Evaluation of Cell Block Diagnostics in Intra-Abdominal Lesions with Radiological and Clinical Correlation in a Tertiary Centre in North Karnataka Region

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ABSTRACT

BACKGROUND

Cytological examination of intra-abdominal lesions is crucial in diagnosis, staging, and prognosis of suspected malignancies. Fine needle aspiration cytology (FNAC) is a routine diagnostic modality for diagnosis. FNAC is a reliable, rapid, cost effective procedure and plays an important role particularly in low resource centres. FNAC along with cell block has shown remarkable result in diagnosis of malignant lesions and its sub-classification using various antibody markers.

METHODS

A total of 102 cases of intra-abdominal lesions were studied over a period of one year in this descriptive study. Ultrasound sonography (USG) guided FNACs were done in 89 cases and ascetic / peritoneal fluid analysis in 13 cases. Cell blocks were made in 95 cases and immunohistochemistry (IHCs) were done in 69 cases of suspected / malignant intra-abdominal lesions to confirm the diagnosis.

RESULTS

Out of 102 cases, cytological smears in 89, fluid smears in 13 and cell blocks in 95 cases were available respectively. Most common intra-abdominal organ involved was liver (21.56 %) followed by retroperitoneum (17.65 %) and lymph nodes (14.70 %). The intra-abdominal lesions were classified into non - neoplastic (16.67 %) and neoplastic lesions (83.33 %). Neoplastic lesions (83.33 %) were further sub classified into benign (15.68 %) and malignant (67.65 %). IHC was done in all malignant lesions for confirmation of primary and / or metastasis. Complete concordance between cell blocks and smears was observed in 95 / 102 cases (93.14 %), while discordances were noted in 05 out of 102 cases (06.86 %).

CONCLUSIONS

Cell blocks play a vital role in cytopathology and as an adjunct to the routine cytology smears and body fluids. Cell buttons are cost effective and simple to make without involving much higher technicality. Cell block sections offer advantages with respect to cellular architecture, archival storage and application of IHCs which help in pin-pointing the diagnosis. Hence, cell blocks must be considered to increase the diagnostic yield and efficacy.

KEYWORDS

Cytopathology, Malignant Lesions, Immuno - Histochemistry, Diagnostic Utility

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BACKGROUND

Cytological examination of intra-abdominal lesions is crucial in diagnosis, staging and prognosis of suspected malignancies. Fine needle aspiration cytology (FNAC) is a routine diagnostic modality for diagnosis.[1] FNACs are reliable, rapid, cost effective procedure and plays an important role particularly in low resource centres. Conventional cytological smears can be challenging in diagnosis, as in differentiating reactive cells from malignant adenocarcinoma cells and in differentiating an adenocarcinoma from mesothelioma. With advent of newer technologies like immunocytochemistry (ICC), immunohistochemistry (IHC), flow cytometry, tumour markers and molecular diagnosis, the accuracy of diagnosis has markedly improved in cytopathology. FNACs along with cell block has shown remarkable results in diagnosis of malignant lesions and its sub-classification using various antibody markers.[2,3]

Usually more than one different preparation, including smears, cytospins, liquid - based preparations and / or cell blocks are routinely used for cytomorphological diagnosis.[4] Cell block technique was first introduced in the year 1896 and gained wide acceptance around 1947 as effective diagnostic tool. Cell blocks offers additional advantages over conventional cytological smears as it shows better architecture, available for several sections and archival storage. Special stains, ultrastructural studies, cytogenetics, polymerase chain reactions and immunophenotyping analysis can be done on cell blocks.[5] Hence, FNACs adjunct with cell blocks is the preferred initial modality of diagnosis over a biopsy or resection. The present study was carried out to study how effective and reliable are these cell blocks in adjunct with FNACs and fluids in diagnosis of Intra-abdominal lesions. The objective was to evaluate diagnostic concordance between cytological / fluid smears and cell blocks with regards to malignancy. Diagnostic discordances were also recorded and sub - classified into major and minor which has significant treatment implications and outcome. Another objective was to evaluate and validate the results of cell blocks using IHCs with corresponding histopathological results.

We wanted to study the role of cell blocks in cytopathology in diagnosis of intra-abdominal lesions and evaluate the utility of cell blocks in FNACs and fluids, and also to apply immuno - markers to enhance the diagnosis.

METHODS

The present descriptive study was carried out for a period of one year from January to December 2s019 in Department of Pathology, Mahadevappa Rampure medical college, Kalaburagi in North Karnataka, India. The study included 102 cases where in cytological smears and corresponding cell blocks were prepared in cases of clinically suspicious Intra-abdominal lesions. Clinical details and radiological correlation were done in all cases. USG - guided FNACs in 89 cases and fluid cytology in 13 cases were done respectively. Cell blocks were available in 95 cases. Cases with inadequate material on both FNACs / fluids and cell blocks were excluded from the study. Smears were stained with Papanicolaou (PAP) and May Grunwald Giemsa (MGG) stain. Remaining material in the needle hub and fluids were submitted for cell blocks. They were made using plasma thrombin / thrombin clot method. The material was submitted in 70 % ethanol and kept for 20 minutes. The sample was centrifuged for 20 minutes at 2700 rotations per minute. Plasma and thrombin were added into the centrifuged pellet. This step enhances the mesh cellular material in the clot. Continuous agitation was done during the procedure to maintain uniform distribution of cells and to prevent uneven concentration of cells in the fibrin meshwork. The cell blocks were subjected to haematoxylin and eosin stains, special stains (PAS, Z - N stain etc) and IHCs were performed wherever necessary.

Statistical Analysis

Sample size estimation was done by using G Power software (version 3.0). Sample size was estimated for chi square test for variance difference.

Statistical analysis was done by using SPSS software (version 20.0). Data was spread on Excel sheet and statistical parameters like mean and standard deviation were calculated. For qualitative data analysis, chi square test was applied and Fischer's - exact test was applied for statistical significance. For quantitative data analysis, t - test was applied for statistical significance. If P - value was < 0.05, it was considered as significant. Accuracy, sensitivity, specificity, positive predictive value, negative predictive value of cytological and cell block correlation were calculated.

RESULTS

A total of 102 cases with suspected intra-abdominal lesions were studied over a period of one year. The average age was 49.04 years (standard deviation is 19.87) with male to female ratio of 1.4: 1. Out of 102 cases, cytological smears and corresponding cell blocks were available in 82 cases (93.13 %). In the remaining 07 cases, only smears were available as cell blocks couldn't be made due to scant material. Remaining 13 cases comprises of smears made up of ascitic fluid with corresponding cell blocks. Probable clinical diagnosis and radiologic imaging studies were evaluated and correlated in all cases. IHC staining was performed in 69 out of 102 cases (suspected malignant, malignant – primaries / or metastasis respectively).

The most common intra-abdominal organ involved was liver (21.56 %) followed by retroperitoneum (17.65 %) and lymph nodes (14.70 %) respectively. The intra-abdominal lesions were classified into non-neoplastic (16.67 %) and neoplastic lesions (83.33 %) respectively. Neoplastic lesions (83.33 %) were further sub classified into benign (15.68 %) and malignant (67.65 %). IHCs were done in all malignant lesions for confirmation of primary and / or metastasis. Concordance and discordance studies were evaluated between cytological / fluid smears and cell blocks with

regards to neoplastic lesions. Complete concordance between cell blocks and smears was observed in 95 out of 102 cases (93.14 %), while discordances were noted in 07 out of 102 cases (06.86 %) (Table – 1). All cases were compared with histopathology (excised / biopsy specimens) as gold standard.

In this study, seven discordant cases were reported on cytology when compared with cell block. Two cases were reported as malignant lesions on FNAC (false positive) which turned out to be benign on cell block. One case of metanephric adenoma was reported as papillary renal cell carcinoma on FNAC. The diagnosis was confirmed on cell block and supported by histopathology and showed strong positivity for WT1 (FIG - 1). Another case of lymphangioma was reported as suspicious for malignancy on FNAC which was confirmed on cell block and on histopathology as lymphangioma with giant cell reactions (Fig - 2).

Similarly, five cases were reported as benign on FNAC (false negative) which turned out malignant on cell block. One case of mucinous cystadenocarcinoma of ovary showed only mucin on FNAC. Another case of transitional cell (urothelial) carcinoma diagnosed on cell block and histopathology was given as inflammatory lesion on FNAC (Fig - 3). One case of uterine leiomyosarcoma was reported as benign spindle cell lesion on FNAC. Similarly, signet ring cell adenocarcinoma of colon showed only haemorrhagic material on FNAC and one case of metastatic poorly differentiated carcinoma showed only necrotic material on FNAC.

Further, the cases were compared between cyto - pathological and clinical / radiological diagnosis, and discordance was assessed (Table - 2). Study observed eleven discordant cases between radiological diagnosis and cell block / histopathological diagnosis. One case of mesenteric cyst was diagnosed as fibroma on cell block and confirmed with reticulin stain (Fig - 4). Another case of ovary was given as cystadenocarcinoma / teratoma which on cell block shows signet ring cell adenocarcinoma (Krukenberg tumour) (Fig - 5). Similarly, two cases of kidney were given as renal cell carcinoma on radiology which turned out to be chronic pyelonephritis and xanthogranulomatous nephritis with cholesterol clefts and chronic inflammatory cells on cell block respectively. Similarly, one case of retroperitoneum leiomyoma / GIST was diagnosed as myeloid metaplasia on FNAC and was confirmed on cell block with presence of megakaryocytes. One case of paraumbilical cyst / lipoma on radiology was diagnosed as metastatic poorly differentiated carcinoma on cytology. One case of pseudo pancreatic cyst on radiology turned out to be pancreatic adenocarcinoma on FNAC and cell block. In the present study, statistical parameters like sensitivity, specificity, diagnostic accuracy, positive predictive value and negative predictive value were calculated for cytological smears and cell block independently and then of combined smears and cell block respectively. As depicted in the Table - 3, the combined efficacy of cytological smears with cell block is of maximum percentage. The increased diagnostic accuracy is also because of use of IHCs on cell blocks.

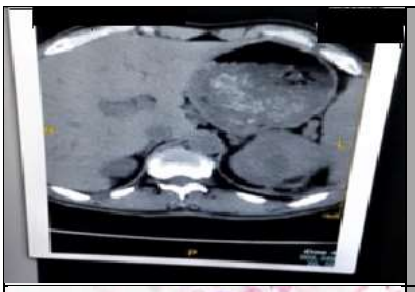

Figure 1A. MRI Shows 11.2 x 9.2 cms Lesion in Upper Pole of Right Kidney Possibly Renal Cell Carcinoma

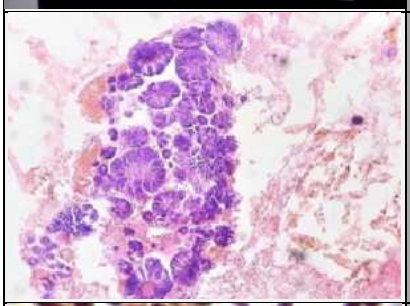

Figure 1B. Cell Block - Cells Arranged in Tubular Pattern with Areas of Haemorrhage and Necrosis

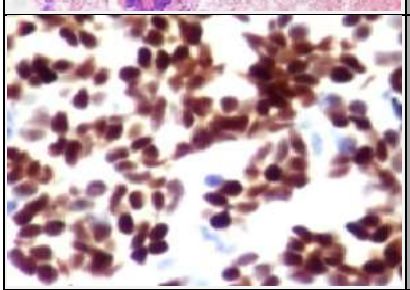

Figure 1C. IHC with PAX - 8 Shows Strong Positivity

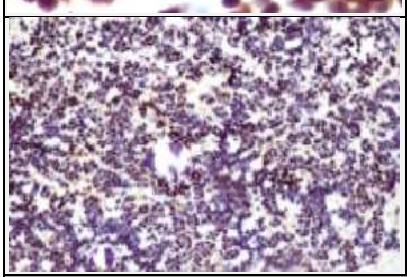

Figure 1D. IHC with WT - 1 Positive

Figure 1. Metanephric Adenoma

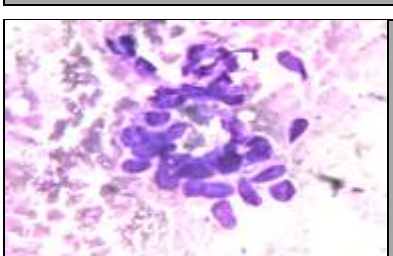

Figure 2A. FNAC Shows Cells Suspicious of Malignancy

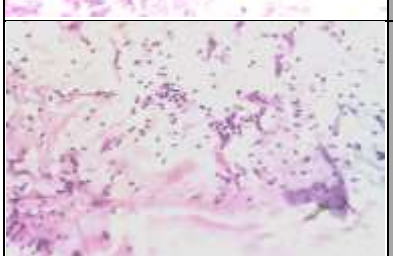

Figure 2B. Cell Block Shows Lymphocytes with Lymph

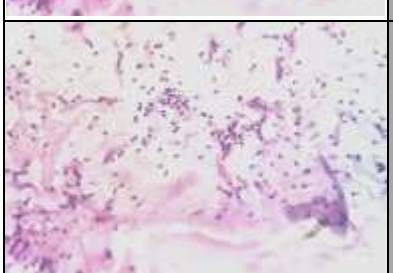

Figure 2C. (H & E) Lymphatic Cyst with Surrounding Giant Cells Reaction

Figure 2. Lymphangioma

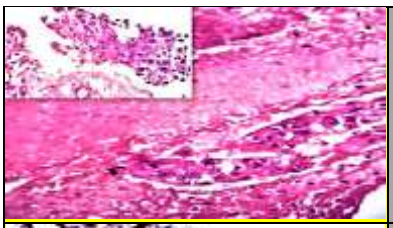

Figure 3A. Cell Block Shows Pleomorphic Cells in Haemorrhagic Background, Inlet Shows Transitional Tumour Cells

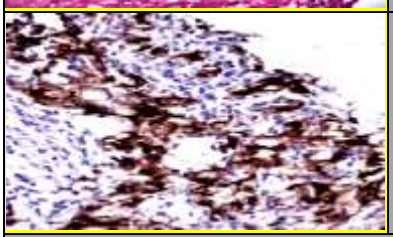

Figure 3B. Tumour Cells are Cytokeratin - 7 Positive

Figure 3. Urothelial Carcinoma

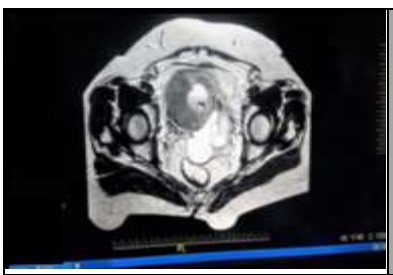

Figure 4A. CT Scan – Well Defined Non-Enhancing Cystic Lesion Measuring 17 × 13 cms – Intraperitoneal Mesenteric Cyst

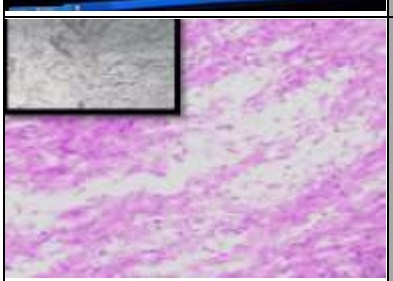

Figure 4B. Cell Block Thin Spindle Cells in Whorl Pattern with Bland Fusiform Nuclei with Oedematous Stroma, Inlet Showing Reticulin Stain on Cell Block

Figure 4. Fibroma

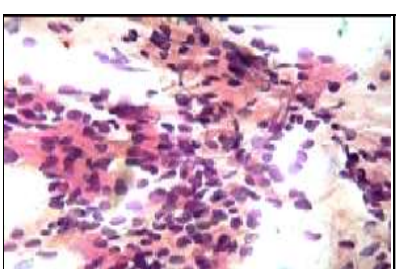

Figure 5A. FNAC (PAP) - Loosely Cohesive Clusters of Cells, Pleomorphic Nuclei, Coarse Chromatin and Prominent Nucleoli B

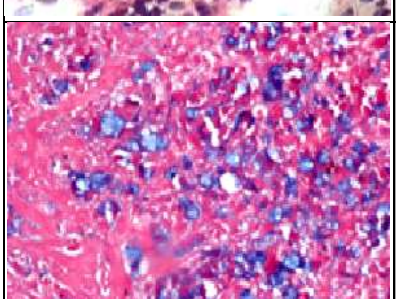

Figure 5B. Cell Block – Alcian – PAS Positive

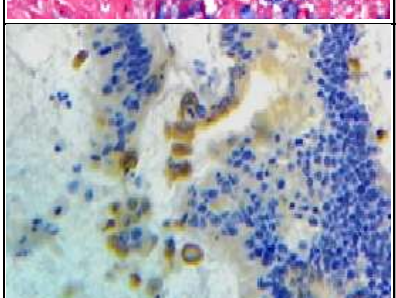

Figure 5C. CK - 20 Positivity on Cell Block

Figure 5. Krukenberg Tumour

Sl. No.	Organ	FNAC Diagnosis	Cell Block Diagnosis	Statistical Analysis
1	Kidney	Papillary renal cell carcinoma	Metanephric adenoma	False positive
2	Pelvis (inguinal region)	Suspicious of malignancy	Lymphatic cyst	False positive
3	Ovary	Only mucin	Mucinous adenocarcinoma	False negative
4	Kidney	Inflammatory lesion	Transitional cell carcinoma	False negative
5	Uterus	Benign spindle cell lesion	Leiomyosarcoma	False negative
6	Colon	Only haemorrhage	Signet ring cell adenocarcinoma	False negative
7	Para-umbilical region	Only necrotic material	Metastatic poorly differentiated carcinoma	False negative

Table 1. Cases Showing Discordance between Cytological Smears and Cell Block

Sl. No.	Organ	Clinical / Radiological Diagnosis	FNAC Diagnosis	Cell Block Diagnosis
1	Liver	Hepatocellular carcinoma?	Hepatic adenoma	Hepatic adenoma
2	Ovary	Mesenteric cyst?	Benign ovarian cyst	Fibroma
3	Ovary	Ovarian tumour – dysgerminoma?	Metastatic ovarian tumour	Krukenberg tumour
4	Ovary	Ovarian cystadenocarcinoma / teratoma?	Serous cystadenocarcinoma	Brenner tumour with serous cystadenocarcinoma
5	Kidney	Oncocytoma / renal cell carcinoma	Inflammatory lesion	Chronic pyelonephritis
6	Kidney	Renal cell carcinoma?	Chronic inflammatory lesion	Xanthogranulomatous pyelonephritis
7	Uterus	Broad ligament fibroid / dermoid cyst?	Benign spindle cell lesion	Lipoleiomyoma
8	Pancreas	Pseudo pancreatic cyst?	Adenocarcinoma	Adenocarcinoma
9	Pelvis	Gastrointestinal stromal tumour?	Metastatic adenocarcinoma	Metastatic adenocarcinoma
10	Retro-peritoneum	Leiomyoma / gist / portal gastropathy	Myeloid metaplasia	Myeloid metaplasia
11	Paraumbilical Region	Lipoma / cyst?	Metastatic poorly differentiated carcinoma	Metastatic poorly differentiated carcinoma

Table 2. Cases Showing Discordance between Clinical / Radiology and Cytology / Cell Block Diagnosis

Diagnostic Modality	Sensitivity	Specificity	Diagnostic Accuracy	Positive Predictive Value	Negative Predictive Value
FNAC / fluid smear	90.90 %	87.50 %	90.14 %	96.15 %	73.68 %
Cell block	98.18 %	93.75 %	97.18 %	98.18 %	93.75 %
Combined smears and cell blocks	98.27 %	100 %	98.59 %	--	--

Table 3. Statistical Analysis of Cytological Smears and Cell Block

DISCUSSION

In cytopathology, cell block is a routine diagnostic modality along with FNACs and fluid smears.[1,6] First introduced in 1896 and gained popularity as diagnostic modality in 1947. Cell blocks have undergone various alterations till now to improve the efficacy with basic principle remaining the same. In recent decades, with advent of IHCs, immunocytochemistry, molecular testings and targeted based approach for neoplastic lesions, role of cell blocks and its application has gained wide applications.[3,7]

Cell blocks can be made from cytological procedures and any fluids (cervical scrapes, serous fluids, urine, exfoliated cells etc). The diagnostic efficacy of cell block is same as that of cytological smears and liquid based cytology or higher when immunocytochemistry / IHCs applied. Various studies

have shown that the role of cell blocks as adjunct to cytological smears and overall improvement in the diagnostic efficacy.[8,9] A good cell block preparation depends on the presence of adequate cellular aggregates after specimen centrifugation.[10] There are various methods for cell block preparations each having its own advantages and disadvantages. Various methods include normal saline, needle rinse method, Shandon T M Cytoblock TM method, tissue coagulum clot (TCC) method, plasma thrombin or thrombin clot method, agar embedding, collodion bag methods, automated cell block method and so on.[5,11]

In this study, plasma thrombin / thrombin clot method was used, with cell free thromboplastin to avoid errors. Continuous agitation was done for uniform distribution of cells during the process. This technique is simple, cost effective with maximum cytomorphology and clean background.[5,12] Optimal results were obtained on cytogenetics, molecular studies and IHCs. The technique can be used for any cell suspensions not fixed in formalin, FNACs and LBCs.[13] Proper precautions have to be taken to avoid contamination of plasma / thrombin.[14] Cell blocks are the preferred choice for immunocytochemistry as they are comparable with surgical biopsies, can use the same control slides, provide the best milieu for morphological interpretation and are without background staining.[15] The preparation of cell blocks as an adjunct for all cytological specimens, particularly fluid specimens, should be considered as a standard practice especially in situations when biopsy is not available for molecular analysis.[16] Cellblocks made from aspiration of specimens shows more tumour cells than background cells in comparison to biopsy.[17]

CONCLUSIONS

Cell blocks play a vital role in cytopathology and as an adjunct to the routine cytology smears and body fluids. Cell buttons are simple to make without involving much higher technicality, are cost effective and limitations of FNACs can be overcome. Cell blocks are superior to smears in showing positivity in higher percentage of cases in suspected and malignant lesions due to reliable applications of immunomarkers. The study was done to show the role of cell blocks in adjunct to cytological smears as early diagnostic modality in intra-abdominal lesions. With recent trends and improved techniques, sensitivity and specificity of this diagnostic modality could be improved.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

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