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ORIGINAL RESEARCH

Study of cell block technique in the cytodiagnosis of serous fluids at a tertiary hospital

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ABSTRACT

Background: Conventional smear is a less complicated procedure than that of cell block technique, it has lower sensitivity due of overcrowding of cells, cell loss, lack of architecture and also abundance of inflammatory cells and scarcity of representative cells contribute to appreciable difficulties in creating conclusive diagnosis on conventional smears. The aim of this study was to check the diagnostic yields of cell block technique in the cytodiagnosis of serous fluids.

Material and Methods: Present study was cross-sectional observational study, conducted in samples of ascitic fluid, pleural fluid, pericardiac fluid that were received in the cytology section, collected from patients clinically and radiologically diagnosed as having serous effusion. After reporting the conventional cytological smear the representative received samples were processed for cell block preparations.

Results: Findings of cellularity differs significantly, indicating better yield of cellularity in cell blocks as compared to conventional smear method. Distribution of findings of background obscured by blood or proteinaceous material differs significantly, indicating less degree of background obscured by blood or proteinaceous material on cell block preparation. Distribution of findings of morphology differs significantly, morphology is better appreciated with the help of cell blocks as compared to conventional smear method. Distribution of findings of architecture differs significantly, indicating better appreciation of architecture on cell blocks. Conclusion: Cell block method, concentrates cellular rich material, and it can increases cellular yield for diagnosis. Cell block increases cellular architecture pattern like acini, cells in sheets, papillae, which has helped to identify possible type or primary site of malignancy.

Keywords: Cell block technique cellular yield, malignancy, cytodiagnosis, conventional smear

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INTRODUCTION

Differentiation between benign and malignant bodily fluid effusions always poses a great diagnostic dilemma. Differentiation usually needs clinical findings, morphological analysis and sometimes immunocytochemistry. Diagnostic possibility is increased if cell blocks are made together with the conventional cytology smears. This may facilitate the clinicians in treating the patient and determining the outcome of the disease process.[1,2]

Conventional smear is a less complicated procedure than that of cell block technique, it has lower sensitivity due of overcrowding of cells, cell loss, lack of architecture and also abundance of inflammatory cells and scarcity of representative cells contribute to appreciable difficulties in creating conclusive diagnosis on conventional smears.[3]

There are several benefits for having a well-prepared, diagnostic CB. Routine hematoxylin and eosin (H & E)-stained sections of a high-quality CB will give both cytologic and architectural detail to support the cytomorphologic findings on direct smears.[4] The cell block technique has additional advantage that multiple sections of the equivalent material can be obtained for special stains and immunohistochemistry.[5] The aim of this study was to check the diagnostic yields of cell block technique in the cytodiagnosis of serous fluids.

MATERIAL AND METHODS

Present study was cross-sectional observational study, conducted in Department of Pathology, Vilasrao Deshmukh Government Medical College, Latur, India. Study duration was of 1 year (November-2018 to Oct 2019). Study was approved by institutional ethical committee.

Inclusion criteria

- Samples of ascitic fluid, pleural fluid, pericardiac fluid that were received in the cytology section, collected from patients clinically and radiologically diagnosed as having serous effusion.

Exclusion criteria

- Patients with marked deranged coagulopathy.
- Patient with skin infection at the site of aspiration.
- Unwilling patients.

The clinical details of patients were recorded (proforma). After reporting the conventional cytological smear the representative received samples were processed for cell block preparations immediately.

In present study, cell blocks were prepared by Plasma thromboplastin method. Fluid samples were centrifuged at 2500 rpm for 15 minutes. Then followed by centrifuging, supernatant was removed and discarded. Then the remaining sediment was mixed with 4 drops of plasma (pooled plasma from a blood bank may be used), that was kept frozen. This plasma was brought to room temperature before use. The stability may be checked periodically by adding two drops of thromboplastin reagent to two drops of plasma, which should clot in about 30 seconds. Followed by few drops of thromboplastin (4-6 drops) were added and mixed. Thromboplastin which was stored at 2^0C and 8^0C was brought to the room temperature before use. The tube containing the above mixture was kept undisturbed for few minutes until clot was formed. If there was no clot formation found, 4 more extra drops of thromboplastin was added until clot appeared. Then the formed clot was scooped out and placed into a filter paper and kept in cassette. The tissue cassette was then fixed in 10% neutral buffered formalin for at least 4 hrs. Then it was processed along with routine histopathological specimens. Cell blocks were made and tissue section of 4-5micron

thickness were taken and stained with H &E. These blocks can be used for IHC whenever needed.

Interpretation of conventional smears and cell block[5] - Each smears obtained by each of the technique was analysed for background, cellularity, cytoplasmic, and nuclear details (cellular morphology), architecture (acini, papillae, cell balls, and proliferation spheres), using the Mair's point scoring system as shown in table no.1and were scored from 0 to 2+ scale.

Table 1: Mair's SCORING SYSTEM

Parameter	**Quantitative description**	**Point score**
1.Volume of Obscuring Background blood or clot	Large amount: diagnosis greatly compromised	0
	Moderate amount: diagnosis possible	1
	Minimal: diagnosis easy, specimen of textbook quality	2
2.Amount of diagnostic cellular material present	Minimal or absent: diagnosis not possible	0
	Sufficient for cytodiagnosis	1
	Abundant: diagnosis simple	2
3.Degree of cellular degeneration and cellular trauma	Marked: diagnosis impossible	0
	Moderate: diagnosis possible	1
	Minimal: good preservation	2
4.Retention of appropriate architecture and cellular arrangement	Minimal to absent: non-diagnostic	0
	Moderate: some preservation e.g., follicles, papillae, acini, syncytia or single cell pattern	1
	Excellent architectural display closely reflecting histology: diagnosis obvious	2

According to the criteria mentioned above, comments were rendered on the quality of the slides by qualitatively grouping them into three categories:

1. Diagnostically unsuitable (0-2)
2. Diagnostically adequate (3-5)
3. Diagnostically superior (6-8)

Patterns reported on conventional smear and cell block

Based on morphology, the conventional smears were categorized as nondiagnostic, inflammatory, benign, suspicious, and malignant lesions. Cell block were categorized into nondiagnostic, inflammatory, benign, malignant. Diagnostically unsuitable cases were categorized under nondiagnostic.

The data on categorical variables is shown as n (% of cases) and the data on continuous variables is presented as Mean and Standard deviation (SD). The inter-group statistical comparison of distribution of categorical variables is done using Chi-Square test. The diagnostic efficacy indices such as sensitivity, specificity, positive predictive (PPV), negative predictive value (NPV) and accuracy is calculated using appropriate Gold standard. p-values less than 0.05 are considered to be statistically significant.

RESULTS

The study included 60 cases satisfying inclusion and exclusion criteria. Of 60 cases studied, majority were from age group 41–50 years (23.3%) & 31–40 years (20 %). The mean age of cases was 50.53±17.63 years. Of 60 cases studied, 14 (23.3%) were male and 46 (76.7%) were female. The male to female sex ratio in the entire study was 1:3

Table 2: Age & gender distribution

Age Group (years)	No. of cases	% of cases
<20	4	6.7
21 – 30	2	3.3
31 – 40	12	20.0
41 – 50	14	23.3
51 – 60	11	18.3
61 – 70	13	21.7
71 – 80	4	6.7
Sex		
Male	14	23.3
Female	46	76.7

37 (61.7%) samples were of ascitic fluid, 22 (36.7%) of pleural fluid and 01 (1.6%) of pericardiac fluid in the study group.

Table 3: Distribution of type of fluid

Type of fluid	No. of cases	% of cases
Ascitic	37	61.7
Pleural	22	36.7
Pericardiac	1	1.7

Of 60 cases studied, 4 (6.7%) had superior, 45 (75.0%) had adequate and 11 (18.3%) had unsuitable quality of conventional smear in the study group. After cell block preparation 19 (31.7%) had superior, 39 (65.0%) had adequate and 2 (3.3%) had unsuitable quality.

Table 4: Distribution of quality of preparation

Quality	Conventional smear		Cell block	
	No. of cases	% of cases	No. of cases	% of cases
Superior	4	6.7	19	31.7
Adequate	45	75.0	39	65.0
Unsuitable	11	18.3	2	3.3

Of 60 cases studied, 11 (18.3%) were non-diagnosable, 16 (26.7%) had suspicious diagnosis, 10 (16.7%) had inflammatory smear, 12 (20.0%) had benign and 11 (18.3%) had malignant findings on conventional smear in the study group. Of 60 cases studied, 2 (3.3%) were non-diagnosable, 11 (18.3%) had inflammatory smear, 23 (38.4%) had benign and 24 (40.0%) had malignant findings on cell block in the study group.

Table 5: Distribution of overall diagnosis.

	Conventional smear		Cell block	
Diagnosis	No. of cases	% of cases	No. of cases	% of cases
Non-diagnosable	11	18.3	2	3.3

Suspicious	16	26.7	0	0
Inflammatory	10	16.7	11	18.3
Benign	12	20.0	23	38.4
Malignant	11	18.3	24	40.0

Findings of cellularity differs significantly, indicating better yield of cellularity in cell blocks as compared to conventional smear method. Distribution of findings of background obscured by blood or proteinaceous material differs significantly, indicating less degree of background obscured by blood or proteinaceous material on cell block preparation. Distribution of findings of morphology differs significantly, morphology is better appreciated with the help of cell blocks as compared to conventional smear method. Distribution of findings of architecture differs significantly, indicating better appreciation of architecture on cell blocks.

Table 6: Comparison of conventional smear and Cell block as per the Mair's criteria.

Parameters	**Findings**	**Conventional Smear (n=60)**		**Cell Block (n=60)**		
		No. of cases	**% of cases**	**No. of cases**	**% of cases**	**P-value**
Cellularity	Minimal or absent	8	13.3	2	3.3	0.001***
	Sufficient	42	70.0	23	38.3	
	Abundant	10	16.7	35	58.4	
Background obscured by blood or proteinaceous material	Large amount	9	15.0	4	6.7	0.002**
	Moderate amount	47	78.3	42	70.0	
	Minimal amount	4	6.7	14	23.3	
Morphology	Marked	3	5.0	0	0.0	0.001***
	Moderate	46	76.7	14	23.3	
	Minimal	11	18.3	46	76.7	
Architecture	Minimal to absent	16	26.7	3	5.0	0.001***
	Moderate	44	73.3	47	78.3	
	Excellent	0	0.0	10	16.7	

Out of 60 cases, On conventional smear method, 11 (18.3%) cases were typed as nondiagnosable. However, among these 11 cases, only 2 were typed as nondiagnosable on cell block preparation. The remaining 9 cases on cell block preparation were of 1 (9.1%) inflammatory, 5 (21.7%) benign, 3(12.5%) malignant.

10(16.7%) cases, both conventional smear method as well as cell block preparations revealed inflammatory lesions. By conventional smear method 12(20%) cases were in benign category, which were confirmed benign by cell block method. 16(26.7%) cases were typed as suspicious, of which cell block revealed, 10(41.7%) to be of malignant category and remaining 06 (26.1%) to be of benign category. On conventional smear preparations, 11 (18.3%) cases were typed as malignant, all of which were confirmed to be of malignant category with the help of cell block method. Of 11 cases with inflammatory diagnosis by Cell block, 1(9.1%) had Non diagnosable and 10 (90.9%) had inflammatory diagnosis by conventional smear. Of 23 cases with benign diagnosis by Cell block, 5 (21.7%) had Non diagnosable, 10 (41.7%) had suspicious and 12 (52.2%) had benign diagnosis by conventional smear. Of 24 cases with malignant diagnosis by Cell block, 3 (12.5%) had Non diagnosable, 6 (26.1%) had suspicious and 11 (45.8%) had malignant diagnosis by conventional smear.

Table 7: Distribution of diagnosis by Conventional smear and cell block in the study group.

Conventional smear	Cell Block										
	Non-diagnosable		Inflammatory		Benign		Malignant		Total (n=60)		P-value
	n	%	n	%	n	%	n	%	n	%	
Non-diagnosable	2	100.0	1	9.1	5	21.7	3	12.5	11	18.3	0.001***
Suspicious	0	0.0	0	0.0	6	26.1	10	41.7	16	26.7	
Inflammatory	0	0.0	10	90.9	0	0.0	0	0.0	10	16.7	
Benign	0	0.0	0	0.0	12	52.2	0	0.0	12	20.0	
Malignant	0	0.0	0	0.0	0	0.0	11	45.8	11	18.3	
Total	2	100.0	11	100.0	23	100.0	24	100.0	60	100.0	

Distribution of efficacy indices such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of inflammatory findings on conventional smear is 90.9%, 100.0%, 100.0%, 98.0% and 98.3% respectively.

Distribution of efficacy indices such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of benign findings on conventional smear is 52.2%, 100.0%, 100.0%, 77.1% and 81.7% respectively.

Distribution of efficacy indices such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of malignant findings on conventional smear is 45.8%, 100.0%, 100.0%, 73.5% and 78.3% respectively.

Distribution of efficacy indices such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of overall findings on conventional smear is 78.6%, 100.0%, 100.0%, 90.3% and 92.9% respectively.

Table 8: Diagnostic efficacy indices of findings of conventional smear

	Diagnostic efficacy indices (%)				
Findings	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
Inflammatory	90.9	100.0	100.0	98.0	98.3
Benign	52.2	100.0	100.0	77.1	81.7
Malignant	45.8	100.0	100.0	73.5	78.3
Overall	78.6	100.0	100.0	90.3	92.9

DISCUSSION

Cell block technique has benefits like it concentrates less quantity of cellular material in one small area which will be evaluated at a glance with all cells lying within the same focal plane of the microscope, and because it uses of histological techniques it offers better cellular morphology higher nuclear and cytoplasmic preservation, intact cell membrane and crisp chromatin details , preservation of Architectural pattern like cell balls, papillae, acini, rosettes and individual cell characteristic ,representing its primary site of malignancy, fragment of this tissue will simply interpreted in a biopsy-like fashion.[3]

In present study, age range was <20-80 year and it correlates with studies of Bhavana et al.,[6] and Shubhada et al.,[3] In present study, maximum number of cases in 41-50 years age group it correlate with the studies of Bhavana et al.,[6] and Shubhada et al.,[3]

As regards gender, 14 (23.3%) cases were male and 46 (76.7%) cases were female. There is slight female preponderance with female to male sex ratio in the entire study was 1.00:0.30.

This finding is comparable with the studies of Bista et al.,[1] having female to male ratio 1:1.05

The higher numbers of cases in male patients were reported in Shivakumarswamy et al.,[7] Bhavana et al.,[6] Shubhada et al.,[6] studies.

In present study total 60 fluid samples were subjected to the conventional smear preparation and cell block techniques. Out of which, 37 (61.7%) had ascitic fluid, 22 (36.7%) had pleural fluid and 1(1.6%) had pericardiac fluid, Which correlate with Ranjana et al.,[8] study, in which out of 300 sample studied, 175 (58.4%) had ascitic fluid, 125 (41.6%) had pleural fluid and no pericardiac fluid. In studies done by Thapar et al.,[5] Bhavana et al.,[6] Shubhada et al.,[3] pleural fluids were studied more than ascitic fluid. This difference is due to random selection of cases.

In Shubhada et al.,[3] study, out of 142 cases, 27(19.01%) had large amount, 114 (80.28%) had moderate amount and 1(0.7%) had minimal amount of volume of blood/clot obscuring background on conventional smear. 18 (12.68%) had marked, 105 (73.95%) had moderate and 19(13.38%) had minimal degree of cellular degeneration and cellular trauma on conventional smear. 141 (99.30%) had minimal to absent and 1(0.7%) had moderate architecture found on conventional smear. This difference is mainly due to random selection of cases. Similar findings were noted in present study.

Pratibha Bista[1] study showed, out of 37 cases , 1(2.7%) had minimal , 20(54.1%) had sufficient and 16 (43.2%) had abundant cellularity on smear. 13(35.15%) had large amount, 13 (35.15%) had moderate amount and 11 (29.7%) had minimal amount of volume of blood/clot obscuring background on conventional smear. Similar findings were noted in present study.

In present study, 4 (6.7%) had superior, 45 (75.0%) had adequate and 11 (18.3%) had unsuitable quality of conventional smear in the study group. In Richa Nathani et al.,[9] study, 15% had superior, 57% had adequate and 28% had unsuitable quality of conventional smear. In Thapar, et al.,[5] study, 54% had superior, 20%had adequate and 26% had unsuitable quality of conventional smear.

On cell block evaluation, Shubhada et al.,[3] noted that out of 142 cases, 20(14.08%) had minimal, 81(57.04%) had sufficient and 41 (28.87%) had abundant cellularity on cell block. 37 (26.06%) had large amount, 98 (69.01%) had moderate amount and 7(4.93%) had minimal amount of Volume of blood/clot obscuring background on cell block. 38 (26.76%) had marked, 81(57.04%) had moderate and 41 (28.87%) had minimal degree of cellular degeneration and cellular trauma on cell block. 112(78.87%) had minimal to absent, 16(11.27%) had moderate architecture and 14(9.86%) had excellent architecture on cell block. Similar findings were noted in present study.

Pratibha Bista[1] study showed, out of 37 cases , 1(2.7%) had Minimal , 13(35.1%) had sufficient and 23(62.2%) had abundant cellularity on smear. In present study, in cell block method, out of 60 cases studied, 19 (31.7%) had superior, 39 (65.0%) had adequate and 2 (3.3%) had unsuitable quality of cell block. In Richa Nathani et al.,[9] study, 25% had superior, 55% had adequate and 20% had unsuitable quality of conventional smear. In Thapar, et al (2012) study, 67% had superior, 21% had adequate and 12% had unsuitable quality of conventional smear.

Distribution of findings of each score differs significantly between conventional smear technique and cell block technique (P-value<0.001) and considered statistically significant. Pratibha Bista[1] and Shubhada et al.,[3] study, findings of each score differs significantly between conventional smear technique and cell block technique, P-value<0.05 and is statistically significant.

In Shivkumarswami et al.,[7] study, Of 60 cases studied, 54 (90%) had negative for malignancy smear, 5(8%) had suspicious diagnosis, 1 (2%) had malignant findings and none was nondiagnosable on conventional smear . 50(83%) had negative for malignancy and 10 (17%) had malignant findings and no cases in suspicious and nondiagnosable category.

Shubhada et al.,[3] study, of 142 cases studied, 102 (72%) had negative for malignancy smear, 19 (13%) had suspicious diagnosis, 21 (15%) had malignant findings and none case was nondiagnosable on conventional smear. 106(74%) had negative for malignancy, 11 (8%) had suspicious diagnosis and 25(18%) had malignant findings and no cases in nondiagnosable category.

In present study, most of cases were in negative for malignancy category with 36.7% on conventional smear and 56.7% on cell block. Similar findings were seen in study done by Shubhada et al.,[3] and Shivkumar et al.,[9] having most of the cases in negative for malignancy group. In suspicious for malignancy, there was no case found on cell block (0%), and positive for malignancy maximum no. of cases were diagnosed on cell block 24 (40%). Similar findings were also noted by Shivkumar et al, .,[9] with maximum no. of malignant cases on cell block.

In conventional smear there is lack of amount of diagnostic cellular material, volume of obscuring background blood or clot, lack of retention of appropriate architecture and cellular arrangement, distribution of cellular material which cannot be studied in single field of examination. In Cell block method various pattern of architecture like acini, cell balls, papillae can be seen, amount of diagnostic cellular material is abundant and slides can be examined in single field of examination which help in making diagnosis. This emphasising the advantage of cell block preparation over conventional smear to improve the diagnosis.[10,11,12]

In the present study diagnostic yield for malignancy was found to be significantly increased by cell block method. The present study identified additional 13 cases malignant lesions by cell block method which was nondiagnostic and suspicious on conventional smear.

Diagnostic yield of cell block method was found to be 21.6% (13 cases). In Shivkumar swami et al.,[9] study, diagnostic yield by cell block technique of malignancy was found to be 15%. In Shubhada et al.,[3] study, by using cell block technique diagnostic yield of malignancy was obtained to be 6.33%. In Shubhada et al.,[3] study, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of overall findings on conventional smear is 78.6%, 100.0%, 100.0%, 90.3% and 92.9% respectively.

It has been seen in varied studies that the cytological examination of fluids by means of smears, but carefully prepared, leaves behind a large residue that is not further investigated which may contain valuable diagnostic material. This residual material is evaluated in a very easy and expedient fashion by treating it as a cell block, embedded in paraffin, and examined additionally to the routine smears. Cell blocks are significantly helpful when the cytological abnormalities are misleading, like in reactive mesothelial cells, or obscure as in occasional well differentiated adenocarcinoma.[13,14,15] The present study has been undertaken to assess the utility of the cell block preparation technique in increasing cytodiagnosis of serous fluids and to know the primary site of malignant effusions.[5]

One of the advantage of combining cell blocks with smears is that the ability to see the histologic correlates of cytologic findings.[16] Some cytologic criteria cannot be translated into histologic criteria thus complementary nature of cell blocks and smears would facilitate avoid the pitfalls of using either cytology or histology alone.[1]

CONCLUSION

Cell block technique is simple as routine laboratory reagents can be used for processing the sample and it is reliable method. Cell block method, concentrates cellular rich material, and it can increases cellular yield for diagnosis. Cell block increases cellular architecture pattern like acini, cells in sheets, papillae, which has helped to identify possible type or primary site of malignancy. In cell block method less dispersion of cell in field of examination, so one can observe large quantity of cellular material in single field which will be efficient for making diagnosis.

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