Original Article

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Pathology Section

Importance of Serosal Fluid Cytology as an Aid to Primary Diagnosis: A Descriptive Cross-sectional Study

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ABSTRACT

Introduction: Cytological study of fluids is an inexpensive, simple procedure and has significant utility in diagnosing neoplastic and non neoplastic lesions. The cytological examination of fluids in combination with physical examination helps identify aetiologic agents, follow the natural process of the disease and monitor the response to the treatment.

Aim: To determine the diagnostic utility of serosal fluid cytology and analyse the incidence of neoplastic and non neoplastic lesions using serous fluid cytology.

Materials and Methods: This was a descriptive cross-sectional study comprising 375 cases conducted in a tertiary care hospital, Sangli, Maharashtra, India, from August to July 2021. Purposive sampling was used to recruit the participants. All the patients with pleural effusions, ascites or in whom Cerebral Spinal Fluid (CSF), pericardial and synovial fluids examination was indicated were included. The provisional diagnosis was obtained from case sheets, including relevant clinical information. Smears were prepared from freshly tapped specimens without adding anticoagulants and were processed by routine, conventional smear technique. The data were analysed using the SPSS version 22.0 for Windows. Numerical variables were reported as frequency and percentage. The chi-square test was used wherever necessary, and the p-value less than 0.05 were considered significant.

Results: The peritoneal fluid was the most common fluid collected in the present study, followed by pleural fluid and CSF. The malignancy rate in the present study was 19 (10.4%) of peritoneal fluid, 6 (5.9%) for pleural fluid, and 2 (4.1%) for CSF. Adenocarcinoma was the most common malignancy found in present study.

Conclusion: Adenocarcinoma was the most common malignancy found in this study, which was in concordance with the research conducted earlier, where gold standard investigations confirmed the findings. In the peritoneal fluid, most of the patients had cirrhosis and tuberculosis. In pleural fluid and cerebrospinal fluid, most of them had tuberculosis and chronic inflammatory conditions, respectively. Previous researchers confirmed similar findings in their studies. It is seen that malignant and benign conditions like tuberculosis can be diagnosed well with effusion cytology.

Keywords: Body cavity, Cytological examination, Effusion

INTRODUCTION

There are three major serosal cavities in the body: peritoneal, pleural, and pericardial, lined by the mesothelial layer. Normally cavities contain minimal fluid, and it has a role in lubrication and protecting the underlying viscera. The Starling law governs fluid accumulation [1]. However, the serous cavities develop spontaneous effusions in pathologic states due to an imbalance between the formation and removal of fluids [2]. It serves as a valuable specimen to evaluate and diagnose underlying pathological conditions, such as malignancies, infections and inflammation, etc., [3].

Ascites is defined as an abnormal collection of fluid in the peritoneal cavity [4]. Dictionary meaning of 'Centesis' is a procedure of perforating a body cavity with a hollow needle for the purpose of extracting fluid. Cytological study of fluids is an inexpensive, simple procedure and has significant utility in diagnosing neoplastic and non-neoplastic lesions [5]. The cytological examination of fluids in combination with physical examination helps identify aetiologic agents, follow the natural process of the disease and monitor the response to the treatment. It also helps in identifying certain atypical cells. For uniform reporting of the serous fluid cytology, the International System for Reporting Serous Fluid Cytology was developed following best international practices [6]. Identifying the malignant cells in the body fluids require careful screening and scrutiny for the distinguishing features of different types of cells in the fluid [7]. Important of body fluids cytology lies in identifying malignant cells, but it also reveals information about the inflammatory conditions of serous cavities, various infections caused by bacteria, viruses and fungus parasites [8]. Consecutive sampling increases the detection rate of cancer cells [9]. The present study was undertaken to assess the diagnostic utility of serosal fluid cytology, analyse the prevalence of neoplastic and non-neoplastic lesions, and finally find out clinically unsuspected neoplastic lesions by body fluids examination.

MATERIALS AND METHODS

The study was a descriptive cross-sectional study conducted at a tertiary care hospital in Sangli city of Maharashtra, India, from August to July 2021. The Institutional Ethical Committee approved this study (BVDUMC&H/Sangli/IEC/273A/17 Dated: 17/08/2017)). Written informed consent was obtained from the study participants at the time of recruitment. Purposive sampling was used to recruit the participants.

Sample size calculation: Pilot testing was done on 30 patients and the overall malignancy rate was calculated as 30%. This prevalence was taken to calculate the final sample size using the W. Daniel formula [10]. A 10% non response rate was added and rounded off to reach a sample size of 375.

Inclusion criteria: All the patients with pleural effusions, ascites or in whom CSF, pericardial and synovial fluids examination was indicated were included in the study.

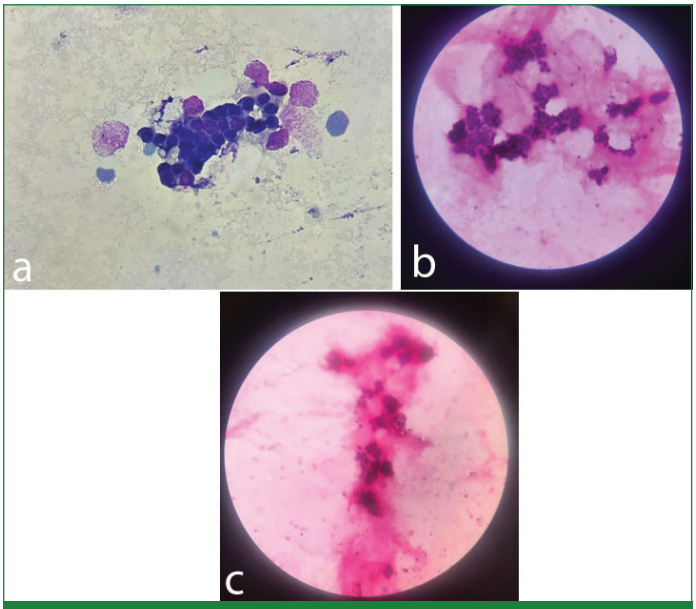
Exclusion criteria: Sample less than 5 mL volume, or received in an unsterile container and the patients who did not consent were excluded from the study.

Study Procedure

The provisional diagnosis was obtained from case sheets, including relevant clinical information regarding age, sex and accompanying clinical features, radiological findings.

The specimen (serosal fluid) was collected in a dry and clean container. The samples were received in the labeled sterile glass bottles with a rubber stopper with filled requisition forms. Smears were prepared from freshly tapped specimens without adding anticoagulants and were processed by routine, conventional smear technique.

The fluid received was centrifuged at 3000 revolutions per minute for five minutes. The supernatant fluid was discarded, and the sediment was transferred with a pipette onto two glass slides. One was air-dried and stained with Giemsa [Table/Fig-1a]. The other slide was immediately fixed in 95% alcohol and stained with Haematoxylin and Eosin (H&E) stain [Table/Fig-1b,c]. For the cell count, an improved neubauer counting chamber was used.



[Table/Fig-1]: a) Malignant cells in peritoneal fluid (Giemsa 40x); b) A 40x view H&E stain; c) Malignant cells in peritoneal fluid (H&E, 10x).

STATISTICAL ANALYSIS

Microsoft Excel was used in creating the database and producing graphs, while the data were analysed using the Statistical Package for the Social Sciences (SPSS) version 22.0 for Windows. Numerical variables were reported as frequency and percentage. The chi-square test was used wherever necessary, and the p-value less than 0.05 were considered significant.

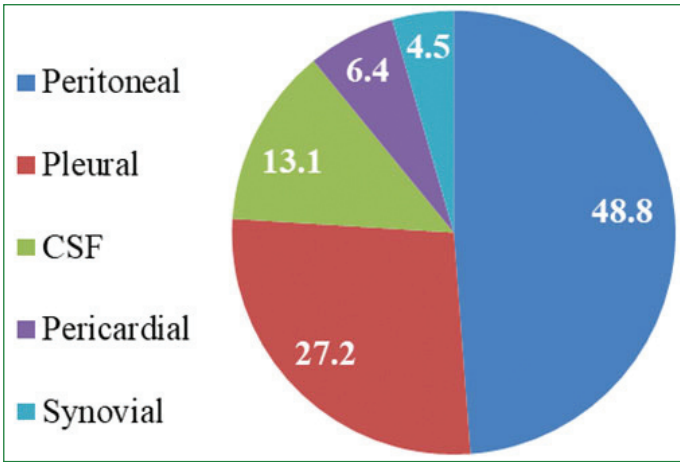
RESULTS

This study assessed 375 samples. The most common specimen received was the peritoneal fluid 183 (48.8%), followed by pleural fluid 102 (27.2%), cerebrospinal fluid 49 (13.1%), pericardial fluid 24 (6.4%) and synovial fluid 17 (4.5%) [Table/Fig-2].

The age group range varied between 3-77 years of age. The maximum number of cases was in the range of 51-60 years, followed by 61-70 years. A maximum number of cases with peritoneal were in the age group of 51-60 years. A maximum number of cases with pleural effusion were in the age group of 31-40 years [Table/Fig-3].

In the peritoneal fluid, majority of the patient had cirrhosis 95 (51.9%), followed by tuberculosis 24 (13.1%) and cancer 19 (10.4%). In pleural fluid, the majority of the cases had tuberculosis 39 (38.2%), followed by acute infective conditions 25 (24.5%). In CSF, the majority of the cases had chronic inflammatory conditions 12 (24.5%), followed by acute infective conditions 8 (16.3%) [Table/Fig-4].

Out of 375 cases, 348 (92.8%) were non-neoplastic lesions, while 27 (7.2%) were neoplastic lesions. The proportion of infective fluid was 140 (37.3%) [Table/Fig-5].



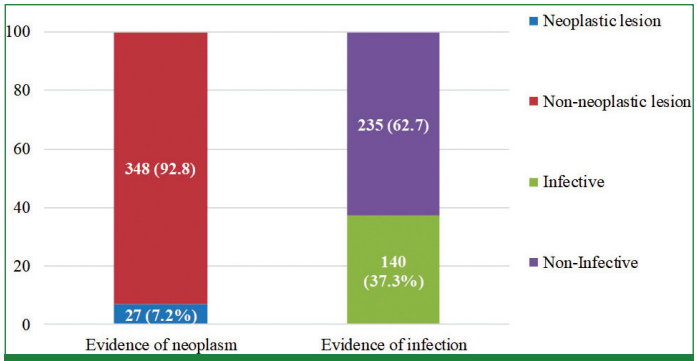
[Table/Fig-2]: Frequency distribution of the cases (N=375).

Characteristics		Type of fluid					Total (%)
		Peritoneal n (%)	Pleural n (%)	Synovial n (%)	Pericardial n (%)	CSF n (%)	
Age (years)	0-10	11 (6.0)	3 (2.9)	1 (5.9)	1 (4.2)	5 (10.2)	21 (5.6)
	11-20	13 (7.1)	4 (3.9)	0	0	0	17 (4.5)
	21-30	19 (10.4)	11 (10.8)	3 (17.6)	6 (25.0)	4 (8.2)	43 (11.5)
	31-40	35 (19.1)	19 (18.6)	1 (5.9)	1 (4.2)	6 (12.2)	62 (16.5)
	41-50	17 (9.3)	16 (15.7)	3 (17.6)	4 (16.7)	4 (8.2)	44 (11.7)
	51-60	36 (19.7)	18 (17.6)	3 (17.6)	3 (12.5)	16 (32.7)	76 (20.3)
	61-70	28 (15.3)	17 (16.7)	4 (23.5)	4 (16.7)	9 (18.4)	62 (16.5)
	71-80	24 (13.1)	14 (13.7)	2 (11.8)	5 (20.8)	5 (10.2)	50 (13.3)
Gender	Male	109 (59.6)	58 (56.9)	11 (64.7)	15 (62.5)	24 (49.0)	217 (57.9)
	Female	74 (40.4)	44 (43.1)	6 (35.3)	9 (37.5)	25 (51.0)	158 (42.1)

[Table/Fig-3]: Distribution of the cases as per their socio-demographic characteristics.

Primary cause of effusion	Frequency (n)	Percentage (%)
Peritoneal fluid (n=183)		
Cirrhosis	95	51.9
Tuberculosis	24	13.1
Cancer	19	10.4
Acute infective cause	8	4.4
Inflammatory conditions	8	4.4
Miscellaneous*	29	15.8
Pleural fluid (n=102)		
TB	39	38.2
Acute infective cause	25	24.5
Cancer	6	5.9
Miscellaneous #	32	31.4
CSF (n=49)		
Chronic inflammation	12	24.5
Acute infective cause	8	16.3
TB	7	14.3
Cancer	2	4.1
Miscellaneous**	20	40.8
Pericardial (n=24)		
Acute conditions	14	58.3
Chronic conditions	2	8.3
Unknown	8	33.3
Synovial (n=17)		
Acute infection	8	47.1
Chronic infection	6	35.3
Unknown	3	17.6

[Table/Fig-4]: Distribution of cases based on the primary cause of the effusion. (Miscellaneous*: Hypoproteinemia/nephrotic syndrome; #Congestive cardiac disease, anaemia; **Chronic conditions)



[Table/Fig-5]: Distribution of cases as per the evidence of neoplasm and infection.

The most common fluid was non-neoplastic 348 (92.8%). Out of 183 cases of peritoneal fluid, 164 (89.6%) were non-neoplastic and 19 (10.4%) were neoplastic. In pleural fluid, less proportion 6 (5.9%) of the neoplastic lesion was observed than peritoneal fluid. In synovial and pericardial fluid, no malignancies were observed. The infective effusion in peritoneal fluid, pleural fluid, synovial fluid, pericardial fluid and CSF were 32 (17.5%), 64 (62.7%), 10 (58.8%), 5 (20.8%), and 29 (59.2%), respectively. The non-neoplastic transudates in peritoneal fluid, pleural fluid, synovial fluid, pericardial fluid and CSF were 144 (87.8%), 90 (93.8%), 10 (58.8%), 21 (87.5%) and 27 (57.4%), respectively [Table/Fig-6].

Characteristics		Type of fluid				
		Peritoneal (n=183)	Pleural (n=102)	Synovial (n=17)	Pericardial (n=24)	CSF (n=49)
Evidence of infection	Infective (N=140)	32 (17.5)	64 (62.7)	10 (58.8)	5 (20.8)	29 (59.2)
	Non infective (N=235)	151 (64.3)	38 (16.2)	7 (3.0)	19 (8.1)	20 (8.5)
Evidence of neoplasm	Neoplastic (N=27)	19 (10.4)	6 (5.9)	0	0	2 (4.1)
	Non neoplastic (N=348)	164 (89.6)	96 (94.1)	17 (100.0)	24 (100.0)	47 (95.9)
Effusion*	Transudate (N=292)	144 (87.8)	90 (93.8)	10 (58.8)	21 (87.5)	27 (57.4)
	Exudate (N=56)	20 (12.2)	6 (6.3)	7 (41.2)	3 (12.5)	20 (42.6)

[Table/Fig-6]: Distribution of type of fluid based on the evidence of neoplasm, infectivity and the type of non neoplastic effusion.
p-value <0.001; *Type of non neoplastic effusion (n=348)

Adenocarcinoma was observed in the majority 24 (88.9%) of the patients. All the cases of cancer in the peritoneal fluid were adenocarcinomas [Table/Fig-7].

Type of cancer	Type of fluid			
	Peritoneal n (%)	Pleural n (%)	CSF n (%)	Total n (%)
Adenocarcinoma	19 (100.0)	4 (66.7)	1 (50.0)	24 (88.9)
Small cell carcinoma	0	2 (33.3)	0	2 (7.4)
Non adenocarcinoma	0	0	1 (50.0)	1 (3.7)
Total	19 (70.4)	6 (22.2)	2 (7.4)	27 (100.0%)

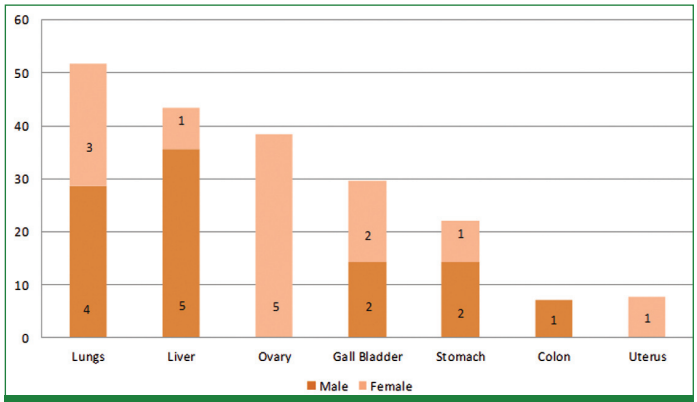
[Table/Fig-7]: Distribution of effusion based on the type of cancer (n=27)*.
*Neoplasm positive/p-value <0.001

The majority of the transudative fluids were non-neoplastic 292 (96.4%), also most of the exudated were non-neoplastic 56 (77.8%). Neoplastic exudates were 16 (22.2%). Majority of males had exudative fluid 13 (8.2%) while among females, majority of them had transudative fluid 203 (93.5%) [Table/Fig-8].

The most common primary cancer site was the liver 5 (35.7%) in males, followed by the lungs 4 (28.6%). The most common primary site in females was ovaries 5 (38.5%), followed by lungs 3 (23.1%) [Table/Fig-9].

Variable		Effusion		p-value
		Transudate (%)	Exudate (%)	
Presence of neoplasm	Neoplastic lesion	11 (3.6)	16 (22.2)	<0.001
	Non neoplastic lesion	292 (96.4)	56 (77.8)	
Gender	Male	14 (6.5)	13 (8.2)	–
	Female	203 (93.5)	145 (91.8)	

[Table/Fig-8]: Distribution of effusions according to the presence of a neoplastic lesion and gender.



[Table/Fig-9]: Distribution of neoplastic lesion based on primary site of neoplasm and gender (M=14, F=13).

DISCUSSION

Effusion cytology dates back to the 19th century. Since then, effusion cytology has gained tremendous importance in the medical literature [11]. The incidence of patients with effusion has increased in the past few years. Hence, it has become essential to study the cytological features of the effusions and provide reliable results for case management [12,13]. The present study is done to analyse the incidence of neoplastic and non neoplastic conditions in various types of effusion. Body fluids are segregated into transudates and exudates. The leading cause for transudative effusion is hypoalbuminaemia, fluid leakage from efferent intestinal lymphatics. Effusion cytology is used to evaluate patients with transudates. The exudates are generally inflammatory, chylous and neoplastic effusion. Neoplastic exudates are predominantly composed of malignant cells. Chylous exudates are composed of lymphocytes, neutrophils, and macrophages [14].

In this study, the most common fluid was the peritoneal fluid (48.8%), followed by pleural fluid (27.2%) and CSF (13.1%). Sherwani R et al., showed similar findings in their study [15]. However, Hathila R et al., found pleural fluid as the most common fluid in their study [16]. The difference may be attributed to more patients with lung diseases having effusion in the present study. The majority of them were non neoplastic (92.8%), the non neoplastic transudates were 94.7%, in concordance with other studies [15,17,18]. Kumavat PV et al., Shulbha VS and Dayananda BS also showed similar findings in their research [12,19].

The malignancy rate in present study was 10.4% of peritoneal fluid, 5.9% for pleural fluid and 4.1% for CSF. In their research, Lobo C et al., showed a higher malignancy rate, i.e., 33% for peritoneal effusions and 31.9% for pleural fluid [20]. Differences in the malignancy rate may be attributed to the different admission rates and differences in the geographical distribution of cancer cases.

In peritoneal fluid, out of 183 patients, maximum numbers were in the 6th decade, unlike other studies [21-23]. M:F ratio was 3:2 showing a higher incidence of peritoneal effusion in males than females, not in concordance with other studies [12,13]. In the peritoneal fluid majority of the patient had cirrhosis (51.9%), followed by tuberculosis (10.4%) and cancer (10.4%). Bodal VK et al., also showed that most cases had cirrhosis (43.6%) [24].

In pleural fluid, out of 102 cases, most were between 31-40 years of age. The male to female ratio was about 3:2, showing a higher incidence of pleural effusion in males, similar to others studies

[22,25]. The majority of them had tuberculosis (38.2%), followed by acute infective conditions (25.5%), and cancer (5.9%). The results were in concordance with others [13,16,17]. However, Wong JW et al., in a study, found that pleural fluid showed the highest proportion for malignant cells [13].

In CSF, the majority were in the age group of 51-60 years (32.7%). The male to female ratio was about 1:1. Most cases had chronic inflammatory conditions (24.5%), followed by acute infective conditions (16.3%). Bae YS et al., suggested that the results should be interpreted in addition to clinical and radiological imaging [26]. In pericardial fluid, the majority was 21-30 years, male to female ratio was 3:1, and no malignant cells were identified, similar to the results shown by Hathila R et al., [16]. In synovial fluid, no malignant cells were identified. Adenocarcinoma was the most common malignancy found in the present study, similar to other studies [27-29]. Jha R et al., and Sears D et al., in research, also found that adenocarcinoma is the most common malignancy [29,30]. The present study confirmed the findings of serous fluid cytology with that of the previous studies conducted on the same line. This study provides evidence for the diagnostic value of fluid cytology with a relatively larger sample size than most of the previous studies.

Limitation(s)

There were some limitations in the present study. Firstly, this was a single center study hence multicentric studies need to be conducted for the generalisation of the results. Secondly, the presence of epithelial cells in the field may potentially cause error in diagnosis.

CONCLUSION(S)

This study concludes that cytological fluid examination fairly correlates with research conducted earlier, where gold standard investigations confirmed primary diagnosis. Methods like cell block and immunocytochemistry additionally improve diagnostic outcomes in cases of serous effusion cytology. It could be suggested that effusion cytology is a simple, safe, and cost-effective diagnostic procedure and can be used as an aid to the primary diagnosis, especially in resource-constrained settings.

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- Manual Googling: Jan 25, 2022
- iThenticate Software: Feb 11, 2022 (10%)

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