

Cell Block Technique: An Effective Tool in Diagnostic Cytopathology

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Abstract:

Background: Surgical biopsies have always been the mainstay for a precise diagnosis of superficial or deep neoplastic lesions, however fine needle aspiration is safe, reliable & minimally invasive. Sometimes fine needle aspiration (FNA) does not yield sufficient information for precise diagnosis and the risk of false negative or intermediate diagnosis always exists. Hence to make the best possible use of aspirate, smear should be combined with cell block (CB) preparation that in turn gives a better morphological & histological diagnosis.

Materials and Methods: A total of 148 patients were included in the study, which were subjected to fine needle aspiration. In addition to the routine smears, CBs were prepared from the residual tissues for all cases & its diagnostic accuracy was analyzed. Further, the use of CBs for immunohistochemistry (IHC) was also established in difficult cases.

Results: Out of 148 cases, adequate material was obtained on fine needle aspiration cytology (FNAC) in 130 cases (87.84%) & on CB in 120 cases (81.08%). Sensitivities of FNAC smears & CB were 94.90% & 98.92% respectively. However, sensitivity of combined FNAC smears & CB was 99.02%. With the combined use of smear & CB, the diagnostic accuracy of the neoplastic lesions approaches to 98.33% & also significantly improves diagnosis & sub-typing of malignancies.

Keywords: Cell block, Fine needle aspiration cytology, Immunohistochemistry

I. Introduction

Fine needle aspiration cytology (FNAC) being safe, minimally invasive and cost-effective method, is widely practiced and has proven to be a very effective means of obtaining tissue from different body sites for diagnosis. However sometimes it does not yield sufficient information for precise diagnosis and the risk of false negative diagnosis always exists.^{1, 2} Disadvantages of FNAC include availability of limited material, lack of tissue architecture, overcrowding of cells and paucity of representative cells.³ In order to overcome these problems, Cell Block (CB) technique has been resorted to make the best use of the available material. This method uses histologic technique for processing & thus, offers one major advantage that multiple sections of the same material may be processed for routine stains as well as for special stains & IHC. The advantage of CB is recognition of histologic pattern of the disease that cannot be reliably identified in smears.⁴

In this study we evaluated the role of CB as a useful adjunct to smears for establishing a more definitive cytopathological diagnosis and for its utility in IHC.

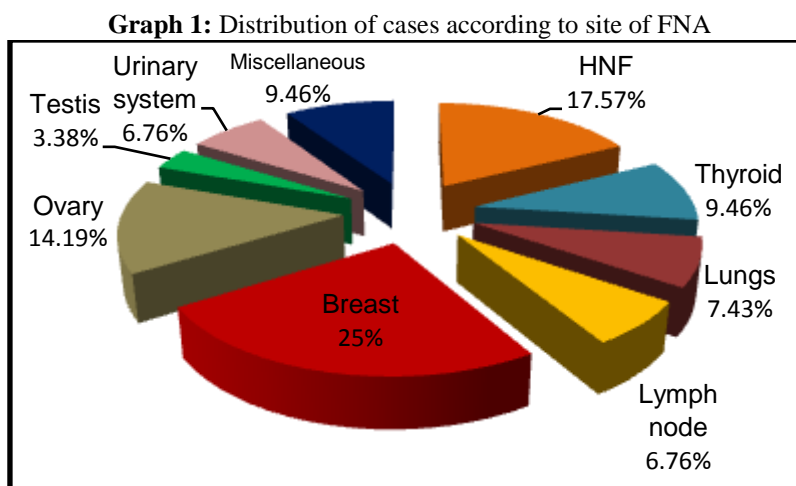
II. Materials And Methods

The present study was a hospital based cross-sectional study carried out in the department of pathology, NKP Salve Institute of Medical Sciences & Research Centre, Nagpur, Maharashtra, over a period of 2 years from November 2013 to October 2015. Patients with clinically suspected neoplastic lesions or diagnosed as cases of malignancy in FNA smears obtained from various sites were included. FNAC was performed using 21, 22 or 23 gauge needles attached to the 10 ml or 20 ml disposable syringes under aseptic condition. Smears were made, and immediately alcohol fixed in 95% ethyl alcohol for Papanicolaou stain & hematoxylin and eosin (H & E) stain. For cell block preparation, the remaining material in the aspirating syringe was fixed in freshly prepared Nathan alcohol formalin substitute (NAFS) consisting of 9 parts of 100% ethanol and 1 part of 40% formaldehyde. It was then centrifuged at 2500/rpm for 10 minutes.

The formed cell button was allowed to fix in NAFS for 30 to 45 minutes.⁵ After re-centrifugation, the cell button was processed as routine biopsy specimen and stained with H & E stain. Histopathology sections were also stained with H & E stain. FNAC smears and CB sections were examined separately for cellularity and reported. The diagnoses were confirmed by histopathology. IHC was done in feasible cases. Considering the cost of IHC, we did the judicious use of IHC markers on CB sections according to location of the tumor and its morphology on CB and/ FNAC smears. The data was analyzed using statistical software SPATA (version 13.0). The Chi-Square test was used for statistical analysis. A p-value of <0.05 was considered to be statistically significant.

III. Results

A total of 148 cases were included in the study of which FNAC smears, cell blocks and histopathology specimens were available. Graph 1 shows distribution of cases according to the site of FNA.



The age of the patients ranged from 2 years to 75 years with a mean of 44.74 years. Majority of the cases were found in 41 to 50 years of age. Out of 148 cases, 67 were males and 81 were females (M: F ratio-1:1.2). Table no. 1 shows adequacy rates on FNAC smears and cell block.

Table 1: Distribution of adequacy between FNAC smears & cell blocks

		FNAC		Total
		Adequate	Inadequate	
Cell Block	Adequate	115 (77.70%)	5 (3.38%)	120(81.08%)
	Inadequate	15 (10.14%)	13 (8.78%)	18(12.16%)
	Total	130(87.83%)	28(18.91%)	148

Out of 26 cases from HNF, 22 cases were adequate on FNAC smears. Of these 22 cases, three cases of oral cavity showed high grade dysplasia on FNAC smears. These three cases were diagnosed as squamous cell carcinoma (SCC) on CB. There were two cases of mucoepidermoid carcinoma, which were diagnosed by CB in both the cases, whereas in only one case by FNAC. Out of 14 cases from thyroid, two cases were diagnosed as anaplastic carcinoma on CB, whereas only one case was diagnosed as anaplastic carcinoma on FNAC. One case revealed only blood on FNAC smears (fig.1), which was diagnosed as follicular variant of papillary carcinoma on CB showing optically clear nuclei, nuclear grooving and follicular arrangement of tumor cells (fig.2).

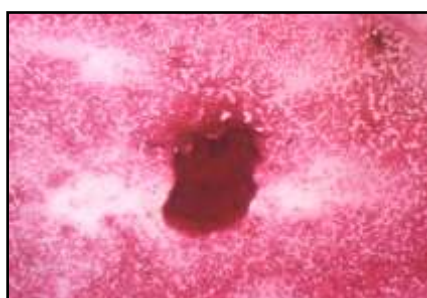


Figure 1: Photomicrograph showing cells entrapped in blood clot on FNAC smear (H&E, X40)

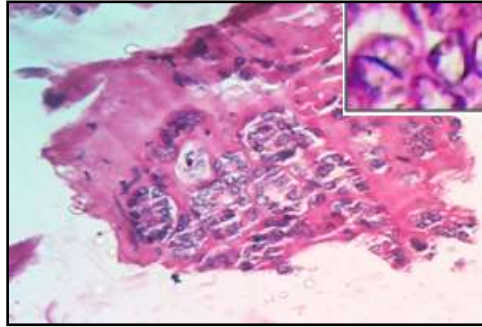


Figure 2: Photomicrograph showing tumor cells with optically clear nuclei, arranged in follicular pattern on cell block (H&E, X400). Inset showing nuclear grooving

A total of 11 cases of lung were enrolled in our study. 10 cases were adequate on FNAC whereas nine were adequate on CB. Two cases were diagnosed as non small cell lung carcinoma (NSCLC) on FNAC smears (fig.3). Out of these two cases, one case turned out to be SCC (fig.4) & the other one adenocarcinoma on CB which were later confirmed by histopathology.

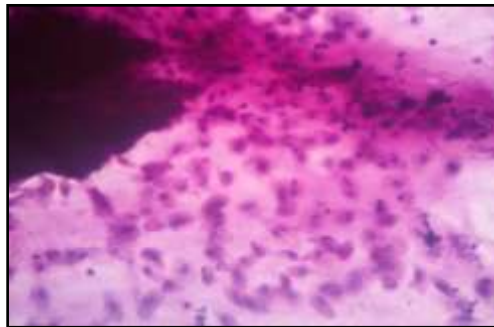


Figure 3: Photomicrograph showing tumor cells with moderate amount of cytoplasm & associated acute inflammation on FNAC smear (H&E, X100)

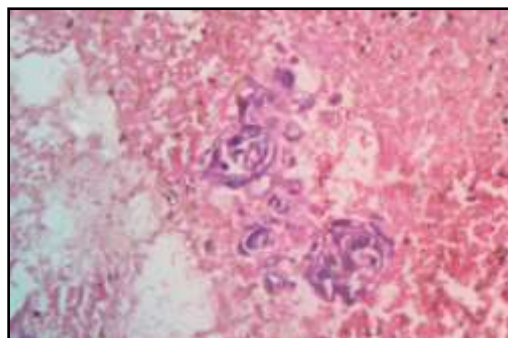


Figure 4: Photomicrograph showing keratin pearl formation in the background of necrosis on cell block (H&E, X100)

Out of 10 cases of lymph nodes, two cases on FNAC smears & three cases on CB were inadequate. One case which was diagnosed as poorly differentiated epithelial malignancy on FNAC, later confirmed as metastatic deposits of SCC on CB. One case of metastatic deposits of adenocarcinoma showed few cells entangled in blood clot while other was inadequate on FNAC. The diagnosis of both of which were possible on CB. However in case of Hodgkin lymphoma, cellular details were better observed on FNAC smear than CB (fig.5 & 6). This is because of the shrinkage artifact due to tissue processing. Thus, although architectural patterns are better appreciated on CB, cytomorphology is excellent on FNAC smears.

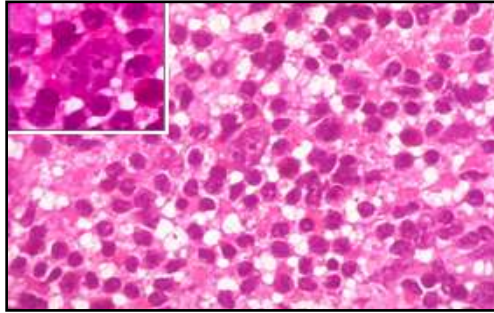


Figure 5: Photomicrograph showing binucleate Reed-Sternberg (RS) cell in a background of mainly small lymphocytes on FNAC smear (H&E, X400). Inset showing RS cell

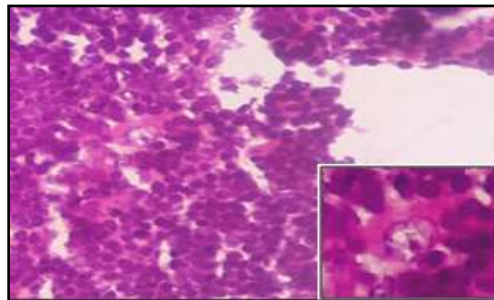


Figure 6: Photomicrograph showing RS cell with lymphoid cells on cell block (H&E, X400). Inset showing RS cell

Out of 37 cases of breast, 34 were diagnosed on FNAC & 32 were diagnosed on CB. One out of 25 cases of infiltrative ductal carcinoma (IDC) (fig.7) turned out to be infiltrative lobular carcinoma on CB and histopathology which showed Indian file arrangement of tumor cells (fig.8). Another case which was suspicious of malignancy on FNAC was later confirmed as IDC on CB & histopathology.

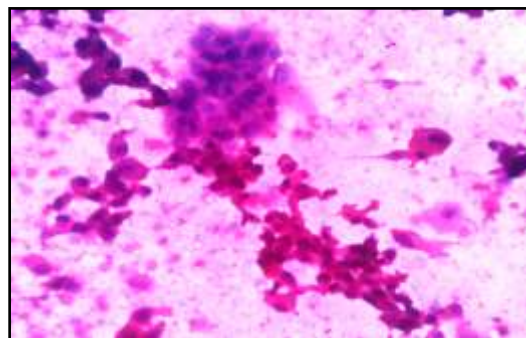


Figure 7: Photomicrograph showing clusters of ductal cells showing crowding and nuclear atypia on FNAC smear (H&E, X100)

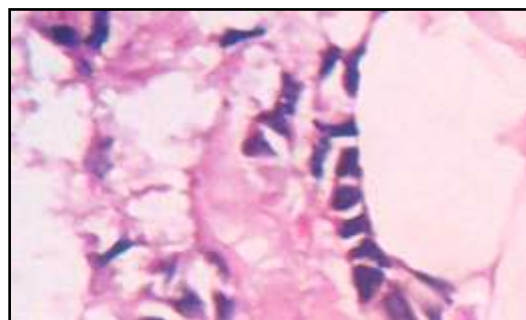


Figure 8: Photomicrograph showing Indian file arrangement of tumor cells on cell block (H&E, X400)

21 cases of ovary were included in this study. One case of borderline mucinous tumor was diagnosed incorrectly as benign cystadenoma on FNAC, which was correctly diagnosed on CB and later confirmed by

histopathology. Two cases which were suspicious of malignancy on FNAC later turned out to be serous papillary cystadenocarcinoma & mucinous cystadenocarcinoma respectively on CB. In case of metastatic signet ring cell carcinoma, signet ring cells were identified on both FNAC (fig.9) & CB (fig.10). But correct identification of primary site i.e. gastrointestinal tract was done on IHC on CB sections, as tumor showed strong diffuse immunoreactivity for CK20 (fig.10).

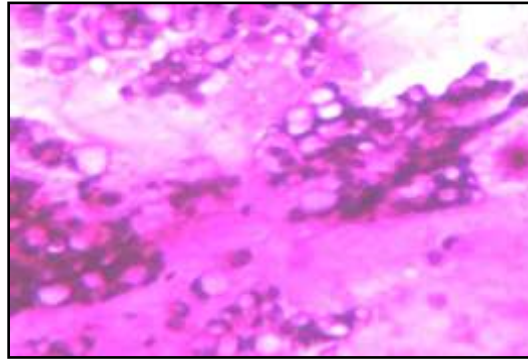


Figure 9: Photomicrograph showing signet ring cells on FNAC smear (H&E, X100)

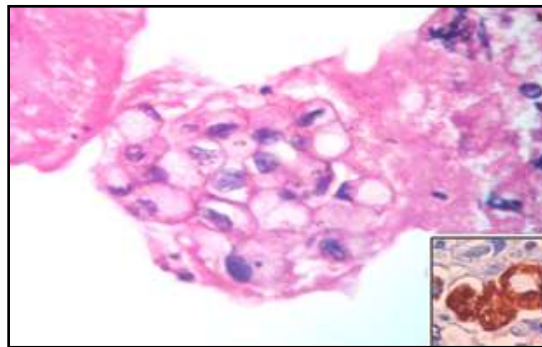


Figure 10: Photomicrograph showing cluster of signet ring cells on cell block (H&E, X400). Inset showing strong diffuse immunoreactivity for CK20

Out of five cases of testicular tumors, one case of embryonal carcinoma was inadequate on CB and it was diagnosed as malignant germ cell tumor on FNAC. Both of these diagnoses did not correlate with histopathology findings.

Total 10 cases of kidney & urinary bladder were included in the study. FNAC smears from Wilm's tumor showed only blastemal component (fig.11), so it was diagnosed as round cell tumor, whereas, on CB, epithelial component in the form of tubule formation was identified in addition to some small round cells (fig.12). Further IHC had added advantage in this case as it showed nuclear positivity for WT1 (fig.12-inset) & negativity for LCA. Another case of papillary renal cell carcinoma was diagnosed on CB, as it showed tumor cells arranged in papillary pattern.

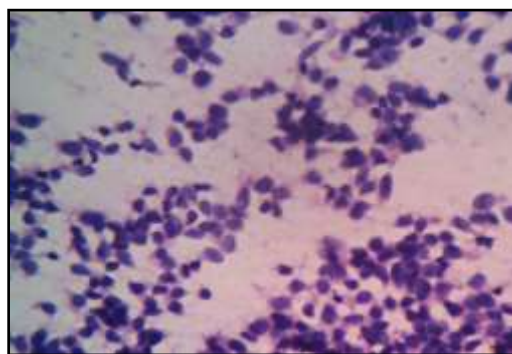


Figure 11: Photomicrograph showing loose clusters of small round cells on FNAC smear (Pap, X100)

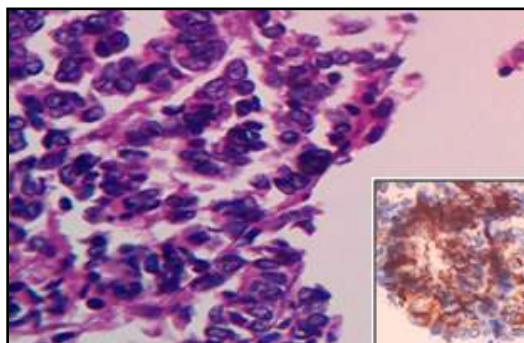


Figure 12: Photomicrograph showing epithelial component i.e. tubule formation on cell block (H&E, X400). Inset showing nuclear immunoreactivity for WT1

Out of 14 miscellaneous cases, two cases of neurofibroma & one case of schwannoma were diagnosed as benign spindle cell tumor on FNAC, which were diagnosed as benign nerve sheath tumor on CB. In case of alveolar rhabdomyosarcoma, small round cells were seen on FNAC (fig.13) but it was diagnosed by IHC done on CB sections which showed nuclear positivity for MyoD1 (fig.14) & Desmin and negativity for CD99 in addition to the small round cells with fibrous septa seen on H & E stain on CB. Another case of malignant melanoma was diagnosed as epithelial malignancy on FNAC which was later confirmed by IHC on CB, as it showed focal positivity for MART1 & both nuclear and cytoplasmic positivity for S100.

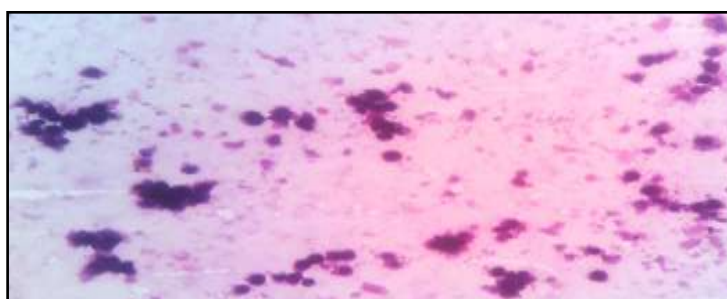


Figure 13: Photomicrograph showing small round cells on FNAC smears (H&E, X40)

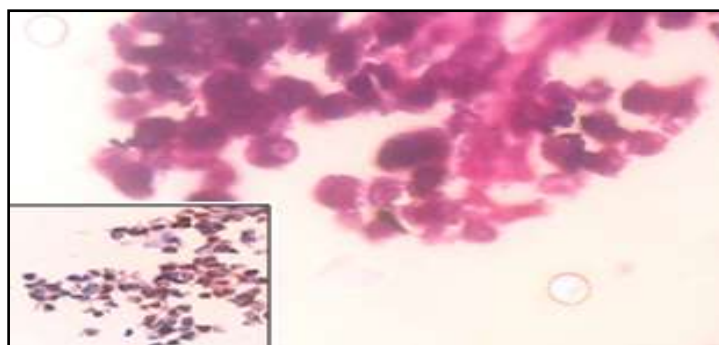


Figure 14: Photomicrograph showing small round cells attached to fibrous septa on cell block (H&E, X400). Inset showing nuclear immunoreactivity for Myo D1

There was 96.67% concordance of diagnosis between CB & histopathology. Table no. 2 shows the sensitivity, specificity & accuracy of FNAC & CB. The sensitivity was increased to 99.02% when FNAC smears were combined with cell blocks. Table no. 3 shows statistical analysis of results on FNAC & combined FNAC with cell block. It showed that when FNAC smears were combined with cell block, there was increase in the diagnostic yield as compared to FNAC smears alone (p value= 0.0006).

Table no.2: Statistical indices of FNAC smears & cell blocks

Indices	FNAC smears	Cell block
Sensitivity	94.90%	98.92%
Specificity	93.75%	96.30%
Accuracy	94.61%	98.33%

Table no. 3: Statistical analysis

Cases	FNAC	FNAC+ Cell block	Total
Diagnostic	104	127	231
Non-diagnostic	26	8	34
Total	130	135	265
Chi Square	11.729		
Degree of freedom	1		
p value	0.0006, HS		

IV. Discussion

FNAC is a simple, safe and reliable method for the purpose of cytological diagnosis. However, its diagnostic efficiency is surpassed by its failure rate that has been mentioned by many to be as high as 45%.⁶ An inconclusive diagnosis on FNAC may be due to poor spreading, air drying artifact and presence of thick tissue fragments despite aspiration of adequate material.⁷⁻⁹ Further in case of highly vascular organs like thyroid, it often yields hemorrhagic aspirate which reduces its diagnostic efficacy.

Bahrenburg was the first to introduce cell block technique almost a century ago, where he used this technique as an alternative to preparation of conventional smear from ascitic fluid.¹⁰ Since then the cell block has been prepared from various specimens like urine, pleural, peritoneal, ascitic and pericardial fluid as well as from tissue scrapings and hemorrhagic aspirates.^{11,12}

Different methods of cell block preparation have been reported.^{5, 13} Basnet S et al. studied total of 49 cases in 2 years, where FNAC smears and cell block preparation were done for all the malignant cases. With combined use of both the techniques, the diagnostic accuracy of tumor diagnosis approached to 100%. Accuracy of cell block diagnosis was found to be 95.91% as against 91.8% of conventional smears.²

In our study, Nathan alcohol formalin substitute (NAFS) i.e. 10% formal-alcohol was used for fixation. This is because the formalin causes proteins to be cross-linked and a gel would be formed, which could not be dissolved in any material used for processing, thus minimizing the cell loss. The advantage of alcohol based fixative is better preservation of antigenicity and cytomorphological features.^{11, 14} Another advantage is that it uses simple chemicals that are readily available in the laboratories. This fixative was also used in the study of Grandhi B et al.¹⁵ and Mohamed D et al.¹⁶

In our study, the numbers of cases with adequate FNAC smears were more as compared to the cell blocks in our study. However, it should not be considered as an evidence of superior diagnostic value of FNAC smears because FNAC smears were prepared first from the aspirate and then the remaining material in the needle hub and syringe was used for cell block preparation. Although cell blocks were inadequate in 28.58% cases from thyroid, they provided additional information in other cases where FNAC smears were inadequate and showed only blood clot.

In correlated cases, though diagnosis on smears and cell blocks were same, cell block showed architectural patterns and additional diagnostic information, which confirmed diagnosis. In adenocarcinoma, glandular formation and in case of squamous cell carcinoma, keratin pearls were better appreciated on cell blocks than FNAC smears. In cases of ductal carcinoma of breast, the cell block showed infiltration of tumor cells in the stroma suggesting an infiltrative pattern of ductal malignancy whereas in case of infiltrative lobular carcinoma, Indian file arrangement of tumor cells was appreciated on cell block sections. Thus, cell blocks gave better idea of tissue architecture.

In our study, FNAC smears & cell blocks showed concordant diagnosis in 97 cases (84.35%) and discordant diagnosis in 18 cases (15.65%). In these 18 cases cell blocks gave additional information and more definite diagnosis. 96.67% cases showed concordant diagnosis between cell blocks and histopathology.

In five cases, FNAC smears were inadequate, while cell blocks were adequate. Out of these five cases, four cases showed only blood on FNAC smears while cell block showed diagnostic material. This may be due to entrapment of tissue fragments in blood clot which was further processed for cell blocks. These included two cases from thyroid, which later diagnosed as follicular variant of papillary carcinoma and anaplastic carcinoma. One case from the lymph node and one from submandibular salivary gland also showed cells entangled in blood clot on FNAC smears. Cell block in case of lymph node revealed metastatic deposits of adenocarcinoma, which showed cell balls with intracytoplasmic mucin whereas in case of submandibular gland, it revealed mucoepidermoid carcinoma showing squamoid, intermediate and few mucus producing cells. One case from ovary revealed only occasional cyst macrophages in proteinaceous background on FNAC smears, whereas the diagnosis of benign mucinous cystadenoma was given on cell block.

In the present study, sensitivity & diagnostic accuracy were high in cell blocks in comparison to the smears with the sensitivities of 94.90% in the smear and 98.92% in the cell block. Diagnostic accuracy of FNAC smears & cell blocks were 94.61% & 98.33%, respectively. In this study, simultaneous use of cell block technique and smear examination increased the sensitivity to 99.02%.

According to Ikeda K et al.¹⁸, compared with the other conventional techniques, the cell block technique is a more sensitive model.

In present study, correct diagnosis was possible in 127 cases (94.07%) when FNAC smears were used along with cell blocks, which was obtained in only 104 cases (80%) on FNAC smears alone. Thus, overall diagnostic yield was 14.07% in present study.

One of the benefits of combining cell blocks with smears is the ability to see the histologic correlates of cytologic findings. Some cytologic criteria cannot be translated into histologic criteria so complementary nature of cell blocks and smears would help avoid the pitfalls of using either cytology or histology alone.¹⁹

In the present study, the observed advantages of cell block technique were-

1. Less cellular dispersion and concentration of cellular material which would help in microscopic examination.
2. Preserved tissue architecture.
3. Multiple sections could be taken for specific stains whenever needed.
4. By using residual material in the needle hub and syringe, maximum utilization of all available material would be ensured.
5. Easy storage of fine needle aspirates as cell blocks.

In the present study, the disadvantages of the technique observed were-

1. As compared to FNAC smears, cellular details were less clear in cell blocks.
2. Increased processing time and hence delay in diagnosis.

V. Conclusion

Cell block technique allows the recovery and processing of minute amounts of cellular material and facilitates the better classification of tumor when reviewed along with cytological smears. The method is simple to perform and no expertise is required to handle the specimen. Cell block bridges the gap between cytology and histopathology. FNAC smears and cell blocks complement each other and our results indicate that combining both the tests improves the diagnostic yield & facilitates accurate diagnosis.

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