DIAGNOSTIC UTILITY OF GELATIN CELL BLOCK OVER CONVENTIONAL CYTOLOGICAL SMEAR

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ABSTRACT

BACKGROUND

Cytological examination of serous fluid is a commonly performed and well accepted procedure. It helps in diagnosis as well as prognosis of the disease, thus helping in management of the patients. But, diagnosis by conventional cytology is often difficult due to presence of reactive mesothelial cells, abundance of inflammatory background, delaying artifact, air drying, poor fixation and leaving behind useful material causes lower diagnostic yield in conventional smear. Cell block technique is one of the oldest and complementary method and takes an intermediate position between histological and cytological method. Cell block preparation increases the sensitivity of detecting malignancies and also has the ability to reduce false-positive interpretations. Gelatin cell block is a simplified, inexpensive and reproducible technique that produces results, which are equivalent to routine cell block and it can be an adjuvant to conventional smear in evaluation of cytological effusions.

MATERIALS AND METHODS

In our study, we analysed 100 fluid samples pleural (65) and ascitic (35) by both conventional cytology (pap and giemsa staining) and cell block technique. We modified cell block method by gelatin cell block technique using gelatin surgical dressing material.

RESULTS

Our results showed that gelatin cell block was superior to conventional cytology by reducing false-negative results and provided more accurate diagnosis. It also reduced the grey zone for suspicious of malignancy. Gelatin cell block were more simplified and reduced the mean time of making of cell block and thus are strongly recommended for cell block method than other methods for cell block.

CONCLUSION

To conclude, the present study showed that cell block technique, which used gelatin as fixative was a simple, reproducible and inexpensive method, which does not require any special instrument or training. This method yielded more cellularity with better architectural patterns. Hence, the gelatin cell block technique can be recommended as an adjuvant in evaluating the fluid cytology for a final diagnosis along with routine conventional smear method.

KEYWORDS

Gelatin Cell Block, Conventional Smear, Serous Effusions.

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BACKGROUND

The cytological examinations of serous effusions have been well accepted and a positive is often considered as a definitive diagnosis.¹ It gives information about various inflammatory and noninflammatory conditions pointing out the aetiology of effusions and list of differential diagnosis. It also helps in staging, prognosis and management of the patients.²

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Aspiration of serous cavities is a simple and relatively non-invasion technique to arrive at diagnosis, but is also diagnostically challenging job. Accurately diagnosing cells as being either malignant or benign reactive mesothelial cells in serous effusions is a common diagnostic problem. The lower sensitivity of cytodiagnosis of effusions is mainly attributable to bland morphological details of cells, overcrowding or overlapping of cells, cell loss and changes due to different laboratory processing methods in conventional cytological smears.³

Distinguishing benign from malignant cellular changes requires meticulous screening and understanding varied reactive changes that can occur. But, the slides received can vary greatly in quality because of cellular damage arising from the method of smearing the material onto the slide, delaying artifact, air drying, poor fixation and leaving behind useful material causes lower diagnostic yield in conventional smear.

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The cell block technique compensates for many of these disadvantages like the residual material can be very useful in increasing diagnostic yield by cell block method. Cell block technique is one of the oldest and complementary method and takes an intermediate position between histological and cytological method. Cell block preparation increases the sensitivity of detecting malignancies and also has the ability to reduce falsepositive interpretations.

Aims and Objectives

The use of cell blocks for processing cytological fluids has been reported since 1947 when Chapmen and Whalen⁴ first described the technique for serous fluids. Many methods have been developed since then like use of agar, use of thrombin clot method. Most methods are time consuming and technically difficult.⁵

To overcome these problems a simple method was developed that requires no special reagents or equipment other than a crouton of gelatin foam such as gel foam that is used for wound dressings. This organic foam is highly absorbent and been organic is compatible with conventional histology processing.⁶ Gelatin cell block is a useful technique. This enables small fragmented tissue to be embedded together without loss of the exfoliated cell into a block. Since gelatin is an animal protein, it can be fixed with formalin and treated as a piece of tissue.

MATERIALS AND METHODS

The study was conducted in the cytology section of Department of Pathology, GMC, Jammu, and comprised of

100 fluid samples of which 65 were pleural fluid samples and 35 ascitic fluid samples.

Each fluid sample was divided into two parts- one part was subjected to conventional smear and other to gelatin cell block method.

Conventional smear technique for conventional method, 5 mL of fluid was centrifuged at 1500 rpm for 15 minutes and two smears were prepared from the sediment. One smear was air dried and stained with giemsa stain and the other was fixed in 95% alcohol and stained with Papanicolaou stain.

Gelatin Cell Block Technique

- 1. First the serous fluid was centrifuged and the supernatant was removed by pipette to leave a deposit of cells at the base of container.
- 2. A crouton of gelatin foam, app 4*4*2 cms was cut from sheet of gelatin-foam dressing material and dropped in the container so as to absorb the fluid (which is encouraged by pipetting).
- 3. Foam left for a period of 30 mins.
- 4. Methylated spirit then poured for 30 secs (it denatures the protein in the fluid to form a film over the surface to seal the cells) and then removed by pipette.
- 5. Formalin put in container and minimum fixation of 6 hrs. done.
- 6. The crouton then placed in tissue paper and processed as normal histology biopsy and stained with H and E stain.



Figure 1 (A), (B), (C), (D), (E), (F), (G). Gelatin Cell Block Technique

Figure 1 (A) Cytology fluid sample after material has been taken for smear preparation. (B) The sample is centrifuged and the supernatant is removed. (C) Small blocks of gelatin foam are cut from a sheet of dressing material. (D) Foam absorbs the fluid to form a solid sample. (E) The solid cell block is wrapped in tissue paper for processing. (F) H and E-stained section; the wall of the gelatin bubbles are deeply oeosinophilic. (G) High-power H and E of lung adenocarcinoma cells.

RESULTS

A total of 100 body cavity fluid samples were studied, of which 65% were pleural and 35% were ascitic fluid samples. The samples belonged to the age range of 21-80 years with predominant cases in the age range of 41-50 years. The females (68 cases) outnumbered males (32 cases) by a 2:1 ratio.

Effusions	Cases	Percentage		
Pleural	65	65%		
Ascitic	35	35%		
Total	100	100%		
Table 1. Total Cases and their Distribution				

Based on morphology, the smears were categorised as benign, suspicious for malignancy and malignant.

The following morphological criteria such as cellularity, arrangement of cells (acini, papillae and cell balls), cytoplasmic and nuclear details were used for giving the cytological diagnosis.

In conventional smear cytology, moderate and marked cellularity was noted in 40.9% and 6.4%, respectively, whereas in cell block study, it was 53.7% and 25.6%, respectively. The difference was statistically significant.

Singly scattered cells were more common in conventional smear while architectural patterns like cell clusters, acini and papillae were more appreciated in cell block preparations.

	Conventional Smear		Gelatin Block Method		
	Number	%	Number	%	
Benign	70	76%	74	74%	
Suspicious	12	8%	-	-	
Malignant	18	16%	26	26%	
Total	100	100%	100	100%	
Table 2. Comparative Evaluation of Conventional Smear and Gelatin Cell Block					

Out of 100 fluid samples, cytological diagnosis by conventional smear of benign was made in 70 cases (70%), malignant in 18 cases (18%) and suspicious for malignancy in 12 cases (12%), whereas in gelatin block technique 74 cases (74%) were diagnosed as benign, 26 cases (26%) malignant and no suspicious for malignancy diagnosis was given.

So, in a total of 100 cases, a difference of diagnosis was noted in 24 cases. Among these, 12 were diagnosed as suspicious for malignancy and 8 malignant cases and 4 benign cases were misdiagnosed by conventional smear method.

By Gelatin cell block method, 8 additional malignant cases were diagnosed representing 30% more diagnostic efficacy. Statistical analysis showed that sensitivity of gelatin cell block study in diagnosing malignant lesions

were 100% while conventional smear showed only 67% in diagnosis of malignancy.

DISCUSSION

Cytological examination of serous fluids is being employed and has also gained acceptance in clinical practice. One of the most common problem in conventional smear cytology is to distinguish reactive mesothelial cells from malignant cells and the difficulty is more enhanced in presence of marked atypia of mesothelial cells. The problem may be compounded by artifacts from poor fixation, preparation or staining technique. Other problems like abundance of inflammatory cells and lack of representative cell population make conclusive diagnosis on conventional smears really difficult. Another limitation of the conventional cytological examination of effusion is that it has a sensitivity of 40-70% for the presence of malignant cell due to overcrowding of cells, cell loss and different lab processing technique.

In the present study, we made an attempt to prepare and analyse both conventional smears and cell block from the same specimen and we modified the cell block method by replacing conventional cell block by gelatin cell block (using gelatin surgical dressing material-gel foam).

Advantages of Gelatin Cell Block Method over Conventional Cell Block

- 1. The mean touch time for the prep of the cell block was 2 mins. 10 secs., which was less than agar block where longer time was required and results more variable.
- 2. The conc. and temp of agar could interfere with the outcome, which was not in case of gelatin cell block.
- 3. The area of gelatin in the sections is very small and the density of the cells is thus greater than seen in agar method.⁶

The cell block concentrated the cellular material into a small area, which was useful in screening the material in lesser time. In 1928, Zemarsky concluded that the cell block method was superior to conventional smear technique. Similar findings were also noted in studies by Dekker et al,⁷ Krogerus et al,⁸ Yang et al⁹ and Thaper et al.³ The other advantage of cell block is concentration of cellular material in one small area that can be evaluated at a glance with all cells lying in the same focal plane of the microscope. It bridges the gap between cytology and histology.

In the present study, in comparison with conventional smear cytology, gelatin cell block preparation have shown statistically significant increase in cell yield, i.e. cellularity. Similar findings were noticed by Bista et al¹⁰ and Udasimath et al.¹¹ Increase in cellular yield is contributed

by utilisation of entire fluid remained after preparation of conventional smear for preparation of cell block.

In the present study, background elements obscuring the cellular details were significantly reduced in cell block preparation than in conventional smear cytology. <u>Udasimath</u> et al study¹¹ findings were similar to our study, whereas according to Bista¹⁰ et al obscuring background was more seen in cell block preparation.

In the present study, architectural pattern was best retained and appreciated in gelatin cell block preparation than in conventional smear cytology. Similar findings were also observed in other studies. Mesothelial cell which mimicked malignant cells were identified as reactive mesothelial cells on CB. The findings were in concordance with Dekker et al⁷ study.

Advantages of the Cell Block Preparation

- 1. Recognition of the histological patterns of disease.
- 2. The possibility of studying multiple sections by routine staining, special staining and by IHC studies.
- 3. Lesser cellular dispersal and possibility of storing the slides for retrospective studies.¹²
- 4. The CB technique is a valuable method, particularly when the IHC staining is required for a battery of markers. The IHC staining, when it is applied to the cell block preparations provides the same accuracy as do the histological specimens.

In a study by Dekker et al,⁷ the rate of recovery of tumour cells by cell block preparation was double that obtained by smear alone. By using cell block method, tumours were subsequently demonstrated in 38% of the patient who had negative or atypical cytological reports. Thapar et al³ showed a diagnosed yield of 20% by cell block preparations. In our study, a difference of diagnosis was seen in 24 cases. Among these, 12 were diagnosed as suspicious for malignancy and 8 malignant and 4 benign cases were misdiagnosed by conventional smear method. By cell block method, additional 8 malignant cases were diagnosed. The present study yielded 30% more malignant cases in serous fluid. These findings were in concordance with other studies like study by Khan et al,¹³ Dekker et al,⁷ Bhanvadia et al¹⁴ and Grandhi et al.¹⁵

In the present study, diagnostic categorisation into suspicious for malignancy was significantly reduced in gelatin cell block preparation in comparison with conventional smear cytology. Similarly, there was statistically significant increase in identification of malignant lesions in cell block preparation than in conventional smear cytology. Bhanvadia et al14 and Grandhi et al¹⁵ showed similar findings. This is attributed to increase in cellular yield and better appreciation of cellular details and architecture in cell block preparation. The degenerating mesothelial can also be misleading. Similar finding were observed in studies by Takagi et al,¹⁶ Vellios et al¹⁷ and Chapman et al.⁴ This problem was overcome by use of cellblock preparation.

Study	Percentage		
Dekker et al (1978)	38%		
Grandhi et al (2014)	5%		
Bhanvadia et al (2014)	10%		
Katti et al (2016)	15.5%		
Present study (2017)	8%		
Table 3. Comparison of Additional Yield of Malignancy by Cell Block Preparation in Various Studies			

CONCLUSION

The present study results showed that cell block technique, which used gelatin as fixative was a simple, reproducible and inexpensive method, which does not require any special instrument or training. This method yielded more cellularity with better architectural patterns. In our study, the diagnoses, which were missed or incompletely diagnosed on routine conventional cytology were diagnosed accurately by this technique. Hence, the gelatin cell block technique can be recommended as a useful as adjuvant in evaluating the fluid cytology for a final diagnosis along with routine conventional smear method.

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