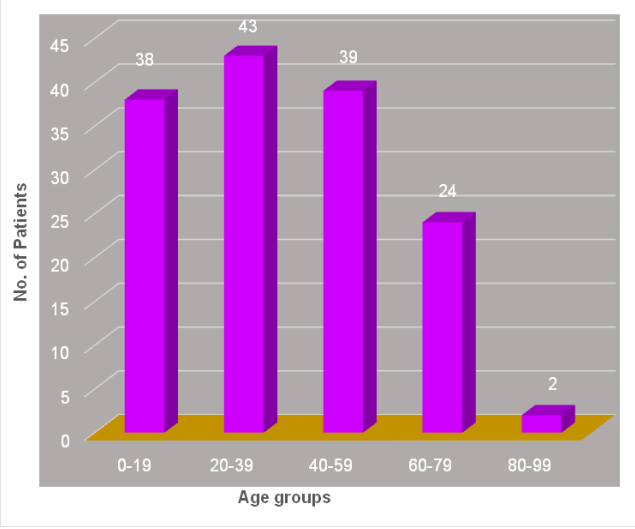

Figure 2. Age distribution of all the cases in the study.

Table 3. Sex distribution of all the cases in the study

Sex	Frequency	Percentage
Male	92	63.01%
Female	54	36.99%
Total	**146**	**100.00%**

Out of total 146 cases studied most cases were in the age group of 20-39 years (29.45%) while few cases were in age group of 80-99 years (1.37%) (Table 2 and Figure 2).

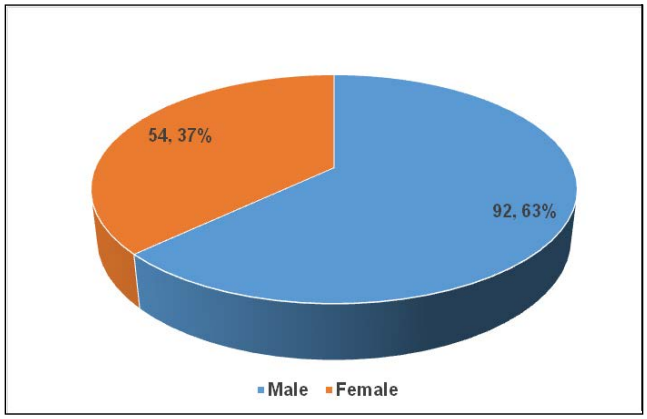

Figure 3. Sex distribution of all the cases in the study.

In this study of 146 cases, 92 cases (63.01%) were male while 54 cases (36.99%) were females.

The male to female ratio observed was 1.7:1. (Table 3 and Figure 3).

Table 4. Prevalence of transudate/exudate among study cases

T/E	Frequency	Percentage
Exudate	33	22.60%
Transudate	76	52.05%
Not Applicable	37	25.34%
Total	**146**	**100.00%**

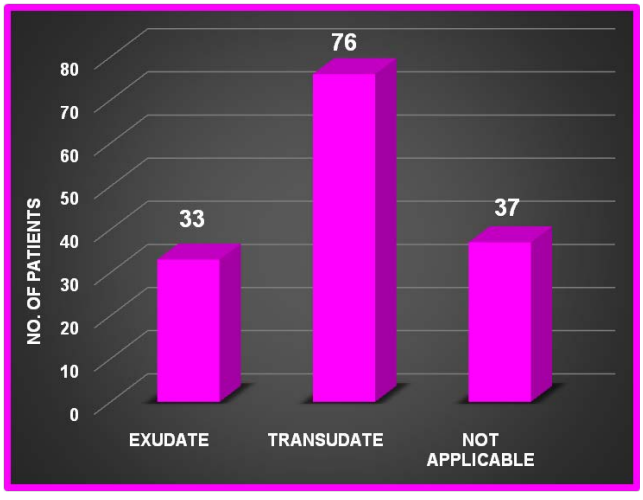

Figure 4. Prevalence of transudate/exudate among study cases.

In the present study, transudative effusion was more prevalent which constituted more than 50% of the cases. (Table 4 and Figure 4)

Table 5. Cytological findings in various body fluids of study cases

Cytological findings	Frequency	Percentage
Benign	134	91.78%
Malignant	9	6.16%
Suspicious	3	2.05%
Total	**146**	**100.00%**

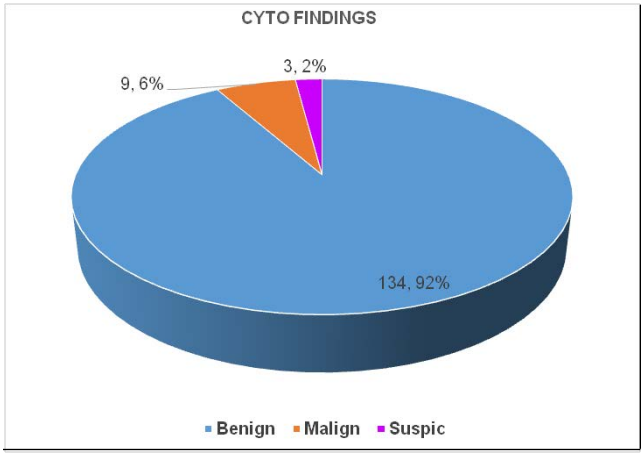

Figure 5. Cytological findings in various body fluids of study cases.

Out of 146 cases studied, cytological diagnosis of benign effusions was more prevalent, this was present in around 92% of cases (Table 5 and Figure 5).

Table 6. Cytological pattern in various body fluids of study cases

Pattern	Frequency	Percentage
3-Dimensional clusters	4	2.74%
Clusters & sheet	2	1.37%
Sheets & cords	3	2.05%
Sheets & singly	1	0.68%
Singly scattered	136	93.15%
Total	**146**	**100.00%**

Among 146 study cases, 136 (93%) cases were having similar cytomorphological pattern (singly scattered), other 10 cases showed various patterns such as 3-dimensional clusters, sheets, etc. (Table 6 and Figure 6).

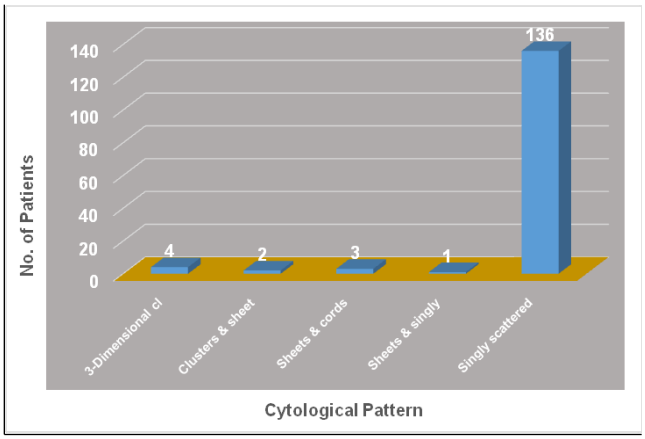

Figure 6. Cytological pattern in various body fluids of study cases.

Table7a. Association of cytological findings with pattern

CY to Findings	Pattern		Total
	Singly Scattered	Other	
Benign	134	0	134
Malignant +Suspicious	2	10	12
Total	**136**	**10**	**146**

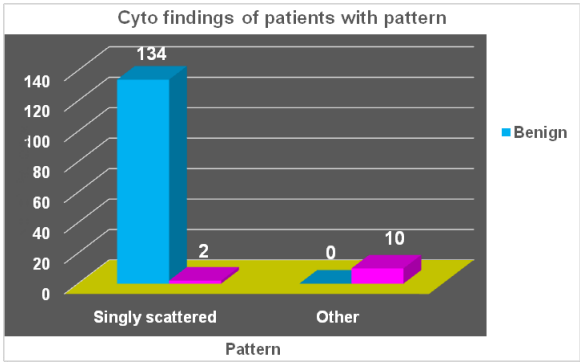

Figure 7a. Association of cytological findings with pattern.

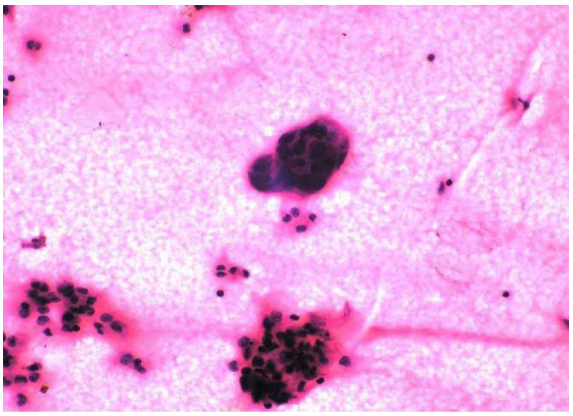

Image 1. H&E: Carcinoma cells in ascitic fluid - Conventional smear (40 X).

Table 7a and Figure 7a shows 134 benign cases, 2 cases as malignant and suspicious of malignancy.

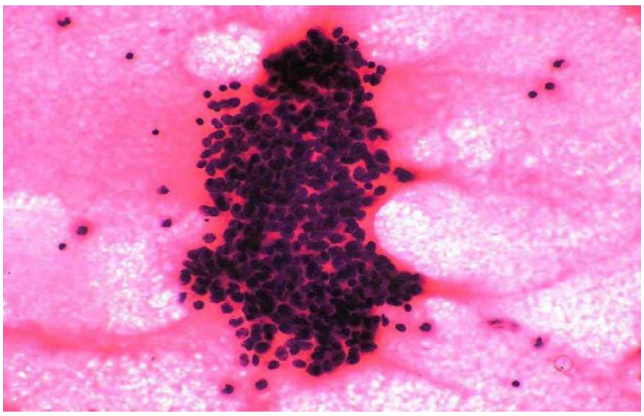

Image 2. H&E: Carcinoma cells in clusters in ascitic fluid - (40 X).

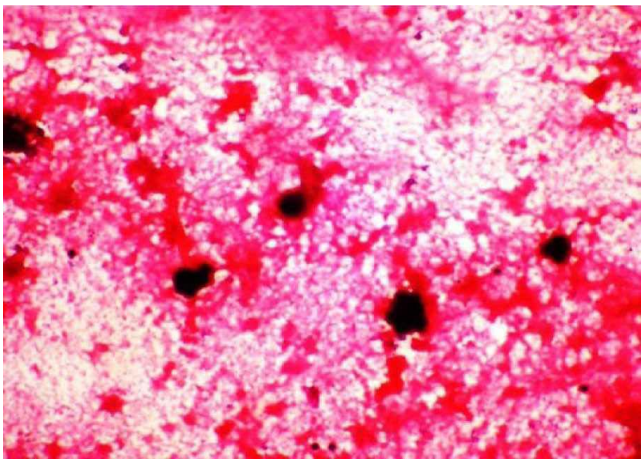

Image 3. H&E: Obscured cell morphology due to high protein content of ascitic fluid in a carcinoma case (40 X).

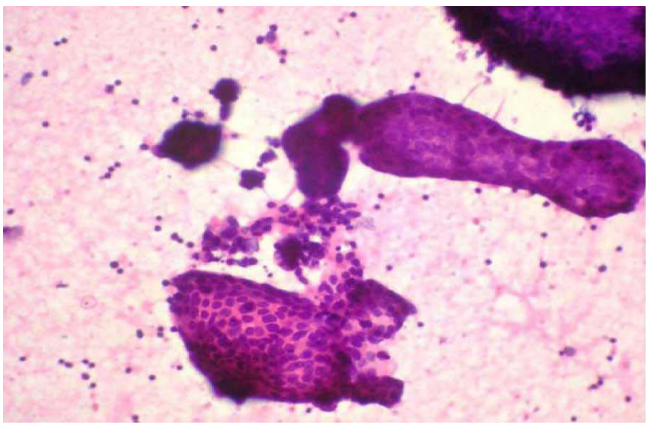

Image 4. H&E: Malignant cells in a adenocarcinoma in sheets & clusters - (40X).

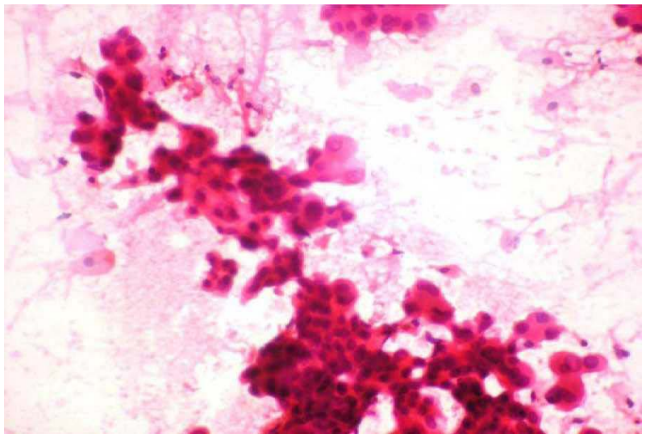

Image 5. H&E: Malignant cells in ascitic fluid in conventional smear (40X).

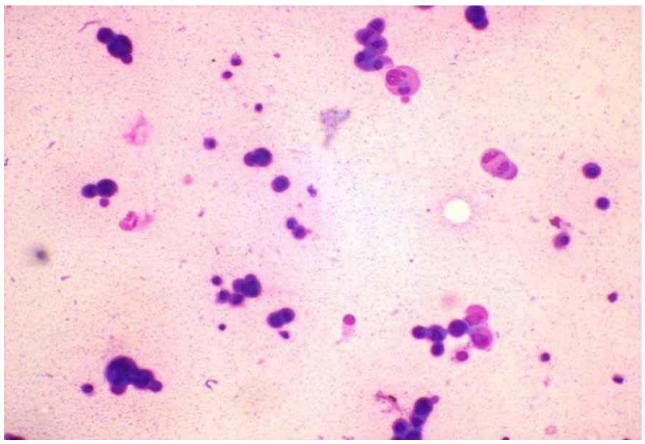

Image 6. H&E: Mesothelial cells in a ascitic fluid - conventional smear (40X).

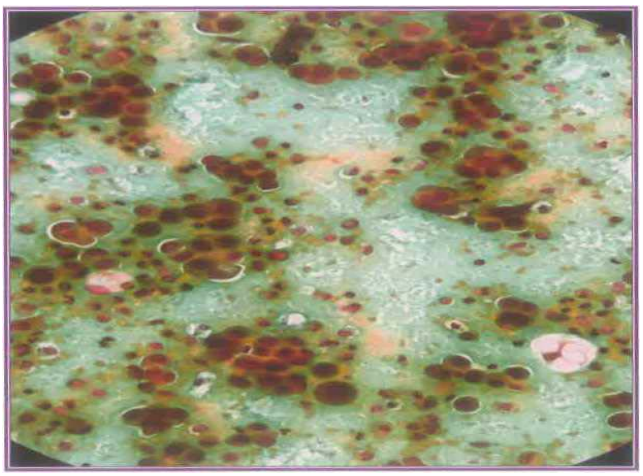

Image 7. Pap stain: Effusion showing reactive mesothelial cells - (10X).

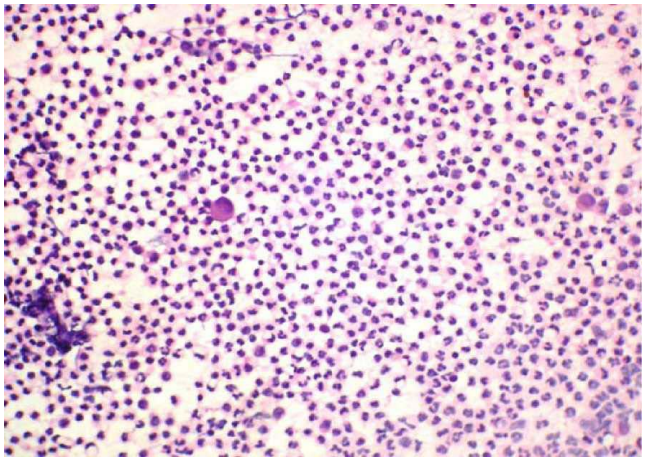

Image 8. H&E: Predominantly polymorphs in pleural fluid – (40X).

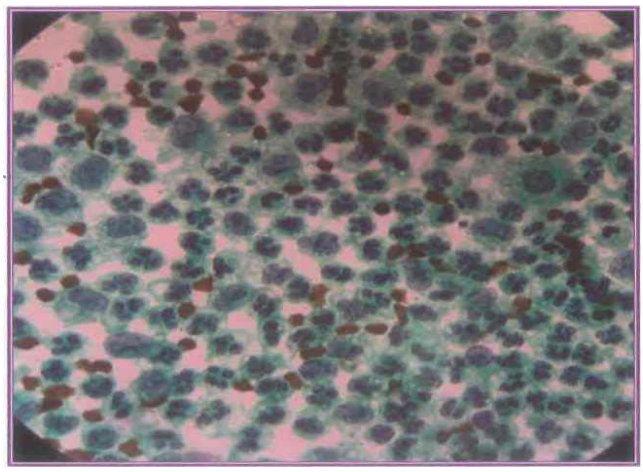

Image 9. Pap: Predominantly polymorphs with histiocytes in pleural fluid – (40X).

Table 7b. Association of cytological findings with pattern (Chi-square test)

Chi-Square Tests				
	Value	**df**	**Asymp. Sig. (2-sided)**	**Exact Sig. (2-sided)**
Pearson Chi-Square	119.88	1	<0.0001	
Continuity Correction	107.17	1	<0.0001	
Fisher's Exact Test				<0.0001

Here P value <0.00001 shows highly significant association between cytological findings and pattern (Table 7b).

Table 8a. Association of ADA with clinical diagnosis

ADA	Clinical Diagnosis		Total
	TB	Non TB	
<45	6	30	36
>=45	14	6	20
Total	**20**	**36**	**56**

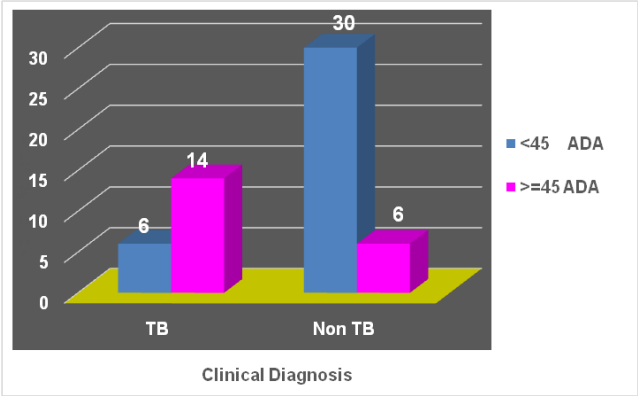

Figure 8. Association of ADA with clinical diagnosis[18].

Table 8b. Association of ADA with clinical diagnosis

Chi-Square Tests				
	Value	**df**	**Asymp. Sig. (2-sided)**	**Exact Sig. (2-sided)**
Pearson Chi-Square	15.929	1	0.000066	
Continuity Correction b	13.691	1	0.000216	
Fisher's Exact Test				0.000113

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.14.

Here P value =0.000066 <0.00001 shows highly. Significant association between ADA and Clinical Diagnosis (Table 8a-8b and Figure 8)

Table 9. Association of ADA levels in CSF and clinical diagnosis

ADA	Clinical Diagnosis in CSF		CSF
	TB	Non TB	
<=10	0	9	9
>10	3	1	4
Total	**3**	**10**	**13**

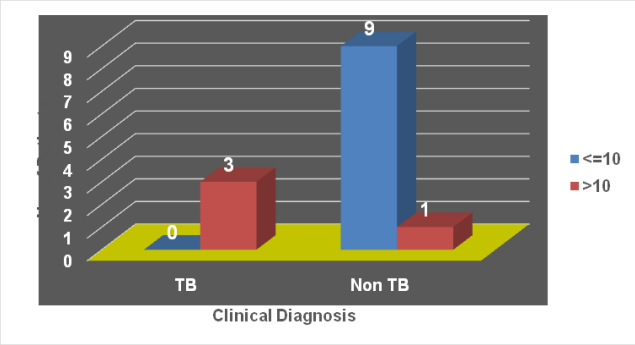

Figure 9. Association of ADA levels in CSF and clinical diagnosis.

Chi square test is not applicable due to small sample size (Table 9 and Figure 9)

Table 10a. Association of cytological findings with Pattern in age groups <=40 years and > 40 years

Age	CY to findings	Pattern		Total
		Singly scattered	Other	
<=40 yrs.	Benign	84	0	84
	Malignant +Suspicious	1	3	4
	Total	85	3	88
>40 yrs.	Benign	50	0	50
	Malignant + Suspicious	1	7	8
	Total	**51**	**7**	**58**

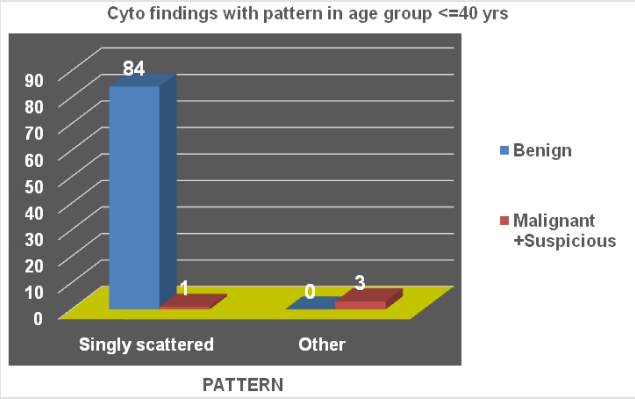

Figure 10(a). Association of cytological findings with pattern in the age groups <=40 years.

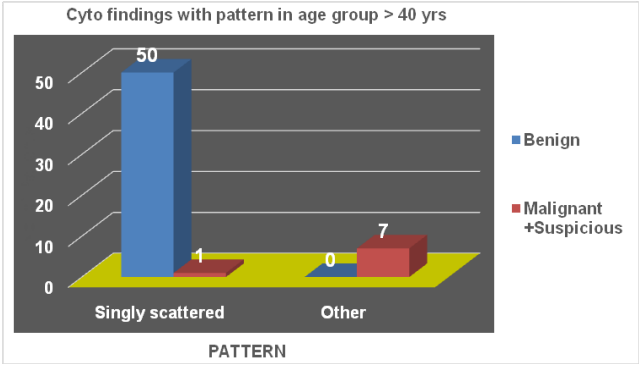

Figure 10(b). Association of cytological findings with pattern in the age group of >40 years.

4. Discussion

Present study was carried out in the cytology section to determine the significance of fluid cytology in the diagnosis of various neoplastic and non-neoplastic lesions. Total 146 cases were studied from August 2015 to August 2017. There were 65 cases of ascitic fluid, 41 cases of pleural fluid and 40 cases of CSF. There were 92 males and 54 females, and their ages were from 0 year to 82 years &the mean age was 41 years. Clinical history with other parameters like findings of clinical examination, routine blood investigation, radiographic findings, levels of various marker levels like (ADA, CA 125, CEA and other serum marker levels) were correlated and final diagnosis was given. In the current study, cytological examination of pleural, peritoneal and cerebrospinal fluid was carried out to find out if the effusion was malignant or non malignant. The results obtained were compared with study by other authors, who have worked previously in this field on routine and cytological examination of fluids and the significant difference and similarities in results are discussed. It is apparent from the study that malignant effusions were highly cellular than benign effusions. Clusters, 3-D balls and papillary patterns are indicative of malignant effusions. Scattered cells mostly indicate benign conditions with the exception of lymphoma.

Investigations of these effusions by cytologic examination are of paramount importance in the diagnosis of diseases/exclusion of neoplasia. A cytologic examination of the fluid performed on the smears of

Table 10b. Association of cytological findings with pattern in age groups <=40 years and >40 years (Chi-square test)

Age groups	Chi-Square Tests				
		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)
<=40 yrs	Pearson Chi-Square	65.22	1	<0.0001	
	Continuity Correction	44.44	1	<0.0001	
	Fisher's Exact Test				<0.0001
>40 yrs	Pearson Chi-Square	49.76	1	<0.0001	
	Continuity Correction	41.85	1	<0.0001	
	Fisher's Exact Test				<0.0001

a. 3 cells (75.0%) have expected count less than 5. The minimum expected count is .14[8].

c. 1 cells (25.0%) have expected count less than 5. The minimum expected count is .97.

P values <0.0001 in both age groups data so we can say there is significant association between Cytological findings and Pattern in the present study (Table 10a-10b and Figure 10a-10b).

centrifuged specimen helps to differentiate between benign and malignant effusions. It also helps in establishing the nature of malignancy in many cases helps in the planning of treatment and reduces the need for invasive procedures and unnecessary surgical intervention thus making the pathologist contribute positively to the clinical diagnosis and management of patients.

The overall sensitivity in the examination of pleural fluid has been reported in the literature to be in the range of 70-90%. A tissue core biopsy shows a sensitivity of only 40 to 70%. However, combination of the two increases the detection rate to approximately 80-90%[5]. In the present study of the 50 samples of pleural fluid studied malignancy could be diagnosed in 4 cases and was ruled out in 46 cases. The histological subtype of carcinoma could also be identified in these cases at cytology. Accuracy of ascitic fluid examination is lower as compared to pleural fluid ranging between 50 to 90% as reported in 1980[6].

Among the ascitic fluid samples, 8 cases were diagnosed as malignant. The rest suggested chronic inflammation with predominantly lymphocytes or reactive mesothelial hyperplasia. Twenty three cases of tuberculous etiology were diagnosed in pleural, ascitic and cerebrospinal fluid. Such a finding of pleural effusions with predominance of lymphocytes in cases of tuberculosis was confirmed by logistic regression method by Elliston et al in 1998. As per the author, a few macrophages and mesothelial cells may be seen in these effusions. The present study confirmed these findings and in correlation with the clinical histories of the patient enabled a diagnosis of Tuberculosis and excluded Viral Pneumonia and the remote possibility of lymphoma. In one case of pleural effusion, an early epithelioid granuloma was observed in the cells & suggested as tuberculous etiology[4].

Levels of ADA in CSF are not only raised in TB meningitis, but also in conditions like other meningeal inflammation and certain intracranial tumors[7]. But it is a test to confirm etiology and has good predictive value[7].

In the study[8] the cut-off value of adenosine deaminase set for tuberculous disease, is not exceeded in non tuberculous disease. ADA estimation is simple, inexpensive, rapid test, a fairly sensitive and specific test (more than 90%), helpful in differentiating tubercular from non-tubercular disease both in pulmonary and extra-pulmonary etiology[9]. Thus this test may helps in early diagnosis, improve the prognosis and reduce spread of disease and complication.

In study[7] cut-off value was 11.39 U/L and has sensitivity of 82% and specificity as 83% in TBM cases. Study[9] has cut-off value as 10 U/L for diagnosis of TBM and found sensitivity 66.6% and specificity 90%. Observed[10] CSF ADA level with a cut-off level of 6.5 U/L may differentiate tuberculous from non-tuberculous meningitis.

In the present study, median ADA levels in CSF were significantly high in TBM. Study of TBM patients in adult age group had similar finding[11].

There are numerous studies in the literature indicating the increase ADA in pleural fluid levels in the Tuberculous Pleural Effusion (TPE)[8]. High values of the sensitivity and

specificity have been reported in the countries with the high prevalence of TPE, particularly in the young patients[12]. In the current study too ADA levels were high in TPE.

In a study[13] evaluating consecutive 410 non tuberculous lymphocytic pleural fluid samples, the level of ADA above 40 U/L was seen only in seven cases (1.71%). They accurately used ADA levels <40 U/L to rule out tuberculosis.

In another study[14] it was reported that the ADA level <50 U/L was used to rule out tuberculosis, but they performed thoracoscopic pleural biopsy on 50 patients with ADA level under 50 U/L and identified tuberculosis in 6 (12%) of these patients. In the study[15] the body fluids included were pleural, peritoneal, pericardial knee joint effusions and CSF.

5. Conclusion

- Cytomorphological analysis of body fluids is an important investigation.
- This investigation may give a clue to diagnose underlying neoplastic or non-neoplastic diseases, which may change prognosis, further management and outcome of patient.
- In cases with malignant effusion it is critical in staging of the malignancy and deciding further protocol of treatment.
- Raised ADA levels can give an important clue to the diagnosis of tuberculous effusions.
- CSF ADA levels are elevated in the TB meningitis cases than non-TBM - viral meningitis cases. It is a simple and inexpensive diagnostic adjunctive test in the rapid and early diagnosis of TBM.

6. Bibliography

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