DOI: 10.5958/j.2319-5886.3.2.060

International Journal of Medical Research & Health Sciences

www.ijmrhs.com Volume 3 Issue 2 (April - Jun) Coden: IJMRHS Copyright @2014 ISSN: 2319-5886

Received: 5th Jan 2014 Revised: 31st Jan 2014 Accepted: 4th Feb 2014

Research Article

THE DIAGNOSTIC UTILITY OF CELL BLOCK AS AN ADJUNCT TO CYTOLOGICAL SMEARS

*Bhavana Grandhi, Vissa Shanthi, Mohan Rao N, Chidananda Reddy V, Venkata Murali Mohan K

Department of Pathology, Narayana Medical College, Nellore, Dr. NTR University of Health Sciences, Vijayawada, Andhra Pradesh

*Corresponding author email: drbhavana.grandhi@gmail.com

ABSTRACT

Objectives: Cytological examination of serous fluids is of paramount importance in detecting cancer cells. Distinguishing malignant cells from benign reactive mesothelial cells in fluid cytology is an everyday diagnostic problem. Cell blocks are valuable when the features in cytology are inconclusive. The motive of this study was to assess the utility of this method in increasing the diagnostic yield of serous fluids. **Methods**: 225 (25%) effusion fluids were analyzed carefully by both smear and cell block technique. **Results**: Among 225 fluids, 139 were pleural, 84 peritoneal and 2 pericardial. In case of pleural fluids and ascitic fluids, maximum numbers of cases were inflammatory. By the cell block technique, 5 additional cases of malignancy in pleural fluids and 7 additional cases of malignancy in ascitic fluids were diagnosed which could not be detected in the cytological smears. In pericardial fluids both cases were inflammatory. Male predominance was noted in case of pleural effusion and female predominance was noted in case of pericardial effusion and ascites. Maximum numbers of cases were seen in the age group of 40-60 years. **Conclusion**: We conclude that the cell block technique when used as an adjuvant to routine smear examination has increased the diagnostic yield because of better preservation of the architectural pattern.

Keywords: Cytological smear, Cell block

INTRODUCTION

Cytological examination of serous fluids is of paramount importance not only in detecting cancer cells, but it also reveals information regarding various inflammatory conditions of serous membranes, various bacterial, viral, fungal infections and parasitic infestations.[1] The involvement of the serous cavities by malignant neoplasms has important therapeutic and prognostic implications. The most common reason to submit an effusion fluid to cytopathology is to determine whether or not it contains malignant cells.[2]

Reporting a cell as malignant or benign reactive mesothelial cell in fluid cytology is an everyday diagnostic problem. The cytological diagnosis of effusions has a lower sensitivity, which is attributed to benign morphology of cells and changes incurred during processing of these fluids. [1]

Cell blocks technique or paraffin embedding of sediments of fluids is almost the oldest methods of preparing material for microscopic examination.[1] Cell blocks are helpful in situations where the cytological abnormalities are ambigious like in reactive mesothelial cells or in occasional well differentiated adenocarcinoma.[3] Apart from increased cellularity, better morphological details are obtained by cell block method as there is a better conservation of architectural features like arrangement of cells , cytoplasmic and nuclear details. [1] Cell block method has many advantages like a number of sections for the same case can be made for further study like immunohistochemistry.[1] The cell block method is

one of the traditional method used for processing cytological material and was described in the literature as early as 1900.[4] For the purpose of fixation, 10% alcohol-formalin is used.The proteins are cross-linked and a gel is formed by the action of formalin, which can't be dissolved in any material used for processing.[5]The present study was done to valuate the utility of this method in increasing the diagnostic yield of serous fluids.

MATERIALS & METHODS

This study included 225 cases (effusion fluids were analyzed, out of which 139 were pleural, 84 were peritoneal and 2 were pericardial) from ASRAM Medical College Hospital, Eluru and Narayana Medical College Hospital, Nellore after obtaining approval by the Institutional ethics committee. Cases included patients who presented with complaints of ascites, pleural effusion or pericardial effusion. The patients were subjected to fluid analysis, by both smear and cell block technique.[5] The presenting clinical features and the laboratory findings were recorded. The fluid sample (ascitic, pleural or pericardial) was divided into two parts. Half of the fluid, about 5 ml was centrifuged, supernatant fluid discarded, smears prepared and stained with H&E and May-Grunwald-Giemsa. Papanicolaou and Leishman stains were used wherever necessary. The remaining sample was subjected to centrifugation at a rate of 1500 rpm. The supernatant fluid was discarded and the sediment or the cell button, thus obtained was fixed for 24hrs in 10% formal-alcohol (combination of ethyl alcohol and formalin) and then processed in a histokinette like a routine histopathology sample. The sections were stained with H&E and special stains like PAS and Mucicaramine were used wherever necessary. The slides were evaluated for cellularity, arrangement, cytoplasmic and nuclear details. A comparative evaluation of smear versus cell block technique was done.

RESULTS

225 effusion fluids were analysed, out of which 139 were pleural, 84 were peritoneal and 2 were pericardial. In a total of 225 fluids received, males were 116 (52%) and 109 (48%) were females. The male to female sex ratio is 1:1.06.The maximum numbers of cases were in the age group of 41-60 years, constituting 77 cases (35%) of the total cases and least common incidence is 0-10 years, constituting only 1 case (0.5%) (Table 1)

The pleural effusion cases were more in males i.e. 85 (61.15%) compared to females, 54 (38.5%) with male to female ratio of 1.57:1. The number of inflammatory cases were more i.e. 127 (91%) compared to malignancy being 12 (9%). Maximum numbers of cases were in the age group 41-50 years and the least number in the age group 0-10 years. (Table 2)

5 (3.60%) smears prepared from pleural fluid were unsatisfactory / suspicious on cytology, where malignancy was picked up by the cell block technique (Figure1) showing that the diagnostic yield is increased by cell block technique. (Table 3)

In ascitic fluid the number of inflammatory cases were more i.e. 69 (82.14%) compared to malignancy being 15 (17.85%) and female to male ratio is 1.54:1. The maximum number cases were in the age group of 51-60 years and the least number of cases in the age group of 71-80 years (Table 4).

7 (8.34%) smears prepared from ascitic fluid were unsatisfactory / suspicious on cytology, where malignancy was picked up by the cell block technique. (Fig 2, 3) (Table 5)

Table 1: Distribution of the sample by age, sex for all fluids

Age group	Pleural		Peritoneal		Pericardial		Total
	M	F	M	F	M	F	
0-10	1						1
11-20	3	3	3	2			11
21-30	19	5	2	2			28
31-40	16	8	5	6			35
41-50	17	12	8	15			52
51-60	11	9	6	27		2	55
61-70	13	10	3	2			28
71-80	9	4		3			16
Total	89	50	27	57		2	225

Table 2: Distribution of the sample by diagnosis and sex for pleural fluids

Diagnosis	Male (%)	Female (%)	Total (%)
Inflammatory	78(61.5%)	49(38.5%)	127(100%)
Malignancy	7(58.35%)	5(41.65%)	12(100%)
Total	85(61.15%)	54(38.85%)	139(100%)

Table 3: Comparison of smear versus cell block in pleural fluids

Category	Smear diagnosis	Cell block diagnosis
Inflammatory	127	127(including no cellularity)
Malignancy	7	7
Unsatisfactory/suspicious	5	5 (Positive for malignancy)
Total	139	139

Table 4: Distribution of the sample by diagnosis and sex for Ascitic fluids

Diagnosis	Male	Female	Total
Inflammatory	28(40.5%)	41(59.5%)	69(100%)
Malignancy	5(33.34%)	10(66.67%)	15(100%)
Total	33(39.28%)	51(60.72%)	84(100%)

Table 5: Comparison of smear versus cell block in Ascitic fluid

Category	Smear diagnosis	Cell block diagnosis
Inflammatory	69	69(including no cellularity)
Malignancy	8	8
Unsatisfactory/suspicious	7	7 (Positive for malignancy)
Total	84	84

In the pericardial effusion cases both were inflammatory and were females in the age group 51-60 years. One had predominantly mesothelial cells and the other had mixed inflammatory cells (Fig 4).

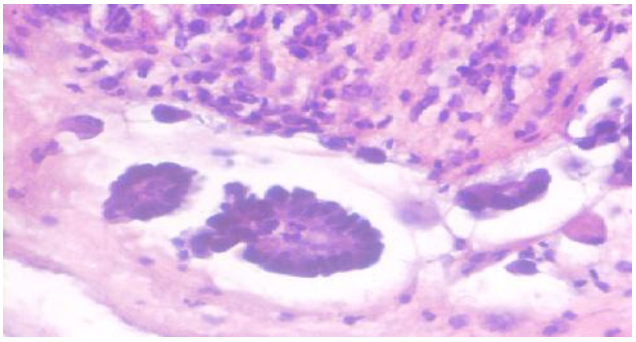

Fig 1: Cell block studied shows tumor cells arranged in acinar pattern; pleural fluid (H & E, 40 x)

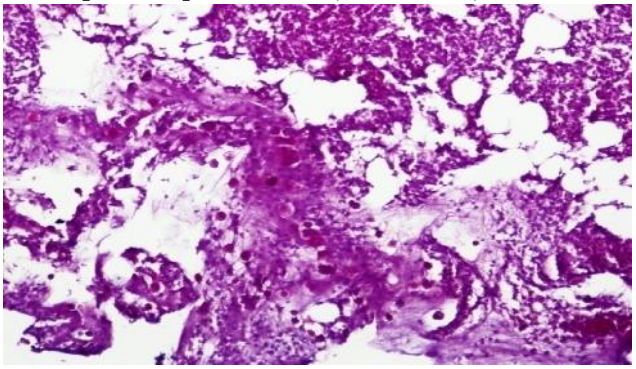

Fig 2: Cell block shows malignant cells arranged in cell balls;ascitic fluid(H&E;100x)

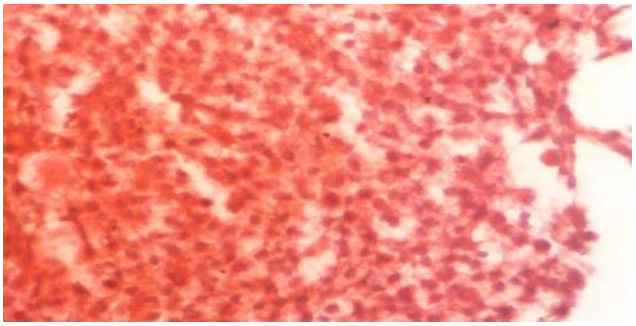

Fig 3: Cell block shows malignant cells; ascitic fluid (mucicaramine;40x)

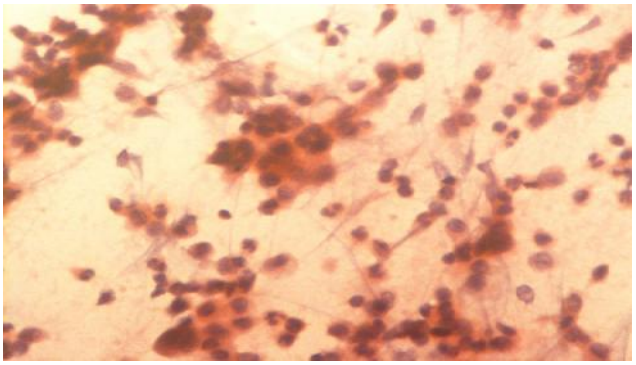

Fig 4: Smear shows mixed inflammatory infiltrate; pericardial fluid (Leishman stain; 100x)

DISCUSSION

The cell block method is the oldest method of processing cytological material ,described by Mandlebaum in 1900 for studying exudate.[4] 10% alcohol-formalin is used for fixation and by the action of formalin,the proteins are cross-linked and a gel is formed which can't be dissolved in any material used for processing.[5]

In the present study of 225 cases of cell block the predominant lesion detected in the various fluids was inflammatory 198 (88%) while malignancy was detected in 27 (12%) cases. The most common effusion was pleural, followed by peritoneal and pericardial effusion. Our results correlated with the studies done by Foot et al[6,7], van de Molengraft et al[8], Khan K et al[9] and Sears & Hajdu[10]. In our study the predominance of pleural fluids can be explained by the high prevalence of tuberculosis in the region of our study (Table 6).

Table 6: Distribution of the cases among various studies

Study done by	A(Pleural)	B(Ascitic)	C(Pericardial)	D(Others)	Total
Foot et al[7]	1301(64.12%)	700(34.5%)	28(1.4%)	-	2029(100%)
Van de Molengraft[8]	171(67.32%)	83(32.68%)	-	-	254(100%)
Khan K et al[9]	32(55.17%)	25(43.1%)	1(1.72%)	-	58(100%)
Sears & Hajdu[10]	1846(61%)	1165(39%)	-	-	3011(100%)
Present study	139(61.78%)	84(37.34%)	2(0.88%)		225(100%)

Table 7: Cellularity of smears: comparison of various studies

Inflammatory cases	Meenu[3] Thapar et al	Melamed[11] et al	Present study
Scanty cellularity	40(33.3%)	21(34%)	7(3.5%)
Predominantly neutrophils	26(21.7%)	13(21%)	43(21.7%)
Mixed inflammatory cells	24(20.0%)	11(18%)	40(20.2%)
Predominantly lymphocytes	16(13.3%)	8(13%)	62(31.3%)
Predominantly mesothelial cells	6(5.0%)	3(5%)	46(23.2%)
Blood		5(8%)	
Total	120(100%)	61(100%)	198(100%)

Table 8: Presentation of malignant ascites in various studies

Data	Archana[1] et al	Steven[9] A et al	van de Molengraft[8] et al	Present study
Clinical Presentation	Ascites	Ascites	Ascites	Ascites
Age group	51-60years	44-75 years	45-65	51-65years
Primary in males	Lung	Lung	Lung	Lung
Primary in females	Ovaries	FGT	Ovaries	Ovaries

Table 9: Age and sex distribution of malignant ascites in various studies

Parameter	Ringerberg[4] QS et al	Khan[3] K et al	Present study
Age group	30-95	41-60years	41-60years
Total	65	15	15
Females	40	15	10
Males	25	0	5
F:M ratio	2:1		2:1

Table 10: Comparison of the diagnostic yield of smear versus cell block in various studies

	Archana[1] et al	Sujathan[19] et al	Present study
Total cases	150	85	225
Inflammatory	77	63	183
Positive for malignancy on smear	29	19	12
Unsatisfactory/negative on smear	10	2	15
Positive for malignancy on cell block	39	21	27
No cellularity on cell block	34	1	7

Cellularity of smears revealed predominantly lymphocytes in 62 (31.3%) cases. In the studies done by Meenu et al[3] and Melamed et al[11]scanty cellularity was seen in 40 (33.3%) and 21 (34%) cases respectively (Table 7).

Leucocytes in pleural effusion are extremely common. In this study, typical pleural effusion caused by chronic inflammation had a high proportion of lymphocytes and very few or no mesothelial cells.

Koss describes that a characteristic feature of mesothelial cells is the flattening of the opposite cell membranes with the formation of clear gaps or "windows", which are most likely because of microvilli separating the cells and are better visualized in air dried smears.[12] Bedrossian insists that in benign mesothelial cells these microvilli are slender, bushy and distributed evenly around the cells whereas in adenocarcinoma, if present they are concentrated at the poles and are short and stubby.[13]

In our study, 23.23% cases (46 cases) of inflammatory effusion had a predominance of mesothelial cells. Mesothelial cells appeared round and had a single central or eccentric nucleus. Some of the groups of mesothelial cells were showing clefts or windows. These mesothelial cells form cell balls, clusters and sometimes take a signet-ring cell appearance thus closely mimicking malignancy. Malignant cells have irregular nuclear membranes, nuclear molding and prominent nucleoli with absence of windows.

In our study, the most common clinical presentation in malignancy was ascites and the commonest site of the primary giving rise to effusion, was ovaries in females and lung in males. 37% of cases were seen in the age group of 41-60 years. The large number in this age group can be attributed to increased incidence of ovarian malignancies (Table 8)

Malignant ascites as the initial evidence of cancer is more likely to occur in women. In the study done by Khan K et al none of the 10 patients were males[3] and in the study done by Ringerberg QS et al maximum number of cases were females (40 cases) when compared to males (25 cases).[4]

In the present study, 15 cases of malignant peritoneal fluid were diagnosed, in which 10 were women and 5 were men with female to male ratio 2:1. The most common age group was (41-60 years) with a median age of 51 years (Table 9)

The cell block is a helpful tool in the interpretation of Grade I adenocarcinoma. These tumors have very few malignant characteristics in smears, while the presence of true acini in the cell block, together with mucin, when stained for PAS is indicative of malignancy.[14]

The cells of adenocarcinoma closely mimic reactive mesothelial cells and the cells of malignant mesothelioma. The typical carcinomatous cells in the cell block are of variable sizes, exhibit nuclear pleomorphism with overlapping of nuclei, prominent nucleoli, occasional multinucleated cells and intracytoplasmic vacuoles. Tumor cells form gland-like or tubular structures with central lumina also referred by some as spheroids or hollow spheres.3-dimensional clusters and complex papillary clusters are also seen. The individual cells have moderate amount of cytoplasm with hyperchromatic and pleomorphic nuclei. The nuclei show granularity of the chromatin, prominent nucleoli and abnormal mitoses.[1]

Cell blocks have a number of advantages as they can be utilized for immunohistochemistry. First, at least ten sections can be cut which usually permits evaluation of a large number of antigens. The storage of cell blocks is easier compared to the smears. The use of cell block sections enables the worker to know in advance the exact nature of tissue available for study. It thus appears that cell blocks have much to offer in the utilization of immunocytochemistry.[15]

In general, Calretinin, CK 5/6, WT1, and Podoplanin are considered to be the best positive mesothelioma tissue markers and CEA, MOC-31, B72.3, and Ber-EP4 the best negative markers for distinguishing between epithelioid mesotheliomas and adenocarcinomas. [16]

D2-40, a recently available monoclonal antibody has been accurate like calretinin and better than cytokeratin 5/6 and WT1 and helps in distinguishing epithelioid malignant mesothelioma versus adenocarcinoma.[17]

Out of 150 cases studied by Archana et al,[1] 39 (26%) were positive for malignancy by cell block method, while by routine method only 29 samples were reported as positive for malignant cells. Thus it was found that there was significant difference between the results obtained by direct smears method and cell block method. 34 cell blocks had no cellularity.[1]

In the study by Sujathan et.al, [18] out of 85 samples studied, 21 (24.71%) cell blocks showed malignancy. Two samples diagnosed as negative for malignancy by smear technique, were diagnosed as malignancy by cell block method. Thus the use of cell block increased the diagnostic yields of malignancy from 19 to 21 samples. Only one cell block had no cellularity out of 85 samples.[18]

In the present study, out of 225 cases, 27 cases of malignancy were detected by using cell block method, while by using routine methods; only 12 cases were diagnosed to be malignant. Only 7 cell blocks showed no cellularity. The reason for the lack of cellularity may be due to technical errors such as inadequate sampling (less than 5 ml of serous fluid sent to the laboratory) or degenerated samples

CONCLUSION

We conclude that the cell block technique when used as an adjuvant to routine smear examination has increased the diagnostic yield because of better preservation of the architectural pattern, particularly in cases where there is a diagnostic dilemma between the malignancy and reactive changes. Immunohistochemistry also gives better results on the tissue in the cell block than cytological smears which will be helpful to arrive at the accurate diagnosis.

ACKNOWLEDGEMENT

I am very grateful to Dr.Vissa Shanthi for helping me in writing this article. I also thank Dr.P.V.B. Ramalakshmi, my teacher who is at present Professor and Head at Maharaja Institute of Medical Sciences, Vizianagaram, Andhra Pradesh.

REFERENCES

1. Archana KB, Parate N, Wadadekar A, Bobhate SK, Munshi MM. Diagnostic utility of cell block preparation in reporting of fluid cytology. Journal of Cytology 2003;20(3):133-35
2. Khan N, Sherwani RK, AFroz N, Kapoor S. Cytodiagnosis of malignant effusion and determination of primary site. Journal of Cytology 2005;20(3):107-10
3. Meenu Thappar, Mishra K, Amit Sharma,Vikas Goyal, Vibhuti Goyal. Critical analysis of cell block versus smear examination in effusions. Journal of Cytology 2009;26(2):60-64
4. Elizabeth A. McGrew. The role of exfoliative cytology in the diagnosis and treatment of malignant disease. Postgrad. Med. J.1961;37:456-67
5. David Hopwood. Fixation and fixatives. In: John D Bancroft & Marilyn Gamble, editors. Theory and practice of histological techniques.5th edition, Churchhill Livingstone, Printed in China.pg63-84.
6. Foot NC. Identification of types and primary sites of metastatic tumors from exfoliated cells in serous fluids. Am. J. Pathol 1954; 30(4): 661-77
7. Foot NC. The identification of neoplastic cells in serous effusions. Am J Pathol 1956; 32: 961-77
8. Fred JJM, van de Molengraft, Peter Vooijs G. The interval between the diagnosis of malignancy and the development of effusions, with reference to the role of cytologic diagnosis. Acta Cytologica 1988;32(2):183-87
9. Sharma G, Singh PK, Singh SN. Myelomatous pleural effusion. Journal of cytology 2005; 22(3) 135-36
10. Donald Sears, Steven IH. The cytologic diagnosis of malignant neoplasms in pleural and peritoneal effusions. Acta Cytol 1987;31(2):85-89
11. Myron R. Melamed. The cytological features of malignant lymphomas and related diseases in effusions. Cancer 1963;16:413-31
12. Effusion in the absence of cancer. In : Leopord G. Koss, MyronR. Melamed, editors. Diagnostic cytology and its histopathological bases:Vol.2,5th ed. Lippincott Williams & Williams, 2006. Printed in USA.pg 919-20
13. Pauri Murugan, Neelaiah Siddiraju, Syed Habeebullah, Debdatta Basu. Significance of intercellular spaces(windows) in effusion fluid cytology:A study of 46 samples. Diagnostic Cytopathology 2008;36(9):628-32
14. Dekker A, Bupp PA. Cytology of serous effusions. An investigation into the usefulness of cell blocks versus smears. Americal Journal of Clinical Pathology 1978:70;855-60
15. Ignatius TM, Kung, Siu-Kwong Chan, Elena SFLo. Application of immunoperoxidase technique to cell block preparations from fine needle aspirates. Acta Cytol 1990;34(3):297 -03
16. Wilentz, Goldman & Spitzer. Asbestos-related Diseases. http://www.mesothelioma.org/understandingmeso/asbestos-diseases.asp

17. Albert Y Chu, Leslie A Litzky, Theresa L Pasha, Geza Acs and Paul J Zhang Utility of D2-40, a novel mesothelial marker, in the diagnosis of malignant mesothelioma. Modern Pathology. 2005;18:105–10
18. Sujathan K, Kannan S, Mathew A, Pillai KR, Chandralekha B, Nair MK. Cytodiagnosis of serous effusions: A combined approach of morphological features in Papanicolaou and MGG stained smears and a modified cell block technique. Journal of cytology 2000;17(2):89-95