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Original Research Article

A case series of comparative study of two cell block methods

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ABSTRACT

Introduction: Cell block method for assessment of body fluids, effusions, material obtained from FNA's is simple, inexpensive and overcomes the pitfalls of conventional cytology such as atypical metaplasia, crowding of cells, reparative changes and staining artifact. Cell block offers long term retrievability.
Materials and Methods: This retrospective study was done from October 2020 to February 2021 at tertiary care hospital. Various samples of body fluids (peritoneal, pleural, cerebrospinal fluid, bronchial wash, synovial fluids, pus, endometrial aspirate, sputum and urine) received in cytology section were studied. The cell blocks by formalin and plasma-thrombin methods were prepared and compared with each other and comparison of their diagnostic accuracy with diagnostic accuracy of conventional smear method was studied.
Results: We used scoring system utilized by Kasichhwa et al. for comparison of formalin and plasma-thrombin methods. The median score of plasma-thrombin method is 5 while that of formalin based method is 4. The diagnostic accuracy of plasma-thrombin method is 69.23% and that of formalin based method is 68.19%.
Conclusion: The cell block helps in providing additional cellular details and nuclear features when studied in conjunction with conventional smear. The plasma thrombin method provides better cellular details but in a low cost setting it is not cost effective. Formalin method is both cost effective and handy, therefore it is recommended that a cell block preparation is always undertaken.

1. Introduction

Age old method of cell block for assessment of body fluids, effusions, material obtained from FNA's is simple, inexpensive, feasible and worthy technique in the world of diagnostic cytology.[1,2] The pitfalls of conventional cytology such as atypical metaplasia, crowding of cells, reparative changes and staining artifacts are overcome by cell block.[3]

Cell block contains residual tissue from body fluids embedded in paraffin that can be cut and stained by same method as used for histopathology. Cell block increases cellular yield, improves cytomorphological features and diagnostic accuracy.[4] Additional ancillary techniques such a immunohistochemistry and molecular techniques can be performed on cell block.[1,5]

Even though utility of cell block is greatly acknowledged cell blocks are not routinely prepared. Since the advent of cell block by Baherenberg in 1985, it has undergone many modifications and alterations. Various cell block techniques have been developed over the years that vary in scope and the type of fixatives, processing and embedding techniques used. Some of the common techniques include tissue fragments by plasma thrombin method, HistoGel method, Shandon cyto-block method, Collodion bag cell

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block method, Bacterial Agar method, inverted filter sedimentation, simple sedimentation etc. [1,6]

Studies have indicated that many laboratories are unsatisfied with their technique for creating cell block. Some of the frequently encountered issues include the technique having high cost, being labor intensive and yielding less than adequate cellular material for diagnosis or ancillary testing. Hence the present study is carried out to compare two different methods of cell block, formalin fixation method and plasma-thrombin (PT) method for cytological diagnosis.

2. Materials and Methods

A retrospective study was conducted in cytology section of Department of Pathology at tertiary care hospital in Mumbai from October 2020 to February 2021.

Samples of various body fluids (peritoneal fluid, pleural fluid, CSF, bronchial washings, tracheal aspirates, synovial fluid, pus, endometrial aspirates, sputum and urine) received in the cytology section were studied.

Samples received were examined for quantity, colour and transparency followed by conventional smear (CS) preparation which were stained with pap and giemsa stain. The remaining fluid was used to make cell blocks (CB) by two different methods i.e. formalin and plasma thrombin method. Samples which were less in quantity (i.e. <2 ml) were not included in the study. Only 91 samples were studied during evaluation due to COVID 19 pandemic.

In formalin based cell block method the fluid was centrifuged at 3000 rpm for 15 min. The supernatant was discarded and the cell button thus formed was kept in 10% formalin overnight for fixation. After fixation, the cell button was wrapped in filter paper and sent to histopathology section where it was processed as routine histopathology sample and haematoxylin and eosin stained slides were prepared.

In plasma thrombin method of cell block preparation the sample was centrifuged at 3000 rpm for 15 min. The supernatant was discarded and 0.5 ml of plasma and 2 drops of thrombin was added. The sample was then agitated quickly. A clot formed within 30 to 60 seconds. The clot was there placed in a filter paper and sent to histopathology section where it was processed as routine histopathology sample and haematoxylin & eosin stained slides were prepared.

Both the all blocks were compared and slides were scored on scale of 1 to 3 using the criteria mentioned in Table 1 which was also used by Kasichhwa et al. in their study. Based on the criteria a minimum score of 3 and maximum score of 9 was given to cell block.

The slides were scored on scale of 1 to 3 for the criteria mentioned inTable 1. Based on the criteria a minimum score of 3 and maximum score of 9 was given to cell block.

Table 1:

Criteria	Score 1	Score 2	Score 3
Cellularity	Paucicellular (occasional cells)	Moderately cellular (few cells to few clusters of cells)	Highly cellular (Abundant cell)
Clarity of cell morphology and nuclear details	Poor	Fair	Good
Recovery of cell clusters and fragments compared to conventional smears	Poor	Comparable to conventional smear	Better than conventional smear

3. Results

Total of 91 fluids were examined in the study, 38 male and 53 females.

The study population comprised of people from the age 05 years to 85 years with mean age of 48 years. The majority of the patients presented in the 5^{th} decade followed by 6^{th} decade.

The distribution of fluids studied is as given inTable 2.

Table 2:

Fluids	No of samples
Peritoneal fluid	26
Pleural fluid	35
CSF	2
PUS	3
Sputum	1
Urine	4
Bronchial wash	4
Endometrial aspirate	3
Other (Cyst fluids/ drain fluids)	13
Total	91

Table 3: Median score of both the cell block techniques

Criteria	Formalin based cell block (Median score)	PT based cell block (Median score)
Cellularity	1	1
Clarity	2	3
Recovery of cell cluster compared to conventional smear	1	1
Total score	4	5

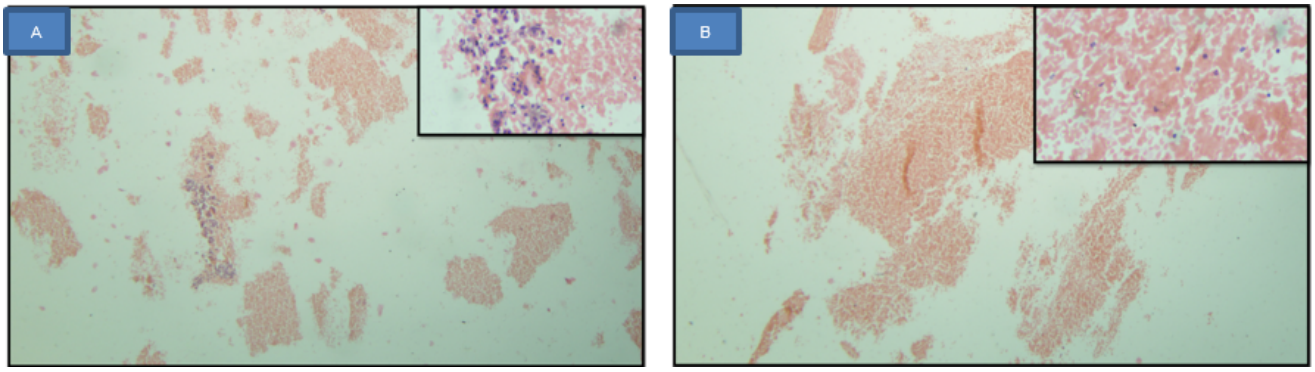

Fig. 1: A,B: H & E, 40x- Cell block of ascitic fluid made by formalin and plasma thrombin method respectively. Section from cell block made by formalin method shows more cellularity compared to that of plasma thrombin method. Inset- 400x magnification of both the sections.

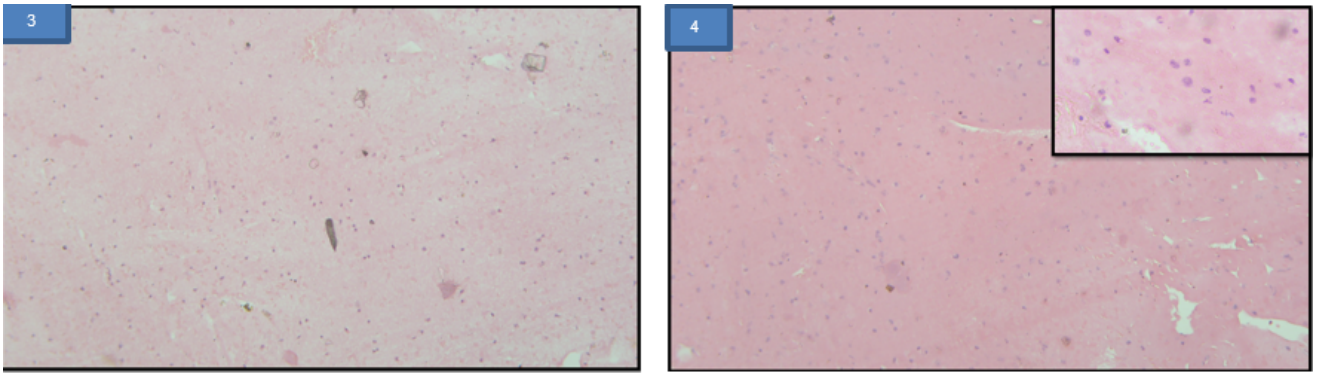

Fig. 2: A,B: H & E, 40x- Cell block of pleural fluid made by formalin and plasma thrombin method respectively. Section from cell block made by plasma thrombin method shows better preservation of morphology compared to formalin method of cell block preparation. **B:** 400x magnification of section from plasma thrombin method shows better preservation of cellular details compared to formalin method

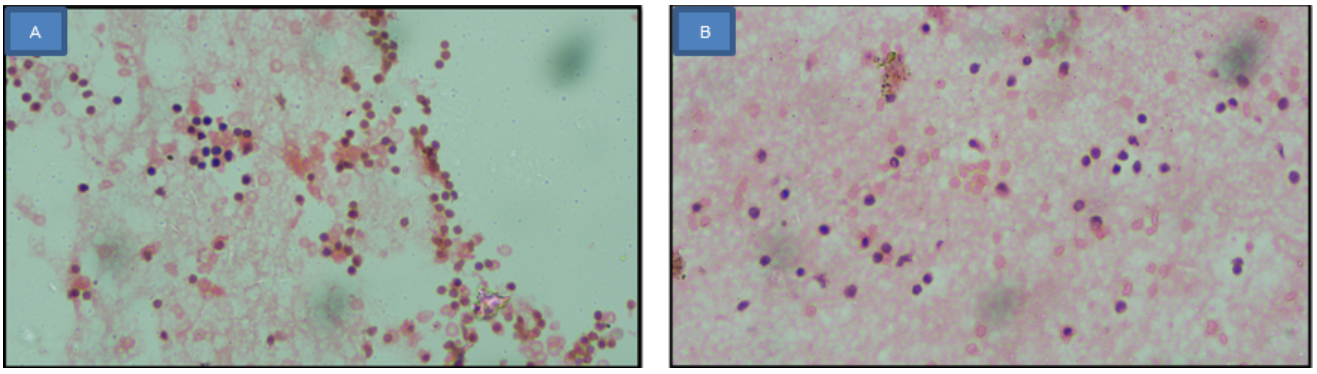

Fig. 3: A,B: H & E, 100x- Cell block of pleural fluid made by formalin and plasma thrombin method respectively. Both the sections show haemorrhage and sparsely preserved cells; but the cellular details are better preserved in cell block made by plasma thrombin method.

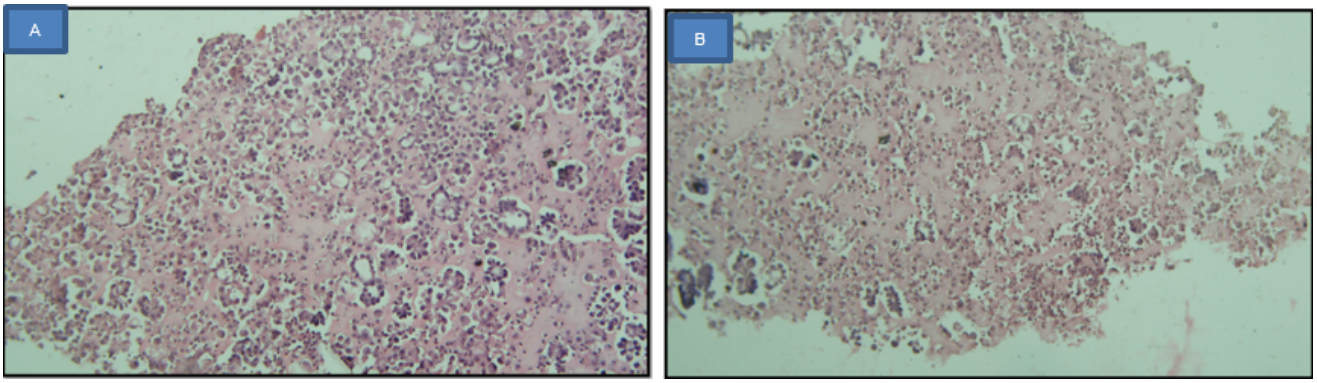

Fig. 4: A,B: H & E, 100x- Cell block of pleural fluid made by formalin and plasma thrombin method respectively showing metastasis of adenocarcinoma. Both the sections show haemorrhage and tumour cells which are arranged in glandular pattern. Both the methods show good cellularity and preservation of cellular and nuclear details

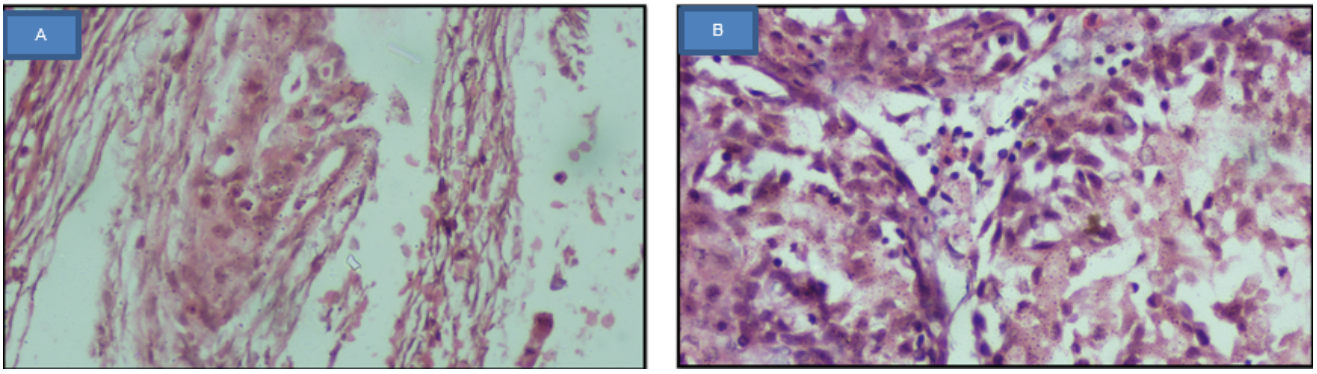

Fig. 5: A,B: H & E, 100x- Cell block of sputum made by formalin and plasma thrombin method respectively showing mucoid material, ciliated columnar cells and mixed inflammatory infiltrates. Plasma thrombin method of cell block shows better preservation of cell morphology.

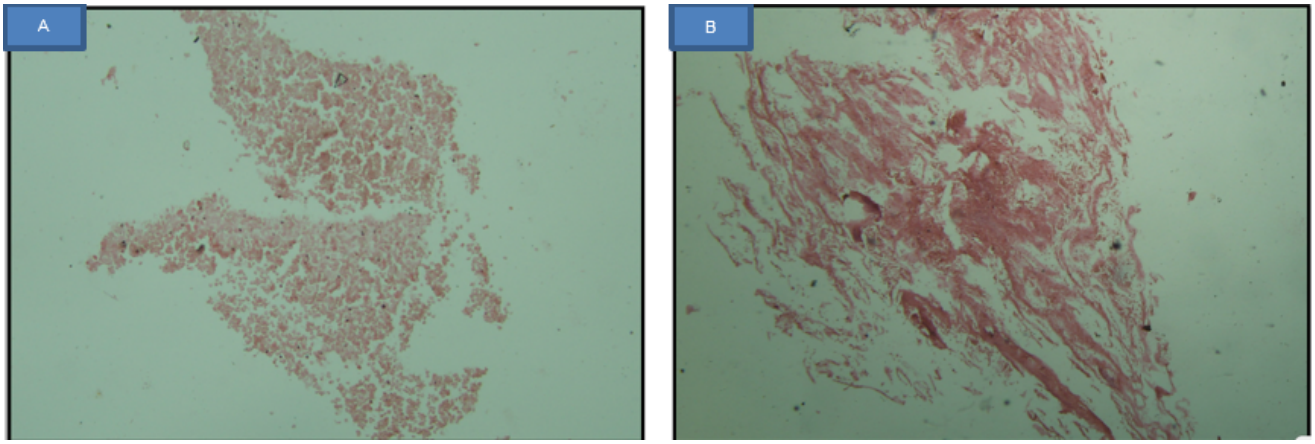

Fig. 6: A,B: H & E, 40x- Cell block of CSF made by formalin and plasma thrombin method respectively showing only haemorrhage

On comparison of total score for the two methods, median score of formalin was 4 & PT was 5 which is significant. Though the median scores were low for cellularity and recovery, the PT based cell blocks had more number of samples (47.25%) having cellularity and recovery score of 2 and 3 than formalin based cell block.

Table 4: Comparison of diagnostic cell block's with diagnostic conventional smears and with other studies

Study	Diagnostic Conventional smears	Diagnostic Cell Block
Nathan et al	84.8%	73.3%
Vinayakmurthy et al	96.96%	68.18%
Present study	73.62%	By Formaline method- 68.13% By PT method- 69.23%

4. Discussion

Cell block is an adjunct to the conventional smear in cytology. They provide additional information regarding cell morphology. As compared to conventional smears, the cell blocks provide clear background so that nuclear details and cell morphology is appreciated nicely. The advantage of cell block is that it aids in diagnosis by facilitating ancillary studies.[1] In our study, we compared the formalin based cell block method with plasma-thrombin method of cell block preparation.

On comparing both the methods, it was observed that plasma-thrombin method was superior to formalin based method in providing cellular details and nuclear features. The plasma-thrombin method showed good amount of cellularity (score of 2 and 3) in 43 samples (47.25%) compared to formalin based method, which showed good cellularity in 41 samples (45.05%).

The plasma-thrombin method, provided better clarity regarding cell morphology than formalin based method. This could be because the formalin pigment obscures the cellular details.

In our study, diagnostic material by plasma-thrombin method was obtained in 63(69.23%) samples out of 91, while formalin based method yielded diagnostic material in 62(68.13%) as compared to the conventional smears which yielded diagnostic material in 67(73.62%) samples. The reference study Vinaykumarmuthy et al[3] obtained diagnostic material in 96.96% samples on conventional smear while the cell block method provided the diagnostic material in only 68.18% samples.

The similar study Nathan et al[7] showed that 84.8% samples were having diagnostic material by conventional smears, while cell block method yielded diagnostic material in 73.3% sample.

The studies Kasichhawa et al,[1] Vinaykumarmurthy et al[3], Nathan et al[7] did the special stains (Zn stain, PAS, Mucicarmine etc) and immunohistochemistry on cell block. Special stains and immunohistochemistry however were not conducted in our study. Nevertheless, it can be conducted at any point in time.

In study Bedrossian et al,[8] plasma thrombin and collodion bag methods were compared which showed equal or better cell preservation in collodion bag method. Similar finding was observed in study Balassanian et al.[5]

Basnet et al[4] observed that diagnostic accuracy of 95.51% when compared with conventional smear, which is very high with respect to our study, but Basnet et al had limitation of less sample size (49).

Study by Saqui A et al[6] concluded that the low yield observed in cell block could be because of variation in preparation, methods and technical skills. Study by Masur et al[9] observed that plasma thrombin method is more effective as compared to the formalin method in producing cellular details and overall preservation of architecture, concordant with our study.

Another study Mishra S et al[10] observed morphology is better preserved in formalin method, but in comparison with agar based method of cell block preparation.

Though the difference was very minimal, the plasma-thrombin method was superior than formalin based cell block method.

5. Conclusion

The formalin based and plasma-thrombin method of cell blocks, both helps providing additional cellular details and nuclear feature when studied with conventional smears. Though the results were not that significant, but the plasma-thrombin method was better than formalin based method. The formalin based method is straightforward, do not need any additional resource and can be done in low resource settings, while the plasma-thrombin method requires the pooled plasma and commercially prepared thromboplastin, which might not be accessible to everyone.

6. Conflict of Interest

None.

7. Source of Funding

None.

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