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Cellular pattern analysis and there cyto histopathological correlation by two technique in serous body fluids

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Abstract
Background: Rendering astir the serous body fluids is slenderly punk by cytology. Now, the cell block (CB) technique is the best method to bonk the cell architecture, easy diagnosis, staging and prognosis.
Aim: This study was conducted with the intent of cell pattern approach to construe serous body fluids cytology and to demonstrate diagnostic accuracy of cell block technique with an accent on diagnostic pitfalls.
Material and Method: Total 192 serous fluids were evaluated, from the year 2017 to 2018. Two techniques were executed in each fluid severally, conventional cytology and cell block. Cytology slides were made after centrifugation or cytospin technique. For cell block preparation, 95% ethyl alcohol was used. Various predominant cell patterns were noted in each case. Overall the final diagnosis was arrived by observing cellularity, architecture, morphology, nuclear and cytoplasmic details and correlated with histopathology in all cases. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were computed.
Result: In conventional cytology, Benign, suspicious for malignancy (SM) and malignant cases were seen in 77.08, 10.94 and 11.98% respectively. Cell block method reveled benign in 76.56% cases and 23.44% in malignant cases. Cytological diagnosis was concordant with histopathological (CB) diagnosis in 167 (86.98%) cases. By cell block malignancy was 11.46% more than CC. Overall sensitivity and specificity were 93.33 and 98.63%, respectively. PPV, NPV and diagnostic accuracy were 95.45, 97.99 and 97.39% respectively.
Conclusion: Cell block method helps to make correct and definite diagnosis and raise the sensitivity. We can narrow or reduced unnecessary or invasive surgeries.

Keywords: serous fluid, cytology, cell block, sensitivity, specificity, diagnostic accuracy

Introduction

The body cavities have a common embryologic origin of mesenchyme and accumulation of fluid within these cavities, referred as serous effusion. Comportment of cancerous cells in the effusion indicates the intimacy of serous membrane [1]. To rule out presence or absence and to study of these cancerous cells, conventional cytology (CC) is the first-line, simple, quick and less invasive screening test. It also point out towards the causes of effusion, diagnosis, proper surgical management, chemotherapy and radiotherapy. So, properly prepared cytology slides are recommended [2, 3]. The simplest approach to the diagnosis is based on the cell pattern and morphology. The cells might be arranged in various pattern including single scattered, sheets, clusters, papillae and cell ball [1, 3]. The limitations of CC are mainly, low cellularity, cell loss and morphology variation which results in difficulty of making conclusive diagnosis [2]. So, In 1895 Bahrenberg introduced Cell Block (CB) technique for routine processing of fluids and in 1917, Mandelbaum devised a technique for the preparation of CB. It is the oldest method [4]. As compare to conventional cytology, several advantages of CB technique are (1) large amount of cells in a small focus, (2) preserve cellular pattern (3) Maintain nuclear and cytoplasmic details, (4) multiple sections of same material can be obtained for special stains and immunohistochemistry [3]. In our study, cytology diagnosis was made on the basis of predominant cell pattern and cell morphology. Further, cell blocks of all samples were made and cyto- histopathological correlation was done.

Material and Methods

We conducted this retrospective study, from 2017 to 2018, in the department of pathology, L.L.R.M medical College, Meerut. A total of 192 samples from three body cavities, namely pleural, peritoneal and pericardial, were included and all scanty, clotted and other than above fluids were excluded from this study. Detail of patients like name, age, sex and registration number, in the requisition form were cross checked with the each labeled sample. A written Performa including all the relevant details were taken. Other clinical history, clinical diagnosis and ultrasonography findings were also noted correlated accordingly. Each sample was divided into two halves. For CC, samples were centrifuged for 5 minutes at 2000rpm and sediment was used to prepare minimum three slides. One air dried for giemsa and two wet fixed smears for Papanicolaou and hematoxylin and eosin stain (H&E) and final diagnosis were reported according to the predominant cell pattern and cell morphology. On the basis of cellularity, arrangement and morphology of cells, interpretation was categorized into three categories: benign, suspicious for malignancy (SM) and malignant effusion. For cell block preparation, samples were centrifuged for 2 min at 1500 rpm, decant supernatant.

We added 95% ethyl alcohol to the button and again centrifuged for 10-20 minutes at 1500 rpm, decant the supernatant. If button is not hard we can repeat the second step again. The cell button was scraped out and wrapped in filter paper and fixed into the formalin. After proper tissue processing slides were made. Comparative evaluation of CS versus CB technique was conducted

Results

Total 192 serous fluids were analyzed in the present study. The common age group of serous effusion was 41-50 years [Table 1]. In the all three fluids and overall males (67.71%) were commonly involved with M: F ratio of 2.1:1. Out of 192 fluids, majority of the samples were from pleural tap 102(53.13%), while the remaining samples consisted 89(46.35%) of peritoneal fluid and 01(0.52%) were pericardial fluid [Table 2]. Cytological findings showed that 148(77.04%) cases were benign, 21(10.94%) cases were suspicious of malignancy and 23(11.98%) cases were malignant [Table 3]. Cell architectural pattern, including single scattered (98/148; 66.22%), sheets (24/148; 16.21%) and clusters (26/148; 17.57%) pattern were predominant in benign fluids while cell ball (13/23;56.52%) and papillae (10/23;43.48%) patterns were common in malignant fluids [Table 4].

All fluids (N=192) were analyzed for cell block technique. Cyto-histology concordance were noted in 167 (86.98 %) cases and discordance were noted in 25(13.02%) cases, out of which 21/25 samples that were reported as SM by CC method, 20/21 samples were diagnosed as malignant effusion and 01/21 as benign lesion by the CB, 3/25 samples reported as benign and 01/25 as malignant by CC method, were diagnosed as malignant and benign respectively [Table 5]. The correlation of CC and CB reveled that the prevalence of malignancy in CC and CB was (23/192) 11.98% and (45/192) 23.44% respectively [Table 6].

Table 1: Age wise distribution of cases

Age (years)	No. of cases	Percentage
0-10	18	09.37
11-20	21	10.94
21-30	29	15.10
31-40	32	16.67
41-50	35	18.23
51-60	33	17.18
>60	24	12.51
Total	192	100

Table 2: Distribution of cases according to the types of fluid

	Pleural	%	peritoneal	%	Pericardial	%	Total	%
Male	68	66.67	61	68.54	01	100	130	67.71
Female	34	33.33	28	31.46	00	00.00	62	32.29
Total	102	53.13	89	46.35	01	00.52	192	100

Table 3: Distribution of cases (N- 192) on the basis of cytological examination

categories	Pleural	Peritoneal	Pericardial	Total
Benign	79 (77.45)	68 (76.40)	01 (100)	148 (77.08)
Suspicious for malignancy (SM)	11 (10.79)	10 (11.24)	-	21 (10.94)
Malignancy	12 (11.76)	11 (12.36)	-	23 (11.98)
Total	102 (53.13)	89 (46.35)	01 (00.52)	192 (100)

Table 4: Distribution on the basis of predominant cell pattern in cytology

	Total	Single scattered	Sheets	Clusters	Cell balls	Papillae
Benign	148 (77.09%)	98 (66.22)	24 (16.21)	26 (17.57)	-	-
SM	21 (10.93%)	-	-	02 (09.52)	12 (57.15)	07 (33.33)
Malignant	23 (11.98%)	-	-	-	13 (56.52)	10 (43.48)
	192 (100%)	98 (51.04)	24 (12.50)	28 (14.58)	25 (13.02)	17 (08.86)

Table 5: Cyto-histology correlation

Cell pattern	Cytology diagnosis		Cell block			
			concordance		Discordance (13.02)	
					Benign	Malignant
Single scattered	98 (B)	51.04 %	96	97.96 %	-	02 (2.04)
Sheets	24 (B)	12.50 %	23	95.83 %	-	01 (04.17)
clusters	26 (B) 02 (SM)	14.58 %	26 -	92.86 %	- -	- 02 (07.14)
Cell ball	12 (SM) 13 (M)	13.06 %	- 12	48.00 %	- 01 (4.00)	12 (48) -
Papillae	07 (SM) 10 (M)	08.85 %	- 10	58.82 %	01 (5.89) -	06 (35.29)
Total	192	100%	167	86.98 %	02 (1.04)	23 (11.98)

Table 6: Analysis of CS and CB methods in total 192 fluid samples

Category	CC	CB
Benign	148 (77.08)	147 (76.56)
SM	21 (10.94)	00
M	23 (11.98)	45 (23.44)
Total	192 (100)	192 (100)

Discussion

Conventional cytology technique is the easy, quick, cost effective and safe technique that provides much larger surface area prepared by centrifugation but judgment of reactive mesothelial cells, macrophages, Benign and malignant effusions is not perfect for better diagnosis that encase lots of things like punctilious masking. Hypocellular and bloody specimens are also challenging. In this study we have demonstrated simple cell block (CB) technique using 95% ethyl alcohol from all serous effusion. CB technique is the complementary and oldest method to increase the cellularity, diagnostic utility and to reduce the false negative result that is high in CC [2, 5]. The objective of this study is to evaluate the utility of routine use of cell block to assess the concordance in diagnosis between conventional smear and cell block

Of the 192 samples of serous effusions the maximum number of the sample was in the age group of 41-50 yrs and accounting for 18.23% of age distribution with M: F ratio of 2.3:1, almost similar to the study of Deepa *et al* [4].

In our study, ascitic fluid (53.13 %) was the common fluid followed by in decreasing order of peritoneal (46.35%) and pericardial (0.52%), which almost comparative with the Few [3, 6-10].

In much previous literature, the final diagnosis of effusions were divided into many formats. Bansode *et al*. Used reported format as negative for malignancy, suspicious for malignancy and positive for malignancy [3].

Geethu *et al*. Classified as malignant cells, atypical cells, suspicious and no malignant cells [12].

Some author's did study under the three groups including benign, suspicious and malignant [6, 7].

We also included three broad categories in the present study, in which maximum no. Of cases is benign (77.09%), followed by suspicious for malignancy (10.93%) and malignant (11.98%), which correlates with the studies conducted by Hathila *et al* [9].

In our study, the cells were analysed into various cell pattern including, single scattered (51.05%), sheets (12.50%), clusters (14.58%), cell ball (13.02%) and papillae (08.86%). Similar finding was found in the study of deepa *et al*.

The cytological diagnosis was encountered on the basis of first predominant cellular pattern that were compared with histopathological examination, whenever possible.

In this study, the predominant single scattered, sheets and clusters pattern were noted in benign cases and only 02/192 cases of SM showed cluster pattern. Cell ball and papillae pattern were commonly noted in malignancy followed by SM. Similar findings were observed in study conducted by Deepa and Hathila *et al* [4, 9].

After compression with cell block, 167/192 cases were concordance and 25/192 cases were discordance.03 of 25 cases that was benign on cytology, confirmed as malignant on cell block technique. 21 of 25 cases were confirmed as one case of benign and 20 were malignant. One case of malignant on cytology confirmed as benign on cell block.

Overall, correlation of CC and CB showed that out of 45 specimens, malignant proven by CB, 23 (11.98%) cases were positive for malignant cells by CS alone. Our study is comparable with the study by Bhanvadia *el al*, [2].

To conclude, the additional yield for the malignancy was found to be 11.46% more by CB as compared to that obtained by CS method. Therefore, we can reduce false negative results and increase diagnostic sensitivity and specificity. CB technique which uses 10% alcohol–acetic acid-formalin as a fixative is simple, safe, inexpensive and reproducible. A combined use of smears and cell block is recommended to raise further diagnostic accuracy.

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