RESEARCH ARTICLE

DIAGNOSTIC IMPORTANCE OF SEROUS FLUID EXAMINATION FOR DETECTION OF VARIOUS PATHOLOGICAL CONDITIONS - A STUDY OF 355 CASES

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ABSTRACT

Background: Cytological examination of exfoliated cells is very challenging and of paramount importance for diagnosis, staging and prognosis as the finding of cancer cells in such a specimen denotes that the patient has advanced and incurable cancer.

Aims & Objective: To study serous effusion for various pathological conditions.

Material and Methods: The study was performed on serous effusions. Serous effusions from pleural, pericardial and peritoneal fluid were included and all other fluids were excluded. Their clinical history and other relevant parameters were noted. Collection was performed with 18-gauge needle under local anaesthesia and sterile conditions. When delay, samples were stored at 2-6°C. Conventional smear and or Cytospin method were performed. Ether alcohol (wet fixed) and air dried smears were used. H & E stain, papanicolaou stain and MGG stain were done.

Results: Out of total 355 cases, 186 were of pleural fluid, 164 of peritoneal fluid and 5 were of pericardial fluid. 288 cases were benign, 24 cases were malignant effusion, 17 cases were suspicious of malignancy and 26 cases were degenerated. Transudate, haemorrhagic and straw coloured fluid were more common.

Conclusion: Benign effusions are common in younger whereas malignant effusions are common in older people. A combined approach to morphology by may-grunwald giemsa, papanicolaou with haematoxylin and eosin stain was better than individual method. Recurrent hemorrhagic effusions are more common in malignant effusions. Conventional smear method can yield good result. Scattered cells are indicative of benign effusions and clusters, 3 D balls, papillary patterns indicate malignant effusions.

Key-Words: Haematoxylin and Eosin; Papanicolaou; May Grunwald Giemsa; Serous Fluid Examination

Introduction

There are three major cavities in the body: pleural, pericardial, and peritoneal, which are lined by mesothelial cell resting on submesothelial stromal matrix tissue containing serous fluid which lubricates the membranes. However, in pathologic states, the serous cavities develop spontaneous effusions due to various causes. This provides a clinically useful specimen for cytological evaluation to diagnose the underlying pathologic process, such as infections, inflammation, neoplasia, etc.[1] Also in many patients the serosal membranes (usually abdominal/pelvic) are lavaged with saline and submitted for cytological analysis for better defining the clinical stage in the patient if malignant cells are observed. Cytological diagnosis by examination of exfoliated cells in serous cavity effusions is one of the most challenging areas in clinical cytopathology. Almost 20% of the effusions examined are directly or indirectly related to the presence of malignant disease, with carcinoma of the lung as the most common underlying culprit.[1] Cytological examination of a serous effusion is important for the diagnosis of cancer, for staging and prognosis of the patient. With the exception of cerebrospinal fluid, in no other type of cytological specimen does the finding of exfoliated cancer cells have such ominous prognostic significance. Cytological examination of cavity fluids may also reveal information about inflammatory conditions of the serous membranes, parasitic infestations, and infection with bacteria, fungi, or viruses. It can also supply evidence of the presence of a fistulous connection with a serous cavity.[2] Cytological examination of effusions is better than biopsy of the serous cavity lining for the diagnosis of malignancy affecting any of the cavities as focal lesions on a serous surface may be missed by biopsy. This leads to false negative results. But in

an effusion, malignant cells exfoliate and accumulate from all surfaces lining that cavity which representation the entire serous cavity. Also effusions are relatively simple to collect. The rate of detection of cancer cells is increased further if multiple effusion specimens are evaluated consecutively.[3]

Materials and Methods

The present study was performed to find the significance of serous fluid cytology in the diagnosis of various neoplastic and non-neoplastic lesions, in the cytology section, department of pathology, govt. medical college, new civil hospital, Surat. Total 355 cases were studied from June 2010 to October 2012. All cases of neoplastic & non neoplastic diseases with serous effusions from body cavity comprising of pleural, pericardial and peritoneal fluid were included and all body fluids other than pleural, pericardial and peritoneal fluid were excluded. There were 186 cases with pleural effusion, 164 cases of peritoneal fluid and 5 cases with pericardial effusion. Their detailed clinical history was noted down in prepared proforma. Other parameters like findings of thorough clinical examination, routine blood investigation, relevant radiographic findings, levels of various marker levels like (ADA, CA 125, CEA & other serum marker levels) were noted for correlation and final diagnosis. Collection is performed with a wide-bore needle, such as an 18-gauge needle, inserted under local anaesthesia and sterile conditions through the body wall into the serous cavity in its most dependent location. The specimen is collected in a clean, dry, large container. Peritoneal or pelvic washing/lavage for staging of gynecological cancers or evaluating the spread of pancreatic and gastric cancers were performed by instilling physiological saline solution into the peritoneal cavity, then withdrawing the fluid for cytopatho-logical evaluation. Although these specimens are processed just like serous effusions, their interpretation criteria need to be modified. The samples of serous effusions received in the rubber stoppered labelled glass bottles, sterile containers, as well as in properly closed large jars in case of large volumes with properly filled requisition forms. In case of delay in submitting after collection beyond working hours, samples were received and stored in refrigerator at temperature of 2-6 ˚C. Sample volume ranged from 5 ml to 2000 ml. Samples were processed by routine conventional smear technique and or cytospin method.

In the current study, freshly tapped specimens without addition of anticoagulant were used for preparing smears. In case of haemorrhagic fluid 0.1 ml of glacial acetic acid was added to haemolyze red blood cells. Ether alcohol was used (wet fixed) for fixing smears as well as air dried smears were used similar to others.[4-8] Haematoxylin and eosin stain, papanicolaou stain and may grunwald giemsa stain were done. Pap staining was helpful in evaluating nuclear chromatin characteristics.

Results

In the present study, total 355 samples were studied. 186 were of pleural fluid, 164 were of peritoneal fluid and 5 were of pericardial fluid. The maximum number of cases were in the age group of 41-50 years (26.19%) while minimum number of cases were in age group of 0-10 Years (0.56%). The range of age of patients was from 9 to 80 years with median age of 44.5 years. The male to female ratio was 1.38: 1.

Table-1: Age Incidence of Cases with Pleural, Pericardial & Peritoneal Effusion

Age (years)	Pleural Effusion	Peritoneal Effusion	Pericardial Effusion
0-10	00 (0.00)	02 (1.22)	00 (0.00)
11-20	14 (7.53)	07 (4.27)	00 (0.00)
21-30	32 (17.20)	24 (14.63)	02 (40.00)
31-40	34 (18.28)	31 (18.90)	01 (20.00)
41-50	45 (24.19)	46 (28.05)	02 (40.00)
51-60	26 (13.9)	35 (21.34)	00 (0.00)
61-70	28 (15.06)	12 (7.32)	00 (0.00)
71 onwards	07 (3.76)	07 (4.27)	00 (0.00)
Total	**186 (100)**	**164 (100)**	**5 (100)**
M:F	**110:76**	**37:127**	**3:2**

Table-2: Cytological Findings in Cases with Pleural, Peritoneal & Pericardial Effusion

Cytological Examination	Pleural Effusion	Peritoneal Effusion	Pericardial Effusion
Benign	157 (84.41)	126 (76.82)	05 (100)
M:F	97:60	28:98	3:2
Malignant	11 (5.91)	13 (7.93)	00 (0.00)
M:F	8:3	1:12	0:0
Suspicious	10 (5.38)	07 (4.27)	00 (0.00)
M:F	2:8	2:5	0:0
Degenerated	08 (4.30)	18 (10.98)	00 (0.00)
M:F	3:5	6:12	0:0
Total	**186 (100)**	**164 (100)**	**5 (100)**

Table-3: Cellularity of Effusions as Seen in Conventional Smear

Cellularity	Type of Fluid					
	Pleural Fluid		Peritoneal Fluid		Pericardial Fluid	
	B	M / S	B	M / S	B	M / S
Low	47	4	43	00	1	0
Adequate	107	10	80	11	2	0
High	3	7	3	9	2	0

B: Benign; M: Malignant; S: Suspicious

Table-4: Microscopic Architectural Pattern of Cells in Effusions in Conventional Smear

Architectural Patterns	Type of Fluid					
	Pleural Fluid		Peritoneal Fluid		Pericardial Fluid	
	B	M / S	B	M / S	B	M / S
Singly scattered	156	8	81	2	4	0
Sheets	1	0	44	6	1	0
Cell clusters	0	4	1	12	0	0
3D balls, Groups, Papillae	0	9	0	0	0	0

B: Benign; M: Malignant; S: Suspicious

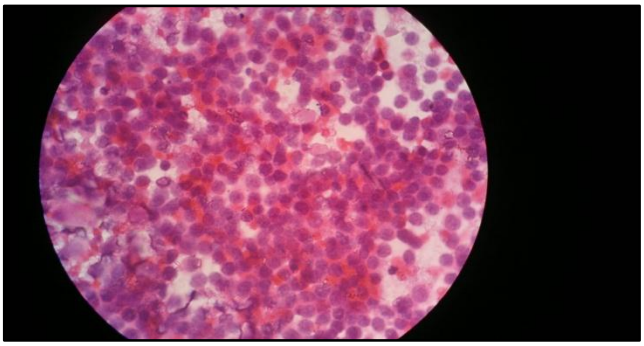

Figure-1: Malignant Effusion in a Case of Lymphoma (H & E)

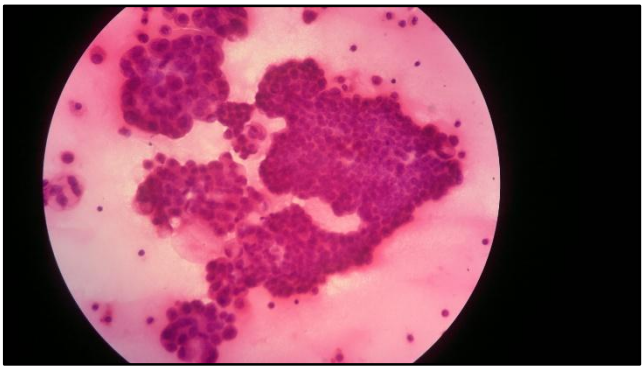

Figure-2: Malignant Effusion showing Sheet & Clusters (H & E)

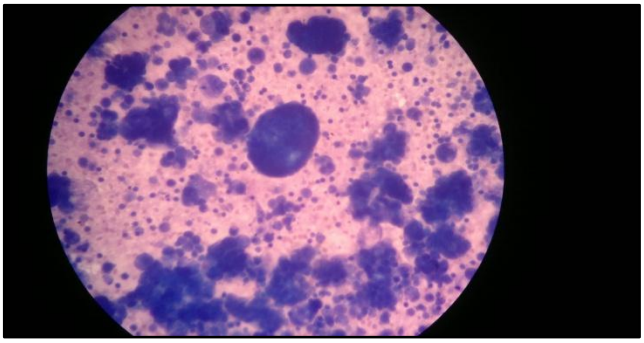

Figure-3: Malignant Effusion showing Proliferation Sphere (3 D Ball) & Clusters (May Grunwald Giemsa)

In pleural fluid maximum numbers of patients were 45 (24.19%) in age group of 41-50 years, followed by 34 (18.28%) patients in the age group 31-40 years and the M: F ratio was 1.4:1. In ascitic fluid maximum number of patients were 46 (28.05%) in age group of 41-50 years, followed by 35 (21.34%) patients in the age group 51-60 years and the M:F ratio was 1:3.4. In pericardial fluid, total number of cases were 5, 2 cases each in the age group of 21-30 and 41-50 years, 1 case in the age group of 31-40 years and the M:F ratio was 3:2.

Naked eye examination of pleural, pericardial & peritoneal fluid (By colour) showed that 37 samples of pleural fluid were straw coloured, 60 were yellow, 83 were hemorrhagic, 05 were white and a single case was chylous and (by appearance) 90 samples were clear and 96 samples were turbid. In ascitic fluid, 29 samples were straw coloured, 49 were yellow, 84 were hemorrhagic and 2 were white and by (appearance) 67 samples were clear and 97 samples were turbid. In pericardial fluid, 3 were yellow and 2 were hemorrhagic and by (appearance) 3 samples were clear and 2 samples were turbid.

On routine examination of pleural, peritoneal & pericardial fluid, it was observed that in pleural fluid, out of total 186 cases, 157 were transudate and 29 were exudate. In peritoneal fluid, out of total 164 cases, 132 cases were of transudate and 32 were of exudate. In pericardial fluid, all 5 cases were transudate.

Cytological findings showed that out of total 355 cases, 288 cases were benign, 24 cases were of malignant effusion, 17 cases were suspicious of malignancy and 26 cases were degenerated.

It was observed that in Pleural effusion: 157 cases were benign, 11 cases were of malignant effusion, 10 cases were suspicious of malignancy and 8 cases were degenerated. In cases of peritoneal effusion 126 cases were benign, 13 cases were of malignant effusion, 7 cases were suspicious of malignancy and 18 cases were degenerated. In cases of pericardial effusion all 5 cases were benign. Table shows that maximum number of males is having lesions involving lung. So, pleural effusion is most common. Also, females and males are having almost equal chances of pericardial effusion.

Data from the table four indicates that in pleural fluid ,156 cases having singly scattered cells were diagnosed as benign, only 8 cases were malignant/suspicious having singly scattered cells. One case was benign having sheet of cells. Cell clusters in 4 cases were malignant/suspicious lesion. 3d balls, groups, papillae in 9 cases indicated malignant/suspicious lesion. Peritoneal fluid showed 81 cases having singly scattered cells diagnosed as benign, only 2 cases were malignant/suspicious having singly scattered cells. 44 cases were benign and 6 cases diagnosed as malignant/suspicious lesion cytologically showed sheets of cells. Cell clusters in 12 cases were malignant/suspicious lesion and in 1 case it was benign. 3 D balls, groups, papillae were not observed. Pericardial fluid shows 4 cases having singly scattered cells diagnosed as benign and Sheet of cells seen in 1 case was benign. About 79% of samples were processed by conventional smear technique alone, 1% by Cytospin alone and rest 20% were processed by both methods.

Discussion

Serous effusion cytology is an important investigation in patients with underlying diseases. Serous fluid examination with correlation of various parameters like clinical history and examination, different serum marker levels, primary malignancy if present and previous cytological diagnosis are very useful for the final diagnosis. Examination of effusion cytology is tricky as morphology of reactive mesothelial cells and malignant cells closely resembles. The distinction between reactive mesothelial cells and malignant cells in cytological smears of serous effusion is a frequent cause of diagnostic difficulty. The present study was undertaken to study the significance of fluid cytology in the diagnosis of various neoplastic and non-neoplastic lesions.

In the current study, most of malignancy was detected on evaluating the first specimen similar to other study. [9] In case of suspicious samples, repeat examination was proved to be positive. This would have probably happened due to delay in receiving the samples and poor preservation in the ward.

The most common effusion was pleural followed by peritoneal and pericardial effusion. The most common site of effusion in the study by Sherwani R et al.[10] was peritoneal followed by pleural and pericardial effusions. This difference may be due to more no. of patient with lung diseases having effusion in current study.

In pleural fluid, 186 cases were studied and maximum numbers of patients were seen in above 3rd decade. Pleural effusion is very common in age group between 30-60 years and the results of present study are comparable with others.[11-13] M: F ratio was 1.4:1, showing higher incidence of pleural effusion occurring in males similar to others.[11,13,14]

In peritoneal fluid, out of 164 patients, maximum numbers were above 3rd decade similar to other studies.[12,15-18] M: F ratio was 1:3.4. This shows a higher incidence of peritoneal effusion in females as compared with males similar to others.[12,15-18] In pericardial fluid, 5 patients were studied and maximum numbers of cases were seen above 4th decade and M: F ratio is 3:2.

Differentiation of transudate and exudate by routine examination of fluid is mainly based on protein estimation of fluid (Transudate less than 3 gm % and Exudate, more than 3 gm %). In our study, out of total 186 cases of pleural effusion, 157 cases were Transudate in nature and 29 cases were exudate in nature. Out of total 64 cases of peritoneal effusion, 132 were Transudate in nature and 32 cases were exudate in nature. Boyer et al, Alexander et al and Garg et al showed similar findings.[12,17,19] All 5 cases of pericardial effusion were transudate in nature.

Current study was performed by conventional smear method & Cytospin method. From the current study it is apparent that conventional smear method is useful for all types of specimen with different cellularity. Cytospin method allows concentrating the scanty cellular sample but not quite useful for highly cellular specimen in which it may led to confusion in interpretation.

Conclusion

Freshly collected samples should be examined as soon as possible without delay as it reduces the

chances of accurate diagnosis. Use of preservatives is not recommended in place of fresh sample analysis. Diagnosis of malignant as well as benign conditions like tuberculosis can be possible. Benign effusions are more common in younger whereas malignant effusions are more common in older people. A combined approach to the morphologic features by may grunwald giemsa and pap with haematoxylin and eosin stain method was found to be better than using one of these methods alone.

Recurrent hemorrhagic effusions are more common in malignant effusions. Conventional smear method can yield good result in all types of effusion ranging from scanty cellular to highly cellular. For highly cellular smears, it is more helpful than cytospin method. Cytospin method is better for scanty cellular smears than conventional smear method. Scattered cells are more indicative of benign effusions with exception like malignant effusions from lymphoma. Clusters, 3 d balls, papillary patterns are more indicative of malignant effusions. Other methods like cell block and immunocytochemistry may improve diagnostic outcome further in cases of serous effusion cytology.

References

1. Ali SZ, Cibas ES. Serous cavity fluid and cerebrospinal fluid cytopathology. New York: Springer. 2012.
2. Bibbo M, Wilbur DC. Comprehensive cytopathology 3rd edi. Philadelphia, PA: Elsevier Saunders; 2008.
3. Shidham VB, Atkinson BF. Cytopathologic diagnosis of serous fluids. 1st edi. London: Elsevier (W. B. Saunders Company); 2007.
4. DiBonito L, Falconieri G, Colautti I, Bonifacio D, Dudine S. The positive peritoneal effusion. A retrospective study of cytopathologic diagnoses with autopsy confirmation. Acta Cytol. 1993;37(4):483-8.
5. Sears D, Hadju SI. The cytologic diagnosis of malignant neoplasms in pleural and peritoneal effusions. Acta Cytol. 1987;31(2):85-97.
6. Sherwani R, Akhtar K, Naqvi AH, Akhtar S, Abrani A, Bergava R. Diagnostic and prognostic significance of cytology in effusions. Journal of cytology 2005;22(2):73-7.
7. Cordozo PL. A critical evaluation of 3000 cytologic analysis of pleural fluid,ascitic fluid and pericardial fluid. Acta Cytol. 1966;10(6):455-60.
8. Johnson WD. The cytological diagnosis of cancer in serous effusion. Acta Cytol. 1966;10(3):161-72.
9. Spieler P, Gloor F. Identification of types and primary sites of malignant tumors by examination of exfoliated tumor cells in serous fluids. Comparison with the diagnostic accuracy on small histologic biopsies. Acta Cytol. 1985;29(5):753-67.
10. Irani DR, Underwood RD, Johnson EH, Greenberg SD. Malignant pleural effusion- A clinicopathologic study. Arch Intern Med. 1987;147:1133-6.
11. Gerbes AL, Jüngst D, Xie YN, Permanetter W, Paumgartner G. Ascitic fluid analysis for the differentiation of malignancy-related and nonmalignant ascites. Proposal of a diagnostic sequence. Cancer. 1991;68(8):1808-14.
12. Ram KN, Sing RS. Diagnostic value of cholesterol in pleural effusion. J Assoc Physicians India. 1995;43(11):748-50.
13. Hymen S, Villa F, Steigmann F. Mimetic aspects of ascites. JAMA. 1963;183(8):651-5.
14. Gil Suay V, Martínez Moragón E, Cases Viedma E, Perpiñá Tordera M, León Fábregas M, Sanchis Aldás J. Pleural cholesterol in differentiating transudates and exudates. A prospective study of 232 cases. Respiration1995;62:57–63.
15. Singh MM, Bhargava AN, Jain KP. Tuberculous peritonitis—an evaluation of pathogenetic mechanisms, diagnostic procedures and therapeutic measures. N Engl J Med. 1969;281(20):1091-4.
16. Garg R, Sood A, Arora S, Bhatia KL, Chawla AS, Gupta R, et al. Ascitic fluid cholesterol in differential diagnosis of ascites. J Assoc Physicians India. 1993;41(10):644-6.
17. Valdés L, Pose A, Suàrez J, Gonzalez-Juanatey JR, Sarandeses A, San José E, et al. Cholestrol: a useful parameter for distinguishing between pleural transudates and exudates. Chest. 1991;99(5):1097-102.
18. Shidham VB, Epple J. Appendix I: Collection and processing of effusion fluids for cytopathologic evaluation. In: Shidham VB, Atkinson BF, (edi). Cytopathologic Diagnosis of Serous Fluids. 1st edn. London: Elsevier; 2007. p. 207-35.
19. Bakalos D, Constantakis N, Tsicricas T. Recognition of malignant cells in pleural and peritoneal effusions. Acta Cytol. 1974;18(2):118-21.

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