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ORIGINAL ARTICLE

UTILITY OF CELL BLOCK TECHNIQUE BY MICROWAVE PROCESSING FOR RAPID DIAGNOSIS IN FLUIDS AND FINE NEEDLE ASPIRATES

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ABSTRACT: INTRODUCTION: The present study was carried out to evaluate the cell block technique prepared out of the residue of fluids and fine needle aspirate (FNA) samples after routine cytological processing. In addition it was processed in a microwave to facilitate early reporting. Aims and **OBJECTIVES:** The aim of the present study was to correlate the cytological findings with those of cell block sections and to establish the microwave processing technique in preparation of paraffin blocks. **MATERIALS AND METHODS:** A total of 100 samples were studied over a two year period. They comprised of 64 fluids and 36 FNA samples. In 88 cases, both cytology and histology were available for correlation. For cell block preparation, the modified plasma-thrombin technique and for microwave processing, the modified Bellotti's technique were used respectively. **RESULTS:** Positive correlation between cell block and cytology for malignant and benign lesions in fluid specimens was seen in 21.87% and 51.56% cases respectively. Positive correlation between cell block and cytology for malignant and benign lesions in FNA specimens was seen in 47.22% and 33.33% cases respectively. The sensitivity and specificity of cell blocks and cytology smears were calculated. Also the use of microwave processing allowed us to give report on the same day without affecting the quality of sections and staining. **CONCLUSIONS:** The present study indicates that even after cytological processing of fluids and FNA specimens, some residue is left behind which may contain valuable diagnostic material which can be processed further as a cell block. In addition, microwave processing gives the added benefit of rapid reports without compromise in the quality of reports.
KEYWORDS: Cell block technique, Microwave processing, Body fluids and FNAC specimens.

INTRODUCTION: The cell block preparation for microscopic evaluation was first introduced by Bahrenburg in 1896. The cell block technique has been in use since 1900 but has been overshadowed by smear technique which has gained considerable momentum since generalized acceptance of Papanicolaou's methods. With the development of excellent cell preparation techniques, the cell block technique has been abandoned by many laboratories and this neglect is not justified.

The present study was based on the premise that cytological examination of fluids and fine needle aspirates, no matter how carefully prepared, leaves behind some residue that is not further investigated and that might contain valuable diagnostic material. In order to evaluate this residual material in a simple and expedient fashion, it was treated as a cell block embedded in paraffin and sections were examined in addition to the routine smears.

Also to improve the turn-around-time in this age of managed care, in our study, the standard technique for tissue fixation and processing was changed to microwave fixation and processing so as to obtain a same-day diagnosis which contributes significantly to patient care.

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MATERIALS AND METHODS: This was a prospective study which was carried out in the department of Pathology at Mahatma Gandhi Institute of Medical Sciences (MGIMS), Sevagram, over a period of two years. In the present study, hundred cases of randomly selected fluids and fine needle aspirates (FNA) constituted the clinical material submitted by the in-patients as well as out-patients of the MGIMS Hospital.

In all these cases, relevant data regarding name, age, sex, registration number of the patient, type of fluid, site of FNAC and the provisional clinical diagnosis were collected on a predesigned proforma. The cytology report was unavailable in 12 cases as the material was directly submitted to histopathology section and processed there only. In the remaining 88 cases, both histologic diagnosis on cell block and the cytology diagnosis on smears were given.

MATERIAL USED: The following material was used to process the specimens- Disposable plastic syringe-10 ml, disposable needle 24 or 26G, glass slides, 10% formalin, conical glass centrifuge tubes, centrifuge machine, Whatman filter paper, cotton thread, domestic microwave oven (BPL-Sanyo), Borosil beaker 250 ml.

Routine wet fixed and air dried smears were prepared from all the specimens and were stained with Papanicolaou and Giemsa stain respectively.

A "Modified plasma-thrombin cell block technique"[1] was followed in all cases. For cases which were sent for FNAC, after making routine smears, whatever material was remaining in the needle and syringe, was thoroughly rinsed in 10 % formalin and taken into a conical centrifuge tube. For fluids, after centrifugation, routine smears for Papanicolaou and Giemsa were made from the sediment. Then the remaining fluids were taken into conical glass centrifuge tubes and 10% formalin was added and allowed to stand for half an hour.

For both fluids and FNA aspirates, the specimens were centrifuged at 2000 rpm for 20 minutes. The supernatant was decanted and 2-3 drops of outdated plasma (obtained from blood bank) was added to the sediment. 2 drops of thromboplastin were added and the fluid was allowed to stand for five minutes. A clot usually formed which was gently collected on a Whatman filter paper which was then folded and tied securely with ordinary cotton thread. This small packet was then transferred to a Borosil beaker, kept in the centre of the microwave and subjected to microwave processing at power 10. A modified Bellotti's technique [2] was used. The steps of processing in the microwave were as follows-

Fixation 10% formalin 2 min
Dehydration 50% ethyl alcohol 2 min
70% ethyl alcohol 2 min
90% ethyl alcohol 2 min
Absolute alcohol 2 min
Absolute alcohol 2 min
Clearing Xylene 2 min
Xylene 2 min
Impregnation Paraffin 3 min
Paraffin 3 min

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After this short processing cycle of 30 minutes, the clot/tissue fragments were embedded in liquid paraffin along with the identification number. The sections were cut as for routine surgical specimens at 4-5 microns and stained with hematoxylin-eosin. Both the smears and sections were examined on the same day. Correlation of diagnosis for smears and cell block was done and the sensitivity and specificity for both the techniques were calculated.

OBSERVATIONS: A total of 100 randomly obtained specimens (64 fluids and 36 FNAs) were processed as cell blocks in the microwave.

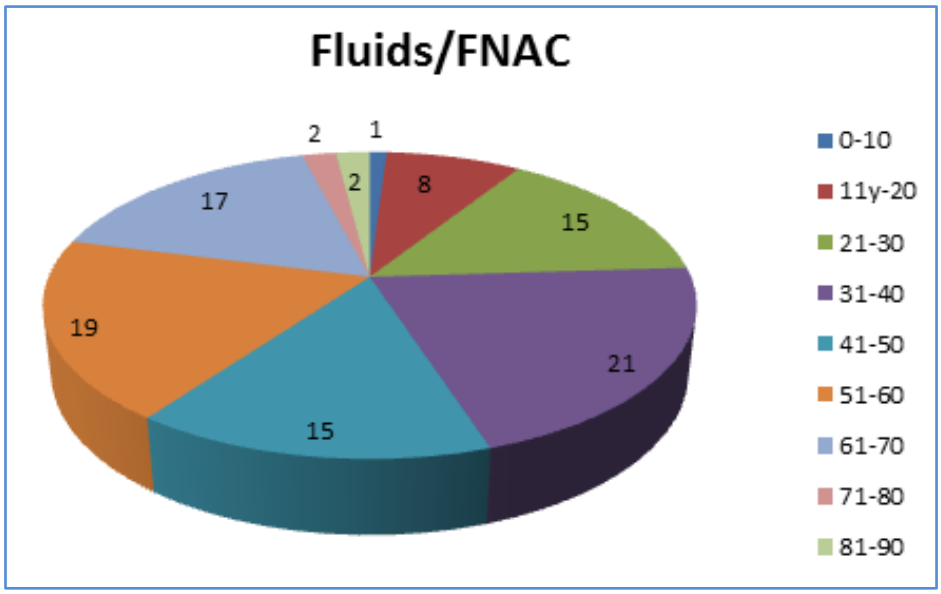

Figure 1: Pie chart for age distribution (in years) in FLUID and FNAC samples

FNAC	Number	Percentage (%)	Fluids	Number	Percentage (%)
Soft tissue	6	16.66	Pleural	40	62.50
Lung	6	16.66	Peritoneal	15	23.43
Thyroid	4	11.11	Synovial	3	4.68
Lymph nodes	4	11.11	Pericardial	2	3.12
Bone	4	11.11	Bladder washings	1	1.56
Breast	3	8.33	Subdiaphragmatic collection	1	1.56
Liver	3	8.33	Scrotal fluid	1	1.56
Mediastinum Miscellaneous	2 4	5.55 11.11	Pseudopancreatic cyst	1	1.56
Total	**36**	**100.00**	**Total**	**64**	**100**

Table 1: Distribution of FNA and fluid specimens

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Fluids	No. of cases	Percentage	FNACs	No. of cases	Percentage
Malignant	15	23.43	Malignant	20	55.55
Benign	49	76.56	Benign	14	38.88
			Inconclusive	2	5.55
Total	**64**	**100.00**	**Total**	**36**	**100.00**

Table 2: Distribution of cases according to histologic diagnosis

Correlation	Fluids		FNAC	
	No. of cases	Percentage	No. of cases	Percentage
Positive correlation for malignant lesions	14	21.87	17	47.22
Non correlation for malignant lesions	9	14.06	1	2.77
Positive correlation for benign lesions	33	51.56	12	33.33
Cases excluded for correlation	8	12.50	4	11.11
Total	**64**	**100.00**	**36**	**100.00**

Table 3: Correlation between histologic and cytologic diagnosis

Type of fluid	Total cases		CB and smear +ve		Smear alone +ve		CB alone +ve	
	No.	%	No.	%	No.	%	No.	%
Pleural	17	100	10	58.82	6	35.2	1	5.88
Peritoneal	4	100	3	75.00	1	25.00	-	-
Bladder washings	1	100	1	100	-	-	-	-
Total	22	100	14	63.63	7	31.8	1	4.54

Table 4: Results of simultaneous use of smears and cell block for malignant fluids in the present study

CB- Cell Block

Type of FNAC	Total Cases		CB and Smear +ve		Smear alone +ve		CB alone +ve	
	No.	%	No.	%	No.	%	No.	%
Lung	6	100	6	100	-	-	-	-
Lymph nodes	3	100	3	100	-	-	-	-
Bone	3	100	3	100	-	-	-	-
Soft tissue	2	100	2	100	-	-	-	-
Liver	1	100	1	100	-	-	-	-
Prostate	1	100	1	100	-	-	-	-
Mediastinal mass	2	100	1	50	-	-	1	50.00
Total	**18**	**100**	**17**	**94.44**	**-**	**-**	**1**	**5.55**

Table 5: Results of simultaneous use of smears and cell block for malignant FNACs in the present study

CB- Cell Block

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Study	Total cases c		CB and smear +ve		Smear alone +ve		CB alone +ve	
	No.	%	No.	%	No.	%	No.	%
Richardson et al	578	100	346	60	164	28	68	12
Present study								
Malignant Fluids	22	100	14	63.63	7	31.81	1	4.54
Malignant FNA	18	100	17	94.44	-	-	1	5.55

Table 6: Comparison of our study with similar study

CB- Cell Block

For Fluids			For FNAC	
Technique	Sensitivity	Specificity	Sensitivity	Specificity
Cell block	81%	100%	100%	100%
Cytology	95%	97%	94%	100%

Table 7: Sensitivity and specificity of cell block and cytology

Soft tissue aspirates constituted most commonly processed FNAC specimens. One case each from thigh swellings in two patients, swelling on sole, lateral abdominal wall lump, forearm swelling and one gluteal region swelling were aspirated. Among bony aspirates, three were from femur and one from a tibial lesion. Of lymph nodes, one FNAC was from inguinal and another from a supraclavicular lymph node. Two cases of anterior mediastinal mass were also aspirated. The miscellaneous group had one aspirate each from prostate, salivary gland, pleura and scrotal swelling.

RESULTS:

1. The most common fluids examined were pleural followed by peritoneal fluids.
2. The commonest age group of patients with effusions was 41-60 years, and for FNACs it was 31-40 years.
3. Two cases of malignancy were picked up, one each in a fluid and FNA sample due to the cell block technique where the smear technique had failed. (Table No. 4 and 5)
4. The sensitivity and specificity of cell block technique to detect malignancy was better than cytology.
5. The use of microwave processing allowed us to give report within one hour of sample collection without affecting the quality of sections and staining.

DISCUSSION: The present study was carried to find the utility of cell block technique processed by microwave and its use as a modality in the rapid diagnosis for fluid and fine needle aspiration specimens.

In our study, we preferred to use more specimens of fluids than FNACs because conventionally the cell block technique is usually used for diagnosis of equivocal cases of malignancies where the smears fail to establish the diagnosis. Most common fluids examined were pleural and peritoneal fluids as these are the sites which are commonly affected by primary or secondary malignant processes.

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The cell blocks were evaluated independently for cytological diagnosis and a diagnosis of malignancy was given in 23.43% of fluids and 55.55% of FNACs (Table No. 2). Two cases with FNAs were inconclusive in nature, because of lack of representative material. In patients with effusions, 8 cases were excluded for correlation between cell blocks and cytology as these samples were submitted only for cell blocks. In 9 cases the correlation between histology and cytology was not observed. In 7 cases the diagnosis was of malignancy on smears but it was of benign nature on cell block. In most of these cases, hyperplastic mesothelial morphology was observed on cell block.

As it was not possible to follow these patients for the true nature of their lesions these cases were taken as false negative by cell block technique. In 1 case each, false negative and false positive results were obtained on cytological examination and these were confirmed by subsequent follow up of patients. In fine needle aspirates positive correlation for malignant lesions was seen in 47.22% of cases, whereas, for benign lesions it was seen in 33.33% of cases. 4 cases were excluded from correlation as the complimentary cytological smears were not available. Only one case showed non-correlation between FNAC and cell block where the FNAC was reported as benign thymoma on cytology and as metastatic adenocarcinoma on cell block. The final diagnosis on clinical follow up was of malignancy.

Dekker et al [3] studied 100 fluid specimens by plasma and thrombin technique. They observed that the cell block technique supplemented the cytological preparation and allowed differentiation of reactive mesothelial cells from tumor cells and vice versa with greater certainty. Rofhaga et al[4] reviewed simultaneous use of cell block and cytology for adequate FNA specimens. They observed 100% correlation between cell block and tissue diagnosis, indicating that the cell block diagnosis was as definitive as conventional biopsy. They also observed that in 55% of cases the smear diagnosis was improved after cell blocks were reviewed.

Yamamoto et al[5] observed that the cell block technique is a reliable method for diagnosis of pancreatic cancers and similarly Kern et al [6] observed that cell blocks were very useful for liver aspirates.

Richardson et al[7] in their study of 2613 specimens found that cell block shows less sensitivity, this however, should not be considered as absolute evidence of superior diagnostic value of smears since material for smears was removed from the specimen prior to cell block preparation. They also observed that smears alone can be negative when only a small fragment of tumor is present in the submitted material. They concluded that evaluation of the entire amount of submitted material can spare the patient from the discomfort and cost of additional procedures. Sangalli et al [8] observed that the hepatocellular carcinoma pattern was appreciable in additional 10 cases with cell block microhistology than on smears alone.

In the present study, we have also observed findings similar to that of other authors [7, 8] that the cell block is a useful technique for diagnosis of malignancy in addition to cell preparation for cytology in fluid specimens. However, in FNACs it is diagnostic on its own.

Dekker et al [9] observed that the advantages of cell block are that there is concentration of cellular material in one small area with all cells lying in same focal plane, helping in better evaluation. Mesothelial cells sometimes aggregate into rosettes and acini on smears but on cell block one can clearly appreciate whether a true acinus is present or not.

Nordgren et al[10] observed certain advantages of cell block technique over cytology in FNACs from lung and mediastinal tumors. In cell blocks the cells were mostly seen as undisrupted

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structures, thereby increasing the possibility of a specific diagnosis. Also further immunohistochemical and special stains could be carried out on sections without thoracotomy, especially for certain tumors and infiltrates which are treated by chemotherapy or radiotherapy and do not require surgical intervention.

The sensitivity and specificity in our study are similar to those of Chiu et al[11] who observed that the diagnostic sensitivity and overall accuracy of cell blocks was significantly better than that of aspiration cytology.

We agree with other workers[6,10] that the advantages of cell block are preserved tissue architecture, availability of multiple sections for special stains and imunohistochemistry, less cellular dispersion which helps in easier microscopic examination, low cost than Tru-cut biopsy and easy storage of the cell blocks. The disadvantages of the technique are increased processing time and delayed diagnosis, loss of cytologic details with respect to smears.

Nathan et al[12] observed lesser sensitivity with cell block than the cytological smears. The cell blocks were nevertheless instrumental in improving results in additional cases both for negative and positive findings.

The authors agree with other authors [8, 11] that the cell blocks not only improve the diagnostic yield but also help in giving an appropriate diagnosis and should be considered in all FNAC specimens whenever possible and in selective cases of exfoliative cytology specimens after review of smears.

The principle [13] of microwave ovens is that they generate nonionizing radiation at a frequency of 2.45 Gigahertz. When dipolar molecules, such as water and polar side chains of proteins are exposed to such electromagnetic fields, they oscillate through 180 degrees at a rate of 2450 million times a second. This kinetic movement produces instantaneous heat which continues till the radiation ceases. The heat produced within the tissue can be controlled by adjusting the energy level and the duration of exposure.

One of the disadvantages of the cell block technique is the lengthy schedule of paraffin processing. To address this problem, we used the 'microwave processing technique' by a modification of Bellotti's technique.[2] With this method the stained slides were available for reporting within one hour of receiving the specimen. We observed that the cell blocks were easier to cut and the cellular material was condensed into a small area.

The quality of sections was excellent allowing detailed study of nuclear and cytoplasmic features and was very much similar to the traditional paraffin processed sections. Similar observations are in concordance with those of Kok et al.[14] and Rohr et al. [15] The tissues fixed in microwave show lysis of RBC if not fixed beforehand in formalin, however, this does not affect the cellular details. Horobin et al[16] studied the hazards and artifacts associated with microwave processing and gave recommendations for safety of laboratory personnel.

From the present study, we finally conclude that cell block preparation by plasma-thrombin technique of fluid and FNAC specimens, in addition to routine cytology, overcomes the diagnostic discrepancies between benign and malignant lesions. Also the problem of delayed reports of cell blocks processed by routine paraffin techniques can be obviated by use of microwave processing without any sacrifices in the section and staining quality.

A positive result and proper typing of the lesions by use of cell blocks will have an obvious influence on patient management which is of utmost importance.

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