VOLUME-9, ISSUE-6, JUNE-2020 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Pathology

ROLE OF CELL BLOCK PREPARATION IN CYTOPATHOLOGIC EVALUATION OF FINE NEEDLE ASPIRATION AND BODY FLUIDS: A DIAGNOSTIC UTILITY

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ABSTRACT

Background: Cell Block preparation with cytological smears from Fine Needle Aspiration Cytology (FNAC) and Body Fluids is simple, reliable, rapid, and cost effective technique to adopt in categorizing lesions whether malignant or benign. Cell block preparation along with conventional smears increases the sensitivity of diagnosis in resource limited laboratories and in guiding towards treatment.

Materials and methods: A total of 92 samples were taken into study, which included FNAC, and Body fluids for cell block preparation over a period of two years. Out of 92 samples, 80 samples were included of which 48 were FNAC and 32 were Body Fluids. 12 cases were excluded from the study due to unsatisfactory sample. Samples were subjected for cell block preparation along with conventional smears. The morphological details and cellular architecture studied and sensitivity of both cell block and conventional smears were determined.

Result: FNAC showed 58.3% sensitivity by conventional smears and sensitivity of 87.5% by cell block preparation. In case of body fluids 62.2% sensitivity was observed by conventional smears and 90.6% sensitivity by cell block preparation. Sensitivity and accuracy of cell blocks over conventional smears were greater in all the smears.

Conclusion: Cell blocks prepared from the residual tissue after FNAC and sediments of body fluids are always useful in establishing more definitive diagnosis, with an advantage that cell blocks can be used for other ancillary tests whenever required.

KEYWORDS : Cell Block, FNAC, Body Fluid.

INTRODUCTION

Cell block (CB) is a luxury when utilized for studying different cytological specimens due to its simple, fast, cost effective, rapid, and reliable technique that can be carried out in different laboratories across the globe and suitable for all types of cytological specimens. [1] It refers to collection of sediments, blood clots or grossly visible tissue remnants from cytological specimens. It is prepared from FNAC, body fluid and every type of cytological specimen available whenever there is a diagnostic dilemma. It can be a useful adjunct to smears for establishing a more definitive diagnosis by use of safe laboratory chemicals for categorization of malignant and inflammatory conditions. It mainly helps in effusion fluids where lower sensitivity of cytodiagnosis of effusion is mainly attributable to bland morphological details of overcrowding or overlapping of cells, cell loss and changes due to different laboratory processing methods.[2]

It has been seen in various studies that the cytological examination of fluids by means of smears, how carefully the smears prepared, it leaves behind large residue that is not further investigated but might contain valuable diagnostic material, Cell Blocks help in diagnosis in those circumstances.[3] In fact it is advisable to study paraffin sections by using CB before discarding specimens which were negative for malignancy by smear examination.[4]

It is especially useful in cases where cytological diagnosis is misleading, as in cases of routine mesothelial cells in effusion cytology, obscuring factors in well differentiated adenocarcinoma.[5] Blood, necrotic materials, and debris many times also interfere in cytological diagnosis. [2,4,6] CB not only increases the positive results but also helps to demonstrate better architectural patterns which could be of great help in reaching a correct diagnosis of primary sites. CB has added advantage that multiple sections of the same material can be obtained for special stains, and Immunohistochemistry. [1,7]

Apart from other advantages, morphological details can also be obtained with CB which includes presentation of the architectural patterns like cell balls, papillary structures and three dimensional clusters, excellent nuclear and cytoplasmic details.[4] Many times fragments of tissue can easily be interpreted in a biopsy like pattern supporting the view that CB should be considered in FNA, body fluids and even in selected exfoliative cases after reviewing smears.[3]

MATERIALS AND METHODS

This study was conducted from October 2010 to September 2012 in the Department of Pathology, V.S.S. medical college and hospital, Burla, Odisha. Cytological specimens of Fine needle aspiration and body fluids after smear preparation, followed by cell block analysis, were performed to evaluate the increase in cytodiagnostic sensitivity. In this study, total 92 cases were considered, from which 80 cases were included for correlation, 12 cases were unsuitable for various reasons.

Out of 80 cases, 48 were FNAC samples, 32 were Body fluid samples. Study of smear examination and cell block analysis was performed and the results were correlated. All patients with clinical history and suspected malignancy, where diagnostic dilemmas were likely to occur, were subjected for CB after obtaining cytological smears. The complete procedure of sample collection, along with side effects was explained, and informed consent was obtained. Samples were collected after preparation of conventional smears. Effusion fluids were collected in 50 ml vial. Gross appearance, clinical and radiological details were also recorded.

Method for Cell Block Preparation:

Following smear preparation, the needles and syringes used to obtain fine needle aspirates were rinsed in 10 ml of 50% ethanol in a specimen container. Any residual clot or tissue in the hub of needles was removed carefully in the laboratory with the aid of another needle and rinsed in 50% ethanol. In body fluids, the supernatant was discarded and sediment was kept after centrifugation. The entire materials were then recent rifuged in a 10-mL centrifuge tube at 4,000 rpm for 6 minutes to

create one or more cell pellets (one pellet in most cases). The supernatant was decanted and the deposit was fixed in freshly prepared alcohol formalin substitute consisting of 9 parts of 100% ethanol and 1 part of 40% formaldehyde.

Fresh working solution is desired because formalin is capable of oxidizing to formic acid after exposure to air and reacting with blood to form acid haematin pigment artefacts. The cell pellets, at the end of 30 minutes of fixation, were re-centrifuged at 4,000 rpm for 6 minutes. These pellets should detach themselves or can be removed easily with a Pasteur pipette following centrifugation. The cell pellets were wrapped in filter paper, placed in a cassette, and stored in 80% ethanol for processing in the automatic tissue processor using a 13- hour processing schedule. The tissues were embedded in paraffin and sectioned at 3-5μm thickness. Routine Haematoxylin and Eosin staining was used on all cell block sections.

RESULTS

The present study is a cross - sectional study conducted over a period of two years from October 2010 to September 2012. 92 samples were taken into study. A total of 80 cases, 48 FNAC and 32 body fluids were considered; 12 samples were unsuitable for cell block preparation due to various reasons i.e. the aspirated material on smear showed only blood elements with no suspicious cells and the lesion appeared to be clinically benign.

TABLE 01: NUMBER AND TYPE OF SPECIMENS FOR CELL BLOCK PREPARATION.

	Malignant	Suspicious of malignancy	Benign	Inflammatory	Total
FNAC (n=48)	21	10	10	07	48
Fluids (n=32)	14	04	04	10	32
Total	35	14	14	17	80

(n = number of cytology samples)

A total of 80 cases of FNAC, and Body fluids were categorized into 4 groups; as 1 - Malignant, 2 – Suspicious of malignancy, 3 - Benign and 4 - Inflammatory. Out of 48 FNAC cases diagnosed cytologically, 21 were malignant, 10 were suspicious of malignancy, 10 were benign, and 07 were inflammatory. In the series of 32 body fluid samples, 14 were malignant, 04 were suspicious of malignancy, 04 were benign and 10 were categorized as inflammatory.

TABLE 02: SENSITIVITY OF SMEARS AND CELL BLOCKS FROM FINE NEEDLE ASPIRATION CYTOLOGY

Serial number	Category of specimen	Smears	Cell blocks
1	Malignant (n=21)	13(62%)	19 (90%)
2	Suspicious of malignancy (n=10)	05(50%)	08 (80%)
3	Benign (n=10)	06 (60%)	08 (80%)
4	Inflammatory (n=07)	04 (57%)	07 (100%)
5	Total (n=48)	28(58.3%)	42 (87.5%)

Table No. 2 shows that 48 samples of FNAC were subjected to both smear and cell block examination and the sensitivity of both samples was compared. Out of 48 cases; in the category of 'inflammatory' (07 cases), 57% sensitivity (04 cases) was observed by smear, whereas sensitivity of 100% (07cases) was observed by cell block method. Out of 10 cases categorized as 'benign', smears showed a sensitivity of 60% (06 cases) where as cell block study showed sensitivity of 80% (08 cases). Out of 10 cases of 'suspicious of malignancy', sensitivity of 50% (05 cases) was observed by smears where as cell block study showed a sensitivity of 80% (08 cases). Out of 21 cases categorized as 'malignant', smears alone showed a sensitivity of 62% (13 cases) where as cell block study showed a sensitivity of 90% (18 cases). Overall, the sensitivity by smears was 58.3% and the sensitivity of cell block was 87.5%. So, an increase of sensitivity by 29.2% in cell block preparation was observed.

TABLE 03: SENSITIVITY OF SMEARS AND CELL BLOCKS FROM BODY FLUIDS

Sl. no	Category of specimen	Total no. of cases	Smears	Cell blocks
1	Malignant	14	09(64%)	12(85%)
2	Suspicious of malignant	04	02(50%)	04(100%)
3	Benign	04	03(75%)	04(100%)
4	Inflammatory	10	06(60%)	09(90%)
5	Total	32	20(62.25%)	29(90.6%)

Out of 32 body fluid samples subjected to conventional smear and cell block examination, the sensitivity was correlated. Out of 10 cases categorized as 'Inflammatory', 60% sensitivity (06 cases) was observed by smears, where as 90% (09 cases) by cell block technique. Out of 04 cases categorized as 'benign', smear showed a sensitivity of 75% (03 cases) and cell block showed 100% (04 cases). Out of 04 cases of 'suspicious of malignancy', sensitivity observed by smear was 50% (02 cases), where as 100% (04 cases) by cell block preparation. Out of 14 cases categorized as 'malignant', smears showed a sensitivity of 64% (09 cases) where as cell block showed a sensitivity of 85% (12 cases). In total, the sensitivity of smears was 62.25% and of cell block was 90.6%. So, an increase of sensitivity by 28.35% on cell block preparation was observed.

TABLE 04: COMPARISON OF SENSITIVITY BETWEEN SMEARS AND CELL BLOCKS

Specimen	Category	Smears	Cell Blocks
FNAC	Malignant (n=21)	13(62%)	19(90%)
	Suspicious of malignancy (n=10)	05 (50%)	08(80%)
	Benign (n=10)	06 (60%)	08(80%)
	Inflammatory (n=07)	04 (57%)	07(100%)
FLUIDS	Malignant (n=14)	09(64%)	12(85%)
	Suspicious of malignancy (n=04)	02(50%)	04(100%)
	Benign (n=04)	03 (75%)	04(100%)
	Inflammatory (n=10)	06(60%)	09(90%)
TOTAL	N = 80	48(60%)	71(88.75)%

Table No. 04 shows sensitivity of smears by 60% and correlated with that of cell block preparation showed a sensitivity of 88.75%. An increase in sensitivity by 28.75% is observed by cell block preparation.

Figure 1a: Cytology of Cervical swelling shows atypical squamous cells in an inflammatory background (Diff- Quick x 400). b. Cell Block technique showing features of keratinizing squamous cell carcinoma (H&E x100)

Figure 2 a. Pleural fluid cytology showing clusters of malignant epithelial cells in glandular pattern (Diff - Quick x 400). b. Cell block preparation showing features of adenocarcinoma (H&E x100)

DISCUSSION

In the present study, all smears and corresponding cell blocks were analyzed and sensitivity was determined. Sensitivity of smears and cell blocks of 48 FNAC specimens was 58.3% from smears and 87.5% from the cell block method, with an increase in sensitivity of 29.2% from the cell block method was observed (Table no.02).

Among the 32 body fluids, the sensitivity by smears was 62.25% and that by cell blocks was 90.6%, with an increase in sensitivity of 28.35% from the cell block method was observed (Table no.03).

Thus the study showed an overall sensitivity of smears as 60% and that of cell blocks as 88.75%. The overall sensitivity of cell block is increased by 28.75% (Table no.04).

The present study correlates the sensitivity of cell blocks with that of smears. Other studies also compared the value of the cell blocks with that of smears. Keyhani - Rofaga et al[8] reported that in a study of 85 cases, 55% of the original smear diagnosis was improved after the cell block examination. The sensitivity of cell blocks varies from 60% to 86%, depending on sampling type and size, type of specimens, and aspiration techniques used. [8,9,10]

Leung SW et al[9] found that all cases with adequate material could be diagnosed on a cell block preparation. The study showed that conclusive diagnostic material was available in 296 (89.4%) cell blocks of total 331cases.[9]

In this study of 80 samples, cell blocks were prepared from residual material after smear preparation. The contribution of cell blocks to the diagnosis also was documented. There is sparse corroborative study in the literature on the routine use of cell blocks, probably due to the different emphasis placed on them in different institutions. Different fixation and processing techniques to maximize the recovery of cellular material from washings, tissue fluids, or fine-needle aspirates with varying degrees of expectation make a valid comparison difficult. Present study adopted conventional method of cell block technique. Other methods of cell block technique, such as cell transfer, various thin layer method, and rapid cell block technique have also been described.

Out of 80 cases, cell block technique shows diagnostic accuracy in 71(88.75%) cases. The present study is in accordance with Barsagade et al who observed diagnostic accuracy of around 87.40%.[11] Maurice et al[12] and Taft et al[13] also compared the cell block technique and smear examination, and concluded that the cell block technique yielded better results than smear.

The advantage of cell block could be interpreted, which included the preservation of architectural pattern like cell ball, three dimensional structures, excellent nuclear and cytoplasmic details and individual cell characteristics (Figure 1 & Figure 2). These observations were in close approximation with the study by Thapar et al.[4]

The study of paraffin sections by using cell block method should be carried out before discarding specimens that are negative for malignant cells by smear examination. Similar conclusions were drawn by Foord and Wetmore et al [5] as they conducted cellular studies of effusions by using smears and paraffin sections. Takagi F et al preferred to study paraffin sections before giving the final diagnosis because it was more accurate and it was easier to demonstrate cellular relationships with the cell block technique.[6]

CONCLUSION

To conclude the present study, an increased diagnostic sensitivity of 28.75% was noted in cell block method. The cell block preparation is simple, low cost, effective method which should be carried out in all laboratories after reporting of conventional smears without discarding the residual tissue which might show important diagnostic advantages and could be kept for special stains and Immunohistochemistry.

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