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UTILITY OF PLASMA THROMBOPLASTIN CELL BLOCK TECHNIQUE IN CASES OF PLUERAL AND PERITONEAL EFFUSION SUSPECTED FOR MALIGNANCY

Dr. Savitri Shukla Junior Resident Department of Pathology Government Medical College Haldwani Nainital, U.K.

Prerena Junior Resident Department of Pathology. Government Medical College, Haldwani, Nainital, U.K.

ABSTRACT

Background- Cell block preparation act as a usefull adjunct on a smear cytology for diagnosis of pleural and peritoneal effusion by using plasma thromboplastin cell block technique. Diagnostic problem arises in day to day practice to differentiate reactive mesothelial cells and malignant cells by conventional smears method, cell block technique is easy required less time and showing improved cytomorphological features and helps to differentiate reactive mesothelial cells and malignant cells and thereby increases diagnostic efficacy and additional benefits when used with routine cytology.

Material & method- Total 168 cases are studied out of which majority of cases were reactive (n=154, 91.7%). Only 14 cases (8.3%) were malignant. Among the reactive effusions, the commonest cause was tuberculosis (n=98, 58.3%).

Result- There is statistically significant increase in the frequency of malignant cells found on cell block technique as compared to conventional smear which is n=14 versus n=8. As 6 cases which were diagnosed as suspicious of malignancy on conventional smear were diagnosed as malignant in the sections of cell block.

Conclusion- Increases in Cellularity, better architecture display and morphological preservation in cell block technique aids in diagnosing the hidden cases of malignancy so it should be used as an adjunct to conventional smears to aid and improve the diagnosis in serous fluid cytology

KEYWORDS : Cell blocks; Cytological smears; Pleural & Peritoneal fluids

INTRODUCTION-

Cytological examination of serous fluid is important as it reveals information about inflammatory conditions such as bacterial, fungal, viral infections & parasitic infestations as well as findings of cancer cells. Cytological examination not only helps for diagnosis of cancer but also for staging and prognosis of diseases. It is a complete diagnostic modality which aims at pointing out the etiology of effusion as well as prognosis of disease[1].

The diagnostic performance of cytological study of pleural and peritoneal fluids attributes to the fact that cell population present in sediment is representative of larger surface areas than that obtained by needle biopsy[2].

Cell blocks was prepared by plasma thromboplastin method. This technique is simple ,cost effective and readily adaptable in routine hospital laboratories. Morphological examination of cell block material provides additional information that is essential to resolve the diagnostic dilemmas[3].

MATERIALS AND METHODS

This study on pleural and peritoneal fluid cytology and plasma thromboplastin cell block technique was undertaken in the department of pathology in Government Medical College Haldwani over a period of two years from September 2014 to September 2016 and 168 cases of pleural and peritoneal effusion were studied. Relevant and available clinical information regarding age, sex, symptoms and accompanying clinical signs were obtained from the patient. An effort was made in this study to immediately process the fluid.

The fluids were examined grossly for volume, color and appearance and findings were noted.

CONVENTIONAL SMEAR

For conventional smear, the fluid was centrifuged at 1500 rpm for 10-15 minutes in test tubes and supernatant decanted. Minimum of three thin smears were prepared from the sediment . Papanicolaou (PAP) , Hematoxylin and Eosin (H&E) and May-Grunwald Giemsa (MGG) staining was done on the smears prepared.

CELL BLOCK TECHNIQUE

- 5ml specimen was centrifuged at 2500 rpm for 10-15minutes
- Supernatant was poured off and sediment was obtained.
- Add 2 to3 drops of plasma to the sediment, mix the sediment and plasma together.
- And add 3 to 4 drop of thrombin solution to the mixture, mix again allow the mixture to clot. Then add 10% tinted formalin.
- Pour the clot and the formalin into a petridish cut the clot into the pieces and allow them to fix.
- The sediment was then wrapped in the filter paper and processed in histokinette as routine histopathological specimen Multiple thin sections of 4-5 micron thickness from paraffin blocks were obtained, stained with Haematoxylin and Eosin stain and examined microscopically.

Scoring and analysis

Two authors independently graded on a semiquantitative basis four different parameters including cellularity, architecture, obscuring blood in the background, cellular degenerative changes according to Mair et.al scoring system[4].Score of 0,1 and 2 was assigned to each smear and cell block preparations.

Table 1-Mair Scoring system-

Criteria	Quantitative description	Point score
Amount of diagnostic cellular material	Minimal	0
	sufficient	1
	Abundant	2
Volume of obscuring background blood	Large	0
	Moderate	1
	Minimal	2
Retention of appropriate architecture	Minimal	0
	Moderate	1
	Excellent	2
Degree of cellular degeneration	Marked	0
	Moderate	1
	Minimal	2

RESULTS

All the samples analyzed were divided into three categories:

positive for malignancy, suspicious and benign/reactive processes. Present study comprised of a total 168 samples which included effusions of body cavities (pleural and peritoneal) fluids. Out of these 131 (78%) were pleural and 37 (22%) were peritoneal effusions.

Exudative effusions outnumbered the transudative effusions both in pleural and peritoneal effusions with 88.5% (n=116/131) and 75.7% (n=28/37) respectively .

In our study the total number of male patients (n=94) were more than female patients (n=74).Therefore, the overall Male: Female ratio was 1.27:1 . In malignant effusions an equal number of cases were seen in both genders.

Majority of the effusions in our study were reactive (n=154, 91.7%). Only 14 cases (8.3%) were malignant. Among the reactive effusions, the commonest cause was tuberculosis (n=98, 58.3%). In the category of malignant effusions a large number of cases did not have a known primary .

Table 2- Pattern of volume of background obscuring blood in the conventional smear and cell block techniques

Volume of obscuring background blood	Point score	Conventional smear	Cell Block
Large	0	2 (1.2%)	1 (0.6%)
Moderate	1	124 (73.8%)	59 (35.1%)
Minimal	2	42 (25%)	108 (64.3%)
Total		168 (100%)	168(100.0%)

The volume of obscuring blood in the background was minimized after preparing cell blocks compared to conventional smears of the same samples. The percentage of score 2 observed was 64.3% in blocks compared to 25% in smears. Similarly the score 0 cases (large amount of obscuring blood) were 0.6% in blocks compared to 1.2% in smears .

Table-3 Pattern of cellular architecture in the conventional smear and cell block techniques

Retention of cellular architecture	Point score	Conventional smear	Cell Block
Minimal/absent	0	5 (3%)	0 (%)
Moderate preservation	1	162 (96.4%)	128 (76.2%)
Excellent architecture pattern	2	1 (0.6%)	40 (23.8%)
Total		**168 (100.0%)**	**168 (100.0%)**

The percentage of excellent **cellular architecture** pattern was increased to 23.8% in cell block from 0.6% in conventional smear .

Table 4- Pattern of cellular degeneration in the conventional smear and cell block techniques

Degree of cellular degeneration	Point score	Conventional smear	Cell Block
Marked	0	5 (3%)	0 (0%)
Moderate	1	163 (97%)	163 (97%)
Minimal	2	0 (0%)	5 (3%)
Total		168 (100.0%)	168 (100.0%)

Although there is slight, 3% minimal **cellular degeneration** found in cell block technique but the percentage of moderate degree of cellular degeneration remains same at 97% in both the techniques.

Table 5 -Comparision of diagnostic cellularity in the conventional smear and cell block techniques

Diagnostic cellularity	Point score	Conventional smear	Cell Block	P value
Minimal/absent	0	16 (95%)	5 (3%)	0.001
Sufficient	1	146 (86.9%)	85 (50.6%)	0.001 (chi sq value is 59.01)
Abundant	2	6 (3.6%)	78 (46.4%)	0.001 (chi sq value is 70.01)
Total		168 (100.0%)	168 (100.0%)	

46.4% cases showed abundant cellularity on cell blocks compared to only 3.6% in conventional smears. The percentage of cases showing minimal/absent. Cellularity decrease to 3% in cell blocks from 95% in conventional smears. Although the proportion of finding of sufficient cellularity is comparatively decreased in cell block to 50.6% than 86.9% in conventional smear, the difference is significant statistically.

Maximum serous effusion cases (n=161) were falling in the category of diagnostically adequate on using conventional smear while diagnostically superior and diagnostically inadequate cases were very few. On cell block however an almost equal number of cases were in the diagnostically adequate and superior category with only one case falling in diagnostically inadequate category.

These figures clearly prove the superiority of the cell block technique and is found to be significant statistically. There is increase in the frequency of malignant cells found on cell block technique as compared to conventional smear which is n=14 versus n=8 and this difference was also found to be statistically significant. The 6 cases which were diagnosed as suspicious of malignancy on conventional smear were diagnosed as malignant in the sections of the cell block. They were classified as suspicious for malignancy (n=6) as nuclear and cytoplasmic details were not clear and vacuolations with eccentric nuclei can be seen even in a degenerating and/or reactive mesothelial cell in conventional smear. The additional diagnostic yield by cell block technique was 42.8% in our study.

DISCUSSION

Many studies support the superiority of cell block technique in the diagnosis of malignancy[5]. ytological examination of serous fluids has increasingly gained acceptance in clinical practice to such an extent that a positive diagnosis is often considered the definitive test that even obviates exploratory surgery[6]. Fluid submitted for cytological examination can be evaluated in many ways. One of the most common and distressing drawback in conventional smear is to distinguish between reactive mesothelial cells from metastatic malignant cells. This is either due to the marked atypia of mesothelial cells caused by the microbiological, chemical, physical, immunological or metabolic insults to the serous membrane due to disease process or due to the subtle cytomorphological features of some malignant neoplasms particularly well differentiated adenocarcinomas . It was easier to differentiate between reactive mesothelial cells and malignant cells on cell blocks which increased the diagnostic yield for malignant effusions, as cell blocks effectively puts both architectural (rossetes, pseudoacini or acini) and morphological features (prominent nucleoli) in their proper perspective. Nucleoli do not appear as prominent in conventional smears and acinar structures can be better appreciated when present in cell blocks. In cases of well differentiated adenocarcinomas malignant character of cells, both architectural and cytomorphological, are better appreciated in cell blocks with presence of true acini[7,8].

Since the introduction of the cell block technique by Bahrenburg,it has been use routinely in the processing of fluids. The technique is simple, safe, cost effective and reproducible even in resource limited settings[9].

The malignant cells in pleural and ascitic fluid are almost always indicative of metastatic tumors as primary malignancies which arise from the mesothelial cells are relatively uncommon. The development of a malignant effusion is an indication of advanced stage of malignancy[10].

We evaluated both conventional smears and cell blocks for cellularity, architectural pattern, predominant cells, volume of obscuring blood and preservation of morphology. A total of 168 specimens were studied which comprised of 131 (78%) pleural and 37 (22%) peritoneal effusions. Therefore the number of pleural fluids was much more than peritoneal. Our results are similar to Subhada et.al [11], Bhanvadia et. al[9],Nair et. al[5] and Shobha et al[12] . However Sujathan et al[13], Joshi et al[14] and Nathani et. al[15] have reported a larger number of peritonial fluids in their study. As far as the provisional clinical diagnosis was concerned, in reactive effusions tuberculosis was the commonest accounting for 58.3% of the cases.

Thapar et al[10] in their study have also shown similar trend reporting 18.3% of the effusions where the underlying pathology was tuberculosis. Joshi et al[14], Shubhada et al[11] and Shukla et al[16]] (33%) have also seen similar results in their study where the major proportion of non neoplastic effusions was due to tubercular pathology. Shobha et al[12] has also reported maximum cases of reactive pleural effusion (52%) having tubercular etiology. Nair et al[5] also reported a similar result in pleural fluid but in peritoneal fluids cirrhosis was the commonest cause followed by tuberculosis. Nathani et. al[15] had maximum cases of cirrhosis followed by congestive cardiac failure and then tuberculosis as the cause of serous effusions in his study. However Luse and Reagan[17] reported underlying congestive cardiac failure as the cause of maximum effusions in their study. This difference may be largely due to the difference in the region where the respective studies were carried out. Studies which are conducted in India and the neighbouring countries which share almost the same geographical terrain and climatic conditions also share common endemic trends for certain diseases especially tuberculosis.

There was a male preponderance seen among the patients with the male: female ratio being 1.27:1. Similar results were seen by Nair et al[5] (M:F=1.55:1) and Joshi et al[14](M:F=1.05:1). In pleural effusions alone males outnumbered the females (M=87, F=44) in our study. Bhanvadia et al[9] (M=61, F=18) and Shivkumarswamy et. al[8] have also shown the same. Peritoneal effusions on the other had a female preponderance in our study, which is similar to what was seen by Bhanvadia et. al[9] but contrary to the results reported Pal et. al[19] in their study where a male preponderance was noted (M:F=1.5:1).The present study showed more of exudative effusions (n=144) than transudative (n=24). However in the study conducted by Bhanvadia et. al[9] the number of transudative effusions (n=91) was much more than exudative effusions (n=59).

Table 6 : Comparison of predominant cell population in reactive effusion

Predominant cell type	Present study	Thapar et al
Lymphocyte	67.5%	13.3%
Polymorph	17.5%	21.7%
Mixed cellularity	10.4%	20%
Mesothelial	1.3%	5%

In reactive effusions we found lymphocytes as the predominant cell in maximum cases (67.5%) where as Thapar et. al[10] has reported polymorphs as the predominant cell (21.7%) in maximum cases .

On evaluating the cellularity of the smears, score 0 (CS0) was observed in 95%, score 1 (CS1) in 86.9% and score 2 (CS2) in 3.6% where as score 1 (CB0) was 5%, score 2 (CB1) was 50.6% and score 3 (CB2) was 46.4% in cell blocks. These results clearly depict the superiority of the cell block technique over the conventional smears. Shukla et. al[16] and Shubhada et. al[11] have also shown a definite advantage that cell blocks have over smears in the cellular yield .

When assessment for retention of architecture was performed, Score 0 was observed in 3% smears which reduced to 0% in block, score 1 was 96.4% in smears and 76.2% in block and score 2 increased from 0.6 % to 23.8% in cell blocks. Shukla et. al[16] has results in congruence with ours, score 2 increased from 20% in smears to 40% in cell block. Shubhada et. al[11] also reports an improvement in retention of architecture in cell blocks in comparison with smears; score1 increased from 0.7%in smears to 11.27% in block and score2 showed a rise from 0% to 9.86% in cell blocks . Cell block preparations revealed better cytoplasmic and nuclear details as compared to conventional smears.

On finally assigning the diagnostic categories to the smears and cell block preparations, our study showed that the diagnostically unsuitable category was reduced from 6.57% to 0.6% in blocks and diagnostically superior was raised from 0.6% to 50% thereby leaving no doubt on the superiority of the cell block technique over conventional smears. Nathani et. al[15] and Thapar et. al[5] also show compatible results in their respective studies .

Cell block concentrated the cellular material into a small area, which made it easier to screen the material in lesser time with cells lying in the same focal plane whereas in conventional smear the cells remain in dispersed form with paucity of representative cells[10,18,19,20].

Sometimes degenerating mesothelial cells appear signet ring cells with large vacuoles and eccentric nuclei which can be misleading as mucin secreting tumours in conventional smears.They may also show prominent nucleoli[19].

Gaps and windows in reactive mesothelial cells were frequently seen in the present study similar to the findings by Bhanvadia et. al[9].

The glandular structures, papillary structures, clusters, 3D balls and demonstration of mucin in cytoplasm of tumor cells was more reliably seen in cell blocks rather than conventional smears. Preservation of architecture and morphology of cells in reactive effusions was also much better in cell block preparations than conventional smears, however there was no additional diagnostic yield in reactive effusions on preparation of cell blocks compared to conventional smears in this study. By using the cell block technique we obtained an additional diagnostic yield of 42.8% . Shobha SN et.al[12]. also demonstrated that there was no helpful diagnostic yield in reactive effusions obtained by cell block on the basis of architectural and morphological preservation.

Thus cell blocks technique is a cost effective method in resource limited laboratories. It not only increases cellularity but also provides better architecture display and morphological preservation increasing the diagnostic yield. It should be used as an adjunct to conventional smears to aid and improve the diagnosis in serous fluid cytology.

CONCLUSION

Cell blocks can be studied in a biopsy like fashion with less cellular dispersal and an added advantage of multiple sections on which special stains can be applied, with a possibility of storing slides and blocks for longer duration.

Apart from this sections from cell blocks increases cellularity with minimal amount of obscuring blood, better preservation of architecture with excellent nuclear and cytoplasmic details which increases the diagnostic yield.

To conclude a combined approach of cell block technique with conventional smears should not only be used for suspicious effusions on conventional smears but it can be used as a routine for all effusions received to find out hidden cases of malignancies. Such an approach is preferable for resource limited laboratories who can use a cost effective method for cell block preparation using plasma thromboplastin.

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