IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 15, Issue 11 Ver.VI (November. 2016), PP 73-77
www.iosrjournals.org

Cytomorphological Study of Body Cavity Fluids in Disease: Conventional Cytology Versus Cell Block

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Abstract
Background: *Exfoliative cytology is a cost-effective, rapid and highly efficient tool for the evaluation of body fluids and should be advised in all effusion cases.*
Aim: *To evaluate the diagnostic utility of cell block versus conventional smear study and to ascertain the primary site of malignancy wherever possible.*
Material and Methods: *All body cavity fluid samples - pleural fluid, ascitic fluid, pericardial fluid and synovial fluid were considered. The samples were processed by routine smear using PAP and MGG stain, and cell block in all cases. For all the cases suspicious of malignancy, PAS stain with and without diastase and Alcian blue stain were done.*
Results: *A total of 100 cases were analyzed. Most of the cases were seen in the age group of 41-50 years, with male to female ratio of 2.7:1. Most common type of fluid was pleural (43%). Fluids were predominantly exudates (74%). On cytological examination of conventional smear (CS) 7% were malignant, while on cell block (CB) evaluation, 17% malignant thereby increasing yield of malignancy by 10%.*
Conclusion: *Fluid analysis and cytology should continue to be the first line of investigation to screen out the malignant and suspicious for malignancy effusion cases. CB method should be incorporated as a complementary tool for improving cytodiagnosis of effusions.*
Keywords: *Cytology, Cell block, Fluids, Pleural, Ascitic, Pericardial, Synovial*

I. Introduction

Exfoliative cytology is a cost-effective, rapid and highly efficient tool for the evaluation of body fluids and should be advised in all effusion cases. The information provided by body fluid analysis serves several functions as it assists the clinician in formulating, in order of priority, a list of differential diagnosis and also allows one to follow the result of therapy.[1]

Accumulation of fluid in the body cavities is a frequent complication of malignant tumours and may be the common presenting sign of malignancy. Its investigation is extremely important since it may give information about the cause, presence of metastatic cells, typing of unknown cases, and the primary tumour sites when unknown or detection of possible recurrence of malignancy in follow-up patients.[2]

Diagnostic problems arise in everyday practice to differentiate reactive or atypical mesothelial cells from malignant cells by the routine conventional smear method. The cytological examination of fluids by means of smears, even though carefully prepared, leaves behind a large amount of residual fluid, that is not further investigated but that might contain valuable diagnostic material. This residual material can be evaluated by treating it as cell block in a simple and expedient fashion, and examined in addition to the routine smears.[3]

The cell block technique will increase the positive results, and will help to demonstrate morphological details by preserving the architectural patterns, which could be of great help in making correct diagnosis of the primary site. The cell block technique has an added advantage that multiple sections of the same material can be obtained for special stains.[3]

II. Materials And Methods

The present study was conducted on 100 cases of serous effusion- pleural, ascitic, pericardial and synovial after obtaining detailed clinical information with regards to age, sex, history, investigations, provisional diagnosis. Fluids thus obtained were first examined by naked eye for physical characteristics and then divided into two halves. Half of the specimen was centrifuged at 2000 rpm for 10 mins. The supernatant fluid was pipetted out and the sediment was transferred to two slides. One was air dried and stained with MGG, the other was fixed in 95% alcohol for a minimum period of 15mins and stained with Papanicolaou stain. For all cases biochemical analysis of protein, sugar and chloride was done.

The other half of the fluid specimen was fixed in a solution of alcohol: formalin (9 parts of 90% alcohol and 1 part of 7.5% formalin) for one hour. After fixation, the specimen was centrifuged at 2500 rpm for

10-15mins. The supernatant was poured off and a further 3ml of fresh alcohol-formalin was once again added to the sediment and kept for one day. Next day the sediment was completely drained off by inverting the tube over Whatman filter paper. The sediment was then wrapped in the same filter paper and processed in histokinette as part of routine paraffin section histopathology. Special stains, including PAS and Alcian blue, were done whenever needed.

A cytological and cell block diagnosis was rendered for each case seen and each individual slide was objectively analysed for cellularity, arrangement (acini, papillae, cell balls, and proliferation spheres), cytoplasmic, and nuclear details.

Data regarding various etiologies of effusion was collected and analysed using statistical tools. Chi-square test was used to find association between spectrum of lesions. SPSS was used for statistical analysis.

III. Results

A total of 100 cases were analyzed. Age ranged from 2-85 years and most of the cases were seen in the age group of 41-50 years (26%) followed by 51-60 years (25%). The least age groups affected were 0-10 years and 11-20 years (2% each). Though male preponderance with male to female ratio 2.7:1 was noted, yet malignant effusions were more common in females.

Majority of the cases were pleural (43%) followed by ascitic fluid (40%). Exudates were more common (76%) than transudates. Exudates were commonly caused by infection, TB and malignancy while transudates were due to chronic liver disease, chronic obstructive pulmonary disease and renal failure.

Cell blocks (CBs) showed preservation of architectural patterns (three dimensional clusters, cell balls, acinar structures and papillary fragments) and better nuclear morphology. CBs also provided enough material for special stains like PAS and Alcian blue.

CSs and CB sections were categorized separately and compared. On CS 83% were benign, 10% were suspicious for malignancy and 7% were positive for malignancy. Out of the 83% cases (83 cases) which were benign on CS, 5 cases proved to be malignant on CB. Out of the 10% cases (10 cases) suspicious for malignancy, 5 cases turned out to be malignant, 4 benign and 1 remained suspicious on subsequent CB. All the 7% cases which were malignant on CS remained so on CB.

On CB 17% cases were positive for malignancy, 1% suspicious and rest benign. CB increased the yield of malignancy by 10% (10 cases). Out of these 10 cases, 5 were suspicious on CS and 5 were benign but turned out to be malignant on CB.

CB showed a sensitivity of 76.5%, specificity of 94%, PPV of 72.2% and NPV of 95.1%. Agreement between the two methods was 91.0 with kappa value of 0.688. Overall the result was highly significant with p value 0.0001.

IV. Discussion

Serous effusion cytology is well documented and accepted as a complete diagnostic modality, to the extent that a positive diagnosis is considered definitive and obviates the need of explorative surgery.[2,4] This is due to the fact that the cells present in body cavity fluids represent a much larger surface than that obtained by needle biopsy.[5] Cytology of body fluid helps to differentiate the causes of effusion including malignancy and also to type the tumour cells in case of unknown primary malignant site.[2] In patients with known malignancy, malignant effusion has prognostic implications which need change in treatment.[2,5] At times, effusion cytology can help in determining the cause of non-neoplastic effusion e.g. certain infectious diseases or in inflammatory conditions like systemic lupus erythematosus, rheumatoid arthritis.[2]

More definitive cytopathological diagnosis can be established by preparing cell block from the residual tissue fluid.[5] This technique is simple, safe, reproducible and cost-effective.[5] Use of cell block increases cellularity with better appreciation of cellular morphology, nuclear and cytoplasmic details and less background staining. It can additionally be used for performing special stains and immunohistochemistry at a later date.[4,5]

In the present study, body cavity effusion was found in age range of 2-85 years. Other studies such as Kumavat et al (2013) had 550 cases in the age range of 1-89 years,[6] Hathila et al (2013) had 355 cases in the age range of 9-80 years.[7]

Serous effusion was observed more in males (73.0%) than in females (27.0%), with a ratio of 2.7:1 which was comparable to the study of Kumavat et al (2013).[6] In our study, majority were pleural fluids (43%) followed by peritoneal fluid samples (40%). Similar findings were noted by Hathila et al (2013)[7], Kumavat et al (2013)[6] and Bhanvadia et al (2014).[5] Few other studies had ascitic fluid as the commonest fluid e.g. Pradhan et al (2006).[2]

Differentiation of transudate and exudate by routine examination of fluid is mainly based on protein estimation and cell count of fluid (Protein >3g% and fluid cell count >1000 cells/cu mm is exudate).[8] In our study, out of total 43 cases of pleural effusion, 34 cases were exudate and 9 were transudate. Kumavat et al (2013) showed similar findings.[6]

In our study on conventional smear examination, 83% cases were benign, 10% were suspicious for malignancy, 7% were positive for malignancy. Out of 83% (83 cases) benign, 38 cases were of pleural effusion, 29 ascitic, 14 synovial and 2 pericardial effusion. Out of 7% (7cases) malignant effusions, 5 cases were of ascitic and 2 of pleural effusion. These findings are concordant with the studies of Shiramba et al (2012),[9] Hathila et al (2013)[7] and Bhanvadia et al (2014)[5] as seen in TABLE 1.

In our study, CB by alcohol-formalin fixation method was attempted on all pleural, ascitic, pericardial and synovial fluids, irrespective of the volume. All the fluids in which precipitate could be obtained, by addition of buffered alcohol-formalin, were further processed for cell block. In malignant and suppurative cases (exudative), CB yielded enough material to come to a conclusion even in fluids with volume <10 ml probably due to high cellularity. But some fluids in which precipitate was not formed, probably due to low cellularity, were excluded. CB confirmed/established diagnosis in 99% cases, while it was suspicious of malignancy in 1% cases.

After the study with cell block method, 4 cases suspicious on cytology turned out to be benign on cell block and 1 case suspicious on cytology remained so on cell block. 5 cases which were suspicious for malignancy and 5 benign on cytology turned out to be malignant on cell block. Thus, our study showed additional yield of malignancy by 10%. This result was similar to the study of Bhanvadia et al (2014).[5] (TABLE 2)

Other studies which have shown additional cases of malignancy on CB- increasing the diagnostic yield were Dekker et al (1978)- 38%,[10] Pal et al (2015)- 24%,[11] Joshi et al (2014)- 13.3%,[4] Bodele et al (2003)- 7%,[12] Thapar et al (2009)- 5.3%[3] and Gayathri et al (2014)- 2%.[13]

In our study, 1 case of tuberculous pleural effusion showed granuloma on CB. Well formed granulomas in peritoneal effusions have been demonstrated by Shobha et al (2013)[14] and have been attributed to tuberculosis, but they have not confirmed these cases by culture or ZN staining, as in our study.

In our study, we noted better morphological details on CB such as preservation of architectural patterns like three dimensional clusters, presence of cell balls, acini, papillary fragments and better cytoplasmic features. Nuclear morphology was better appreciated in CB when compared to CS. Background staining of RBCs was immensely reduced in cell block compared to CS and making inflammatory and malignant cell features better appreciable. Similar findings were noted in various studies.[3,12,15]

The overall efficacy of CBs in our study showed a sensitivity of 76.5%, specificity of 94%, PPV of 72.2% and NPV of 95.1%. Other than bronchial lavage and peritoneal wash, all pleural, ascitic, pericardial and synovial fluids irrespective of the volume, were subjected to the CB procedure. Various authors have suggested various advantages of CB over CS in effusions. In our study, among the benign fluids (82 cases), CBs showed low sensitivity, but high specificity and negative predictive value.

The diagnosis of malignany in effusion is important clinically as it indicates advanced disease with poor prognosis and helps in deciding further management. In our study, 17 out of 100 cases were positive for malignancy. In 16 cases, histological typing was possible. 13 cases were of adenocarcinoma (76.4%), 2 cases were of malignant mesothelioma (1 ascitic and 1 pleural) and one case of ascitic effusion was of Chronic lymphocytic leukemia (CLL). This was in concordance with other studies of Pradhan et al (2006)[2], Gupta et al (2012)[8] and Shiramba et al (2012)[9], as all had adenocarcinoma as the most common subtype with few cases of mesothelioma and lymphoma/leukemia.

Khan et al (2005) used cytospin and cell block technique to study 58 malignant effusions and determined primary site by correlating clinical features, radiological and cytological features with 89.7% accuracy. In remaining 10.3% cases cytologic features were inconclusive and primary site could not be associated with malignant effusion. In their study, the most common primary site was lung (29.3%), followed

In our study, out of 13 cases diagnosed as adenocarcinoma on CS and CB, primary could be determined in 9 cases (69.2%), while in 4 cases (30.8%) primary was unknown. Ovaries were the most common primary site of malignancy (4 cases), followed by lung (3 cases). One case had primary in colon and 1 in endometrium. This was in concordance with the study of Gupta et al (2012).[17]

V. Conclusion

In developing countries like India, where health facilities are inadequate, and cost of investigations and management is often unaffordable, fluid analysis and cytology should continue to be the first line of investigation to screen out the malignant and suspicious for malignancy effusion cases. CB method, by alcohol-formalin fixation method, should be incorporated as a complementary tool for improving cytodiagnosis of effusions. CBs are more useful in diagnosis of malignancy by better preserved architectural patterns and thus bridging the gap between cytology and histopathology.

Definite diagnosis of serous effusion can be accomplished cytopathologically in the majority of cases and is very important to clinician and surgeon for staging and further management of patients.

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Figures And Tables

Table 1: Comparison of cytological diagnosis in various studies

Site of effusion	Cytological diagnosis	Bhanvadia et al (2014)[5] %	Hathila et al (2013)[7] %	Shiramba et al (2012)[9] %	Present study %
Ascitic	Benign	35.4	35.8	42.4	29.0
	Malignant	5.3	3.6	7.3	5.0
	Suspicious	5.3	2.0	-	6.0
	Degenerated	-	5.0	-	-
Pleural	Benign	40.7	44.2	39.1	38.0
	Malignant	6.7	3.0	9.9	2.0
	Suspicious	5.3	2.8	-	3.0
	Degenerated	-	2.2	-	-
Pericardial	Benign	1.3	1.4	1.3	2.0
	Malignant	-	-	-	-
	Suspicious	-	-	-	1.0
	Degenerated	-	-	-	-
Synovial	Benign	-	-	-	14.0

Table 2: Analysis of additional yield of malignancy on cell block in various studies

Sl no	Authors	Additional yield %
1	Thapar et al (2009)[3]	5.3
2	Joshi et al (2014)[4]	13.3
3	Bhanvadia et al (2014)[5]	10.0
4	Dekker et al (1978)[10]	38
5	Pal et al (2015)[11]	24
6	Bodele et al (2003)[12]	7.0

7	Gayathri et al (2014)[13]	2.0
8	Present study	10.0

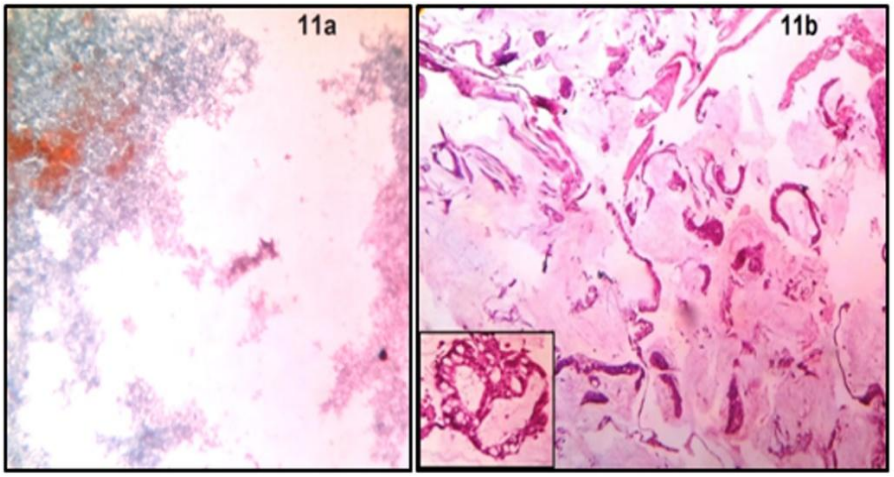

Figure 1- Ascitic fluid- Conventional smear(left)- Photomicrograph showing necrotic material with occasional cells (PAP stain 400X)

Ascitic fluid- Cell block(right)- Photomicrograph showing pseudomyxoma peritonei. Inset: showing acinar arrangement of tumour cells (H&E 100X; inset H&E 400X)

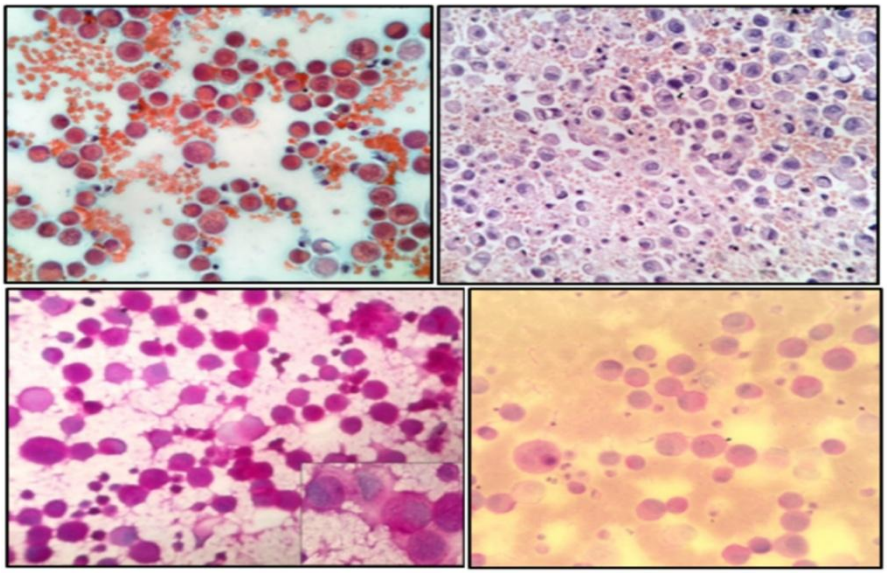

Figure 2- Pleural fluid- Conventional smear(top left)- Photomicrograph showing malignant cells (PAP stain 400X)

Cell block(top right)- Photomicrograph showing malignant mesothelioma (H&E 400X) Malignant mesothelial cells positive on PAS stain (bottom left) (PAS stain 100X; Inset- PAS stain 400X), and negative on PAS with diastase (bottom right), confirming diagnosis of malignant mesothelioma (PAS-D 400X)